THESIS

QUANTIFICATION OF VARIATION IN BIOLOGICAL INPUT MATERIALS AND ITS EFFECT ON MANUFACTURE AND PROCESS CONTROL

Submitted by

Jamie A. Thurman-Newell

Wolfson School of Mechanical, Electrical and Manufacturing Engineering

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Doctoral Committee:

Supervisor: David J. Williams

Supervisor: Jon N. Petzing

Internal Examiner: Nicholas Medcalf

External Examiner: Julian Braybrook

Abbrevations

ATMP: Advanced Therapy Medicinal Products
CD: Cluster of Differentiation
CD34: A cell surface marker attributed to Haematopoietic Progenitor Cells
cCD34: collected CD34+ cells, prior to processing
CLIA: Clinical Laboratory Improvement Amendments
CMCF: Cell Manipulation Core Facility (a laboratory at DFCI)
CP: Process Capability (C_P), indicator of process capability
CPK: Process Capability Index (C_{PK}), adjustment of CP for the effect of a non-centred distribution
CTQ: Critical to Quality (attributes)
DFCI: Dana Farber Cancer Institute
GCSF: Granulocyte Colony Stimulating Factor
GMP: Good Manufacturing Practise
cGMP: US GMP ('current' GMP)
GvHD: Graft versus Host Disease
FDA: US Food and Drug Administration
HPC: Haematopoietic Progenitor Cell
HSCT: Haematopoietic Stem Cell Therapy
MHRA: Medicines and Healthcare products Regulatory Agency
MNC: Mononuclear Cells
NHSBT: National Health Service Blood and Transplant
QA: Quality Assurance
QC: Quality Control
Raw Material: Additional components added to the therapeutic product such as anti-coagulants
RBC: Red Blood Cell
RM: Regenerative Medicine
SOP: Standard Operating Procedure
SROC: Spearman's Rank Order Correlation
Starting Material: The active, biological therapeutic component of the product
tCD34: transplanted CD34+ cells, post-processing
TNC: Total Nucleated Cells
WBC: White Blood Cell

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Publications and Presentations

Publications

The following are peer-reviewed publications as result of original and novel research presented in this thesis.

Thurman-Newell, J. A., Petzing, J. N. and Williams, D. J. (2013), Quantification of variation in biological input materials and its effect on clinical outcome and manufacture. Presented at the Fourth Doctoral Training Centre Joint / Conference 2013, 12th July 2013, Sheffield University. (https://dspace.lboro.ac.uk/2134/13278)

Thurman-Newell, J.A., Petzing, J.N. and Williams, D.J. (2014), Quantification of variation in biological input materials and its effect on clinical outcome and manufacture. Cytotherapy , 16 (4) S109. doi: 10.1016/j.jcyt.2014.01.403

Thurman-Newell, J. A., Petzing, J. N. and Williams, D. J. (2015), Quantification of biological variation in blood-based therapy – a summary of a meta-analysis to inform manufacturing in the clinic. Vox Sanguinis, 109: 394–402. doi: 10.1111/vox.12288

Thurman-Newell, J. A., Petzing, J. N. and Williams, D. J. (2016), A meta-analysis of biological variation in blood-based therapy as a precursor to bio-manufacturing. Cytotherapy, 18 (4): 686-94. Doi: 10.1016/j.jcyt.2016.01.011.

Two further publications are currently in progress: one from a single centre clinical dataset (using results from **Chapter 4**, and one from the healthy baseline database (using results from **Chapter 5**). These form a novel quadrumvirate exploring biological variation from multiple perspectives.

Presentations

Poster Presentation. 12/09/2013. Sheffield, UK. Poster presentation of select results within chapters 2 and 3 at the Fourth Doctoral Training Centre Joint Conference at Sheffield University.

Poster Presentation. 24/04/2014. Paris, France. Poster presentation of select results within Chapters 2 and 3 at the Annual International Society of Cellular Therapy Meeting

Biological Variation Seminar. 09/04/2015. Stevenage, UK. Formal presentation of the results within Chapters 2, 3 and 4 to the Cell and Gene Therapy team at GlaxoSmithKline.

Poster Presentation. 02/06/2015. Amsterdam, The Netherlands. Poster presentation and discussion of select results within Chapters 2, 3 and 4 at the 2015 PDA Europe Advanced Therapy Medicinal Products Conference.

Oral Presentation. 14/09/2015 – 15/09/2015. Cambridge, UK. Oral presentation of select results from Chapters 2, 3 and 4 at the Pluripotent Stem Cell Platform's Comparability: Manufacturing, Characterisation and Controls Workshop. Results and feedback from this presentation have been incorporated into a UKRMP PSCP Report entitled **Comparability: Manufacturing, Characterisation and Controls** (accepted 17/05/16)

Oral Presentation. 30/09/15 – 02/10/15. Washington, USA. "Quantification of Variation in Biological Input Materials and its Effect on Clinical Outcome and Manufacture", Invited speaker at IBC's Cell Therapy Bioprocessing and Commercialisation meeting, presenting select results from this thesis.

Oral Presentation. 18/11/15 – 19/11/15. Boston, USA. "Analysis of Biological Variation in Blood-based Therapy: A Precursor to Bio-Manufacturing", Invited speaker at MIT's BioMAN Summit on Cell and Gene Therapy Products: *Meeting the Biomanufacturing Challenges. (* http://web.mit.edu/cbi-events/2015-bioman-summit/index.html)

‘It is much more important to know what kind of patient has a disease than to know what kind of disease a patient has’

– Caleb Hillier Parry (1755 – 1822)

“When you can measure what you are speaking about, and express it in numbers, you know something about it, when you cannot express it in numbers, your knowledge is of a meagre and unsatisfactory kind; it may be the beginning of knowledge, but you have scarcely, in your thoughts advanced to the stage of science.”

— William Thomson, 1st Baron Kelvin (1824 – 1907)

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Chapter 1 Introduction

1.0 Regenerative Medicine

Regenerative medicines (RMs), including cellular therapy, are set to revolutionize healthcare by improving patient quality of life and reducing costs due to their substantial long term health benefits. They are unique in that they can replace or regenerate the original cells or tissue and restore normal function [1].

One particular RM is stem cell therapy, which contains a self-renewing progenitor cell with the capability to differentiate into any cell type of the body due to their unique ability to proliferate into specialized cells and tissues. This has exciting possibilities for treatment of chronic disease and injury.

Current, existing therapies include long term drug regimes, cadaveric/xenogeneic organ transplantation and medical devices; which manage rather than cure diseases, have considerable immunological implications, or do not grow to adapt to a growing patient or condition, respectively.

RMs such as stem cells offer considerable advantages in avoiding these long term regimes by restoring natural function – such as in diabetes patients [2]. Immune rejection and graft-versus-host disease (GvHD) can be avoided by using transplanted tissue grown from the recipients own cells [3]. Joints can be rebuilt with biological materials that will grow and maintain themselves over the lifetime of the patient, rather than needing to be replaced as they wear out.

Additionally RMs can be used to offset side effects of current therapies – such as the use of peripheral blood progenitor cell transplantation after aggressive radiotherapy to repopulate the patient's bone marrow [4]. Unmet clinical need, such as Parkinson's Disease [5], may also be treated via the use of regenerative medicine.

1.2 Manufacture

However, there is much to be done before a viable and robust industry can be established to support regenerative medicine [6][7]. Improvements must be made in our understanding of the highly-regulated manufacturing processes and how to optimize them, and how to quantitatively characterize biological therapies from both a manufacturing perspective and from a therapeutic viability perspective.

The challenge for cellular therapy is even greater, as we are still developing tools for measuring cellular characteristics, and living materials have a greater degree of complexity than anything prior in the traditional pharmaceutical sense.

As pharmaceutical therapies are defined in terms of their active ingredient so must cellular therapies. Definition and characterization of the therapeutic agent not only satisfies regulatory requirements, but allows a greater understanding of mode of action and dose-response relationships. Biological raw materials (such as those required from cellular therapy) have a greater complexity, sensitivity and plasticity [8] than their more traditional pharmaceutical counterparts – partly due to the inherent variation of their source: human beings.

As a consequence of the development of civilization and technology, humans can live in remarkable extremes of conditions, and consciously alter them to suit themselves. Depending on culture or location, we vary by diet, lifestyle, age and weight amongst a myriad of other characteristics, in addition to the ethnicity [9] defined by our genetics. These all become important factors to consider when we begin to approach ourselves as sources of starting material – in particular, by how much do we vary in respect to the raw material in question and how much does this variation affect the final product and its clinical outcome?

This is in addition to the more traditional concerns of engineers when defining and controlling a manufacturing process. Currently, both input variation and process variables are poorly controlled in cellular therapies – determination of measurements such as cell confluence or viability are largely

assessed on an individual basis based on operator experience, much akin to when traditional engineering training used Masters and Apprentices to control variation by the passage of craft or trade skills [10]. Progress is being made here though, with the use of Standard Operating Procedures (SOPs), and element of standards of practice – one of the seven tactics suggested by Byrnes for reducing variation [11]. Nevertheless, there are established tools, from traditional manufacturing and engineering which may be applicable to a biological RM process.

Significantly producing a commercial-scale cell therapy will require a manufacturing process that is either designed around biological variation, designed to control it or reduced prior to manufacture. This is a precursor to control strategy design, a key step in understanding the dose/response relationship, and an element in both the application of Good Manufacturing Practice (GMP) [12] and in designing a scalable process.

Therefore these processes will either need to be designed for biological variation, or absolutely controlled [13]. Firstly, the range of deviation at input must be established – the baseline amongst the donor population. Having this will allow stratification of variation in terms of donor characteristics, such as weight or age, and what effect these have on the quality and efficacy of the final product. This may highlight key variables that have a significant impact on patient benefit – the Critical-To-Quality (CTQ) attributes of the input material.

1.3 Variation

Variation in manufacturing process will depend on five factors: humans, environmental changes, machine and measurement variation, differences in methodology and fluctuation in the raw material. We can reduce operator variation using SOPs, mechanisation and automation, as demonstrated by *Liu et al* [14], which will be key to development of a robust and cost effective process. Operator variance can be reduced by use of mechanisation – such as the introduction of multi-channel pipettes, but automation is the ultimate step in reducing the influence of the operator (assuming that automation is robust and repeatable in its own right) . This also has the added

benefit of forcing us to systematically approach process improvement and design, using data to drive improvement, with the robotic guarantee that the only deviation in the process will be a factor of the raw material (and any human error in programming or machine failure).

Another unique factor of RM is the importance of the living cell as a product, something that has not yet needed to be considered in the pharmaceutical industry, and the sensitivity of the cell to environmental and automation factors (e.g. shear stress as a function of mixing). The therapeutic potential of the cell relies on an extremely complex interlocking system of chemicals, organelles and proteins – of which a slight change can be enough to make the cell unviable for its intended process.

1.4 Blood

To account for the extent and distribution of variation, a representative exemplar must be chosen to benchmark current and future cellular therapies. This can then be used to qualitatively determine the critical steps in the manufacturing process and measure them quantitatively.

There are currently very few cellular therapies on the market [15], the main and most established being bone marrow transplantation and peripheral blood progenitor cell transfusion. These involve isolation, processing, implantation and registry of outcomes, without the manufacturing and regulatory complications of an expansion step, and are therefore well suited as case studies to explore the impact of biological variation. As donor information is taken at the initial stages and patient response is recorded in the form of a transplant registry, they offer a unique opportunity to provide information as to the baseline variation.

Haematopoietic Stem Cell Transplantation (HSCT) utilises Haematopoietic Progenitor Cells (HPCs) derived from blood or bone marrow; raw materials inherently variable due to donor-specific factors such as geography, ethnicity, gender and quality of life. HSCT utilizes the therapeutic potential of these progenitor cells to differentiate into various cell types within the body and can be used for a range of clinical applications such as recovery therapy; where the patient's own cells are used to revivify their bone marrow after particularly harsh cancer treatment.

As this type of HSCT is classified under FDA guidelines as 'minimally manipulated', the effects of variation can be studied in the isolation, processing and implantation steps, and the subsequent outcome, without the regulatory and manufacturing burden of an expansion step – making HSCT an ideal exemplar to benchmark this type of RM and to inform approaches to manufacturing advanced therapy medicinal products (ATMPs) that are more than minimally manipulated.

As HSCT is a minimally manipulated product the variation attributed to the expansion step will be out of scope of this work. In addition, variation as a function of genetics will be acknowledged as a possible contributor but will be outside of the scope of this work due to time and resource constraints, as a result of the added complexity.

1.5 Problem Statement

For cellular therapy to become a mainstream, frontline option, the regulator, clinician and patient need to be satisfied about its long-term efficacy, cost benefit and safety – especially when in competition with existing, proven alternatives.

Cellular therapies are currently produced in small numbers, for relatively few patients in a clinical setting. This approach does not have the capability to produce in volume, to meet future demand. Given the limited space, budget and manpower of our clinical centres there will be a requirement for mechanisation and/or automation.

The extent and range of variation within cellular therapy remains anecdotal, therefore the key novel outcomes for this research will be the qualitative and quantitative understanding of variation. This will aid the understanding of the complexity of manufacturing and product development as a result of living starting materials. Only by understanding the process sufficiently and demonstrating the ability to manufacture a fully defined product at a consistent standard will any RM meet regulatory requirements.

Understanding the variation that is encountered in the starting material, and the process, means an understanding of the tolerances and the specification that a machine or a process needs to be

designed around – to tolerate or control. This is the primary rationale behind the quantification of biological variation.

Consequently, this thesis will

- Review current research into biological and stem cell therapy related variation, and identify key sources of information and data for analysis
- Use this identified data to quantify biological variation in starting materials and the therapeutic product, using a blood-based stem cell therapy exemplar, both overall and as a function of allogeneic and autologous donors.
- Compare this variation globally to a specific single clinical processing site.
- Compare this variation between 'healthy' and 'sick' donors.
- Identification of potential sources of variation, triage in order of influence, and provide informed suggestions for solutions and / or research directions to solutions.
- Use public forums to gauge stakeholder appreciation and spread understanding and awareness of the importance of the fundamental engineering and scientific principles required to make regenerative medicines a successful long-term endeavour.

This will then propose the most prudent research and development direction in tackling biological variation in blood-based therapy, whilst identifying and justifying areas of lower value or research priority.

1.6 Thesis Structure

This thesis is comprised of 6 chapters, which present the background and context for the research and the novel content of the research.

- **Chapter 1** is an introduction that presents the context of the work, the aims and objectives, and the structure of the thesis.
- The literature review in **Chapter 2** describes the rationale and importance of quantifying variation, establishing confidence in the novelty of this research and providing the vocabulary and background to appreciate the challenges behind the headline breakthroughs.
- **Chapter 3** describes the variation within a **global, unwell** sub-population using an extensive literature meta-analysis based on the PRISMA guidelines This is intended to give a background basis of variation from multiple centres in multiple countries. The level of overall variation in HSCT is given, and stratified into allogeneic and autologous therapy.
- **Chapter 4** describes the variation within a **localised, unwell** sub-population, from the Dana Farber Cancer Institute (DFCI), Harvard, Boston, by data mining product and process records for patient and product measurements. This also includes an in-depth discussion about the challenges of manufacturing in the clinic, and potential observed sources of variation such as operator variation, process protocols and measurement techniques.
- **Chapter 5** describes the variation within a **national, healthy** sub-population, using data provided by the UK Biobank. This includes discussion of the effect of physical metrics such as weight and age on biological metrics such as white blood cell count, and some notable observations about appropriate sampling, 'big data' and direct comparisons between this healthy dataset and the relatively 'unwell' dataset collected from DFCI.
- **Chapter 6** will summarise the findings of this thesis and discuss future work and direction.

Chapter 2 Focused Literature Review

This review aims to discuss the effect of variation on blood based cellular therapies including bone marrow transplantation (BMT), peripheral blood progenitor cells (PBPC) and umbilical cord blood (UCB). There will be a particular focus on PBPCs. The effect of variation from the source, process and operator and its effect on graft characteristics and clinical outcome will then be discussed. Other factors such as dosage, clinical practise variation, and the subsequent effect on clinical outcome – patient benefit – will be examined.

Figure 2.0 is a generic HSCT process map around which the following review has been structured. Key sections have been highlighted and page numbers given for corresponding sections.

The following topics will be discussed:

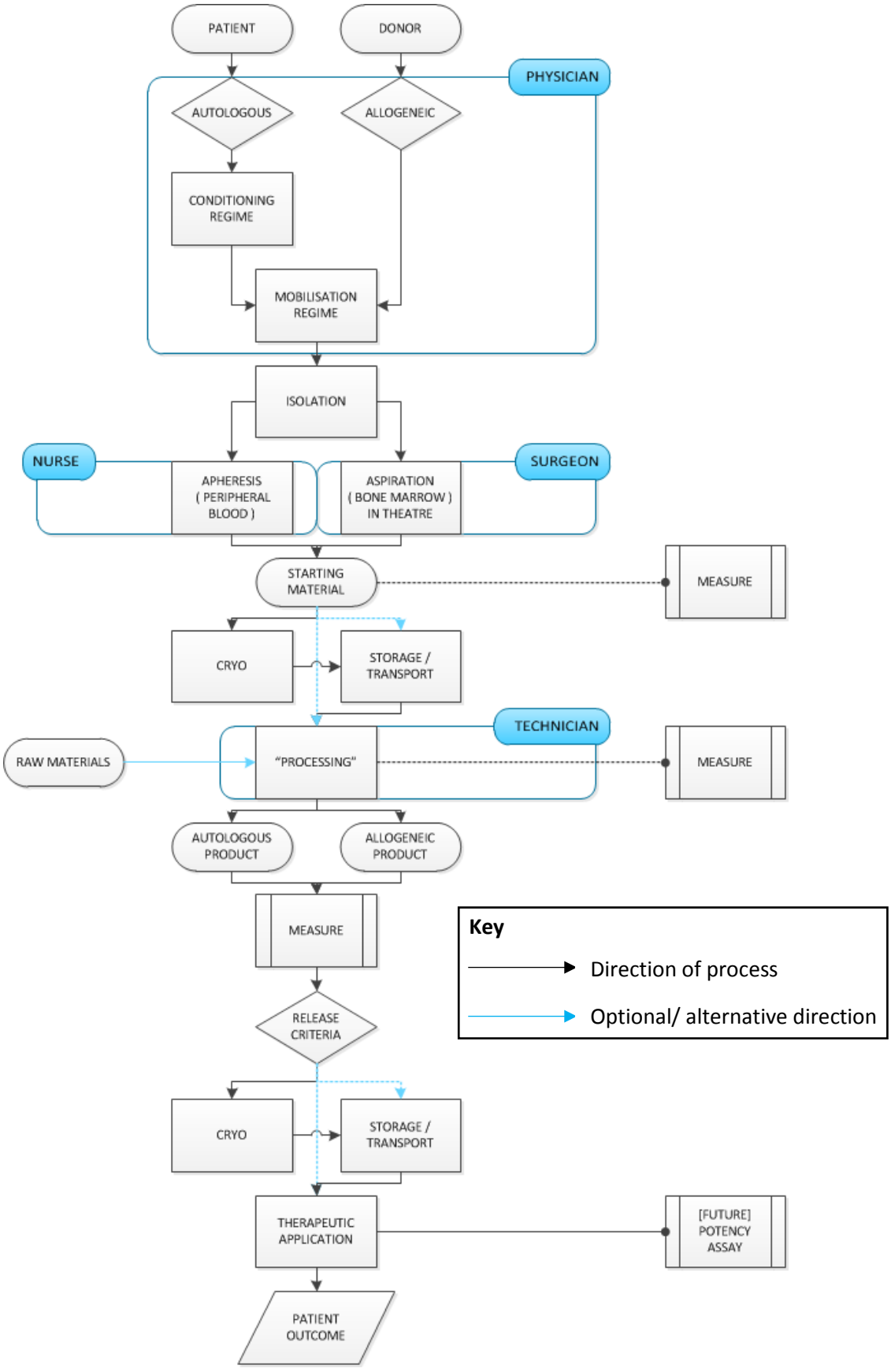


Figure 2.0: Generic Process Map for HSCT

2.1 Regenerative Medicine

Regenerative Medicines (RMs), including Cellular Therapies, are set to revolutionise healthcare by improving patient quality of life and reducing costs due to their substantial long term health benefits. They are unique in that they can replace or regenerate the original cells or tissue and restore normal function [1].

The keystones of cellular therapy are stem cells. They are the self-renewing progenitors of all specialised cells and tissues within the body. They also have the unique ability to proliferate into these specialised cells and tissues. Primarily, stem cells are involved with embryonic and foetal growth, growing and forming all the structures of the human body. However, recent evidence has shown that 'niches' of stem cells exist within the adult body and contribute towards repair [16]–[18].

Current, existing therapies include long term drug regimes, cadaveric/xenogeneic organ transplantation and medical devices; which manage rather than cure diseases, have considerable immunological implications, or do not grow to adapt to a growing patient or condition, respectively. RMs such as stem cells offer considerable advantages in avoiding these long term regimes by restoring natural function – such as in diabetes patients [2]. Immune rejection and graft-versus-host disease (GvHD) can be avoided by transplanted tissue grown from the recipients own cells [3]. Joints can be rebuilt with biological materials that will grow and maintain themselves over the lifetime of the patient, rather than needing to be replaced as they wear out. Additionally RMs can be used to offset side effects of current therapies – such as the use of peripheral blood progenitor cell transplantation after aggressive radiotherapy to repopulate the patient's bone marrow [19]. Unmet clinical need, such as Parkinson's disease [5], may also be treated via the use of regenerative medicine.

With the threat of radiation exposure due to atomic energy and weaponry in the late 1940s, there was considerable effort put into protection or recovery of bone marrow, which is particularly vulnerable to irradiation. Bone marrow was first shown to have a therapeutic effect in 1957 [20]

where a transplant provided haematological reconstruction in two leukaemia patients following radiotherapy. Early progress of bone marrow transplantation was slow due to consistently low survival rates, because of the high incidences of GvHD.

GvHD is a complication following transplantation where the recipients' body identifies the donor tissue as foreign and attacks it. It has two forms; acute and chronic. Acute GvHD is relatively short-term, manifests within three months and includes abdominal pain and skin rash. Chronic GvHD can last a lifetime, manifest after three months and includes weight loss, fatigue and vision impairment.

With the discovery of the Human Leukocyte Antigen (HLA) system [21] – the cell-surface marker system that controls the immune system's 'friend or foe' response – there was a significant increase in survival rates, as donors and recipients could be matched. By 2006 over 50,000 BMTs had been carried out, and today over 40,000 haematopoietic stem cell transplantations per year are carried out, worldwide [22].

Current routine cellular therapies, such as Haematopoietic Stem Cell Therapy (HSCT), are produced in a batch-to-batch manner, typically in a clinical laboratory environment, under special legislation such as the European Hospital Exception Clause [23] and the FDA's Investigational New Drug Exemption [24]. To meet future demand an appropriate bio-manufacturing process is likely to be required to replace some or all of the current manual processing. A quality-by-design approach to process control that is designed around or controls the variation inherent to biological raw materials is a key prerequisite to biological manufacturing at scale and an ongoing challenge for the entrepreneurs, manufacturers and regulators.

HSCT is one of the few cellular therapies currently in routine use [15], and is well established worldwide. The therapeutic potential of this therapy originates from the haematopoietic progenitor cells. HSCT utilises the unique properties of these progenitor cells, isolated from peripheral blood, bone marrow or cord blood for clinical applications such as the revivification of a patient's bone marrow following potent chemotherapy or radiotherapy. It also allows cancer patients to receive

higher doses of chemotherapy than their bone marrow would usually tolerate. Infusion of haematopoietic stem cells occurs in a similar manner to a simple blood transfusion, intravenously over a period of time.

Between 1990 and 2000, out of 44,165 allogeneic HSC procedures in Europe, 75.9% were used in the treatment of leukaemia (i.e. acute myeloid leukaemia and myelodysplastic syndrome), 12.9% were used in the treatment of non-malignant disorders (i.e. thalassemia, Fanconi Anemia), 8.9% for lymphoproliferative disorders and 0.6% for solid tumours [22].

As pharmaceutical dose is defined in terms of grams per kilogram and purity of active ingredient, so must HSCTs. Blood-based therapies are characterised by their cell content, specifically cells per kilogram of patient weight, and may include total nucleated cells (TNC) and CD34+ cells as a measure. The number of TNCs / product is the more traditional, commonly reported parameter for cell dose and represents the number of cells present excluding red blood cells and platelets. A more specific characterisation uses the Cluster of Designation (CD) cell surface marker system. Clusters are involved in critical cellular functions, and are therefore indicative of particular cell types, enabling identification of specific cell types. CD number 34 is a particular marker found on haematopoietic progenitor cells (although not all CD34+ cells are HPCs [25]).

The prevalence of HSCT makes it an ideal exemplar to benchmark the variation encountered in cellular therapies. It occupies a unique regulatory niche in that it is ‘minimally manipulated’, so has the potential as a case study for informing the process design for more complex, future biological manufacturing of products that are more than minimally manipulated and fall into more challenging regulatory classifications such as Advanced Therapy Medicinal Products (ATMPs) [26]

2.1.1 Autologous versus Allogeneic

HSCs can be harvested from both autologous and allogeneic donors.

There are two methods of HSCT: allogeneic and autologous. Autologous therapy involves removing and returning one person's cells, and tends to be used as a 'rescue therapy' after harsh chemotherapy or radiotherapy. Allogeneic therapy involves two people; a donor and a patient, and it is dependent on matching the donor to the patient immunologically using the Human Leukocyte Antigen (HLA) system [27]. The degree of HLA-matching will determine the probability of graft rejection and GvHD. It has the benefit of utilising cells from a healthy donor, rather than an ill donor, and tends to be used to replace a defective marrow or immune system.

Autologous donors are also the recipients of the HSCs – such as when a person's cells are returned to them after particularly harsh chemotherapy or radiotherapy as a 'rescue therapy'. Autologous donors will therefore tend to be 'unwell' individuals.

Allogeneic therapy has the potential to be more effective and cost effective, as allogeneic stem cells extracted in large numbers from healthy donors could be physiologically, metabolically and genetically more stable [28]. An allogeneic model of manufacture has the potential to offer more consistent production and supply, by banking and expanding healthy cells of an ideal set of characteristics. However allogeneic therapy is associated with immunological complications, graft rejection (Host versus Graft Disease) and rejection of the recipient (Graft versus Host Disease) [29].

2.1.2 Procedures

The source of HSCs can be further stratified into three cell sources: bone marrow, peripheral blood and cord blood.

Sourcing HSCs from bone marrow is known as *aspiration* and is a surgical procedure, performed under local or general anaesthesia [30]. Bone marrow is removed from the *iliac crest* in the hip bone (or more rarely, the sternum) via an aspiration needle. Several aspirations may be required to reach the required volume. As this is an open, surgical procedure, there tends to be more severe complications and risks, such as infection of the bone marrow.

Sourcing HSCs from peripheral blood is known as *apheresis* and uses a specially designed machine that filters the circulating blood, separating the stem cells from the whole blood and returning the remaining volume to the donor [31]. HSCs circulate in the whole blood in low concentrations, but this number can be increased with the use of mobilisation agents such as granulocyte colony-stimulating factors (G-CSFs) which stimulate the bone marrow to release HSCs into the circulating peripheral blood. This is a relatively closed procedure, using two needles in an out-patient clinic, but infection is still a risk, as is cytotoxic shock from any additives used in the machine, such as acid citrate dextrose. Peripheral blood appears to have a greater risk of both acute and chronic GvHD compared to bone marrow [32] [33]. *Demiriz et al* bids us to bear in mind the following when choosing peripheral blood sourced stem cells over bone marrow sourced stem cells; chronic GvHD risk increases in addition to indication-specific benefits and drawbacks, such as survival rate increase for *advanced* stage chronic myeloid leukaemia (CML) patients and survival rate decrease in *chronic* phase CML patients [34]. However stem cells harvested from peripheral blood resulted in improved cell yield and faster engraftment, as well as a decrease in immediate transplant-related complications and reduced cost, compared to bone marrow sourced stem cells [35].

Sourcing HSCs from umbilical cord involves extraction of the cord blood from the umbilical cord and placenta shortly after birth [36]. Traditionally, these organs were discarded after birth, but now represent a considerable source of therapeutic stem cells. There is minimal risk to the donor, as

these are naturally occurring by-products. Cord blood units can be a good alternative to unrelated transplantations with mismatched HLA tissue types [34].

Given these source-specific benefits and drawbacks, there also appears to be indication specific benefits, such as bone marrow being the preferred source for unrelated donor transplantation in the treatment of serum amyloid A (SAA) [37]. This hints at a much more complex, and interesting interaction between cell type, and function, with specific indications and disease modes of action.

There is evidence that these sources produce functionally different sub-populations of cells. In *Lathers et al* cultures from peripheral blood and cord blood had entirely different allo-stimulatory capabilities – therefore their effectiveness at stimulating an anti-tumour immune response [38].

2.2 Manufacturing Issues

2.2.1 Defining HSCTs

Blood-based cellular therapies are defined in terms of cell dose. This can include TNCs, mono-nucleated cells (MNCs) and CD34+ cells.

TNC is the most frequently reported parameter in the literature for cell dose, and is usually reported as the number of cells administered per kilogram of recipient bodyweight. This cell population includes all cells that contain a nucleus which excludes red blood cells and platelets, and has historically been used to access the adequacy of peripheral blood progenitor cell grafts [4]. Unfortunately, TNC is a measure of cell identity and does not discriminate between live and dead cells so a further measure that is indicative of cell quality is required.

Colony forming assays [39], Trypan Blue staining [40] and 7AAD DNA staining [41] are used to determine the viability of the cell. The former two methods can be subjective analytical techniques, whilst the latter is a more objective analysis, assuming that dead cells will not have intact cell membranes and therefore expelled DNA can be measured and quantified.

2.2.2 CD34+ Cells

A more specific measure of identity is the CD marker system. Clusters of Designation (CD) can be involved in cell signalling or adhesion, and as a side-effect of their function can be used to identify cell lineages and types by testing for cell surface molecules using immunophenotyping of cells.

A cell is measured for having been positive or negative for a particular marker. Cell surface molecules are involved in critical cellular function, and are therefore indicative of specific cell types, enabling identification of specific cell types. CD34 for example, is found on haematopoietic stem cells – but not exclusively as endothelial cells also test positive for CD34 [42][43][44], and tumours of an epithelial origin[45][46]. As succinctly summarised by *Ivanovic et al* [25]:

> *'It should…be repeatedly stressed the fact that the majority of haematopoietic stem and progenitor cells express CD34+ does not mean that all CD34+ cells are stem cells or progenitors.'*

Therefore, a CD34+ cell population represents a heterogeneous population and is not a definitive measure of haematopoietic stem cells. A further specific marker is CD number 133 [47], which can be found on haematopoietic progenitor cells – but also endothelial and epithelial cells.

Currently CD34 is the most accepted and widespread measure of haematopoietic stem cell content, and whilst it is not an absolute measure of the number of stem cells, it is commonly treated as such (an analogue) for the purposes of cell dose and starting material specification.

2.2.3 Potency Assays

> *"Living cells are complex entities and only limited control over cell propagation and manipulation in vitro is possible."* [48]

There is a great need for reference standards, potency assays and surrogates for potency [49][48].

The US Food and Drug Association (FDA) has identified four expectations for the characterisation of cell based therapies; identify, purity, safety and potency [50]. Further measures of cell product characterisation can be found in Table 1 of *Bravery et al* [51] and include physicochemical

characterisation, biological characterisation, potency, comparability testing, biocompatibility, stability and stability testing, and release assays.

TNC and CD34+ cell counts are not indicative of quality or efficacy, but are measures of identity. Unlike pharmaceutical drugs, such as aspirin, where identity is indicative of efficacy, cells are living, plastic entities that are capable of changing form and function, and potentially dying.

Reference standards are artefacts of a known composition used to enforce comparability and standardisation, such as the International Prototype Kilogram for weight. The IPK is a platinum-iridium ingot kept by the International Bureau of Weights and Measures in France. Reference artefacts are a particular challenge for cellular therapy given the *in-flux* and complex nature of the cell itself, and the mutability and fragility of its physical form [51]. Even artefacts of physical measurements (i.e. second, metre, kilogram, ampere, kelvin, and candela) are prone to change or degradation over time and are shifting towards energy constants [52], that are less likely to change over time - so artefacts representative of cells may well be of particular difficulty.

Potential reference cell lines would need to be consistent and representative of the quantifying characteristic – whatever measure or measures of quality are identified for that particular cell type, and be able to reproduce these characteristics in spite of the potential for these cell types to change phenotype and life cycle, in addition to storage considerations. A demonstration would have to be made that these cells can be grown in multiple facilities across the globe, or a small number of sites can reliably produce and send out reference samples that are comparable. The number of variables involved in this endeavour are considerable.

An alternative would be a reference artefact, a man-made construct that exhibits specific characteristics that are reflective of appropriate measures of quality – such as cell surface markers in a given ratio that reflects a given cell state and identity.

The keystone concept for these reference materials is the identification of appropriate measures of quality that can be replicated artificially, or in a controlled manner, that are representative of the state of the therapeutic agent, which on application will exhibit a desirable patient outcome. Identification of these measures of quality are reliant on the relationship between cell characteristics, patient safety and therapeutic efficacy. A defining factor in this relationship is controlling the variation to an extent that it has minimal effect on patient safety or benefit.

2.3 Variation

> *"Various aspects of cells in culture or in vivo are in constant flux, including changes in mitochondria, methylation profiles, and other epigenetic changes as well as genomic changes. Many of these changes are of little consequence, but other [rarer] events such as loss of a tumor suppressor or activation of an oncogene can be highly significant in terms of both efficacy and safety. Both researchers and regulatory authorities need to determine how to distinguish trivial changes from potentially dangerous alterations, and whether appropriate tests are available or even feasible."* [53]

As the source of HSCs, human beings are inherently variable due to a multitude of factors that affect physical and biological characteristics, such as age, weight, diet, lifestyle and quality of life. This variation is important as it determines the quality and quantity of the starting material used in production of a therapeutic, and has an impact on the final product and therefore patient outcome.

The cornerstones of manufacturing are the ability to make the same product repeatedly, at multiple locations, to a given set of standards and specifications.

This is in addition to the more traditional concerns of engineers when defining and controlling a manufacturing process. Currently, both input variation and process variables are poorly controlled in cellular therapies – determination of measurements such as cell confluence or viability are largely

assessed on an individual basis based on operator experience, much akin to when traditional engineering training used Masters and Apprentices to control variation by the passage of craft or trade skills [10].

Progress is being made here though, with the use of Standard Operating Procedures, an element of standards of practise (SOPs) – one of the seven tactics suggested by *Byrnes* for reducing variation [11]. Additionally, there are established tools, from traditional manufacturing and engineering which can potentially be applied to a biological process.

Operator variance can be reduced by use of mechanisation – such as the introduction of multi-channel pipettes, but automation is the ultimate step in reducing the influence of the operator. Automation has the added benefit of forcing us to systematically approach process improvement and design, using data to drive improvement, with the robotic guarantee that the only deviation in the process will be a factor of the starting material (and any human error in programming or machine failure of course).

2.4 Spread and Distribution of Biological Variation

Biological variation, as a function of the donor, can be defined as either intra or inter-donor variation.

Intra-donor variation (*within-person*) is the effect of changes within a specific donor due to certain internal or external circumstances, such as changes in blood composition due to seasonality, circadian rhythm, exercise, extent of specific illnesses and the particular method of isolating the raw material [55][56].

Inter-donor variation (*between-person*) is the most visually apparent of sources and concerns the differences between individuals such as age, weight, lifestyle choice and ethnicity [57][58][59].

This donor variation affects the quantity and quality of the starting material for a given cellular therapeutic, the therapeutic building blocks for the medicinal agent. However, this is not the only factor influencing biological variation, because the starting material is but the beginning of a chain of

events that will lead to the final therapeutic product, involving a number of processes and personnel intervention.

These events are illustrated in **Figure 2.0**, a generic process map for HSCT, designed to illustrate the many factors involved. These include the physical isolation of the starting material, the processing of the starting material and any other ingredients required, the influence of the operator and the measurement system, cryopreservation, transport and storage where necessary, clinical practise and dosage, the disease state of the patient and the resultant patient outcome.

2.5 Manufacture and the Practice of Medicine

Clinical practice is of particular importance, as without a standardised method of application, any effects of standardisation or control of earlier steps may not be felt by the patient. Additionally, it may be difficult to build up statistical confidence in the outcome of a specific therapy, if there is no commonality for comparison. Furthermore, clinical practise introduces two further sources of variation, the so called 'wanted' and 'unwanted' variation [60].

Wanted variation has already been described as intra- or inter-donor variability, but unwanted variation is that which is caused by not applying the 'best' practise in the clinic and the most up-to-date protocols and is a result of the skill, knowledge, willingness and resources of the clinic [61][62]. This is highly dependent on the skills and resources of the given therapeutic centre, and is sensitive to geographical variation. This type of variation is particularly important from a patient perspective and the pursuit of clinical trials, as this leads to variation in the quality of treatment and the potential outcome and survival chances of the patient [62].

Several potential sources of variation will now be discussed.

2.5.1 Source

Human beings are inherently variable due to a multitude of factors that affect physical and biological characteristics, such as age, weight, diet, lifestyle and quality of life.

As the source of starting material in allogeneic therapy, donors could potentially be stratified in terms of desirable qualities that are indicative of controllable cell numbers or more efficacious cells.

However, currently the criteria for donors is highly variable: in *Karp et al*, major blood collection centres were evaluated in 17 different countries, and found a wide range of donor inclusion criteria, including minimal deferral time after pregnancy or tattoos, minimum donor weight and amount of whole blood taken during collection [63]. Whilst each centre will have its justification for its criteria, as allogeneic therapies are drawn from a worldwide pool of donors, to match the disparate tissue matching criteria of the patient, donations from different centres will not be equivalent. *Ekhurt et al* suggests a number of criteria leading to permanent and temporary postponement in allogeneic donor selection which they have listed in their paper under *Table 1* and *Table 2* [64].

In one large scale South Korean study, different blood groups yielded different cell numbers within their cord blood units, in particular blood group 'O' showed an increased number of TNCs, CD34+ cells and CD34+cells/TNC cells compared with any other blood group [65].

2.5.2 Age

Age is a universal characteristic measurement of humans, and it is our cells that define this age (or to be more precise, our telomeres [66]). As age affects the quality or characteristics of our cells, it is reasonable to assume that age will affect the quantity and quality of HSCs isolated from a given donor.

This is highlighted within mesenchymal (adult) stem cells in *Maijenberg et al* where age is associated with different stem cell subsets being prevalent depending on the age of the patient – $CD271^{bright}$ CD146+ is the main subset in adults, whilst $CD271^{bright}$ CD146- is the main subset in paediatric and foetal bone marrow. This suggests a more complex relationship than a simple linear relationship between cell numbers and age, more indicative with the complex state changes that come with age – particularly between paediatric and adult donors.

This difference between paediatric and adult donors is also shown in HPCs, illustrated by a marked increase in mobilised CD34+ cells compared to any other age group [67][68][69]. This may be

representative of an ‘unsettled’ growing system, with a higher demand for stem cells, compared to the more steady-state adult biological system.

Further evidence for the relationship between donor age and the peripheral blood content of the starting material is inconclusive. A number of studies has concluded that there is no significant correlation [70][71][72], whereas others have shown a marked decrease in HPCs with age [73], particularly in donors over 38 [74] and over 55 years old [75][68]. However, in umbilical cord blood, maternal age and race had no effect on yield [76].

The aforementioned have focused exclusively on cell number, but as was identified earlier, cell number is not indicative of cell quality. *Baxter et al* used colony forming unit assays with mesenchymal stem cells to determine that as the donor’s age increased, the number of colony forming units decreased – indicating that as the donor increases in age the ‘quality’ of the cells decreased (or at least their ability to form colonies) [77].

Although this is not a definitive consensus, it appears that younger donors produce higher quality cells in higher numbers, compared to their older, adult counterparts. This is appropriate given the emerging potential use of paediatric HSCs in reduction of morbidity and mortality, and applied uses in gene therapy, autoimmune diseases and inherited metabolic disorders [78]. This conclusion might promote the use of PBSCs over BM stem cells for older patients, as PBSC donations tend to contain higher proportions of CD34+ cells. Age may therefore affect the variation of *quality* in HSCT.

2.5.3 Weight

Weight is another universal characteristic and is of particular therapeutic interest, as dosage and stem cells are typically measured against patient weight. However, this is a simplistic measurement of body mass, as it does not take into account body type, corresponding height or fitness levels – the same weight may be representative of a shorter, larger individual *and* a taller, thinner individual, or different levels of physical health.

With respect to processing and sourcing stem cells, it is the *donor* weight that is important for analysis of the effect of weight. Recipient weight, as more widely reported, is important for determining cell dose and location of the graft, but for isolation of starting material and correspondence between weight and characteristics of the graft, the donor's weight is the key metric (of course, in autologous therapy, the donor is the patient). This could potentially explain the reason for the considerable lack of donor characteristics in the literature, with the focus being on the patient and their benefit.

Larger individuals will require more blood to supply nutrients to this greater mass. This might be as a result of a greater level of exercise, and therefore it can be expected that the cardiovascular system has grown to accommodate and cope with this volume. Conversely, this mass might be a result of the obesity epidemic in the Western World [79], and this greater body mass and blood volume may be married to a cardiovascular system that is unable to cope over a long period of time leading to disease and dysfunction. This appears to correlate with the results from *Keser et al* who postulate that regular exercise increases the number of stem cells mobilised during autologous stem cell transplantation [80]. In particular, anaerobic training increases CD34+ counts in peripheral blood over aerobic and control groups [81].

It is reasonable to assume that a larger mass will result in a larger volume of blood, and therefore a larger population of cells, in particular red blood cells. Studies have demonstrated that there is a significant positive correlation between the weight of the donor, the density of the cell harvest for bone marrow [82] and peripheral blood [58], but specific cell populations have yet to be related specifically to weight in this review.

Importantly for obese patients, *Muller-Ehmsen et al* has demonstrated that obesity is linked to a decrease in circulating progenitor cells in peripheral blood compared to donors with less body fat [83]. This suggests that whilst increasing body mass results in an increase volume, and in some cases number of cells, specific populations of cells may be intrinsically linked to the capability of other

organs, and that being of greater mass is no guarantee of a greater quantity of stem cells – in fact being over a certain BMI may actively limit the number of stem cells. Furthermore, obesity may be linked to lower survival rates compared to non-obese patients [84] (although this was refuted for multiple myeloma, where BMI does not appear to affect progression-free survival, overall survival or mortality [85]).

Weight appears to be a more direct influence for umbilical cord blood content. *Al-Sweedan et al* gives evidence for a significant positive correlation between TNC count and neonatal weight, and between CD34+ cell numbers and neonatal / maternal weight [86]. This is potentially an interaction between the increased number of CD34+ cells in younger patients, and the cell/mass ratio that could be expected. Furthermore, *Strohsnitter et al* found that an incremental increase in cell concentration happened for every 500 g increase in neonatal weight [87], and another research group determined that birth weight also increased the median number of CD34+ cells [88]. Umbilical cord blood appears to have a much less complicated relationship with weight than its more mature counterparts in peripheral and bone marrow sourced blood, and must be associated with the rapid growth and proliferation present in the new-born biological system. Weight therefore may affect both the *quality* and *quantity of HSCTs.*

2.5.4 Gender

Gender is another fundamental distinction and is indicative of disparate body composition, chemistry and form. Research has shown that certain blood components, such as neutrophils and platelet counts are much higher in male donors compared to female donors, in all ethnic groups [59]. This may imply that a given culture from either gender will be functionally different to the other due to different ratios of nucleated cells, such as the strength of the GvHD response in allogeneic therapy due to different numbers of white blood cells. However for HSCT specifically, *Zamora-Ortiz et al* found no significant difference in cell numbers between genders, regardless of age, diagnosis or whether the HSCT was autologous or allogeneic [89].

Morishita et al suggests that post-HSCT, different genders require a different rehabilitation approach with female donors responding well to exercise and male donors responding well to a rehabilitation programme that includes a psychological element [90]. This is possibly more indicative of the difference in mindset / social programming than physical differences between gender, and would affect the clinical variation that will occur in the application of the therapeutic at the end of the product process.

2.5.5 Ethnicity

There is an assumption that country of origin is indicative of ethnicity: the key difference is that race is a geographic and social term, whilst ethnicity is a genetic term, and significant ethnic overlap can occur within populations due to immigration and emigration. For example, British citizens originate from a wide range of different countries and their respective gene pools and ethnic variances.

> *"…geography reflects race, ethnicity, social circumstance, lifestyle and culture attitude."* [62]

It is important to define stratification by ethnic background as a result of genetic differences rather than socially defined race or belonging. That is not to say that geographical location will not have an effect: local water and air quality, prevalence of disease and access to medical services will all affect the health of the donor, and therefore their cell quality and quantity on donation, but in terms of inter-individual variation, ethnicity must be defined by genetic origin.

Ethnicity is a potential source of variation between donors. Multiple studies have demonstrated fundamental and statistically significant differences in blood composition [59] such as lower CD34+ cells collected in Caucasian donors, compared to African-American, Hispanic or Asian donors [91] and higher proportions of CD34+ cells in Hispanic and Caucasian umbilical cord blood units [92]. Furthermore African-American donors appear to have lower CD34+ yields compared to other racial groups in some collection sites [93]. A large study of 10,776 donors with consistent mobilisation and controlling for age, gender, BMI and apheresis year found increasing CD34+ yields in obese African-

American and Asian/Pacific Islander donors, and Hispanic male donors compared to Caucasian donors, with the greatest mobilisation yield across ethnic groups being most prominent in males in higher BMI categories [94]. This may be due to the disparate access ethnic groups have to autologous and allogeneic HSCT compared to Caucasian donors, and may be skewing these results [95].

Due to the requirement for HLA tissue matching between donor and patient, it is unlikely a specific sub-population will become the 'best' source of HPSCs – particularly an ethnic group – but rather that the process and protocols for isolation of these cells may have to take into account variation as a function of donor ethnicity.

2.5.6 Mobilisation

Mobilisation is the individual drug and drug regime used to increase the number of circulating peripheral blood progenitor cells. HSCs are present within the circulating peripheral blood at a very low amount (0.04%) [96][97]. Mobilisation agents stimulate the bone marrow to increase this circulating volume of HSCs, before isolation and collection for therapeutic use. The use of a mobilisation agent significantly increases the TNC and CD34+ cell yield [98]. Donors have been categorised as 'low' or 'high' mobilisers depending on how many CD34+ cells their apheresis procedure has yielded – in fact it has been suggested that different mobilisation regimes be used for different categories of mobilisers [99].

This is a direct influence on the variation in quantity of cells collected from a given individual and may well be one of the most important sources of variation – especially as the recommended drug and regime is widely disputed in the literature [100][101][102][103][104][105].

This becomes of increasing importance when the inter-individual variations are considered. Basal levels of CD34+ cell counts vary from individual to individual and do not appear to fluctuate over time [106]. This is consistent with lymphocyte counts, which over an inter and intra-day study did not appear to fluctuate [107]. This means that not only are mobilisation regimes and drugs widely

disparate, but the amount of original starting material is also, affecting the clinician's decision to use mobilisation drugs and when, and how much these will mobilise between two given donors.

Furthermore, recovery after mobilisation and donation is key to successful long term patient outcome. One study noted that post-PBSCT and conditioning a number of measured outcomes were adversely affected and not all these changes were reversed after 100 days – indicating that nutrition is of key importance for recovery post-PBSCT, and therefore greater chance of a positive long term patient outcome [108].

A sister metric to mobilisation is conditioning. This is treatment applied prior to HSCT for specific indications, such as chemotherapy and radiotherapy. Because these damage the bone marrow it has been suggested that the quality and quantity of HSC products will be affected as a result. *Jiang et al t*ested the quality of stem cells via their homing capacity and long-term haematopoetic reconstitution and found that stem cell collections from 'poor mobilisers' to be equivalent to 'good mobilisers' [109]. However, *Ivanovic et al* found that cells obtained from 'good' mobilisers were associated with a much higher rate of proliferative and metabolic activation (albeit with a decrease in their expansion rate) [110] indicating that it is important to define the appropriate quality metric that is related to a specific patient outcome.

In summary, the mobilisation regime is a key influence on both the *quality* and *quantity* of stem cells collected from the donor. This is of particular importance as this determines the characteristics of the starting material, prior to any further processing or manipulation that may decrease or increase either metric of variation. Further work in the medical community is needed to specifically link quality metrics with patient outcome, and define a universal standard for stem cell *quality*.

2.5.7 Isolation

Sourcing HSCs from bone marrow is known as *aspiration* and is a surgical procedure, performed under local or general anaesthesia. The greater the volume of marrow extracted, the greater the number of TNCs isolated [111]. However, large aspirations from a single site do not produce as many

TNCs as multiple aspirations from multiple sites [111], [112]. This is a function of the structure of bone, being made up of a honeycomb of 'trabecular spaces' that house a finite amount of marrow. As these spaces are drained of bone marrow, they fill with peripheral blood and contaminate the aspiration. Peripheral blood, being naturally low in progenitor cells will dilute the bone marrow and decrease the yield of progenitor cells (by increasing the total volume) [113]. *Muschler et al* recommends 2 ml aspiration from each site to prevent this [114]. It is also important to ensure the procedure itself does not reduce the *quality* of the cells isolated, for example aspiration needle diameter does not affect the viability of bone marrow cells isolated [115] therefore needle diameter can be designed for patient benefit and safety.

However, multiple aspirations increases the time the patient is under anaesthesia, increases the physical puncture holes in the bone, and increases the donors blood loss both in total and as the peripheral blood fills the trabecular spaces [116] so a compromise must be reached between optimisation of isolation for quantity of cells and the risk to the patient as a result of the procedure. For example, *Lannert et al* used a needle with five holes instead of one, and although the yields were comparative, the operation time was greatly decreased because the collection rate was significantly increased (therefore requiring less anaesthesia, or invasive manipulation).

The timing of aspiration also appears to have an effect on collected cell numbers, an important observation for maximising patient benefit against the risks of the procedure. In one study, equal volumes of bone marrow were aspirated, but an average of 6 fold higher yield in CD34+ cells occurred when the bone marrow was aspirated during the daytime and late afternoon, with a lower yield during the night [117].

Sourcing HSCs from peripheral blood is known as *apheresis* and uses a specially designed machine that filters the circulating blood, separating the stem cells from the whole blood and returning the remaining volume to the donor. Optimisation of CD34+ cell collection is important to minimise cost and donor toxicity. The number of CD34+ cells harvested in an apheresis process will depend on the

CD34 concentration prior to harvest, the percentage drop of CD34 during harvest and the collection efficiency of the cell separator [118]. Collection efficiency does not appear to differ between different blood volumes which result in CD34+ yields equivalent to apheresis blood volumes in blastoma patients [119].

Different apheresis machines yield different cell numbers, benefits and drawbacks. Lower white blood cell (WBC) contamination was found in the COM.TEC and Spectra Optia systems compared to the traditional COBE Spectra, but at the expense of higher product volume and longer apheresis time. Higher collection efficacy and lower product volume favoured the newer Spectra Optia [120]. This implies improvement in understanding of the process and product requirements over time, and that the use of different equipment will yield different results – one of the key sources for variation between different centres is their access to different machines. Further studies confirm this advantage of the COBE Spectra over the Optia in collection efficiency at the cost of a longer apheresis time [121][122]. This is an advantage as long as the time taken for apheresis does not negatively affect the patient benefit, or incur greater costs as a result of the longer procedure, either directly or indirectly.

Timing of this collection can be a key factor in cell number – *Sung et al* shows that by waiting for a higher level of peripheral white blood cell count (4,000 / µL compared to 1,000 / µL) increases the number of CD34+ cells collected as a result, in children [123]. This may suggest, at least for paediatric patients, that WBC count can be a predictor of CD34+ cell count.

Previous treatment however, such as radioimmunotherapy, significantly impairs the mobilisation of HSCs in follicular lymphoma compared to patients who did not receive radioimmunotherapy [124]. This implies that with autologous therapy, previous treatment regimens are going to be significant indicators of mobilisation, and the number of collection procedures that will need to take place to reach the required dose – which may affect the quality of cells, and the recovery of the patient.

Sourcing HSCs from umbilical cord involves extraction of the cord blood from the umbilical cord and placenta shortly after birth. Babies delivered by caesarean section had more CD34+ cells and volume, but less TNC [76] which may be indicative of a less stressful environment for mother and child.

2.6 Process Design and Automation

2.6.1 Process

As shown in the previous section, isolation of HPCs requires human intervention, processing and (depending on type and intended use) storage and transport. These stages are heavily influenced by the operator, the equipment used and the protocol followed. It is important to control these factors to reduce the amount of variation introduced into an already variable material and potentially control or reduce the starting material's variation.

Traditional sources of variation within the process included operator error, systematic error, differences in protocols, technique and equipment, time between isolation and engraftment (shelf-life), even the temperature of the operating theatre has been reported to affect aspiration volume [125].

The following sections will discuss various elements of the process and how they could potential influence the quality and quantity of HPCs isolated and/or therapeutically delivered to the patient.

2.6.2 GMP and Manufacture

"What has become clear over the last decade is that no universal cell type or generalized method of cell delivery will be universally applicable for therapy." [53]

Currently HSCT is supplied by small scale, clinical laboratory processing methods, highly subjective to individual skill and expertise, access to process and analysis equipment and limited by the resource and space available to the hospital.

Academic and transfusion centres lead this small scale processing of regenerative medicines, however they have a tendency to lack expertise in regulatory matters, are too optimistic about deadlines and are not as rigorous with documentation and complex protocols [126]. Improvement of this may involve utilizing 'on campus' cross disciplinary capability, or outsourcing to contract research organisations.

To meet future demand for cellular therapies, and future therapies such as advanced therapy medicinal products these will need to be produced at a greater scale, that will require more consistent and controlled processing methodologies to both provide safe and efficacious therapies, but demonstrate confidence in the process and the product to both the regulator and the patient. This is a fundamental challenge in the field of regenerative medicine: the translation from laboratory to large scale manufacture.

Manufacturing at scale will need to either accommodate or control the variation as a function of the starting material, and therefore will need to identify sources of controllable or uncontrollable variation and optimize the process accordingly [127]. *Liu et al* has already demonstrated that manual culture of human osteosarcoma (HOS) cells has a poor process capability compared to an equivalent automated process, as a result of the operator dependent variability [14]. Automation, or a degree of mechanization will have a greater degree of repeatability and standardization, as they are not subject to the motivations and interpretations of human operators, and can run at a larger scale and for longer than an equivalent staff.

For autologous processes, expensive kit works on a 1-kit-1-product basis, meaning that a high degree of capital costs is locked in equipment that can only be utilized for one product at a given time.

Regulatory agencies require that ATMPs be manufactured in a strictly controlled environment, free of contaminants. These standards are defined as Good Manufacturing Practices (GMP). Although this varies between the USA's Food and Drug Administration and Europe's European Medicines Agency, this is essentially manufacture within a clean room environment, which is a significant financial investment.

2.6.3 Operator

The operator is only human.

Cell culture can be alluded to being a subjective art form, dependant on the individual's knowledge, technical skill and attitude. It has been likened to 'gardening and cooking' [128] in that the outcomes are largely dependent on who is in control. This is similar to when the trade craft system (Masters and Apprentices) was used to control manufacture before industrialisation.

Operator variation is undesirable when producing a cell-based therapeutic product, because the efficacy of the product is dependent on the consistency of the process and therefore reliant on the technique and judgement of the operator. This may well be affected by the individual's willpower and motivations – in fact a study demonstrated that the time of day, the individuals motivation and mood, may have important knock-on effects for patient benefit [129].

Human operators vary in skill and can demonstrate an increase in this skill over time – the effect of learning. *Lannert et al* demonstrates this effect, where the bone marrow collection volume increases and was maintained over time following a protocol change [130], demonstrating a 'settling in' period for manual operators. This is undesirable in cellular therapy because change in product quality or quantity may affect patient outcome or recovery, therefore the use of automation would remove this 'learning period'.

At the clinical end of the process the operator is still a key component, although these operators are now clinicians and nurses rather than technicians and scientists. Nurses are instrumental in ensuring low rates of infection and maintaining wellbeing following HSCT. *Bevans et al* compared 1,500 nurses in 7 countries via an online questionnaire and although standard protocols were followed, some discrepancies between countries do exist, such as timing or application of less efficient or new practises [131]. This implies a different standard of care, and as a result different patient outcomes between the clinical centre and/or country of transplantation. *Frassoni et al* studied this further by

examining the variation in outcome as a function of different, but equally competent centres, and demonstrated that different centres had different survival rates and incidences of GvHD for comparable patients, depending on which centre they visited [132]. This may be due to centres specialising in different clinical indications, different caseload between centres or differences in equipment and training, but demonstrates that there is sufficient variation in simply the *application* of these therapies to have a noticeable effect on clinical outcome – and this is especially important given the highly variable nature of the material these processes begin with.

Automation can dramatically reduce the level of operator variation [14], however the robot is only as good as the commands of the operator. If the incorrect commands are given, the process will be consistently inefficient. There is much work to be done before automated cell culture can completely replace manual culture, although this is a promising, if costly, future solution to the question of operator variation.

In conclusion, the human element is a significant contributor towards the biological variation found in cellular therapies, although the methods for reducing this variation are traditionally recognised and not unfamiliar to a manufacturing and process engineer.

2.6.4 Cost

Cost is one of the key hurdles holding back development of tissue engineered products [133]. Many regenerative medicines are highly personalised and given that several analytical tests are destructive, it is not always feasible to make further products beyond the first for testing, or store material for later analysis. Industrial R&D, academic facilities, and in particular clinical laboratories have limited funding and resources for exhaustive testing and investigative studies to identify sources of variation – a potential reason for limited research and recognition in this area, and a justification for this body of work.

In an ideal world, these biological materials would be fully classified and explored prior to application, but with the rise of sensational headlines and compassionate use, there is considerable

pressure for a potentially premature release of experimental therapeutics. These are expensive in of themselves (see below) and so extra costs associated with exploratory research is unlikely to be justified. One potential solution of this is the use of a stem cell therapy results database, which could collate processing, product and patient outcome data into an investigational, diagnostic and prognostic tool that reallocates the investigatory burden from the clinic to third party researchers.

In the Netherlands in 2012, the cost per patient of autologous stem cell transplantation were estimated to be €45,670 and allogeneic sibling stem cell transplantation at €101,919, with costs for unrelated donors being considerable higher at €171,478 for allogeneic matched donors and €254,689 for allogeneic umbilical cord blood with considerable costs being attributed towards hospital in-patient days, laboratory costs and donor search costs [134].

The cost per patient in the US between 2007 and 2009, over a 100 day period for autologous HCT were on average $99,899 ($73,914 – $140,555) and $203,026 ($141,742 - $316,426) for allogeneic HCT, with the majority of costs being associated with the initial hospital in-patient days [135].

The uncertain success of regenerative medicine products has produced a commercial 'valley of death' that can potentially discourage investors and large industry [136], so much so that authorities in several countries have established specific organisations to facilitate translation of regenerative medicine products – such as the California Institute for Regenerative Medicine in the US, the Forum for Innovative Regenerative Medicine in Japan and the Cell and Gene Therapy Catapult in the UK.

2.6.5 Regulations

"There will therefore no doubt be regulatory issues that are specific to a particular cell type, particular modality of action, and specific method of delivery." [53]

Cell therapies, and other regenerative medicines are regulated on a case-by-case basis. These can be as 'minimally manipulated' such as HSCTs or as 'advanced therapy medicinal products' that are more than minimally manipulated, amongst a number of other classifications, such as medical devices.

The main regulatory bodies in the US (FDA) and EU (MHRA, EMA) differ enough to mean that regulatory approval in one state is not indicative of approval in another, and changes to process and protocol may result in a different product or outcome for the patient with these complex therapeutics.

2.7 Scale

The ability to produce cellular therapies at scale is a vital capability to supplying new regenerative medicines, especially if it becomes a viable frontline therapy. This requirement comes with a number of unique challenges.

2.7.1 Multiple Site

> *"…the data illustrate the feasibility of a mandatory data collection and data analysis system within a complex field of medicine. This provides a basis for a network for collaboration and exchange, a prime prerequisite for quality improvement."* [137]

This is the challenge of repeatability and comparability: is a product manufactured in two factories identical in form and function from the perspective of the regulator and the products efficacy? This will require comparability of process, control over variation and the identification and implementation of a product specification - that may prove challenging if the mode of action of cellular therapies is unclear.

Marschner et al warns that a large difference between regions in multinational clinical trials should be expected due to chance variation [138]. *Mentz et al* goes further to identify that trials across multiple regions include differences in patient characteristics, medical practise patterns and health policies, as well as concerns over unsatisfactory quality oversight [139].

However, if the centre data is adjusted for each centres particular mix of patient indications, the quality between centres in Switzerland was deemed to be equivalent [137] (mortality rates were

different between centres, but some effort was made to explain this effect using the indication mix and treatment population).

Gee et al demonstrates that small scale manufacture of autologous bone marrow mononuclear cells can be made comparably across multiple sites (n = 5) by using automated systems, standardised protocols and a centralised facility running quality control. This was achieved partly due to a standardised 'training' regimen for the manual cell culture. Further studies have shown that adherence to a given protocol can reduce the variation in results of CD34+ cell enumeration across multiple centres [140], indicating the importance of standard operating procedures.

> *"One memorable example of statistical chance in subgroup analyses comes from the ISIS-2 trial. The authors presented a cautionary note when they showed that patients born under the Gemini or Libra astrological signs had higher mortality after randomization to aspirin than similar patients randomized to no aspirin."*
>
> [139]

2.7.2 Cryopreservation and Storage

Cryopreservation is an essential factor in long term storage of cell therapies due to their living nature. Usual preference and protocol within clinical centres is to infuse within 48 hours of collection, but this assumes that the patient can be prepared in time, the facility has the capacity and workload to meet the demand and that the patient's conditioning regime / medical treatment is timed to facilitate this.

Overnight storage at room temperature has been shown to decrease the quality of HPCs [141].

Cryopreservation is used in this instance to enable a better time frame and schedule for HSCT – to make sure the dose collected is adequate and the apheresis is timed with the patient's in-patient days and conditioning regimen. *Parody et al* found no difference in viability, engraftment or speed of recovery by using frozen PBSCs over fresh PBSCs, but a greater incidence of acute GvHD in the frozen product likely due to the cryoprotectant [142]. *Kim et al* also confirms this similarity between fresh

and frozen PBSCs in terms of outcome [143], therefore it is reasonable to assume that cryopreservation does not introduce variation from a patient outcome perspective – although the cryoprotectant media is still a source of concern. Cryopreservation uses a cryo-protectant media, dimethyl sulfoxide (DMSO), that is toxic to the patient, but is necessary for keeping cells viable after thawing.

Cryopreservation reduces the number of TNCs and CD34+ cells within a graft, and the viability of the graft falls [144]. Given the already variable nature of these cells, it is important to measure cells post-thaw to ensure the dose has fallen below the risk-benefit threshold since the pre-freeze measurement. This can be quite difficult because immediate testing may not capture apoptotic (dying) cells. However, from a long-term storage perspective, *Winter at al* demonstrated that once frozen viable cells numbers and viabilities did not significantly change over time – with a consistent stability for up to 15 years in cryo-storage.

Delaying cryopreservation after isolation is a risk associated with the high workload, limited facilities of clinical centres or a geographical distance between collection and processing centres. Starting material could be left at room temperature whilst awaiting cryopreservation protocols. *Fry et al* examined the maximum time HPCs can be stored without losing their potency and found much higher cell recoveries post-thaw in samples that had been maintained at a refrigerated temperature prior to cryopreservation [145]. This suggests that there is a key protocol disparity between centres, and highlights the importance of refrigerated 'waiting room' storage, or refrigerated transport between sites, because being kept at room temperature in the interim is affecting cell quality and quantity, and increases the risk of an unsuccessful transplant.

2.7.3 Perishability

Much like many other foodstuffs and medicinal products, cellular therapy is perishable and has a 'use-by date'. With the product being a living cell, there will be a limited amount of time within which the product must be used fresh before it becomes detrimentally affected. Cryopreservation may alleviate this somewhat.

An example of this 'use-by-date' is illustrated in *Bieback et al* where the presence of stem-like cells could not be established within the umbilical cord after 15 hours from the point of birth [146]. HSCs appear to have a half-life within the patient of 12 hours [147].

Age of the graft is historically associated with the deformation of red blood cells in storage – the 'storage lesion' [148] which is infrequently reported in stem cell registries [149]. It is unclear whether the storage lesion affects cell quantity or quality in a manner other than because of cell death.

2.8 Confidence in Therapy

"Real knowledge is to know the extent of one's ignorance"

- Confucius, 551-479 BC

2.8.1 Clinical Variation

The primary purpose of a therapeutic is to alleviate, treat or cure a patient's symptoms, disease or damage. A potential future challenge will be the uniform application of these therapies, that they are used as intended in a comparable manner under the practice of medicine – there is a clear difference even in current medicine between what is defined as best practice and what actually happens [150].

Reasons for this difference in practice include differences in patient and indication population, lack of awareness of the latest medical knowledge, lack of clear guidance or a lack of organizational support [151]. This is an issue because clinical variation means differences in the standard of care between centres and patients will have different outcomes depending on the clinical centre, their particular clinical team, and/or country [62]. Adherence to general guidelines is sporadic, and is dependent on the indication and particulars of the individual patient [152].

Clinical variation in isolation is known to be an issue between individuals and centres;

"The same well-trained operation team was used throughout, so that variations in techniques between physicians and the fact that some physicians collect BM more rapidly or collecting larger volumes from each aspiration side should not influence the results." [130]

Clinicians are used to adapting therapy to suit a patient's needs, but this makes building up the evidence to support future investment, use and confidence in these new regenerative medicines more difficult – and therefore therapies need to be comparable, traceable and results kept consistent and repeatable.

This new field of medicine offers the opportunity to 'start-from-scratch' when it comes to how these therapies are defined, controlled and applied. One of the main reasons for lack of this control of adherence is worryingly that the clinical centres have 'neither the will nor the resource' to control clinical variation [61]. In some instances, clinicians may not have the understanding or have not been provided with the training in new medical techniques and therapeutics. With respect to improvement of different centres and clinicians practices, it has been noted that (for red blood cell infusions) clinicians are:

"…not particularly interested in learning more about inappropriate transfusions [because] they believe their current practices are not deficient, and that it is others who need to be encouraged or educated to change their practices" [153]

Loberiza et al has made the case that if clinical variation is an issue, then it would be prudent to identify whether the volume of procedures within a clinical centre affects the patient outcome, because this would make a viable case for centres of excellence i.e. restricting procedures to certain centres ensuring the best quality of care [154].

With cellular therapies still being in its infancy and vulnerable to scandal and setbacks, it is of paramount importance that they are applied comparatively to be able to measure mode of action, effect of dose, and identify areas of risk and side-effects. *Lindhal et al* even goes on to implore his

colleagues to document and review their practice to establish routines and guidelines based on available evidence [60]. It is important that clinical centres work together to achieve this uniformity, and they are not unfamiliar with cross-collaboration (this may be more viable in the UK's NHS system, than in the USA's more independent business orientated environment);

> *"The short time intervals mean that the clinical unit, the apheresis unit, and the laboratory must work together efficiently (and may need to be located within the same area) to optimize PBSC collection in poor mobilizers"* [99].

Good will alone will not drive this process improvement [155], and needs to be driven top-down by the regulator and by the academic and medical community by providing overwhelming evidence as to its benefits. This may be as a result of redesigning clinical pathways [156] or the drive of particularly charismatic individuals [157].

2.8.2 Dose

Dose in cellular therapies will be highly dependent on the ability to characterise the biological product.

In pharmaceuticals, a given tablet is guaranteed to contain a given amount of a given active ingredient – to be within the limits set out in the product specification - and cellular therapies must be no different, at least from a patient benefit perspective. Dose should define the therapeutic effect and how it is used in terms of disease severity, or location of engraftment.

The current consensus within PBPC transplantation is that a higher dose reduces mortality and stimulates an earlier recovery rate in certain cell types compared to a lower dose [158][159][160][161]. Recommendations vary between $2x10^6$ CD34+ cells [158] to $5x10^6$ CD34+ cells [160]. Lower doses appear to have a greater risk of mortality and a lower survival rate [162], potentially due to the same risks of the procedure but with less patient benefit as a result of a lower dose-effect. Higher doses appear to cause fever and an increased risk of GvHD [163].

2.8.3 Patient Outcome

Clinical outcome can be defined as the perceived and physiological benefit given to the patient by a particular therapeutic; in the case of haematological malignancies such as those treated by HPCs, this may include short and long term survival, rate of relapse and incidences of chronic or acute graft versus host disease.

In 2011, *Passweg et al*, demonstrated it was unclear whether donor age had an effect on graft failure rates, incidence of GvHD or survival rates of the patient [164], however increasing *patient* age was shown to increase the mortality risk after HPC transplantation [165]. As previously shown, age of the donor affects the characteristics and qualities of the isolated cells so it is reasonable to assume there will be some effect of donor age on outcome, but this will only be further determined by more data or a greater understanding of the particular mode of action for HPCs.

Similarly, information regarding the effect of donor ethnicity on clinical outcome is inconclusive. While *Kollman et al* and *Ustün et al* show that racial mismatch did not affect incidence of GvHD, relapse or survival rates between different ethnicities [166][167], *Ballen et al* demonstrated a minor increase in survival rate for Caucasian children over black children in umbilical cord blood transplantation [168]. Further work is required to isolate whether ethnicity has no effect, or whether this has a combinatorial effect with regards to age of the donor. If ethnicity transpires to have no effect on outcome (whilst it has already been established that different ethnic groups have different cell populations) light may be shed on whether the composition of the graft is important, or the quality of the cells themselves.

Donor age appears to have no detrimental effect on PBSC transplantation for leukaemia, represented in terms of engraftment, maintenance of cell populations after one year, or risks of acute GvHD (in a single centre study) [169].

Information regarding donor gender is equally intriguing. In *Gahrton et al*, female donors are recommended for female patients to reduce to risk of negative outcomes, whilst there is no greater

risk for male patients being treated with female or male donor tissue, in myeloma [170]. There is insufficient evidence to draw definite conclusions on the significance of this, although the same paper suggested a sensitisation effect of females to males during pregnancy, and therefore having T cells keyed towards male antigens prior to donation. There is currently no data regarding the impact of weight, lifestyle or diet of the donor on clinical outcome.

In conclusion, information regarding donor impact on clinical outcome is inconclusive, because attractive as it may be to draw conclusions from one or two studies. Further work is required to determine the effects of changing donor characteristics, and would be determined by examining the effect of the graft characteristics on the clinical outcome, then determining what factors causes those specific graft characteristics.

2.9 Summary

The journey from input to clinical outcome is a minefield of multiple factors and influences that can affect the characteristics and quality of the grafted tissue. There is an understandable focus on the patient and patient characteristics, but the recipient is but one (albeit critical) consideration in a chain of events leading from the characteristics of the donor, through mobilisation and isolation, manufacture and processing, transport and storage before application in the clinic. The human element introduces considerable variation into this process, from the inescapable variation in biological tissues and between patients/conditions, to operator variance, clinical application and administration and the universal application of standards and protocols.

Here, it has been suggested that donor characteristics are a key factor in controlling or selecting graft characteristics, and that in turn these graft characteristics affect the clinical efficacy and safety of the final product. Variation in protocol, operator skill and techniques has also been shown to be another factor, one that is perhaps more easily controlled than biological variation itself, because the process is something we are historically well suited to control – given our experiences in traditional engineering. Further work is required to identify and stratify the critical steps in the process, and a greater onus on the *donor* characteristics and their effect – an issue thus far is that

very few studies are *directly* focused on the biological variation issue.

This analysis of the literature has shown that there has been little/no work that has addressed the critical issue of quantification of the variation of the input to regenerative medicine manufacturing. Consequently, as outlined in the introduction, this thesis will address this to make a novel contribution via detailed studies of the literature, using large public databases, and by deep analysis in a major international clinical manufacturing setting. This begins in the next chapter with a systematic meta-analysis.

Chapter 3 Systematic Literature Meta-Analysis

3.0 Chapter Aims

This chapter aims to quantify the variation in a global, multi-centre population, characterized by the reported data presented by the academic literature. The chapter aims to establish a baseline of variation across multiple centres for a unhealthy population.

Figure 3.0 is a generic process map for an exemplar HSCT product, which has been included here to indicate the primary focus of this chapter with respect to the process as a whole (indicated in **orange**).

The following topics will be discussed:

Chapter Aims and Introduction	3.0 and 3.1
Systematic Meta-Analysis and Methodology	3.2, 3.3 and 3.4
Results	3.5
Further Stratification	3.6
Source of Variation	3.7
Limitations of the Meta-Analysis	3.8
Conclusions	3.9

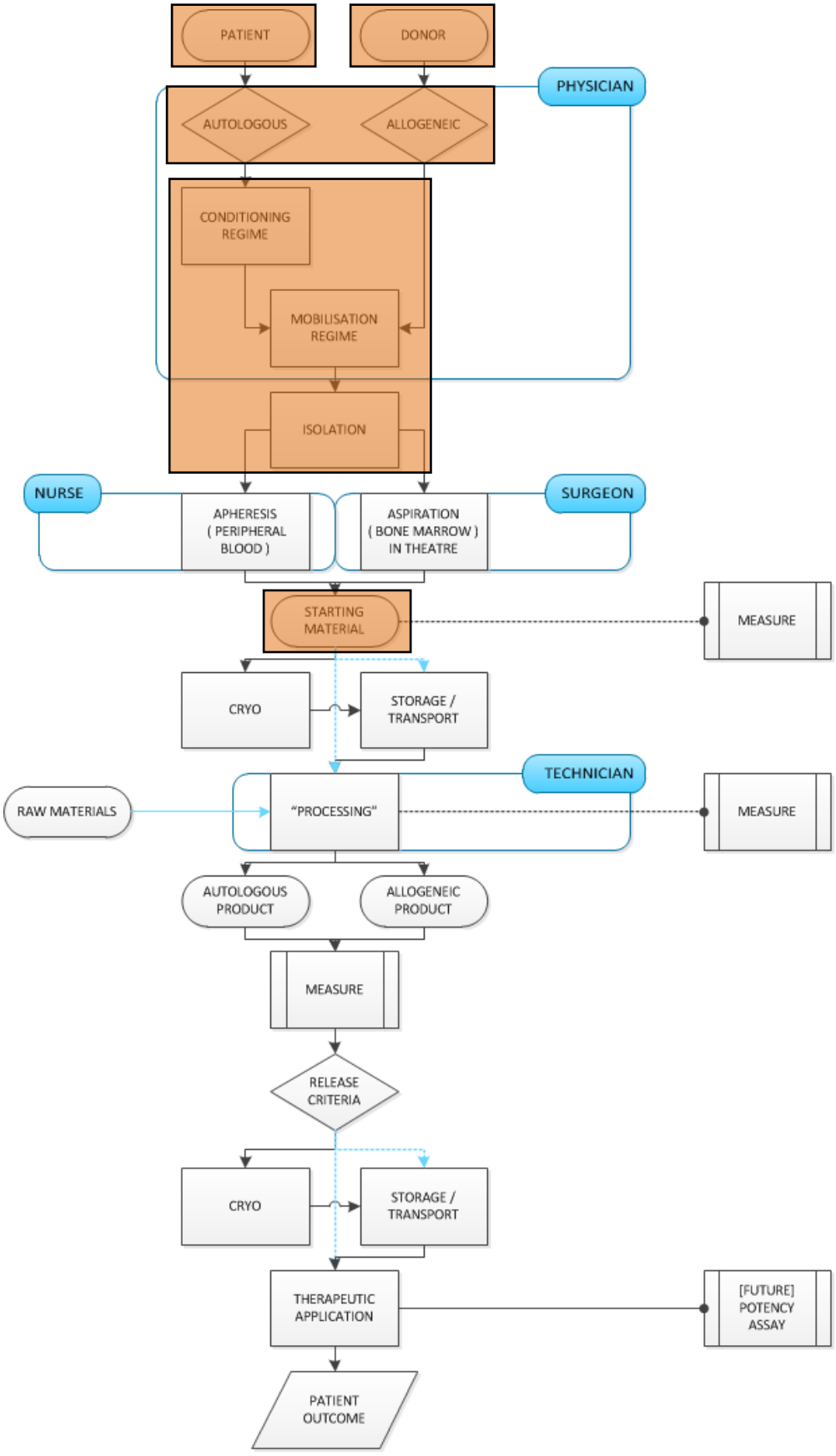


Figure 3.0: Generic Process Map indicating in orange the focus of this Chapter

3.1 Introduction

Understanding the variation that is encountered in the starting material, and the HSCT process, means an understanding of the limits and the specification that a manufacturing machine needs to be designed around – to tolerate or control the biological variation – because only by a degree of mechanisation or automation can cellular therapy achieve the scale necessary for frontline medicine status.

Chapter 2 reviewed the literature surrounding biological variation. This Chapter will use the medical literature to establish a baseline for biological variation as reported on a global scale.

This Chapter is a systematic literature study, using specific guidelines and search terms to extract cell measurements and donor information from the medical literature to benchmark the current overall level of variation in Haematopoietic Stem Cell Therapy. It also intended to identify further specific sources of variation and inform further research.

The information in this Chapter exists in the public domain, but has not been presented as an amalgamated whole prior to this Chapter.

3.2 Quantifying Variation

Quantifying variation will require elucidation of the baseline variation for the process input / output, and identification of causes of variation within this process, and their magnitude. This will identify Critical-to-Quality attributes and measurands that are key contributing factors towards the quality and efficacy of the final product, and determine the extent of common and special cause variation. As Lord Kelvin stated 100 years ago and still holds true today;

> *'…when you can measure what you are speaking about, and express it in numbers, you know something about it; but when you cannot express it in numbers, your knowledge is of a meagre and unsatisfactory kind'* [171]

Control or reduction of variation decreases the number of product defects and increases product quality at a reduced cost [172]. Additionally, standardisation is a vital component to Good

Manufacturing Practice. This control may facilitate the production of a consistent product, with a known efficacy, at scale. For a biological therapeutic this means maximising patient quality of life and longevity, whilst minimising costs.

Understanding biological variation requires both a measure of the allowable variation that does not impinge safety or efficacy, and the achievable variation with current technology and skills. This can be represented as a process capability index (C_{PK}) equal to the quotient of the allowable variation and the achievable variation, and compares the output of a given process with the specification set for the product. These ratios demonstrate the ability of a process to produce a product within set specification limits and compares the observable behaviour of the process with the customer set specification. It is a ratio of the distance from the process centre to the nearest specification limit divided by a measure of the process variability (see **Equations 1 – 3** below) [173].

$$Capability = \frac{Allowable\ Variation}{Achievable\ Variation} \quad (\boldsymbol{Equation\ 1})$$

$$C_P = \left(\frac{upper\ specification\ limit}{6\sigma}, \frac{lower\ specification\ limit}{6\sigma}\right) \quad (\boldsymbol{Equation\ 2})$$

$$C_{PK} = min\left(\frac{upper\ specification\ limit - \mu}{3\sigma}, \frac{\mu - lower\ specification\ limit}{3\sigma}\right) \quad (\boldsymbol{Equation\ 3})$$

C_P is a measure of the process capability assuming the mean is centred between specification limits, and assumes process output is normally distributed. C_{PK} is the C_P assuming the process mean may not be centred between the specification limits. The standard C_{PK} level for a process in six sigma quality control is 2.0 [174]. C_P demonstrates the manufacturer is capable of meeting the tolerances, but C_{PK} demonstrates that the product meets the tolerance, by taking into account the process mean. C_P is the tolerance width divided by 6 standard deviations (process variability). The minimum of the two ratios is used because it gives the worst-case scenario. In the context of normal manufacturing an acceptable C_P is 1.33, and generally the higher the better [174].

To use tools such as the capability indexes, the process should be in control before assessing capability. Variation is never eliminated entirely, and consists of two broad categories; common cause and special cause variation. Common cause variation is expected inherent variation as a function of the starting material and the process involved. Special cause variation is unexpected variation due to external factors or unaccounted variables – such as machine failure causing a product to deviate from tolerance. A process with only common cause variation is stable and predictable. Another regulatory challenge faced for cellular therapies is the comparability question, demonstrating that the process remains the same after a change – which at its extreme is doing the above, at multiple locations, whilst still remaining cost-effective [175].

This will require an understanding of the variation in the starting material, the patient / donor population, the effect of processing and clinical practice, and will ultimately inform strategies to account for this variation. This reflects the requirements of the MHRA [176]. Additionally, consultation with prescribers and surgeons is a pre-requisite to setting an achievable specification (the limits of variation the product can meet without negatively affecting patient outcome) and designing a manufacturing process with the appropriate ability to cope with this variation (its tolerance range).

3.3 Establishing a Baseline

Considering the anecdotal evidence encountered by the author of the extent of biological variation, the next stage was to gather the experiences of clinical, industrial and academic bodies, evaluate the extent of the challenge and inform the methodology for the subsequent, data-driven steps.

One outcome of these investigations has been the production of two generic process maps for HSCT, one of which has been used to illustrate the contents of each Chapter of this thesis (**Figure 3.0**). **Figure 3.1** is the precursor to this graphic, and includes hypothetical sources of variation. These two figures illustrate a typical procedure within a clinical laboratory environment for processing a single HSCT product.

Figure 3.1 illustrates several potential sources of variation. Process maps from Anthony Nolan and SOPs from the Dana Farber Cancer Institute were used to design these two Figures, and they were changed and improved as the research progressed. Discussion with key industrial stakeholders from GSK, NHS Blood and Transplant, Terumo BCT, the Dana Farber Cancer Institute, the Anthony Nolan Trust and delegates at international conferences, identified and expanded upon potential sources of variation and the reasons behind their influence.

As a result, several clinical and open-source datasets were identified for data mining, including the European Bone Marrow Transplant (EBMT)'s and National Health Service Blood and Transplant (NHSBT)'s databases. Whilst exploring these avenues of research, one source of potential information on variation was the medical literature available in the public domain. This publicly available, free information could be used to established a baseline of the overall variation in HSCT and be used as a benchmark for comparison.

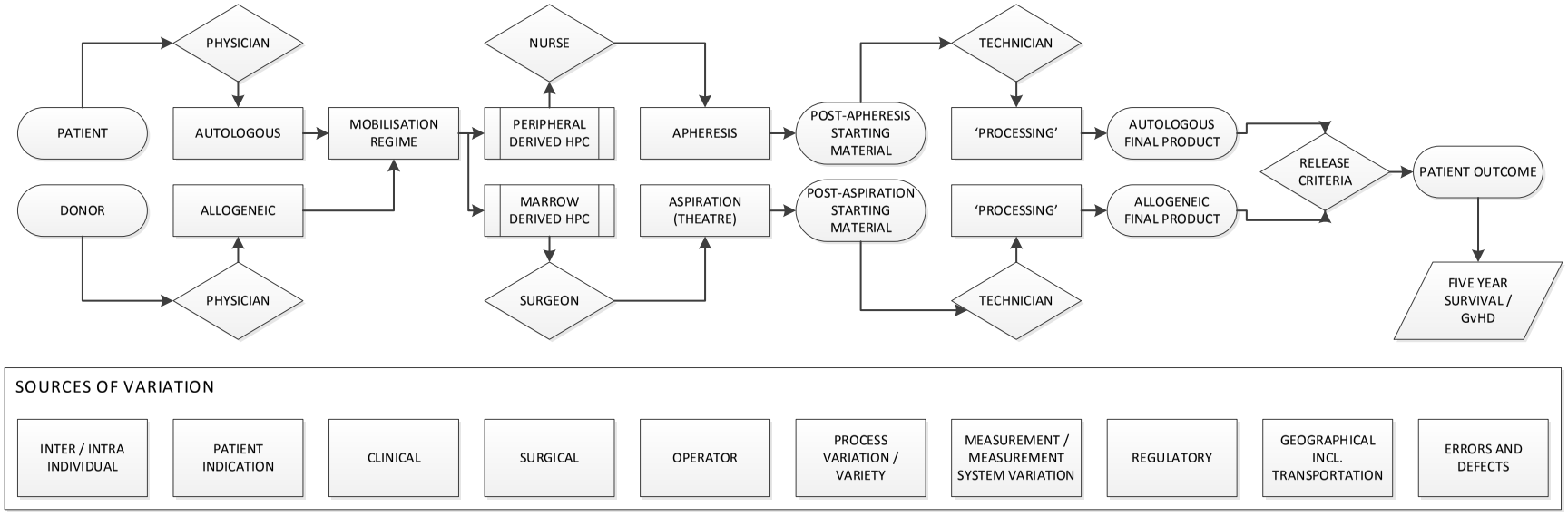


Figure 3.1: Generic Process Map for HSCT

3.4 Systematic Meta-Analysis

This systematic meta-analysis aimed to examine the literature of HSCT for a number of key predetermined variables. The primary objective of this analysis has been to gain a baseline understanding as to the extent of biological variation in collected and transplant cell metrics, under the practice of medicine, because this therapy is applied and manufactured in a hospital setting. Secondary objectives were data-dependent and included the effect of different processes, indications and donor / patient characteristics on the aforementioned cell metrics.

It was hypothesised that sufficient information may be present within the medical literature to complete a preliminary investigation into the extent of biological variation – sources, collected cell characteristics, product / dose characteristics, process specifics and patient outcomes – if the nature of such a dataset and its caveats were understood. The results of this investigation would identify key areas of interest, aspects requiring further refinement, and promote critical process development discussion between industrial, clinical and academic bodies.

During the literature review (**Chapter 2**) this hypothesis was tested when a handful of the papers read included information about cell numbers and distributions. For example *Chevallier et al* reported median proportion, number and volume of bone marrow, cord blood and leukapheresis product [177]. This also included range data; the minimum and maximum number recorded in the study. This information, alongside mean and median results could be used to benchmark the variation encountered in various blood and graft sources.

After this discovery, a study was initiated to systematically examine the literature further with the aim to expanding this database of biological information that would inform the spread and distribution of biological variation.

3.5 Methodology

This meta-analysis was guided by the principles prescribed by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (PRISMA [178]) to ensure the approach and results were robust and systematic. One deviation was the use of a single operator (the author) for

extraction and analysis, so there may exist a bias that could have excluded some data-containing studies. A number of journal papers may have been excluded due to finite search terms, limitations of the database, and data published in languages other than English. Secondary objectives arose from a first appraisal of the literature and were aimed at examining correlations between human factors, processing methods, and the cell characteristics of the harvested material (see **Table 3.0**). The online databases Web of Science and PubMed, were used to search the literature, using a number of pre-determined keywords, Medical Search Headings (MeSH) and publication dates (see **Table 3.1**). Articles were restricted to English language unless a native translation was provided, and only refereed journals were used (conference proceedings were excluded for example, as many did not contain numerical data that could be extracted). The abstracts of the resultant studies were then screened for likelihood of containing cell data – for example those that were comparison / outcome studies or clinical trials. Eligible publications were obtained in full and examined manually for patient, donor and graft characteristics guided by the variables in **Table 3.0**. These characteristics were identified by previous discussion and mind-mapping. The primary characteristics were mandatory for studies to pass through to the data extraction stage. Qualitative data such as methodology (where recorded) was reduced to single word / numerical data. The name of the first author and a unique identification number was used to mark papers used for future reference.

Data was extracted from the full article into a spreadsheet within Microsoft Excel. Both Excel and IBM SPSS 22.0 were used for data analysis. Literature that yielded data was downloaded and stored for future record alongside its unique identification number. Some papers yielded more than one observation, such as when comparisons were made between cell source, or mobilisation regime.

Primary Study Characteristics	Secondary Study Characteristics	Tertiary Study Characteristics
Number of donors Donor gender Donor age* Donor weight (kg)* Donor ethnicity Number of patients Patient gender Patient age* Patient weight (kg)* Patient ethnicity Patient conditioning Autologous or allogeneic therapy Source of stem cells Collected TNC, MNC, CD34+, CFU-GM and viability** Transplanted TNC, MNC, CD34+, CFU-GM and viability**	Donor mobilisation drug Donor mobilisation regime Day of aspiration / apheresis Study start and end date Number of centres involved in study Country of study Patient indication Patient prior medication Patient prior stem cell therapy	Named collection equipment Named processing equipment Named analytical equipment CD34+ elucidation method Number of aspirations / apheresis procedures / donor Apheresis flow rate used (ml / min) Target apheresis volume (ml) Duration of apheresis (mins) Target apheresis CD34+ cell count Number of times donor complete blood volume was processed Number of grafts / transfusions per patient Collection aims for TNC, MNC and CD34+ cell populations Volume of Collection (ml / kg)

*At time of procedure
** mean, median, standard deviation, upper and lower ranges

Table 3.0: Variable Table

	Search Terms	Database	Search Results	Studies with Data
A	Autologous, haematopoietic, stem, cell, therapy (2003-2013)	Web of Knowledge	298	31
B	Autologous, haematopoietic, stem, cell, therapy (1992-2002)	Web of Knowledge	105	6
C	Allogeneic, haematopoietic, stem, cell, therapy (2003-2013)	Web of Knowledge	1183	36
D	Allogeneic, haematopoietic, stem, cell, therapy (1992-2002)	Web of Knowledge	125	4
E	Stem, cell, comparison (2003-2013)	Web of Knowledge	585	9
F	Stem, cell, outcome (2003-2013)	Web of Knowledge	894	40
G	Stem, cell, blood (2010-2015)*	Pubmed	458	30
H	Stem, cell, blood (2005-2009)*	Pubmed	486	71
I	Autologous, peripheral (2010-2015)	Pubmed	1321	42
	*Clinical trials only	**Total**	**5458**	269

Table 3.1: Publication Search Terms

3.5.1 Statistical Tools

Normally distributed data (**Figure 3.2**) is required for a number of standard statistical tools, such as t tests and ANOVA. Depending on whether the data is normally distributed, or non-normally distributed, different statistical tools that are designed for those distributions, may be required –

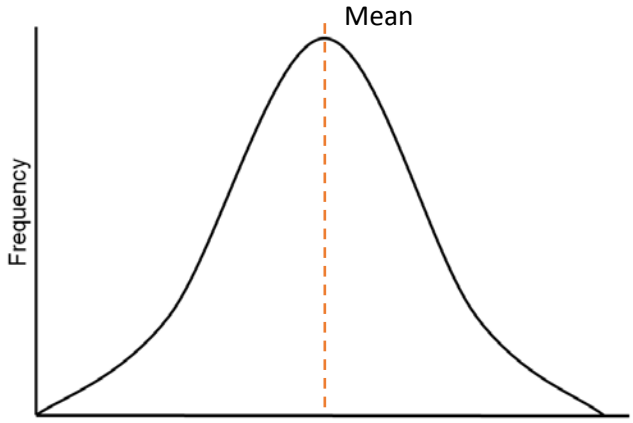


Figure 3.2: A normally distributed dataset, centralized around the mean

using the incorrect statistical tool may provide misleading results and conclusions.

Non-normally distributed data (**Figure 3.3**) occurs when there are too many extreme values, when the data is derived from more than one process, or operator, when round-off errors occur or when there is insufficient resolution on measurement devices, when data values are close to zero or when the data follows a different distribution such as exponential or binomial [179]. Normality can be tested statistically using Shapiro-Wilk's test [180].

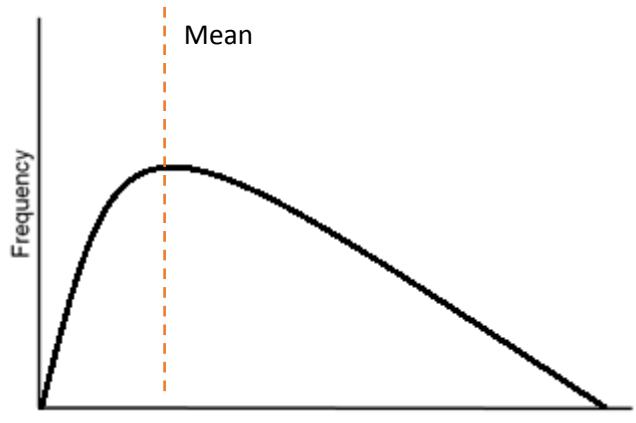


Figure 3.3: A skewed, non-normally distributed dataset

Non-normally distributed data can either be transformed mathematically into normally distributed data, or alternative statistical tools can be applied directly to the non-normal data.

The shape of the distribution can be described with two metrics, skewness and kurtosis. Skew is the measure of symmetry, and kurtosis is a measure of the shape of the distribution. A skew of 0 is symmetrical, whilst a positive skew represents a long tail to the right and a negative skew represents a long tail to the left. A kurtosis of 0 is a Gaussian, normal distribution, with a positive kurtosis being indicative of a more peaked distribution and a negative kurtosis being indicative of a flatter distribution than the Gaussian. Neither of these measures have units. The Shapiro-Wilk's test is an alternative to the skew and kurtosis of a distribution – if the data tests significant then the data is normal, if it doesn't the data significantly deviates from a normal distribution [180].

The cell metrics of this dataset such as TNC and CD34+ cells were tested for normality using a Shapiro-Wilk test, and their distribution using skewness and kurtosis.

3.6 Results and Discussion

The primary output of this meta-analysis were several figures that demonstrate the extent of variation in transplanted cell dose found within the literature. There was insufficient data within the meta-analysis sources to produce a similar demonstration for cell content of the starting materials. TNC and CD34+ cell count was the most prevalent cell characteristic reported.

This meta-analysis represents a specific sub-population of the data available in the public domain, derived from summaries of transplant case studies, clinical trials and comparison studies. This is not an exhaustive representation, but was obtained by examining 5,458 peer-reviewed journal articles between 1980 and 2015. This resulted in 269 articles that contained 491 observations including several donor, centre and graft variables.

Data drawn from this meta-analysis originated from multiple global sources, clinical centres, clinicians / surgical teams, addressing different indications and derived from different patient and

donor demographics. Stratification into each of these subsets was not possible due to the limitations of the literature, however the dataset was scrutinised using Pareto analysis as a factor of location and indication of study.

Pareto Analysis is one of the Seven Tools of Quality in the manufacturing sector, so named after the seven weapons used by the warrior monk (Sōhei) Benkei to triumph in battle [181]. The Pareto principle being commonly known as the 80/20 rule – the observation that 20% of the causes determine 80% of the problems [174]. The patient indication and the study country were examined using this principle to identify the factors with the highest influence – the most prevalent country or indication in the database for example. In this case each variable (indication / country) is plotted in descending order from highest to lowest contribution with an overlay of percentage cumulative contributions for indication (**Figure 3.4**) and country (F**igure 3.5**) respectively.

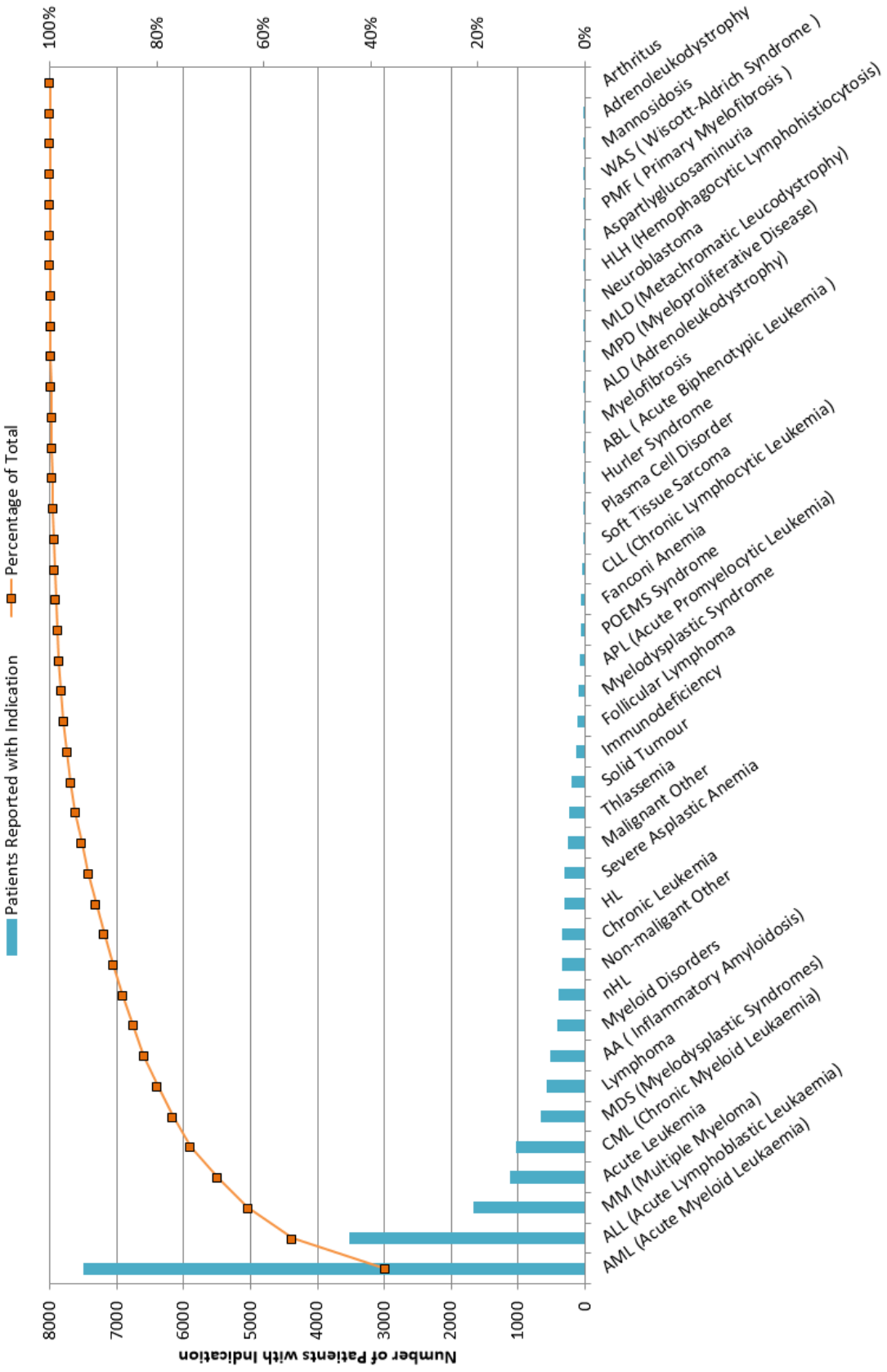


Figure 3.4: Pareto Analysis of Indications within the Meta-Analysis

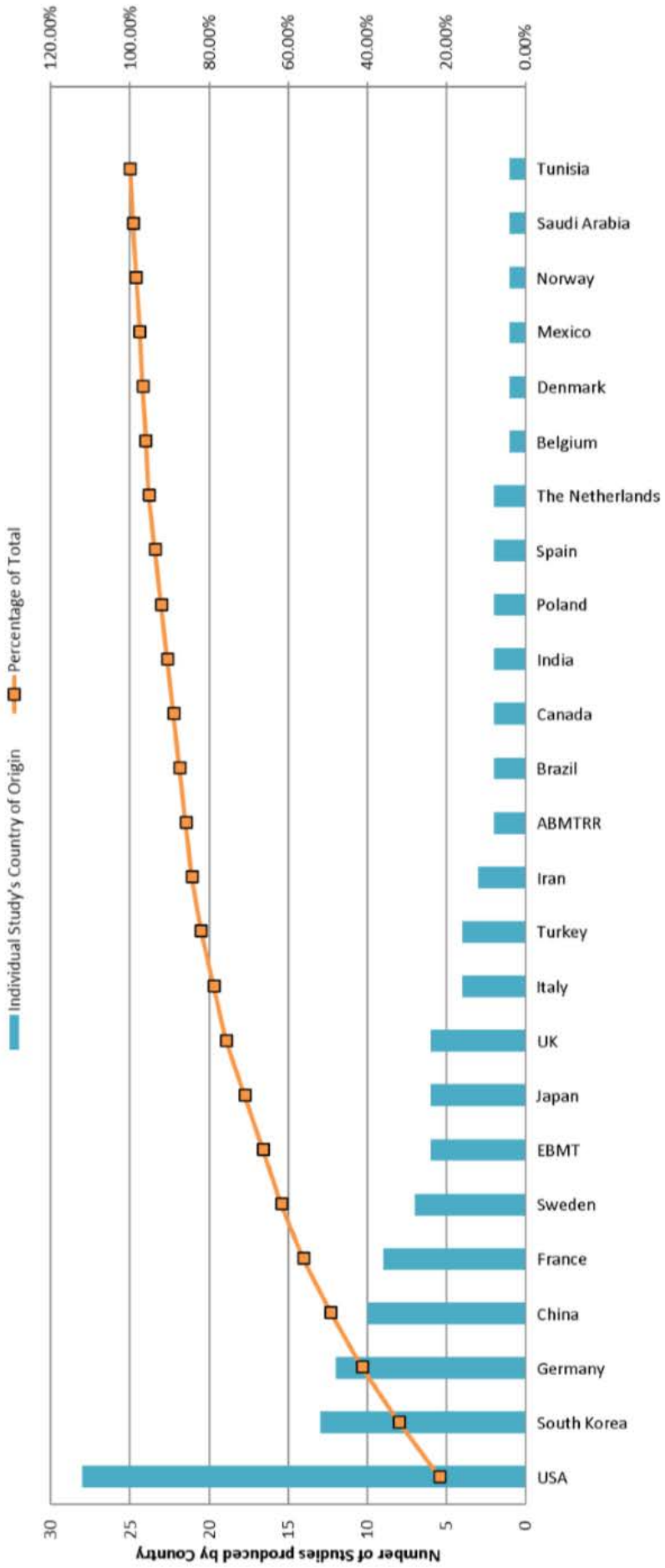


Figure 3.5: Pareto Analysis of Centres by Location within the Meta-Analysis

The content of the starting material is reported here as Collected CD34+ cells (**cCD34**) and content of the therapeutic dose is reported here as Transplanted CD34+ cells (**tCD34**), because these were the most common measures of cell content encountered and as such are the primary measurands used here to represent variation. The majority of studies reported their cell metrics as cells / kg (patient weight).

Figure 3.4 is a Pareto chart for the indications included in this meta-analysis and from this it can be determined that leukaemias (blood cancer) make up the majority of the dataset, which is to be expected given that HSCT is primarily used for treating such indications. This suggests that the majority of the data found in other sources - NHS datasets, university hospital research for example – will be focused on this indication. As a result clinical centres or organisations specialising in this area are likely to have further useful data, and will be approached for further work (see **Chapter 4, DFCI**).

Figure 3.5 illustrates strong contributions to this dataset from the USA, Germany, China and South Korea. EBMT represents contributions from the European Society for Blood and Marrow Transplantation, publications that were carried out as part of a multi-centre working party or group under their umbrella. This is a unique variable, as all other points in this analysis are representative of a single centre or country.

3.6.1 Overall Variation in the Product

Figure 3.6 plots the median TNC count (y-axis) against the median CD34+ cell count (x-axis) of the given cell dose for a given literature study. These two metrics have been plotted against each other as CD34+ cells are a sub-set of the TNC population, and it was intended to identify whether one is indicative of another in terms of cell number. Points represent the median cell count for a given study, and the lines represent the range of cell count within the given study. The range in dose given to patients within each study is represented by the lines spreading out from these points, and has been produced on a logarithmic scale to demonstrate the range as a 'cloud'. The variables in **Figure**

3.6 were plotted pairwise and is therefore limited to those studies that provided both median and range data for both TNC count and CD34+ cell count.

This key figure emphasizes that the variation in HSCT dose in any given study from this dataset can be between one and four orders of magnitude about the median. As HSCT dose is determined on a case-by-case basis as a result of a minimal cell number (as long as the dose is above this number, it is prescribed), the variation shown in this diagram not only shows the variation as a function of posology, but as a result of biological, process and operator variation also (given that variation is not currently actively controlled for, other than by minimal collection criteria and minimum dose criteria).

This difference in cell dose raises concerns about comparable levels of efficacy and treatment within individual studies, and will only complicate attempts to discern the definitive mode of action for HSCT and the dose / response relationship. It is important to note that in some cases the transplant itself is only one component of the treatment plan. When it comes to larger scale production, this level of variation would not permit a stable and predictable manufacturing process and would be unacceptable within an equivalent non-biological process. However, there are a number of considerations regarding this dataset that must be taken into account;

Limited data reported in many of the given studies meant that stratification according to patient indication or demographic, and donor metrics such as age, weight or ethnicity was not possible. To identify any common and special cause variation due to donor / patient metrics, further stratification that would allow this was not possible. Cell dose was inconsistently reported as either cells / kg patient, or donor bodyweight. Due to the presence of paediatric or bariatric donors / patients, several data-points may be skewed.

Methodology was rarely reported, including isolation technique, patient mobilisation drug, regime, prior conditioning or apheresis procedure, for example. Considering the significant effect these can have on the cell content of the raw input material, these are important variables to stratify the

effect of differing processes on the variation. Whether fresh or cryopreserved cells were used was unclear in several cases; a further important stratification in the context of product efficacy and yield post-cryopreservation. However the substantial variation encountered in **Figure 3.6** can be attributed to a number of broad categories (albeit at an unknown magnitude and detail).

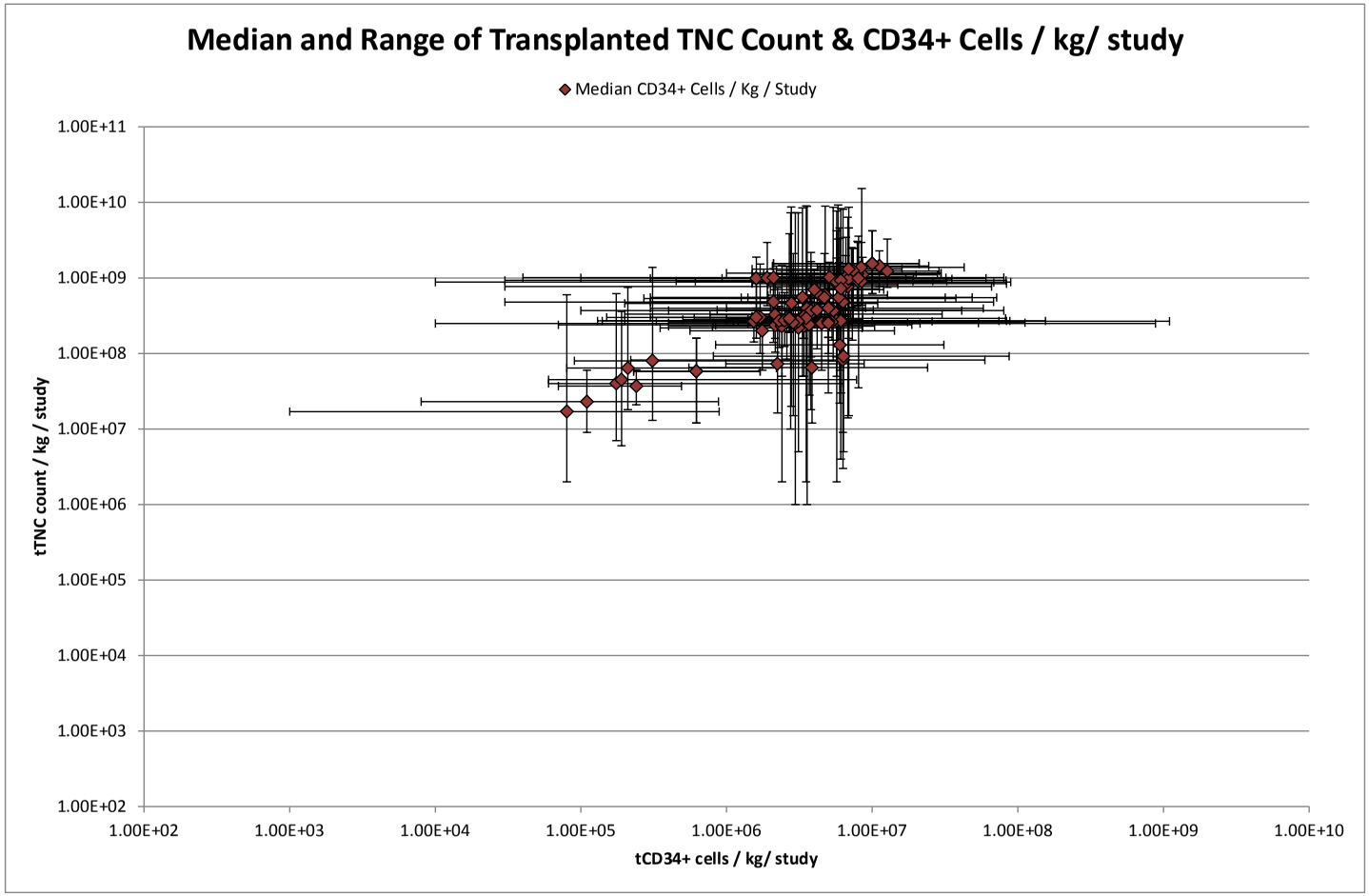


Figure 3.6: Transplanted Total Nucleated Cell Count against CD34+ Cell Count, of the product, recorded in the meta-analysis.

3.6.2 Overall Variation, stratified into autologous and allogeneic sources

Under the practice of medicine, the variation currently encountered in HSCT (and represented by this sub-population) can be between one and four orders of magnitude of the median amount. As an analogy, this is the difference between a pharmaceutical dose of 1 g and 10 kg.

The range of both **cCD34** and **tCD34** was calculated for each study sample. An open-high-low-close (OHLC) chart, traditionally used to plot financial fluctuations, was used to plot each respective samples' median, minimum and maximum **cCD34** / **tCD34**. This format was used for biological data because it is a visually succinct method of demonstrating the extent of variation with respect to the median and with other studies. **Figure 3.7** and **Figure 3.8** represent the overall variation for collected (n = 102) and transplanted (n = 275) CD34+ cell count. Point (i) on **Figure 3.8** exhibits no obvious characteristics that would differentiate itself from the other studies (paediatric donor or a cord-blood sourced transplant for example). All data points were plotted according to their unique ID numbers.

A number of studies have reported a minimum of zero collected or transplanted cell counts, which are very particular extremes. It is unlikely these are true values of zero but more likely insufficient cells were mobilised and were deemed to be unviable for transplant. These values, as much as they must be considered as a representation of an individual's personalised medicine, skew the overall analysis of variation considerably. For the purposes of this analysis, collected/ transplanted cell counts of zero have not been used, to avoid this misleading skew.

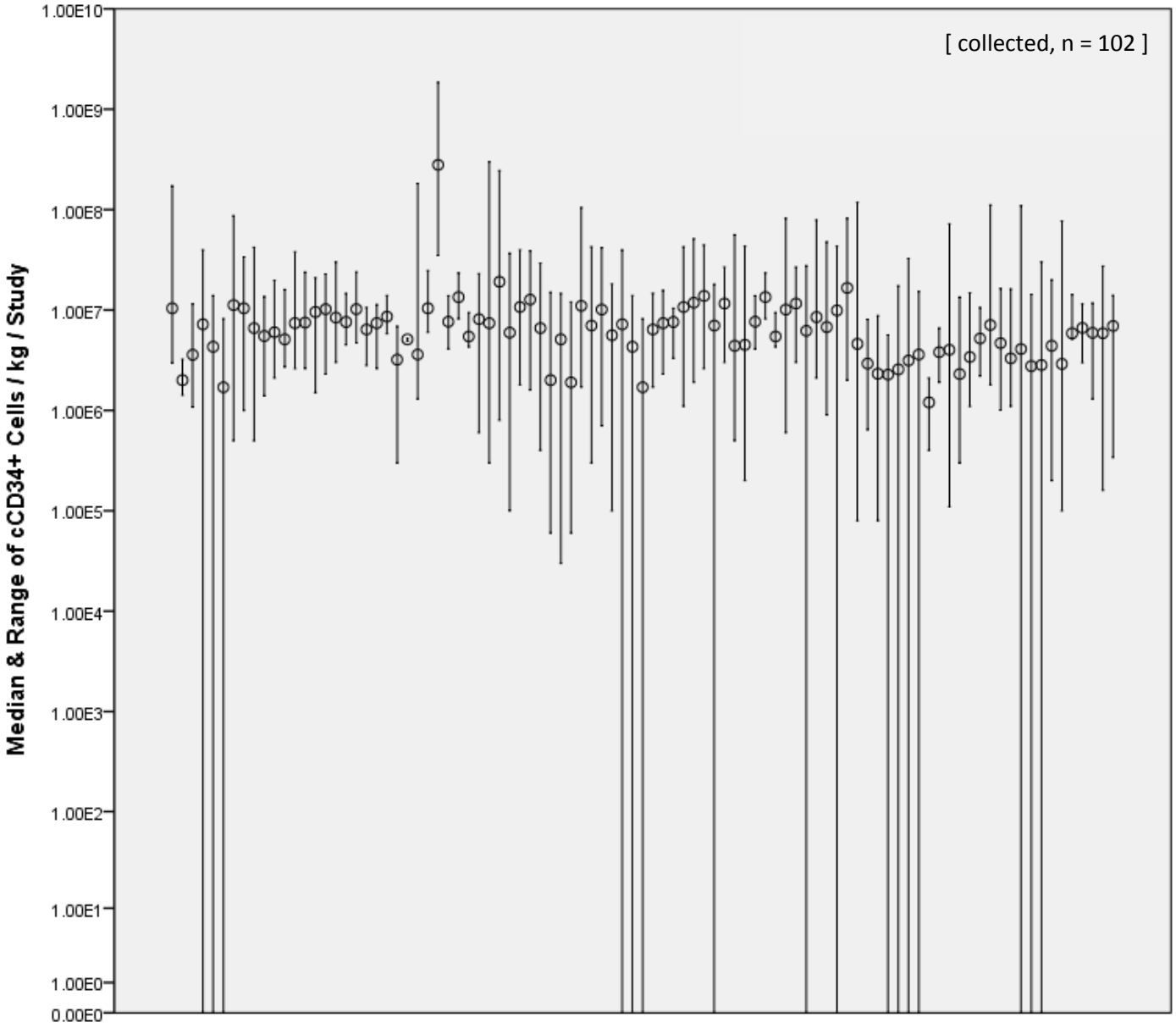


Figure 3.7: An OHLC of CD34+ cell count within the Starting Material, as reported within the literature

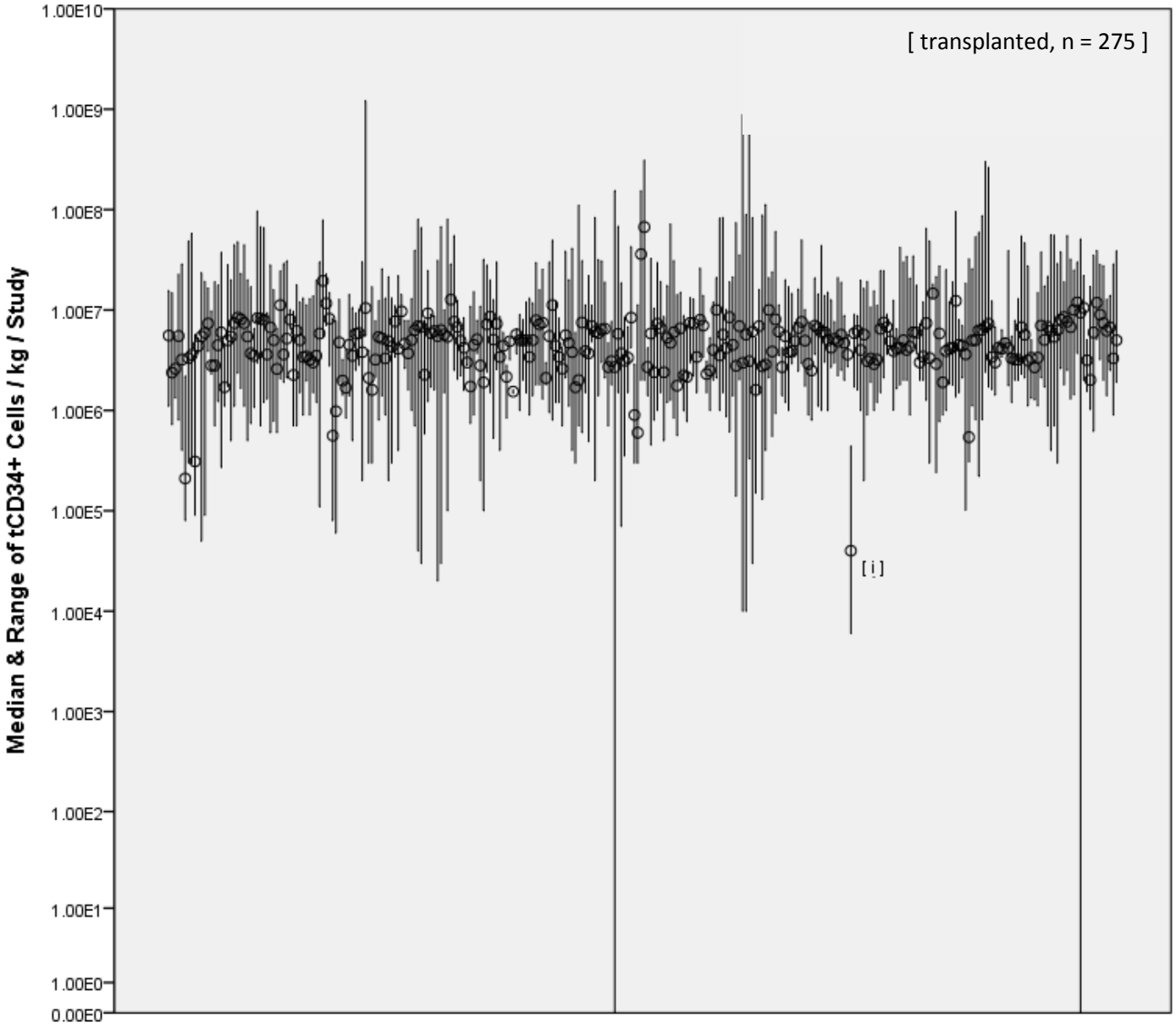


Figure 3.8: An OHLC of CD34+ cell count within the final product, as reported within the literature

3.6.3 Stratification of the Overall Variation into Collected and Transplanted

Allogeneic derived material can vary up to 6 orders of magnitude of the median cell count, whilst Autologous derived material can vary up to 4 orders of magnitude. Further stratification is required to discern the mechanism for this and to determine its impact and relevance.

The population was split into autologous or allogeneic transplant and the stem cell source (bone marrow, peripheral blood, cord blood, mixed, and not reported). Only bone marrow, peripheral blood and mixed sources contained statistically significant numbers of data-points, therefore only these groups were included. Mixed source represents patients treated with both peripheral blood and bone marrow.

A considerable number of cases included in the dataset had minimum CD34+ cell count values of zero. For the analysis that follows, these have been removed so as to not skew the range of variation – this can be up to 9 orders of magnitude of transplanted CD34+ cells if values of zero are included.

The variation in collected CD34+ cell count (**Figure 3.7**) is up to four orders of magnitude of the median mobilised amount (3.00×10^4 cells / kg to 4.45×10^8 cells / kg, n = 102).

The variation in transplanted CD34+ cell count (**Figure 3.8**) is up to six orders of magnitude of the median dose (1.00×10^4 cells / kg to 1.21×10^9 cells / kg, n = 275). Point [i] exhibited no identifying features such as younger age, or lower weight that would explain its significantly lower median and range of CD34+ cells – a clear example of the variation within this starting material.

Figure 3.9 stratifies transplanted CD34+ cell count into cell source and transplant type. For autologous therapy the range in CD34+ cell dose was between 6.00×10^4 cells / kg and 3.00×10^8 cells / kg, (n = 110). For allogeneic therapy the range in CD34+ cell dose was between 1.00×10^3 cells / kg and 1.21×10^9 cells / kg, (n = 188). The variation is two orders of magnitude higher in the allogeneic transplant fraction than in the autologous fraction.

Autologous bone marrow derived material appears to be under the most control (3.0×10^5 cells / kg to 2.1×10^7 cells / kg, n = 9), but is determined from only 9 results which is not a statistically reliable sample compared to the other sub-sets. However, autologous peripheral blood appears to be within 2 and 3 orders of magnitude of the median dose (2.0×10^5 cells / kg to 3.0×10^8 cells / kg, n = 54). This is considerably more manageable than the variation demonstrated in allogeneic peripheral blood – up to six orders of magnitude (6.0×10^3 cells / kg – 1.21×10^9 cells / kg) – a key starting material for HSCT.

It may be noted that a number of studies reported median cell counts that fells below the average count represented by other studies. As a result, cord blood derived and paediatric data (a total of 10 and 18 data points for cCD34 and tCD34 respectively) were removed from this analysis, so these values are a demonstration of the extremes of the inherent biological variation in adult bone marrow and peripheral blood –derived material only.

A summary of the variation in CD34+ cell count, stratified into cell source and transplant type can be found below in **Table 3.2.**

CD34+ Cell Count	N	Range (cells / kg)
Collected, Overall	102	3.00×10^4 to 4.45×10^8
Transplanted, Overall	275	1.00×10^4 to 1.21×10^9
Transplanted, Autologous	110	6.00×10^4 to 3.00×10^8
Transplanted, Allogeneic	188	1.00×10^3 to 1.21×10^9
Autologous, Peripheral	54	2.00×10^5 to 1.10×10^8
Autologous, Bone Marrow	9	9.00×10^4 to 2.10×10^7
Autologous, Mixed	9	6.00×10^4 to 2.57×10^7
Allogeneic, Peripheral	72	$1.00\ 10^4$ to 1.21×10^9
Allogeneic, Bone Marrow	40	1.00×10^4 to 1.10×10^9
Allogeneic, Mixed	55	2.00×10^4 to 8.00×10^7

Table 3.2: Summary of CD34+ Variation in the Meta-Analysis
The difference between stratified and overall N values are due to missing values – such as papers reporting allogeneic median and ranges, but not cell source, for example

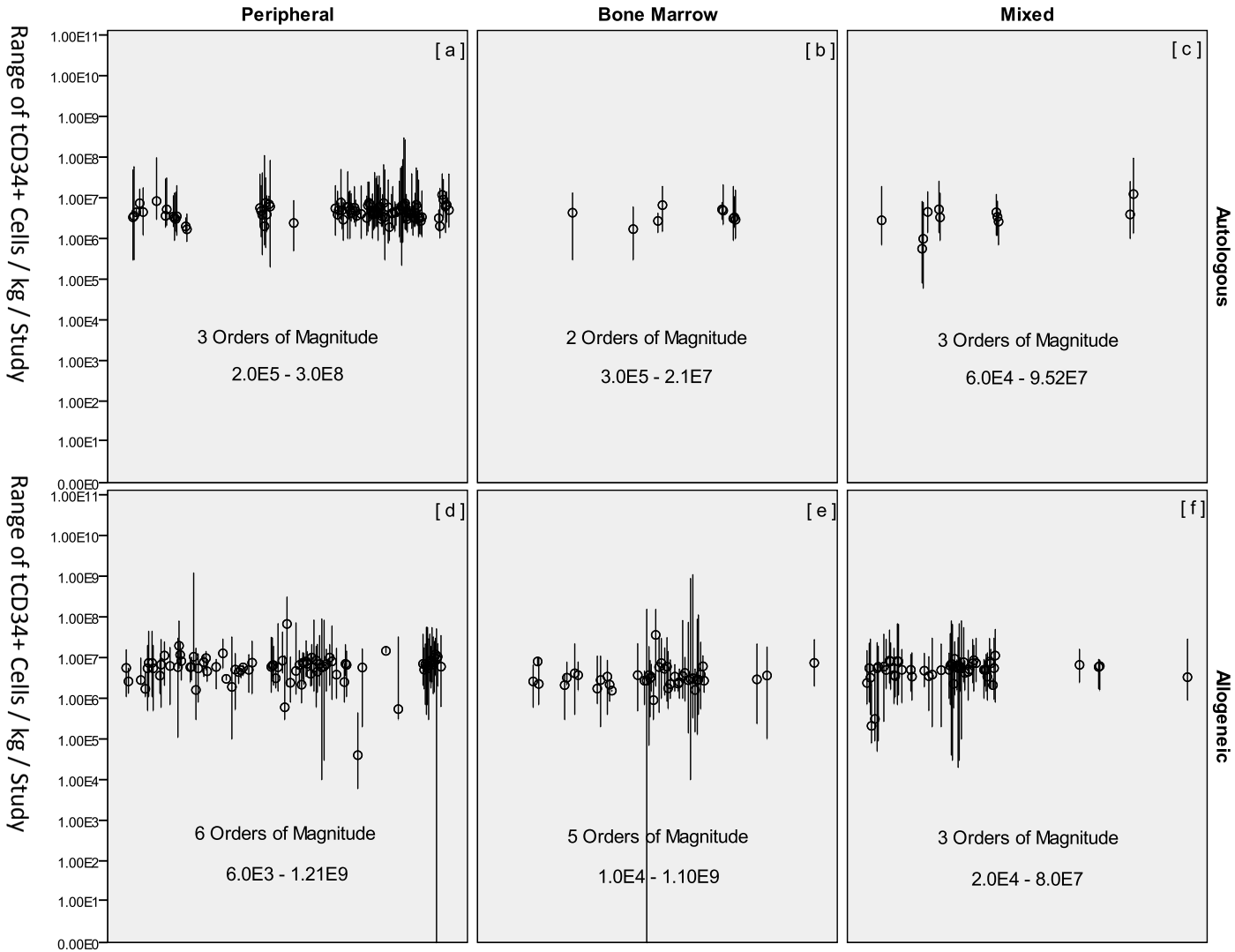


Figure 3.9: An OHLC of CD34+ cell count within the final product, stratified into autologous and allogeneic sources, and derived from peripheral blood, bone marrow or mixed sources

3.6.4 Variation as a Function of Time

Overall, there has been limited improvement, or requirement for improvement in our ability to control the variation encountered in HSCT over the thirty years included in the meta-analysis population.

Figure 3.10 represents the range of collected CD34+ cells and **Figure 3.11** represents the range of transplanted CD34+ cells transplanted, ordered by time. Time has been represented by the year in which the individual studies commenced. This is the stage where the protocols were established and therefore represent the technology / methodology typical of the time. The mean length of study was 7 years, and this explains the relative lack of data found between 2010 and 2015 because studies within this timeframe will still be in progress and not yet reported. Point (i) on **Figure 3.10** exhibits no obvious characteristics that would differentiate itself from the other studies.

A Spearman's Rank-Order Correlation (SROC) was carried out to assess the relationship between CD34+ cell ranges (collected and transplanted) over time, and alleviate the Cluster Illusion [182] from data interpretation. The Cluster Illusion is the human tendency to see patterns amongst random data. Only paired observations were used for this analysis. SROC was chosen as the range of collected / transplanted CD34+ cells was not normally distributed, as confirmed by visual inspection of a histogram and a Shapiro-Wilks test ($p > 0.05$). Preliminary analysis showed the relationship to be monotonic, as assessed by visual inspection of a scatterplot. There was a weak negative correlation between the range of CD34+ cells collected / transplanted and the year a given study commenced, $r_s = -0.295$, $p < 0.003$ / $r_s = -0.178$, $p < 0.003$.

There are a considerable number of other variables that may contribute to a correlation or not (such as incremental improvements in technology, or institutional experience) rather than just study start year, however, some improvement was expected, as a factor of operator learning, improved regulations, procedural improvement in line with clinical best practice or incremental increases in the resolution of measuring equipment or improvement of processing technology. Additionally, the

number of unknowns (of which some are summarised later) are enough that any correlation could be argued to be insignificant or circumstantial. However the data still shows that, within this sub-population, there has been very little improvement (or apparent drive to improve) in the community's ability to control variation over time.

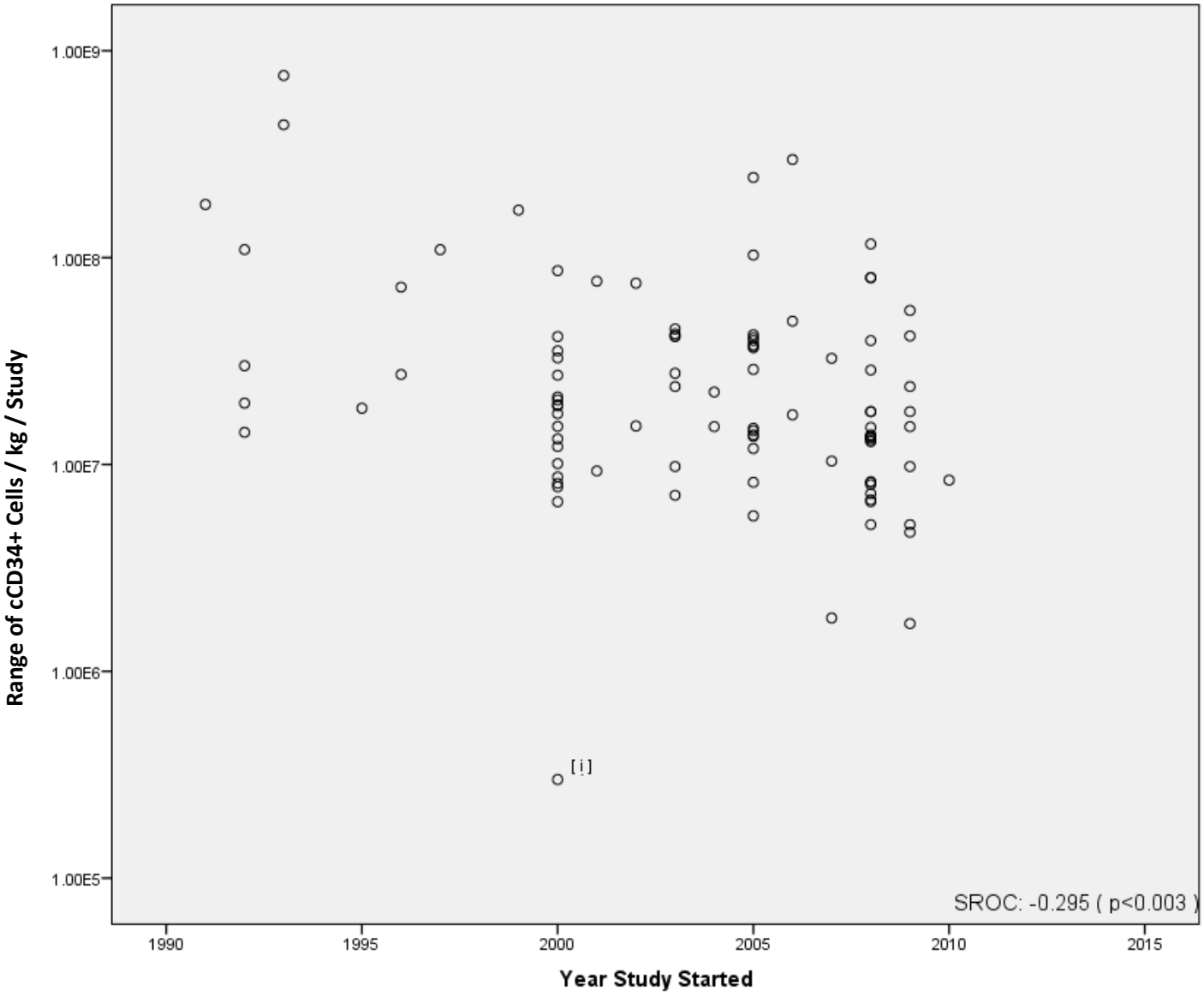


Figure 3.10: Range of collected CD34+ cell count against the year of study starting

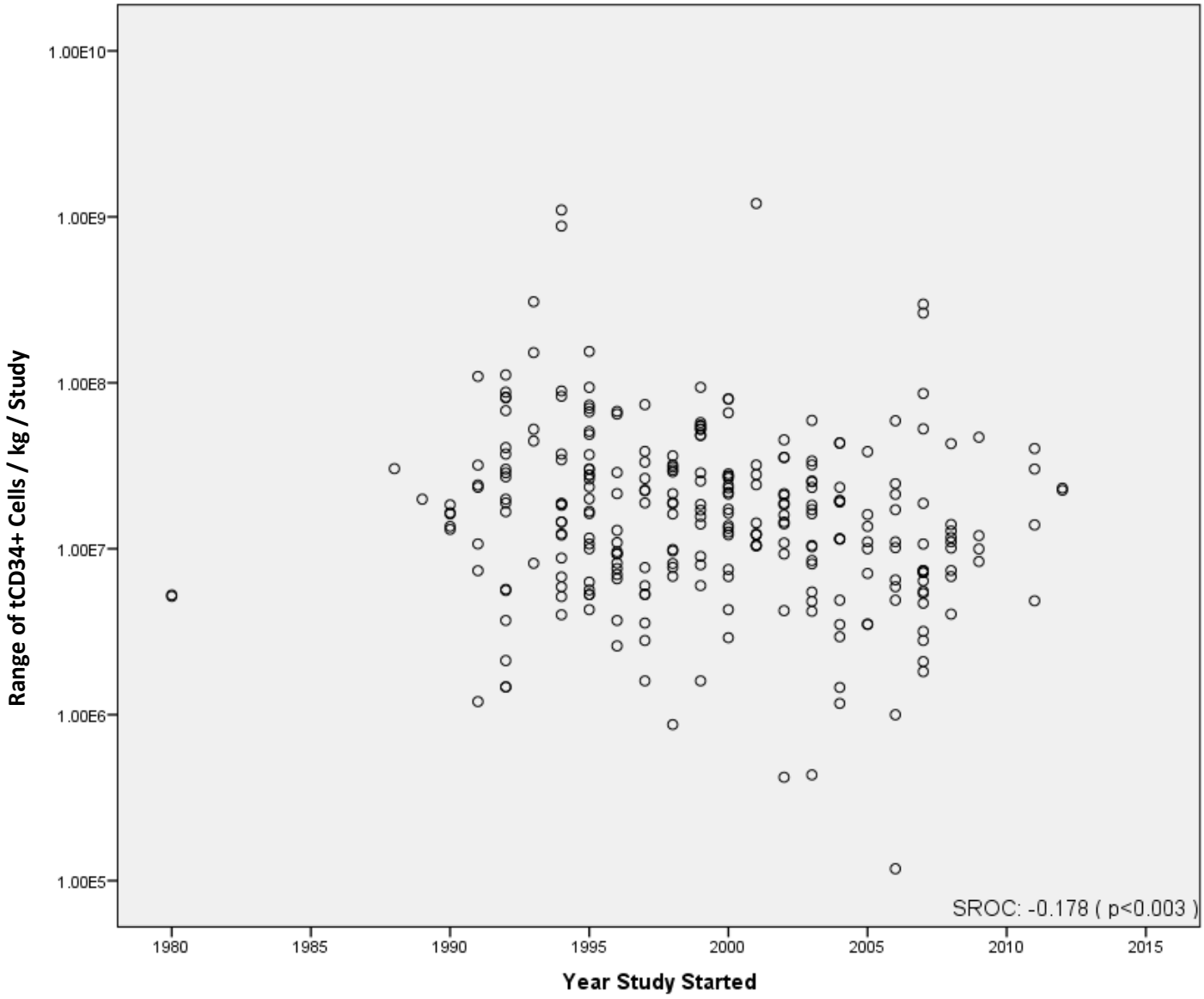


Figure 3.11: Range of transplanted CD34+ cell count against the year of study starting

3.6.5 Variation as a Function of Country

This meta-analysis contains observations from 32 different countries and by multinationals, which are represented by 143 clinical centres. There is insufficient resolution to discern any specific advantage in terms of variation between any clinical centre or country.

The geographical location and clinical centre of each study was recorded. A Pareto Analysis was carried out on the meta-analysis database to discern the leading contributors within this dataset. This identified ten countries that had significant presence. This was subsequently used to plot another OHLC chart (**Figure 3.12**), segregated into a number of high impact countries. Only tCD34 has sufficient data-points within the dataset to plot with significance.

For each country, the number of centres contributing towards the result has been annotated. It is difficult to discern differences between countries without much greater resolution, as each of these centres will likely operate under different practices / equipment / staff and target different indications and patients. It could be interpreted however that Germany, with 11 centres, has a significantly greater control over variation than Sweden, with 2 centres, but it is difficult to make concrete observations.

China in particular appears to have the most control over its variation, compared to other countries. An unfortunate stigma surrounds Chinese research (so much so, that rules have been issued by the Chinese authorities regarding dishonesty in science publishing [183]), but it is entirely possible that this control is as a direct result of the massive investment China is making into science and technology. In fact, China now spent more on research and development than the EU in 2013, and is expected to outspend the USA by 2020 [184].

There is however enough evidence here to suggest that there exists a dichotomy in practice between countries that results in varying levels of variation, and some countries appear to have much greater control over this than others. Italy is also particularly good. As a result, it is likely there are cultures of practice or technology that could be adopted to improve the global biological

variation challenge. Alternatively, larger countries such as China, may be incorporating a larger sample size of clinical centres.

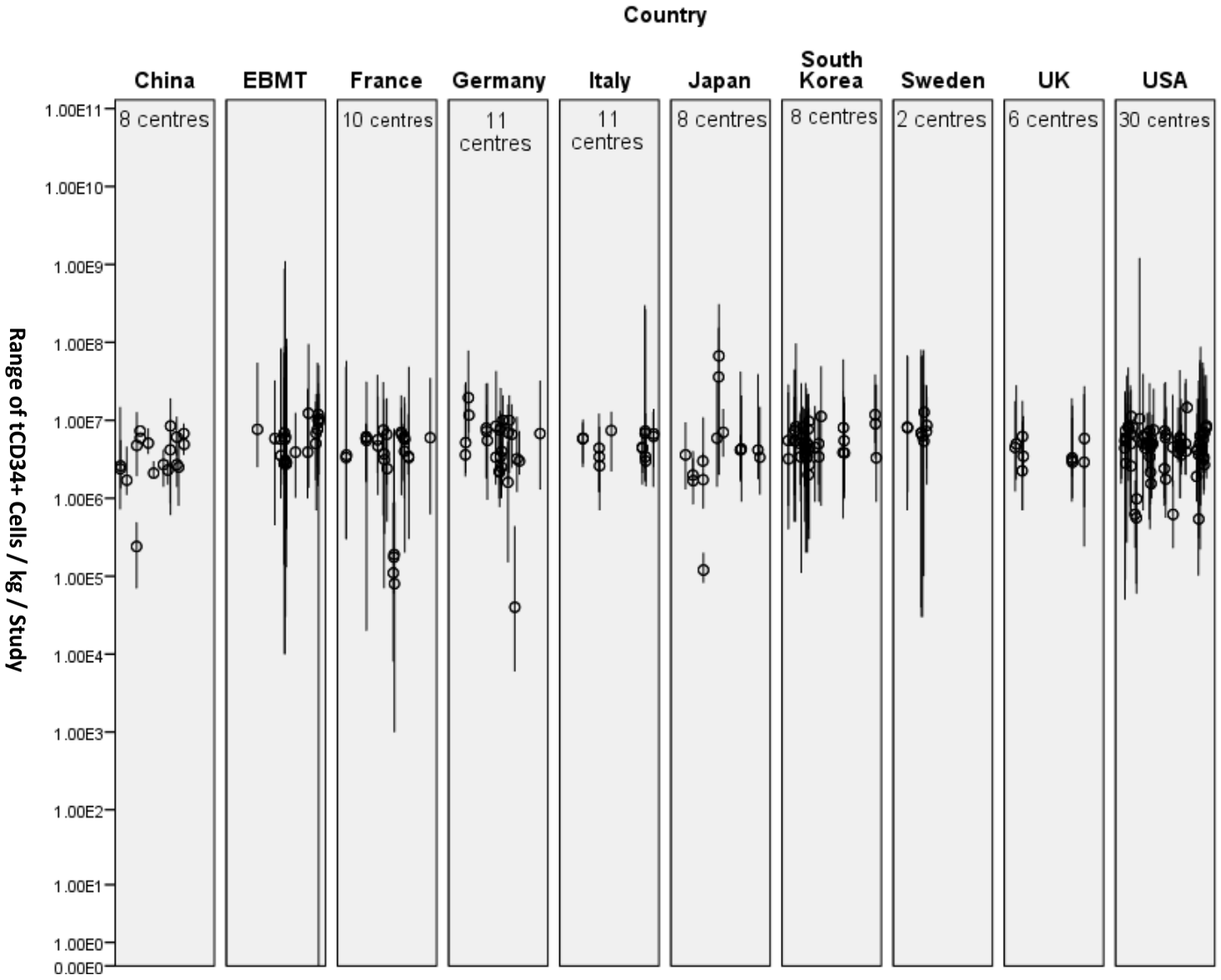


Figure 3.12: An OHLC chart showing the median and range of transplanted CD34+ cells against the location of each study

3.6.6 Variation as a Function of Study Size

As the number of donor / patients involved in a study increases, the amount of variation also increased. This suggests that using the current method for manufacturing HSCTs will produce products that are difficult to control when larger scale production is required. As previously stated, HSCTs are produced at a local, small scale level, typically in single batches in a clinical environment. To meet increasing demand, and to produce future therapeutics at scale, it may be necessary to replace some or all of the current manual processing methods.

Figure 3.13 is a stratified scatter diagram of the range of tCD34 / kg / study against the number of donors in the study. SROCs were carried out for each subsection and have been included in this diagram (**a – f**). These statistically significant correlations demonstrate that as the size of the study increases (and therefore the number of donors and products involved) the total variation per study increases ($p < 0.01$).

From a variation perspective, this not only indicates that the current lab-based production methods find it increasingly difficult to process large volumes of product in a consistent manner, but will most likely be unsuitable for large scale production of a future therapeutic, without substantial variation in the output.

The correlation between number of patients or donors per study and the range of tCD34+ cells is summarized in **Table 3.3** below.

Transplant Type / Cell Source	N	SROC Value	Sig. Value
Autologous, Peripheral	86	-0.355	p<0.05
Autologous, Bone Marrow	9	-0.667	p<0.05
Autologous, Mixed	13	-0.749	p<0.05
Allogeneic, Peripheral	72	-0.456	p<0.05
Allogeneic, Bone Marrow	39	-0.803	p<0.05
Allogeneic, Mixed	51	-0.422	p<0.05

Table 3.3: Summary of the Correlation between study size and biological variation as a function of tCD34+

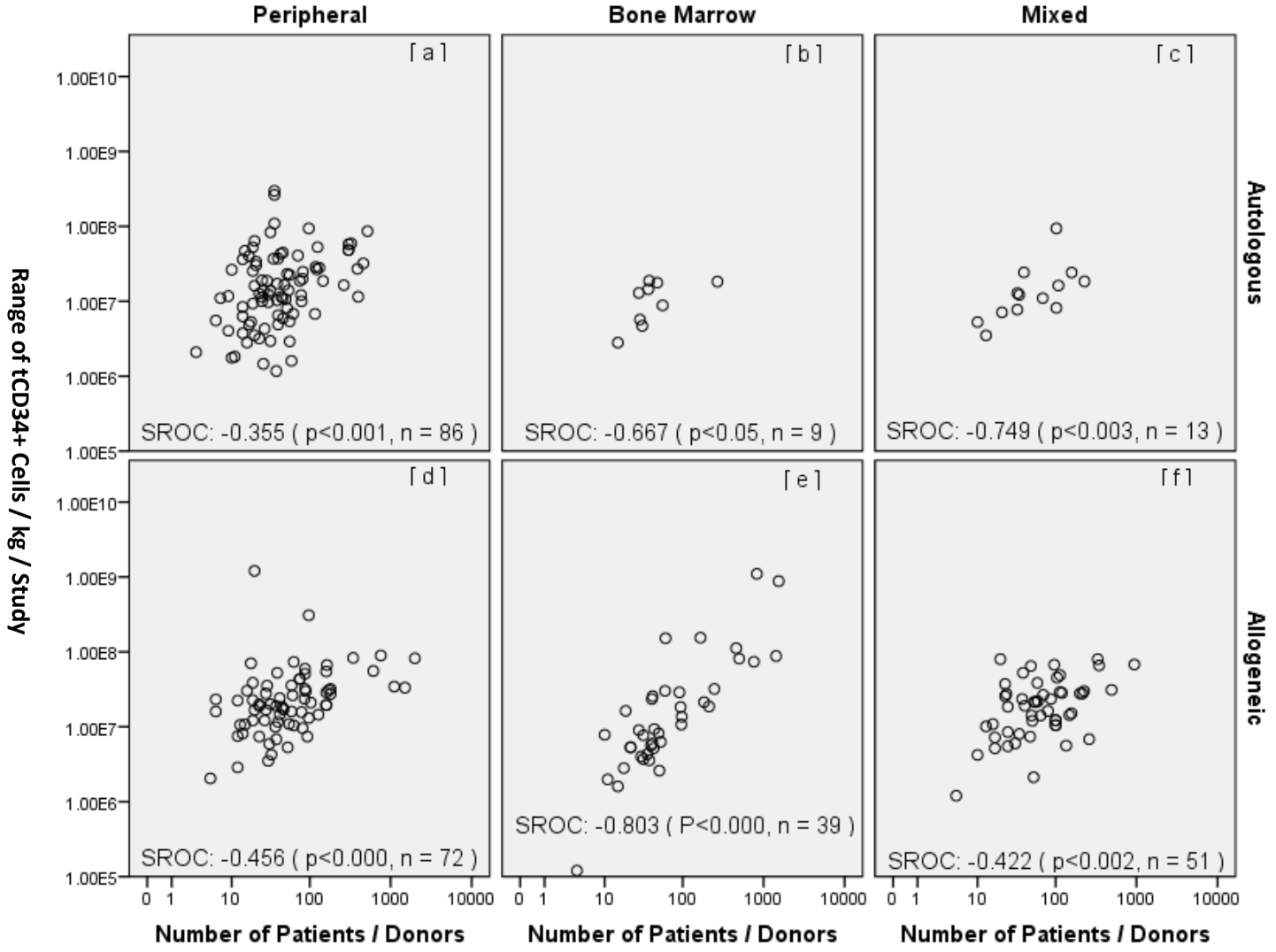


Figure 3.13: Range of collected CD34+ cell count against the study size

3.7 Limitations of the Meta-Analysis

Due to the disparate and aggregated nature of how data is reported and has been collected in this study, a number of limitations must be considered when regarding the results presented here.

3.7.1 Outliers

Traditionally outliers represent products that have fallen outside of specification, and are unfit for purpose in one way or another. In biological therapeutic manufacturing, 'outliers' can be representative of the heterogeneity of an individual's biological state (and/or the state of their personalised medicine), so cannot be disregarded or discarded. As such, any bio-manufacturing process or medical mode of action must be capable of dealing with these outliers. The significant extent of variation demonstrated here includes these extremes, which in the absence of an alternative solution, must be incorporated into any control strategy.

Several papers encountered during this study chose to report inter-quartile ranges, not true ranges. As a result, these were not included in this meta-analysis, because this removes 50% of the reported variation and consequently is not a complete picture of the extent of biological disparity with which a process would have to accommodate.

3.7.2 Sampling Variation

Considering the disparate nature of the raw data of this study it can be difficult to identify errors without comparison to the original source material. Every effort has been made to ensure that the database represents the values reported in the literature.

Additionally, there may be an element of researcher bias with respect to the studies chosen in the selection phase, and which studies have been judged to not contain useful data. This may have resulted in data-containing papers being overlooked. For example, as noted above studies that reported interquartile ranges instead of ranges were disregarded. However, given the overall spread of the data from the reported sources the author does not anticipate any significant shift in statistical behaviour.

3.7.3 Duplicate Data

Inclusion of data from conglomerate groups such as the EBMT means there is a risk of overlapping data. Because there was no access to the underlying raw data it is impossible to know whether the same donor / product information contributes towards the data reported in multiple papers. This is a problem that using dedicated raw data from a single clinical centre would not encounter. In this context effort has been made using SPSS to identify and remove duplicate study data from this database by cross examining median / range data between database entries. Several EBMT based studies were removed at the early stages for having overlapping data.

3.7.4 Patient versus Donor

Donor metrics such as weight were rarely reported, in particular in allogeneic therapy. In autologous therapy the donor is also the patient, and therefore the autologous literature (with a natural focus on the patient) provided more information regarding the source of the starting material.

This study uses secondary data (e.g. medians, ranges) not raw data, so it would unwise to normalise for certain variables such as weight. However, as cCD34 and tCD34 were reported as cells per kg of patient bodyweight, ideally they would have to be first normalised to donor bodyweight – (In autologous therapy, this is not an issue as the patient is also the donor) – this would be an instance for allogeneic therapy of adding distance from the true value.

3.7.5 Paediatric Analysis

This meta-analysis contained a total of 18 paediatric donor-based studies and therefore a separate analysis regarding children has not been made, or a comparison between adult and paediatric cCD34 and tCD34. However importantly, autoimmune disease, inherited metabolic disorders and gene therapy are all prime targets for paediatric therapy [78] and an understanding of the distribution of variation, and the differences between adult and children would advance our understanding of age-targeted therapeutics.

A notable discrepancy between countries and organisations is the definition of the paediatric patient. This can vary between 16 and 19 years of age between example definitions such as the European Medicines Agency [185], UN Conventions [186], England's National Service Framework [187] and the Children Act 1995 of Scotland [188]. In terms of this meta-analysis the definition of paediatric in Article 1 of the United Nation's Convention for Rights of a Child was adopted, identifying all patients up to the age of 18 as paediatric, unless otherwise stated in the particular journal paper.

3.6 Mobilisation / Conditioning Regime

Mobilisation refers to the regimens and pharmaceuticals used to mobilise an individual's stem cells from the bone marrow into the peripheral blood. Conditioning refers to the previous and ongoing treatment the individual is receiving for their current indication.

These are difficult to quantify as they vary on a case-by-case basis, in terms of dosage, regime, drugs, clinician [189] and a combination of these. Additionally, details were not uniformly reported. These regimes are crucial factors [190] in the content and quality of the starting material for HSCT and proper characterisation and stratification is vital to a better understanding. This would be difficult to achieve without raw data.

3.7 Apheresis / Aspiration Processes

Following mobilisation there are the physical methods used to extract the starting material from an individual, whether it be via apheresis (extraction from the peripheral blood in a clinical setting) or aspiration (extraction from the bone marrow in a surgical setting).

Details regarding the instrumentation used for apheresis, the flow rates, number of procedures or extractions were rarely reported. These factors all influence the number and quality of cells produced [56], so to make an accurate statement of the contributing variation sources, these need to be considered in any subsequent work.

Furthermore, it is not clear from the literature whether cCD34 is taken from the total collected cell count, or from a particular stage – or how many extractions were required to meet this target. It is common clinical practise to pool individual products to reach the specification set by the clinician, but it is unclear which (if any) of the reported metrics are single products or pooled products.

The number of collections is also a key factor, particularly for patient health [108]. Multiple bone marrow aspirations are painful and repeatedly expose the donor to infection, and repeated apheresis procedures reduce the immediate effectiveness of the immune system. Consequently, each single donor who is identical in every variable to another donor, who has contributed multiple times, is a different variable and must be accounted for.

3.7.8 Cryopreservation and Transport

It is unknown the extent and effect to which cryopreservation and transportation of product has had on the database, because it was not reported. It is known that cryopreservation negatively affects the total number of cells within the product [144], so this is a 'hidden' variable within this meta-analysis. It is accepted that cryopreservation causes losses in TNC, viability and CD34+ cell numbers [144] but it is unclear as to whether this loss, in addition to biological variation, will cause the product dose to fall below the prescribers safety / dose specification because many product measurements are taken pre-cryopreservation.

3.7.9 Multiple Centres

Naturally the database can be further split into countries, and furthermore into clinical centres. 143 centres or groups published data that contributed towards this meta-analysis. In the case of conglomerations such as the EBMT, it is impossible to discern the individual centres contributions from the literature study alone. There are several centres with multiple observations within the dataset, but in insufficient numbers to make meaningful statistical observations regarding the variation between and within centres. The ideal circumstance would be the use of raw data from single site clinical centres to allow this comparison.

3.7.10 Raw Materials versus Starting Materials

Another 'hidden' variable are the additional chemicals added to the product such as anti-coagulants. The UK MHRA has defined these chemicals as 'raw materials', whilst the biological component is the 'starting material' and it is important to understand the effect both have on each other and the process.

Definitions are a crucial part of scientific and regulatory rigour, but tend to vary between disciplines and locations which is a reason why rulings (such as the EU's ruling on the definition of starting and raw materials) are important. An observation made during this meta-analysis is the use of the term 'optimised' to describe comparisons of variables or formula. This is not indicative of an optimised process, but a process improvement step; to label a process as optimised is to attach a very high ceiling of rigour and precision to the result and should be interpreted to mean a process is the best it can possibly get with current technology.

3.7.11 Reported Data

As the source of starting material, people can be stratified in terms of desirable qualities by the use of donor criteria. However this is not universally standardised; *Karp et al* evaluated all the major blood collection centres in 17 countries and found a wide range of set donor criteria [63]. This included minimal deferral time after pregnancy or tattoos, minimum donor weight, haemoglobin levels, upper age limit and the amount of whole blood taken during donation. This was reflected in the wide range of variables reported (and not reported) in the literature.

3.8 Conclusions

This Chapter has identified a source of information regarding biological variation within HSCT within the medical literature, and has used this source to identify the range and spread of biological variation across this global, unhealthy population

This meta-analysis of publicly available data has been developed to provide a broad-scope demonstration of the challenge that biological variation imposes on the potential for controlling large scale bio-manufacture and will impose when manufacturing Advanced Therapy Medicinal Products. The primary output from this analysis is a series of results that represent the extent of biological variation in both collected and transplanted material for a clinical sub-population defined by the data available in the public domain.

This meta-analysis has determined that the current variation encountered under the practice of medicine for hematopoietic stem cell therapy (limited by the constraints of this sub-population) can be up to 4 and 5 orders of magnitude of the median dose (if the instances where no cells were collected or transplanted are discounted). This is similar for the collected cell content. However, if the records where 0 cells were collected / transplanted are included, this would increase to 9 orders of magnitude. This level of variation would be unmanageable from a bio-manufacturing perspective.

Additionally, comparing the ranges of collected and transplanted CD34+ cells over time, there is little evidence to suggest an improvement in the community's ability to control variation in HSCT over the last three decades. There is a weak negative correlation between collected CD34+ cells and study start year, which may represent the improvement in apheresis technology, but the range of transplanted CD34+ cells remains inconsistent over the thirty-year time period examined in this meta-analysis. This has potential implications for comparable efficacy and patient outcome, and the exploration of HSCT's mode of action.

It is clear that to make the next decisions on an appropriate bio-manufacturing strategy, a stratifiable source with greater resolution than is exhibited in this meta-analysis will be required; such as patient databases and centre-specific clinical records. Clinical centres or organisations specialising in this area are likely to have further useful data, and will be approached for further work (see **Chapter 4, DFCI**).

Without the raw data that underlies the literature, quantification of the sources and extent of variation contributed by each variable will remain informed speculation.

Consequently, the next step in this exploration is the acquisition and analysis of high quality datasets from single site clinical sources and petitioned national health resources, where the data represents individual cases, not summaries. This will allow stratification of the contributions of variables such as donor age or weight, process parameters and indication (in the case of autologous therapy, where the donor is also the patient) and an increased rigour and quality in the reported results. This will also allow access to absolute numbers of cells per collection / product as opposed to cells / kg. Additionally, using datasets from specific centres will allow normalisation of the variation with respect to centre specific variation, such as geographical, surgical, clinical and operator variation to a degree, because it is reasonable to state these should remain constant within a single centre.

This chapter has also highlighted several potential concerns with the current reporting methodology for stem cell therapy. The amount of methodology and clinical practice data available within the public domain is limited, and can make it challenging to cross-reference studies and findings without seeking out the original data. A possible solution is to propose a reporting framework within stem cell therapy research with several agreed, predetermined and useful metrics that must be reported as standard – even if they occupy the 'supplementary' information section of online resources if it cannot be naturally written into the publication. One potential challenge with this is the highly personalized nature of regenerative medicines, particularly within oncology where at the clinicians discretion individual patient regimes may be changed, and therefore in some instances reporting this data may not be straightforward (for example, different patients receiving different doses or types of chemotherapy drug compared to their trial or case study cohorts).

To meet this challenge cross-disciplinary collaboration between medical and engineering fields will be crucial [191]; sharing their combined experience and reaching compromises between the priorities will allow for identification of the sources of variation and the strategies required to

accommodate and control this variation for production to accommodate large numbers of patients – either in large volumes in concentrated facilities or by more distributed approaches. Variation cannot be eliminated completely, but it will need to be brought to a state where the differences in the product do not negatively affect patient outcome, and is a key step in establishing product specifications and achievable manufacturing limits.

Chapter 4 Single Centre Variation

4.0 Chapter Aims

This chapter aims to quantify the variation encountered in a single clinical centre, characterized by the product and patient records available at the Dana Farber Cancer Institute in the USA. A particular aim of this research was to reduce the number of confounding variables, such as patient age and weight, and examine the capability of a large centre with respect to the control of variation.

Figure 4.0 is a generic process map for an exemplar HSCT product, which has been included here to indicate the primary focus of this chapter with respect to the process as a whole (indicated in **orange**).

This chapter is split into two parts; a qualitative discussion surrounding issues and challenges encountered whilst on site and in discussion with hospital staff, and a quantitative section surrounding the data mining and analysis of DFCI's Cell Manipulation Core Facility (CMCF) patient and product records.

The following topics will be discussed:

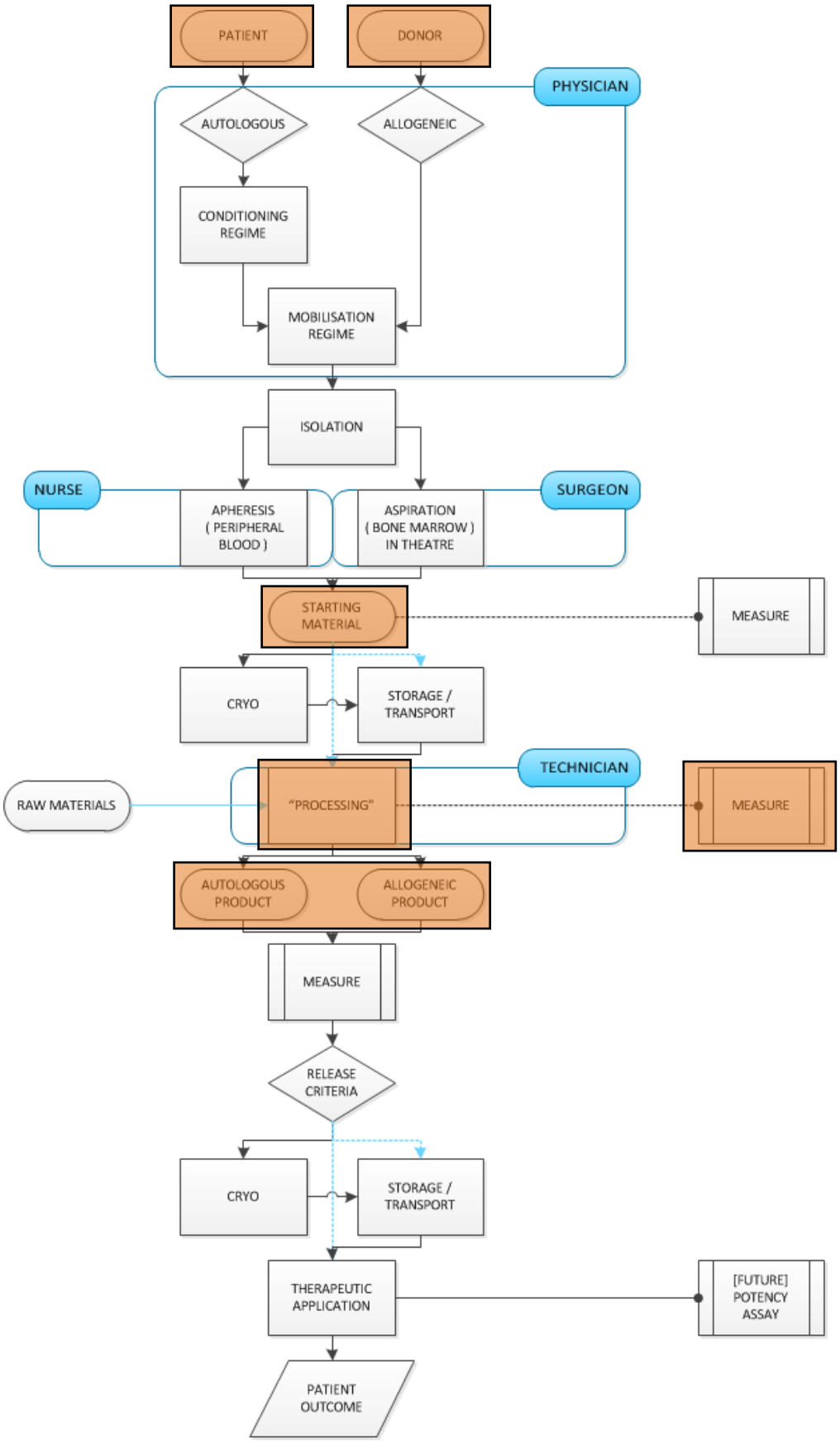


Figure 4.0: Generic Process Map indicating in orange the focus of this Chapter

4.1 Introduction

Understanding the variation that is encountered in the starting material, and the HSCT process, meant an understanding of the limits and the specification that a manufacturing machine needs to be designed around – to tolerate or control the biological variation – because only by a degree of mechanisation or automation can cellular therapy achieve the scale necessary for first line medicine status. **Chapter 3** examined the extent of variation on a global scale. This Chapter will explore the extent and sources of variation within a single clinical centre.

This Chapter is a case study of the Connell and O'Reilly Families Cell Manipulation Core Facility (CMCF) at the Dana Farber Cancer Institute (DFCI) in Boston, Massachusetts. It reviews each area of the process (step by step) in order to identify challenges in each area. This is followed by a statistical analysis of process and product characteristics as collected on site, followed by a discussion concerning the challenges variation imposes, and potential solutions.

The information in this chapter is unprecedented in the public domain, and is part of an ongoing quality improvement programme at the hospital with the underpinning hypothesis and expectation that this single clinical centre should have dramatically improved control over variation than that shown in the Meta-analysis.

DFCI is a teaching and specialist hospital that has been ranked fourth in the USA for cancer treatment [192]. It has a throughput of over 300,000 visits per year and is involved in approximately 700 current clinical trials.

The CMCF is a facility within the DFCI that produces cell therapies - from processing of haematopoietic stem cells, to generation of tumour vaccines and preparation of immune cell populations. It produces approximately 1,000 products per year, with 3,696 products being produced within this facility between 2010 and 2013.

The CMCF carries out several product preparations and processes. This research focuses on the isolation and processing of peripheral blood derived haematopoietic stem cells.

The research is the result of two site visits, in addition to a number of external communications and extensive post-visit analysis and review. The first visit in 2013 was a week-long feasibility study to understand the processes involved in manufacturing a cell therapy in a clinical environment. The second visit in 2014 was a data mining exercise, where over a period of two weeks a database on product and process variation was manually collated. The time between these two visits was used to identify key data and improve understanding of the CMCF's process. Access to electronic records and data was not available at the time for ethical reasons so data was manually transferred from physical records to the database – physical records contained a minimal amount of patient information, but the electronic databases would have allowed access to patient records and medical history.

4.2 Methodology

The purpose of the qualitative analysis was to interview and explore the experiences of those involved in the process, the culture surrounding biological manufacturing, and the day-to-day running of such a facility. This was completed by informal interview and experience within the centre.

The purpose of the quantitative analysis was to build upon the data collected in the previous literature meta-analysis. Where that was representative of a global picture of variation, this analysis was based upon a *single* site's records. The meta-analysis identified characteristics important in determining the extent of variation – such as transplanted CD34+ cell count – and these characteristics informed the requirements for the CMCF database.

After the 2013 visit, the CMCF sent redacted examples of their patient and product records. Alongside the meta-analysis characteristics, these were used to design a database to facilitate the manual data mining process whilst on site. During the 2014 visit, these records were scrutinized, characteristics were identified and transferred manually to this database. Due to time constraints, some of these characteristics were triaged such as the Haematology Lab's flow cytometry results.

4.21 Disclaimer

It must be stated that any variation within Haematopoietic Stem Cell Transplantation is not indicative of the *quality* of the product provided by CMCF, or the expected patient benefit thereof. In this instance HSCT is a secondary therapeutic, used to alleviate the side effects of the primary therapeutic – chemotherapy / radiotherapy - which will have a more significant effect on patient outcome.

4.22 The Anthony Nolan Trust

In 2013, between visits to DFCI, two weeks were spent at the Anthony Nolan Cord Processing Facility in Nottingham, shadowing their process and informally interviewing their technicians on protocols, procedures and perceived sources of variation. This included receipt of cord blood units, volume reduction and separation of the buffy coat layer of the blood that contains HSCs using Biosafe's SEPAX cell separation system, and the freezing / thawing process. This visit lead to the creation of **Figure 4.0** which has been used throughout this thesis. This facility is not a transplant centre such as DFCI but a cord blood bank, receiving fresh cord units in the morning, selecting several units from these based on quality criteria, then processing and freezing the therapeutic fraction. For comparison, reference will be made to the different procedures and priorities between this cord blood bank and the single clinical transplant centre at the DFCI.

4.3 Qualitative Analysis

What follows is a review and discussion of each key area of the HSCT process (step-by-step) in order to identify the sources and challenges of biological variation.

4.3.1 Patient / Donor

Humans are the source of starting material. Depending on whether the therapy in question is allogeneic (donor) or autologous (patient), this source will be distinctly different.

Autologous therapy will primarily address, and be derived from, a sick population, and come with a number of caveats concerning cell quality and health—not to mention the effect on patient health by isolating and removing these cells. Additionally, autologous therapy requires the patient to be matched specifically to their own tissue, and from a manufacturing perspective, you cannot just discard material that falls out of specification.

Allogeneic therapy is derived from a relatively healthy population that has been screened for a number of quality related metrics. Allogeneic therapy allows for screening and stratification of the donor. This is likely to be the route for gene therapy, or 'off-the-shelf' cell therapies - where "one batch will fit all". This is a gross oversimplification considering the number of cell lines that may be required for an effective haplobank of cell lines (potentially between 50 – 150 [193]) that can match to the majority of the population. Theoretically, their diet and lifestyle could be improved prior to a donation, whereas an autologous donation is from a sick patient, undergoing a number of chemical or radiological regimes, with a much more limited environment, diet and lifestyle than an allogeneic donor might be expected to have.

During the visit to the CMCF it was unclear whether there was a defined separation between paediatric and adult patients. The definition appeared to be subjective – and is in line with the wide variety of definitions discussed earlier (**Section 3.7.5**) and remains an open question.

4.3.2 Starting Material

There is a prescribed difference in terminology between raw and starting material. It is natural for a physical scientist or engineer to associate 'raw material' with the initial building blocks for a therapy - and this is true, to a degree. However, the author has been careful to distinguish between the two, especially in light of recent EU / MHRA guidelines [176] that refer to the isolated cells as the starting material, and the additional reagents / additives (such as acid citrate dextrose) as raw materials.

Considering the disparity between fields such as medicine and engineering, it is useful to have a lexicon with clear compatible definitions. An example occurred during the literature meta-analysis, where protocols were described as having been optimised, but the English Oxford Dictionary definition of optimised is;

'Make the best or most effective use of (a situation or resource)'

Determining that a particular protocol or reagent is statistically or patently superior to another is laudable, but not optimised as might be defined by an engineer (when referring to a process). Referring to a process as being optimised, inspires a much different standard of quality depending on whom you speak to. It is a loaded term, with specific connotations.

4.3.3 Conditioning / Mobilisation

Conditioning refers to a procedure that a patient will undergo prior to HSCT, for example the radiotherapy for cancer treatment. Mobilisation refers to a procedure to draw out and mobilise Haematopoietic Stem Cells (HPCs) from the donor's bone marrow, into the peripheral blood. Mobilisation agents such as granulocyte colony stimulating factors or G-CSFs [194] are used to promote the release of these HPCs by stimulating the bone marrow to produce granulocytes and stem cells.

Given a similar patient group, different doses of mobilisation drug may potentially result in different numbers of cells isolated as a result. In some instances, several types of drugs may be used, as certain patients may be more responsive to one type over another – making reporting of trial

methodology complicated. Different conditioning regimes or intensities, number of previous treatments or ongoing treatments could potentially affect the quality of cells isolated, the number of cells isolated, and the health of the patient – as the isolation procedure may remove cells from the patient (albeit temporarily) that were fundamental to their ongoing disease response and/ or recovery.

A blood test, and clinical judgement determines whether or not these procedures are carried out. If the circulating stem cell population from this test is not to a given level (a one-sided specification) then a pharmaceutical (a G-CSF) will be applied so that the donor exceeds this threshold. This is subjective to the clinician in question [195]. Some have defined this criterion as being below 10 cells / µL of sample blood; others have defined this as below 5 cells / µL of sample blood. Mobilisation agents significantly increases the number of circulated stem cells, so this clinical variation becomes a concern, especially as many therapeutics are known to be formed by 'pooling' individual collections to meet the minimum dose (pooling is when multiple apheresis products are combined to reach the proscribed clinical dose). However, pooling is unlikely for ATMPs so this current solution will not translate to future therapies.

4.3.4 Isolation - Aspiration / Apheresis

Isolation refers to the extraction of material from the donor. Aspiration is an invasive procedure and involves surgery to extract material from the donor's bone marrow. Apheresis is a procedure usually completed in clinic, and filters the desired cells from the donor's circulating peripheral blood.

The minimum collection criterion is usually 10 ml of bone marrow for every kilogram of donor bodyweight - however there is general reluctance to stick closely to this rule, likely due to the surgeon's discretion with respect to the individual contributor's health due to "outliers" and the clinical approach of reducing preventable death

4.3.5 Physician

Compared to the 94 centres within the literature meta-analysis, it is likely that the CMCF as a large and high performing centre, will adopt a consistent culture of practise, due to comparable SOPs,

equipment, facilities and oversight, and therefore it is expected to exhibit a much lower degree of variation.

Each physician has the freedom to monitor and alter their treatment, alongside best clinical practise and knowledge (practice of medicine). It is recognised that although there will be a similar practise culture within a centre, each individual clinician may apply the same therapy differently.

4.3.6 Measurement / Quality Control

The purpose for this section on measurement is to highlight that before any variation as a result of the starting material can be considered, before the process adds or removes any variation, before the clinicians apply the therapeutic, there exists a degree of variation between the measured value and the true value. Without a representative and appropriate measurement system, any further conclusions must be taken into consideration that a degree of the variation may be to the measurement system.

Appropriate sampling is a primary concern when carrying out measurements. In the case of destructive testing, such as the Tryphan Blue viability assay, it is not possible to test the complete starting material, so a sample is taken and it is assumed that this sample will be representative of the whole. With such a dynamic, heterogeneous population as a bag of cells, this is potentially difficult. However, inappropriate sampling can mean the measurement is incorrect so the sampling methodology is important.

The products that were encountered by the author were contained within plastic transfer bags, with several seals and tubes to assist aseptic transfer and integration with equipment. Sampling can take the form of a blister pack that is attached to one of these tubes (in the case of Anthony Nolan), manually depressed and allowed to withdraw material, or a syringe (in the case of CMCF). It must be made clear that these samples are meant to be representative of the whole - the system within the bag is essentially an emulsion of liquid and cells, prone to settling, clumping and clotting and the sample that is taken may not represent what is in the bag. Considering that this sample is used to

make power calculations on what is in the full bag (e.g. there are 100 cells in 1 ml therefore 100,000 cells in 1 litre).

Measurements are taken for a specific purpose such as volume of chemical for a specific reaction for example. This may be more complex for cellular therapy as the mode of action is not yet clear: it is unsure whether the cells themselves are the therapeutic agent and how to appropriately measure cell identity or phenotype.

Currently the CD marker system is used, which uses cell surface markers to determine the identity of the cell but these markers are not definitive. The CMCF uses a particular definition of stem cell - haematopoietic progenitor cells. They use a specific protocol, embodied by a proprietary product - BD Biosciences Stem-Kit [196] - for their CD34+ cell enumeration. This kit is applied via flow cytometry. Paraphrasing *Ivanovic* [25] in **Section 2.2**, all haematopoietic stem cells are CD34+ but not all CD34+ cells are haematopoietic stem cells.

Tryphan Blue is used to determine the population of cells that are alive or dead (with limited resolution and definition to the term 'alive'. 7AAD [41] is a more complex test and can determine cell damage. This test is sensitive to DNA, and assuming a given cell is alive, its cell membrane will be intact and no DNA will be detected. If the cell is dead or dying the structural integrity of the cell membrane will not be intact, and increasing levels of DNA will be detected.

Flow cytometry is an analytical instrument used in biological measurement [197], that suspends the cells of a given sample in a stream of fluid and passes them through an electronic detection apparatus. These cells are tagged for particular markers, such as the CD identity markers. This process is called "staining" and is carried out by attaching chemicals to cell surface molecules, then attaching a fluorescent marker to this chemical, which can then be detected by the cytometer. Part of the analysis involves a process known as 'gating'. This step is where the results are displayed on a 2D graph on a computer, and the operator will subjectively group ('gate') different cell populations.

Automated gating software is available, but CMCF technician opinion of this software currently varies between it being 'terrible' to 'impractical and imprecise'.

Additionally, depending on the source of the starting material (bone marrow or peripheral blood), the results can be easier or harder to interpret. Bone marrow, for example, is harder to gate than peripheral blood, because there is significant overlap between different cell type populations - thus is open to much greater operator variation.

Considering that TNC count has been the accepted measure of dose for cellular therapies in the past, and now CD34+ cells are an accepted measurement, it could be said there has been an increase in the resolution of what is being measured. It is still a heterogeneous population, and until greater specificity is attained, it may be difficult to ascertain the therapeutic potential of cellular therapy and draw up appropriate dosage or mode of action.

Another example of this specificity is the use of WBC counts. This is part of the QC testing process that also includes specific cell measurements such as red blood cells, platelets and haematocrit. The current accepted measurement for WBC count can be inappropriate when there is a high fat content within the product, such as with samples taken from obese patients. This skews WBC measurements so another more appropriate measurement is taken—*differential* white blood cell count, that takes this extra fat content into account. This differential WBC count is currently not an accepted measurement within the CMCF protocols and QA/QC requirements. Therefore each time it is required to be used it is being flagged incorrectly as 'out of specification' (incorrectly because processing will remove the fat content). Until this is approved by the clinical board, this exception must be continually made.

To counter this potential measurement variation the operators at the CMCF are required to take part in Clinical Laboratory Improvement Amendments (CLIA) testing [198], once per year as part of a requirement by the Centres for Medicare and Medicaid services (CMS). The core of this testing is a requirement for the technician to achieve a value within 80% of the CLIA standard. However, if

viewed from a different perspective, this implies +/-20% of variation around a given value is allowed, and still considered acceptable.

4.3.7 Middle, Edges and Outliers

Conventional manufacturing is focused on the mean and the range of the products which may also be the case for biological manufacturing. The unique nature of the cell as a starting material may introduce a number of changes that may need to be made as to our approach.

Using data collected from the CMCF process records, a histogram of transplanted CD34+ cell count has been plotted (**Figure 4.1**). The mean and median values are shown to illustrate the statistical advantage of the median over the mean in skewed datasets. Note the long, skewed tail on the RHS. This figure raises two critical issues. One is the issue of statistical outliers, and the second is the distribution of the dataset and its effect on the statistical tools that can be used.

Firstly, the mean will be unsuitable as biological measurements such as CD34+ cell count is not normally distributed and not centred around the mean, therefore the median is more suitable for heavily skewed datasets such as that shown in **Figure 4.1.** Traditionally manufacturing is focused on the median and range of a given process measurement, but this figure demonstrates that a third consideration will need to be included for cellular therapy manufacturing – outliers (which would be discarded in traditional manufacturing).

4.3.8 Outliers

In a traditional manufacturing sense, outliers refer to out of specification products. In statistics, outliers are usually removed to allow for more robust analysis. They may refer to errors, defects or abnormal events. In the cellular therapy world, however each outlier is either a representation of an individual's state of health, or an irreplaceable patient-keyed product. Either way they cannot be removed or discarded. Any process for a given therapy must be able to accommodate these outliers which includes any future automated process or system.

Consider **Figure 4.2**, which represents a basic box and whisker plot. Box plots are highly descriptive summaries of the spread, quartiles and median of a given population. However, note the extreme points for lowest and highest values are exceeded by outliers, normally indicated in SPSS by circles for ‘out’ values and crosses for ‘far out’ values. These data points are part of the population, not outliers and therefore the box plots are not designed for when outliers are crucial parts of the data. However, they can be used to indicate where much of the dataset lies, and its distribution – the interquartile range and the median.

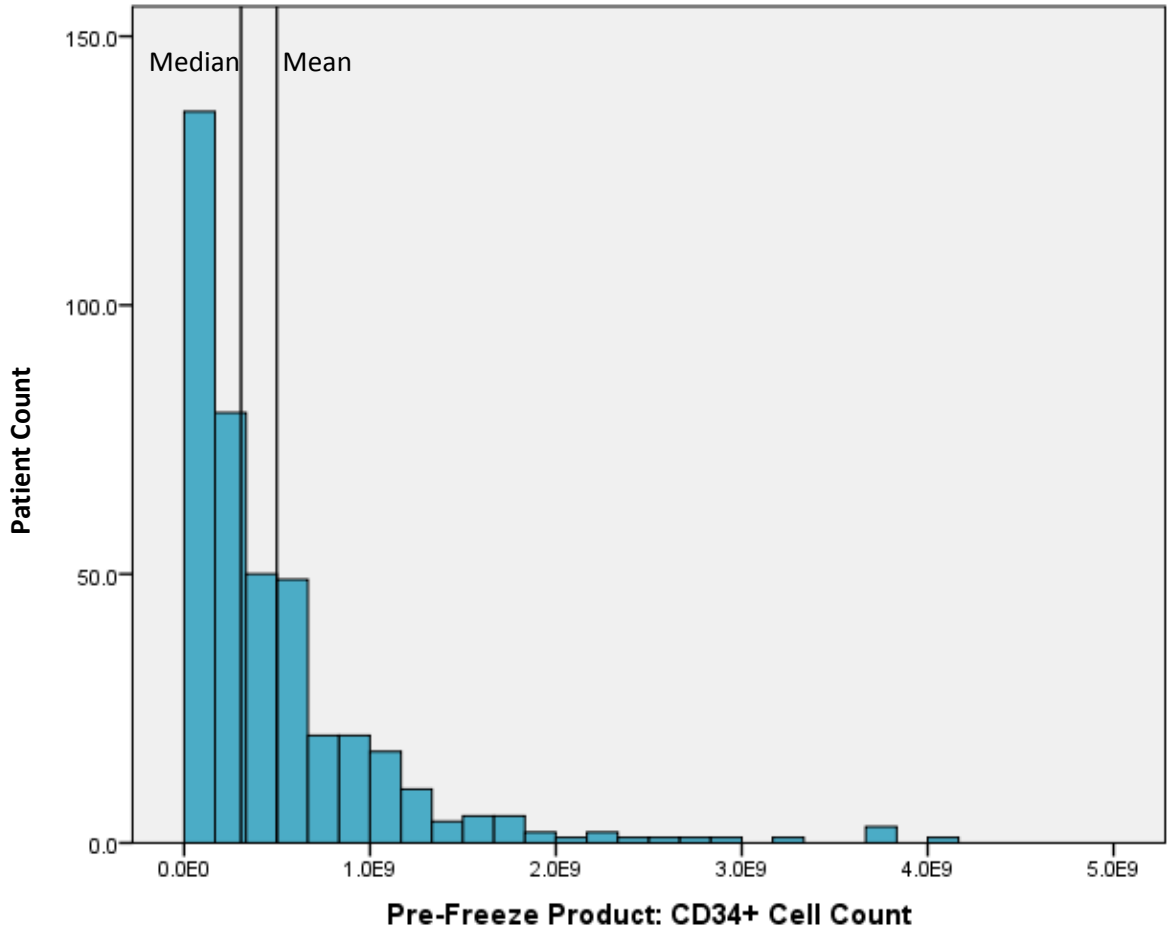


Figure 4.1: Distribution of CD34+ cells within the pre-freeze product, demonstrating a non-normal distribution.

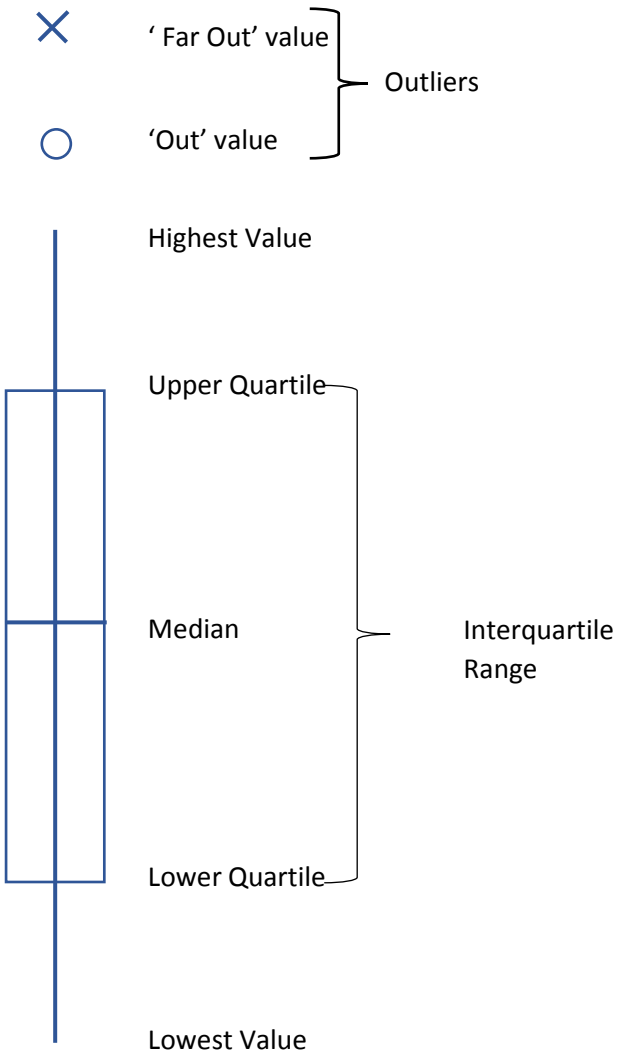


Figure 4.2 – Example Box and Whisker Plot

This raises issues for statistical analysis, because a primary assumption of many statistical tests (such as the t test or ANOVA) is that all significant outliers are removed, which may be problematic when these 'outliers' represent an individual's state of health.

Additionally, cell metrics are not normally distributed (**Figure 4.1**). This may be exacerbated by a minimum collection criterion, and that these cell types have more situationally-dependant behaviour than red blood cells (RBCs will be present in a given living person at all times, WBCs will vary depending on health and requirements). This skew means a different statistical approach may be required. There is also a question as to how the product behaves therapeutically at the 'long tails' of these distributions – this is of particular interest to the Regulators who expect the process to perform consistently across the whole distribution. This is an unusual system for manufacturing and process engineers to face, and may require an original approach to statistical process design.

4.3.9 Process Capability

Process capability is a process design tool used to measure the state of a manufacturing process[199].

$$Capability = \frac{Allowable\ Variation}{Achievable\ Variation} \qquad \textbf{Equation 1}$$

So then allowable variation is set by the specification, in this case, the requirements of the clinician. This is difficult to set currently, because the complete specification for a cellular therapy is unknown - this would require an understanding of the mode of action for cellular therapy (which will be challenging as blind/blind trials of cell therapies are unethical). For this example, there is a one sided specification in the form of a minimum cell dose - the minimum number of cells to be considered efficacious.

Achievable variation is that of the equipment and protocols, and the tolerances that can be routinely set on those protocols. It represents the current technological ability of the process.

To demonstrate the potential difficulties determining process capability for a biologic, what follows is a worked example, using data from the CMCF, and their one-sided specifications. (A caveat is that these calculations are usually reserved for processes *under control* with an understanding of both common- and special-cause variation within the process.)

The formula for calculating process capability is;

$$C_{PK} = min\left(\frac{upper\ specification\ limit - \mu}{3\sigma}, \frac{\mu - lower\ specification\ limit}{3\sigma}\right) \qquad \textbf{Equation 2}$$

Where μ is the estimated mean of the process, and σ is the standard deviation of the sample.

HSCT does not have a two-sided specification. There are upper limits postulated by the academic community based upon incidences of Graft versus Host Disease (GvHD), but the current requirement set by the clinician is a single sided specification, so Equation 2 must be replaced;

$$C_{PL} = \frac{\mu - lower\ specification\ limit}{3\sigma} \qquad \textbf{Equation 3}$$

The lower specification limit is known from SOPs. At the Anthony Nolan Trust this was 3 x 10^6 cells per kilogram of patient's weight, and at the DFCI, this was 2 x 10^6 cells per kilogram of patient's weight.

This difference in value may be because the CMCF prescribes a higher cell count to account for losses during transport / cryopreservation, or because of the different immediate priorities of a transplant centre versus a cell bank such as Anthony Nolan, or due to differences in therapy and indication between centres.

Figure 4.3 describes the required minimum dose set by the clinicians at DFCI during 2014. Note the prevalence of the minimum prescribed dose in the SOPs (2.00 x 10^6 cells / kg) and subsequent higher doses as prescribed by the clinician for a particular patient and/or indication.

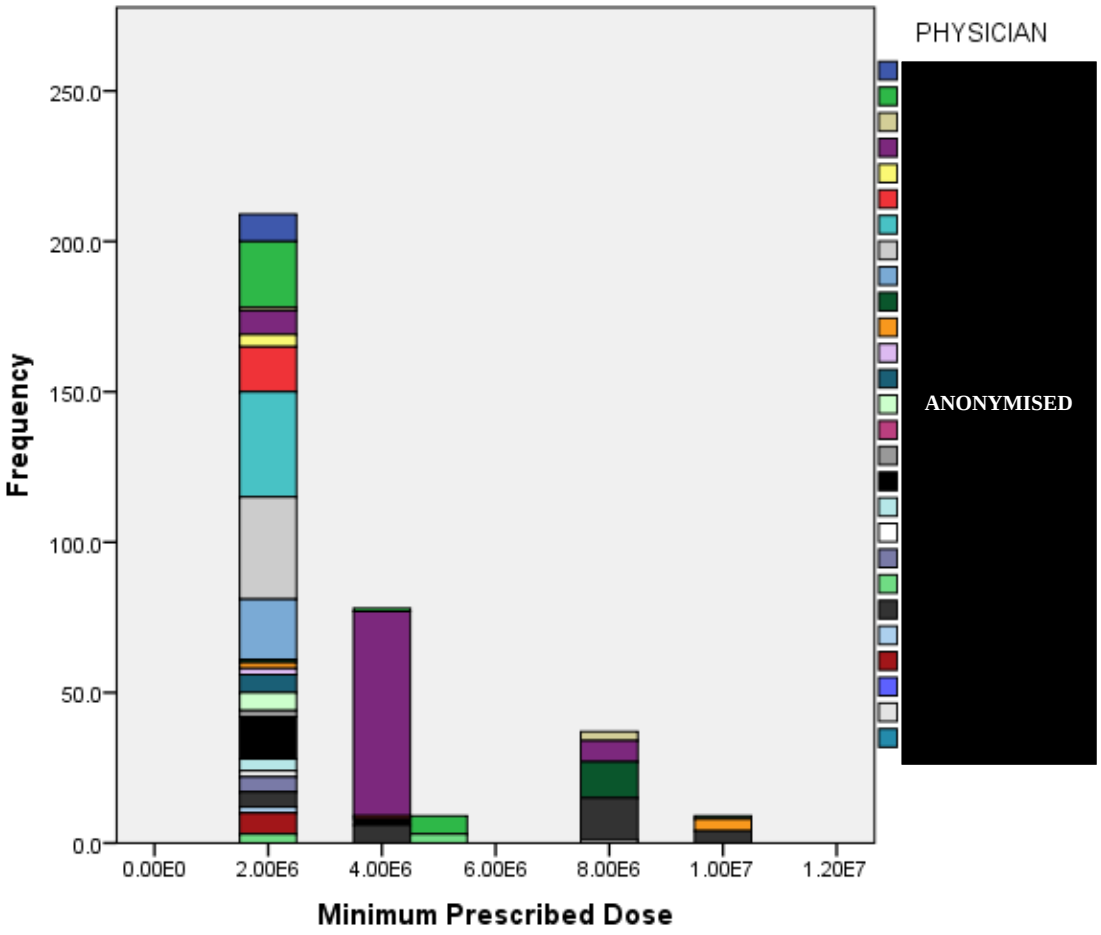


Figure 4.3: Minimum CD34+ cell dose prescribed, stratified into individual clinicians

Each colour on **Figure 4.3** represents a different clinician's requirement for a given patient. As the most common dose was 2 x 10^6 cells / kg were the most often used, it will be used as the lower specification limit in Equation 4. Additionally, this Figure suggests that there is not a set formal HSCT dose, just a minimum below which the usefulness of the therapy will not outweigh its risks.

$$C_{PL} = \frac{\mu - 2E6}{3\sigma} \qquad \textbf{Equation 5}$$

The mean and the standard deviation of the process are now required. However, CD34+ cell count is known to be non-normally distributed (**Figure 4.1**) and as such will need to be mathematically transformed to determine the normally distributed mean and standard deviation.

Data is transformed by performing a deterministic mathematical function on each data point. A systematic examination [200] resulted in log(x) being the transformation that brought the distribution of the data closest to normality (**Figure 4.4)**. This also requires the specification to be appropriate for the transformation (e.g. the specification must also be in a log(x) format).

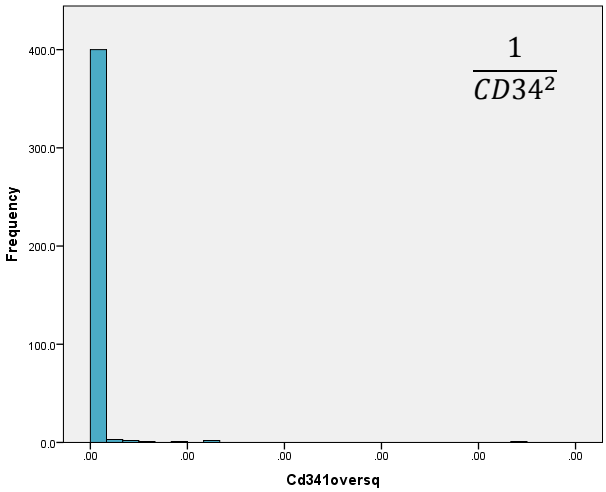
$$C_{PL} = \frac{\mu - 2E6}{3\sigma} = \frac{4.99E8 - 2.00E6}{3\,(\,5.97E8)} = \frac{8.70}{3(8.78)} = \frac{8.70}{26.33} = 0.33 \qquad \textbf{Equation 6}$$

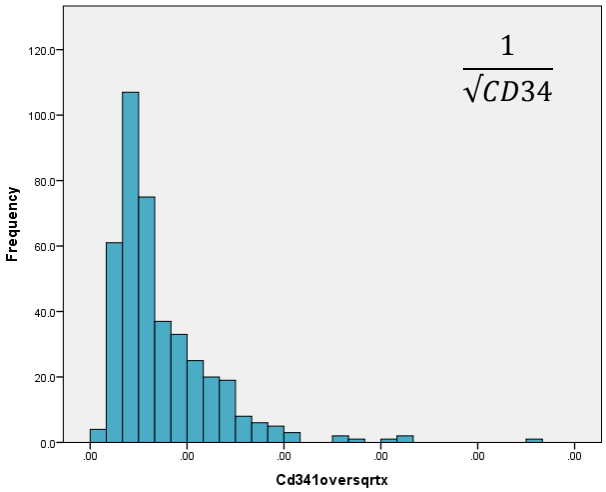
Where σ is the standard deviation and μ is the mean of the sample population. The standard deviation for the DFCI data is 5.97x10^8, and the mean for the DFCI data is 4.99x10^8. Both these values, and the minimum collection criteria (2.0x10^6) will need to be transformed by log(x). These values will therefore be 8.77, 8.70 and 6.30 respectively.

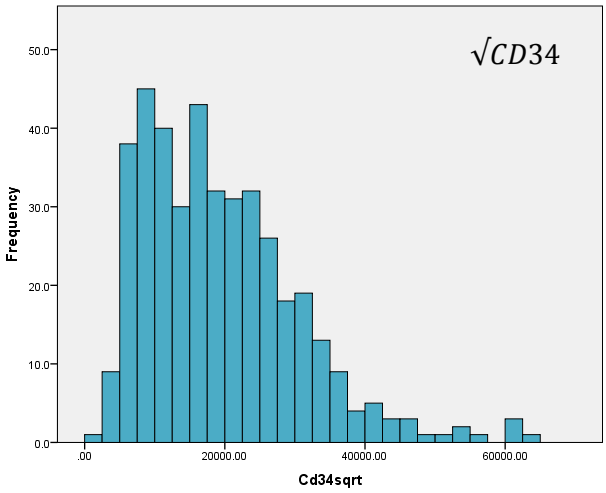
As a result, for a one-sided specification of 2.0x10^6 cells / kg, this process would theoretically have a C_{PL} of 0.33.

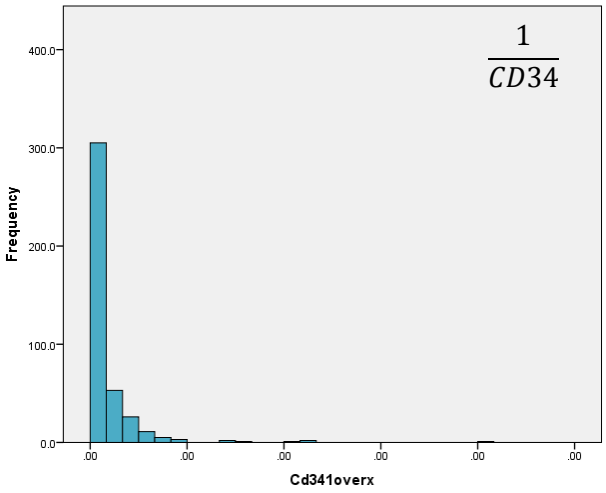
Within traditional engineering a C_{PK} value of 1.50 for a double sided specification or 1.45 for single sided specification[201] or more is usually considered acceptable. Previous work has shown that manual culture processes has a C_P of 0.55 compared to an automated culture that had a C_P of 1.32 [14].

Using the sigma conversion tables [202] (**Figure 4.5**) this has an equivalent sigma level of 1.000 and is therefore indicative of 691,500 defects per million products. These tools are either unsuitable for use with such variable starting materials, or the starting materials are so wildly variable that these products will regularly fall outside of specification. It should be re-iterated that these calculations are normally not performed until the process has been determined to be in a state of statistical process control – if it is not, it is not stable and cannot be usually predicted using capacility indices.









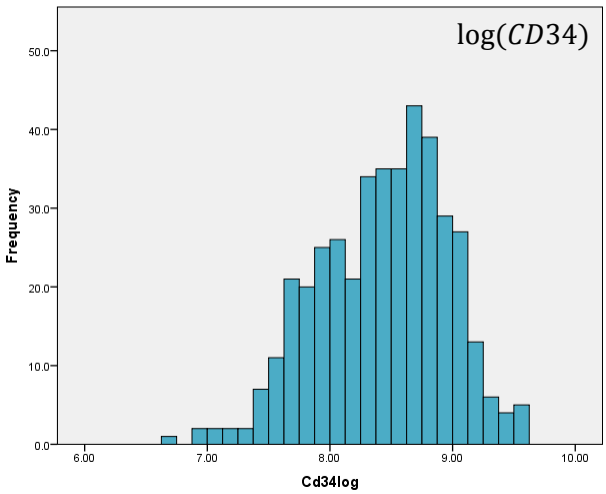


Figure 4.4: Mathematical transformations of CD34+ cell count, for relative normality.

Sigma Conversion Table		
Defects Per Million Opportunities	Sigma Level (With 1.5 Sigma Shift)*	Cpk (Sigma Level / 3) With 1.5 Sigma Shift*
933200	0.000	0.000
915450	0.125	0.042
894400	0.250	0.083
869700	0.375	0.125
841300	0.500	0.167
809200	0.625	0.208
773400	0.750	0.250
734050	0.875	0.292
691500	1.000	0.333
645650	1.125	0.375
598700	1.250	0.417
549750	1.375	0.458
500000	1.500	0.500
450250	1.625	0.542
401300	1.750	0.583
354350	1.875	0.625
308500	2.000	0.667
265950	2.125	0.708
226600	2.250	0.750
190800	2.375	0.792
158700	2.500	0.833
130300	2.625	0.875
105600	2.750	0.917
84550	2.875	0.958
66800	3.000	1.000
52100	3.125	1.042
40100	3.250	1.083
30400	3.375	1.125
22700	3.500	1.167
16800	3.625	1.208
12200	3.750	1.250
8800	3.875	1.292
6200	4.000	1.333
4350	4.125	1.375
3000	4.250	1.417
2050	4.375	1.458
1300	4.500	1.500
900	4.625	1.542
600	4.750	1.583
400	4.875	1.625
230	5.000	1.667
180	5.125	1.708
130	5.250	1.750
80	5.375	1.792
30	5.500	1.833
23.4	5.625	1.875
16.7	5.750	1.917
10.1	5.875	1.958
3.4	6.000	2.000

Figure 4.5: Sigma Conversion Table[202]

To summarise;

- Cell measurements are non-parametric, and therefore requires statistical transformation to be applied, so these tools can be used. Note that the process capability equations require mean, and standard deviation, traditionally associated with normal distribution and symmetrical spread from a central point. These equations do not consider the spread, and distribution of these metrics.
- An outcome-based specification based on either dose/response or mode of action does not exist, so current process capability calculations are based on minimum numbers of cells—which can be compensated for by pooling multiple products

Currently the traditional process capability equations may be unsuitable for HSCT. It is also unclear whether this 'optimum' minimum C_{PK} is appropriate for biologics, or whether alternative tools will have to be derived to consider the non-normally distributed, outlier containing datasets of biological manufacturing.

4.3.10 Protocols

Protocols refer to the Standard Operating Procedures (SOPs) used when manufacturing a product. These should guide an operator through the process in a way such that it can be replicated irrespective of the user. Cell culture remains mostly manual culture and is therefore subject to operator variation in its three main forms; inter, intra and learning (see **Chapter 3**).

Protocols will be subject to a certain degree of variation due to the subjective judgement of the operator. For HSCT the COBE 2991 cell processor is used at the CMCF for product washing, plasma depletion and red blood cell depletion. This is a centrifuge system similar to that found on the SEPAX cell separator (as used by Anthony Nolan), except the separation by COBE 2991 at the CMCF is controlled manually, rather than automatically. The distinction between the three main cell groups of blood (plasma, buffy coat, and 'heavies' such as red blood cells) is determined by eye, and is referred to in an SOP as 'within about' a small fraction of an inch. Therefore, the number of stem cells separated is dependent on the judgement of an operator, of which there will be multiple operators in a given facility, whose judgement may vary across time and shifts.

4.3.11 Technicians

Technicians, in their role as operators, are one source of operator variation.

By examining variation at the single clinical centre (characterised by the CMCF) a limited personnel pool is assumed, all of whom should be trained to a comparable level. However, there will be differences in operator skill and experience and it is up to the facility to ensure that this difference does not reflect in the quality or efficacy of the product. A key observation was that it was considered by the technicians to be difficult to learn new processes, because each operator carried out the procedure differently, sufficiently enough that they could all not easily follow it. This implies that the SOPs are currently insufficiently detailed to capture the resolution needed for repeatability and reproducibility. More process understanding is required to reduce the level of expertise required to understand and follow the SOPs. It was also noted that technicians would benefit from

the use of process maps or visual aids to represent and guide the process - also indicative of insufficient SOP detail.

This observation could be considered reassuring, because it implies that a significant degree of between-product variation may be due to operator variation. This is because some variation is not inherent to biological materials, but due to the human factor which the field has proven historically it is able to improve upon (e.g. the mechanisation of firearms manufacturing and the automation of car manufacturing).

When asked, the technicians defined the greatest source of variation as that of donor mobilisation - what they saw as the source for wildly disparate numbers of cells and volume of material. Conversely the managers see the greatest source of variation as being the technicians, and see automation as key to reducing variation. It must be noted that variation has not been seen as a high priority, partially because HSCT is a secondary therapeutic, but also because deviations from their specification have been solved by pooling products.

4.3.12 Cryopreservation

Depending on the location of the donor, or the hospital, transportation of starting material or product may be required. Given the nature of the product, this will require cryopreservation to maintain viability, which is known to damage cells [144]. Product data from CMCF is recorded pre-cryopreservation. In some cases, the product was not preserved, but given to the patient locally and in other instances the product may have been frozen and stored. As this process affects the cells - either killing them, pushing them down an apoptotic pathway (towards dying) or otherwise affecting their function - it can be assumed that the pre-cryopreservation cell numbers, will differ from the actual product given to the patient. Additionally, from an efficacy perspective, the quality of the product prior to cryopreservation will be different to that post-thaw. Consider the distribution in **Figure 4.1**, if there was a set specification, how many products would fall below this specification post-cryopreservation, given that the process will reduce the number of living cells?

The thawing process is also subjective, and potentially even more damaging. A water bath is used for thawing, and it was noted that technicians massaged the product to speed up the thaw. Considering the acicular nature of ice crystals, it is not unreasonable to consider the damage that might be caused [204].

4.4 Quantitative Analysis

Disclaimer: This is a limited dataset. Due to time constraints of the amount of data that could be practically transferred within the two-week time window. Useful conclusions can be drawn, but the extent to which the data can be stratified is limited. There are a number of figures presented here that appear to demonstrate trends between variables, at least visually. Without proper stratification or normalisation for a network of other potential confounding variables, it is important to recognise that these trends may be inaccurate.

This section investigates and discusses the data mined from the CMCF and the conclusions that can be drawn with respect to variation, and its effect on manufacture of future cellular therapy. It consists of a more detailed methodology section is in addition to a summary of what the database contains, then a section by section analysis of each Figure, their purpose and the lessons learned.

This type of data has never been publicly reported and statistically analysed and provides ranges of variation and potential correlations with variables that may be clinically important or have implications for manufacturing and process controls.

4.4.1 Methodology

The limitation of the meta-analysis was that the journal papers did not provide enough resolution for more detailed analysis because important metrics were disparately reported. A single centre eliminates the differences in equipment and practise culture that can be found in the previous global ‘picture’, and reduces the number of operators and clinicians involved, reduces the pool of donors, and may limit the number of indications (and the method for which they are treated). This gives a baseline for manufacturing engineers in terms of the ‘best-case’ scenario from which to work from.

Prior to the data mining exercise, a database was set up in Microsoft Access and was then transferred into IBM SPSS 22. Microsoft Access was abandoned on site at the CMCF, because it slowed the transfer process down and was much harder to add or remove variables as they were encountered. This database was informed and set up using the variables established in the meta-analysis, and was then further refined by redacted patient records sent by the CMCF. **Table 4.0** is a list of these variables.

Directly from Contributor	"Hemolab" / QC	Starting Material	Pre-Freeze Product	
Autologous / Allogeneic	Collection Date	Initial Volume	TNC Count	Processing Type
Indication	Received Date	ACD Volume	TNC Count / kg	Unique ID
Blood Type	WBC	QC Volume	CD34+ Cell Count	Source
Adult / Paediatric	Haemoglobin	Supernatant Volume	CD34+ Cells / kg	Protocol
Weight	Haematocrit	Post-expression Volume	Volume	Physician
Height	Myeloblasts	Plasmalyte Added		Product
Age	Immature Neutrophils	Initial Dilution, cell count, and viable cell count		Collection Date
Gender	Neutrophils	Tryphan Viability		Collection location
Initial / Product Flow	Eosinophil			Minimum and maximum cell dose
White Blood Cell	Polys			Processing date
WBC x Dilution	Basophil			Processing Tech
Red Blood Cells	Lymphocyte			Calculation Tech
Haemoglobin	Promyelocyte			Analysis Tech
Haematocrit	Monocyte			TNC Recovery %
Mean corpuscular volume	Myelocyte			Temperature
Mean corpuscular haemoglobin	Meta			Expiration Date
Basophils	Mean corpuscular haemoglobin concentration			
Immunoglobulin	Platelets			
	Red cell distribution width			
	Platelet Size			
	Lymphocyte			
	Monocyte			
	Eosinophil			

Table 4.0: List of Variables extracted from the CMCF Database

- ACD Volume is the volume of acid citrate dextrose used (an anticoagulant)
- QC Volume refers to the volume taken from the total product for QC testing.
- Supernatant volume is the volume removed from the product during centrifugation.
- Post-expression volume is the amount remaining after the supernatant is removed.
- Plasmalyte is raw material added to the post-expression product to make up to a given volume

Haematology laboratory analysis / quality control analysis / initial flow / product flow were all secondary but useful information. It was hypothesised that these could be used in a comparison with similar data from the UK Biobank to establish where DFCI's patient and donor population sat on a relatively healthy UK baseline population. However due to time constraints this was ultimately given a lower collection priority in favour of starting material and process related variables, specifically those that related to collected and pre-freeze TNC count and CD34+ cell count. The aim of this triage was so that greater volumes of the 'core' characteristics could be collected and greater resolution on the ranges and distribution of the variation could be presented here.

4.4.2 Statistical Analysis

The unique deliverable of this chapter is the statistically robust number of data points used to analyse variation, in this Chapter and in **Chapter 5**, where previously only very small datasets could be used, such as the super-orphan treatment of ADA SCID with 15 patients [205].

Firstly, the spread of the data was analysed using scatter graphs and box & whisker plots. These give details as to the mean and median of the population, the minimum and maximum values (including the range), the standard deviation and the variance.

Given that the majority of the biological data presented here is non-normally distributed (see **Figure 4.1)** the median is a more useful descriptive statistic than the mean, as it is more sensitive to skewed data. Furthermore, in these skewed datasets a larger mean than the median indicates a positive skew in the data, whilst a larger median indicates a negative skew in the data.

The minimum, maximum and range data is also presented. This is the most basic representation of the variation encountered, as these values represent the smallest and largest cell populations within a given time frame. They demonstrate the extremes of biological variation, and the potential extremes that a corresponding manufacturing process will need to be capable of addressing.

The standard deviation is a measure of dispersion of the data. This determines how tightly the dataset is grouped around the mean. A high standard deviation represents data that is spread out

over a wider range of values. Variance is a measure of spread of the data, and determines how far each number in the set is from the mean. A large variance indicates that numbers in the population are far from the mean.

Variance has been included as it is not based upon the mean (which may be skewed in non-normally distributed data) but based on the distances from a given data point to another, so may be a more representative measure of spread in non-normally distributed data.

Independent samples median tests, independent Mann-Whitney tests (for two independent groups, such as gender) and independent sample Kruskal Wallis (for multiple independent groups) tests have been used to measure whether the median or the distribution of population differs significantly between groups [206]. The Kruskal-Wallis test is the non-parametric equivalent of the one-way ANOVA, that determines whether there are statistically significant differences between two or more groups of an independent variable on a continuous or ordinal dependent variable.

There are several assumptions that must be met to use the K-W test;

- One dependent variable measured on a continuous or ordinal level
- One independent variable consisting of two or more categorical independent groups
- Independence of observations - no relationship between the observations in each group - e.g. different participants in each group.
- Distribution of scores for each group of your independent variable e.g. males and females, have the same or different shape (having the same shape means having the same variability).

A statistically significant K-W test means that the differences in distribution between groups is significant. Further tests can be done to determine the extent of this significance.

4.4.3 Database Contents Summary

The final database is summarized in **Table 4.1**.

The database contains information relating to 410 products, processed between the 2nd January 2014 and the 5th September 2014. There were 287 autologous products for patients between the ages of 6 months and 76 years old, and 123 allogeneic products from donors aged between 3 months and 69 years old. There were 11 paediatric patients.

Out of the 410 products, 212 were volume reduction processes and 51 were not manipulated (filtered, but otherwise untouched). 147 products involved more bespoke processes such as gene therapy. Further details of the indications being treated were not readily available in the product records however, **Figure 4.6** is a Pareto diagram of the known indications. This is only representative of a small (n = 101) section of the population, but mirrors the literature meta-analysis (**Figure 3.4** in **Chapter 3**) in that the majority of patients are undergoing treatment for leukaemia (blood cancers). This limitation is due to privacy concerns surrounding access to patient records.

Processed Products	**N = 410**	
Autologous	287	6 months – 76 years old
Allogeneic	123	3 months – 69 years old
(Including Paediatric)	11	
Protocols Used	**N = 410**	
Volume Reduction	212	
Filtered, manipulated	51	
Other, bespoke	147	

Table 4.1: Contents of the DFCI-LU Database

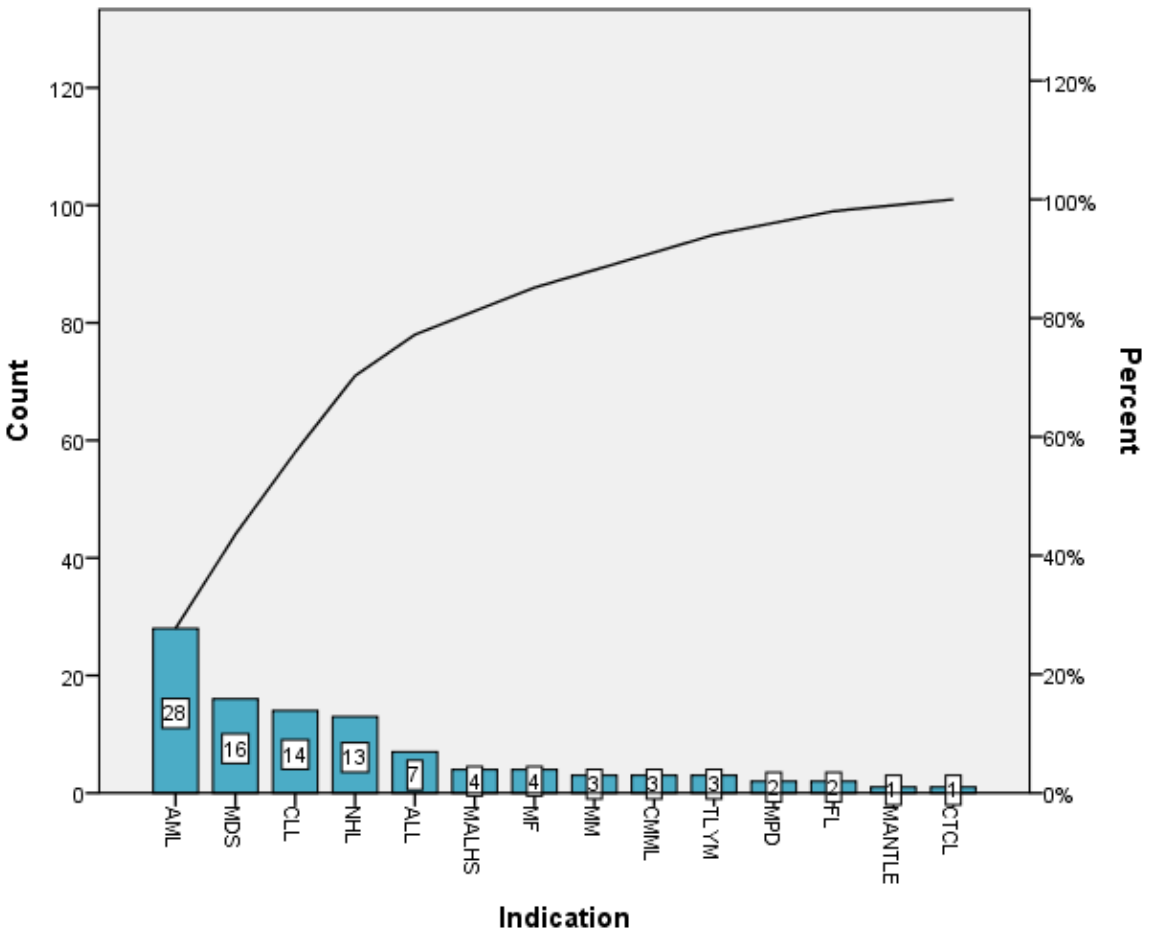


Figure 4.6: Pareto Analysis of Indications reported in CMCF database.

- **AML**: acute myeloid leukaemia
- **MDS**: myelo dysplastic syndrome
- **CLL**: chronic lymphocytic leukaemia
- **NHL**: non-hodgkins lymphoma
- **ALL**: acute lymphoblastic leukaemia
- **MALHS**: a gene therapy target
- **MF**: mycosis fungoides
- **MM**: multiple myeloma
- **CMML**: chronic myelomonocytic leukemia
- **TLYM**: a gene therapy for leukemia
- **FL**: follicular lymphoma
- **MANTLE**: mantle cell lymphoma
- **CTCL**: cutaneous t-cell lymphoma

4.5 Results

The primary objective of this analysis was to determine the extent of biological variation encountered in a single centre, in a statistically robust manner.

The secondary objective of this analysis was to determine how processing and donor metrics such as protocol or patient weight and age affect the variation encountered in the subsequent starting material and final pre-freeze product. It is important to note this was not intended to be an analysis of the relationship between these metrics, but rather to capture their respective effect on the extent of biological variation.

Four variables were chosen as measurands of biological variation, determined as important from the literature data and clinical discussion. These four metrics were:

- Volume of the Starting Material, post-apheresis
- TNC Count of the Starting material, post-apheresis
- TNC Count of the Product, pre-freeze
- CD34+ Cell Count of the Product, pre-freeze

CD34+ cell content of the starting material was not measured by the CMCF so it is currently unclear as to the effect of the various processing steps on the yield of CD34+ cells in the final product.

Each of these four metrics have been examined for overall spread and distribution, split into both autologous and allogeneic sourced material, and examined with respect to patient age, weight and gender (so the subsequent results will focus on autologous therapy only). Allogeneic sourced data has been collected but in significantly smaller numbers as this externally donated material is obtained off-site and so limited information was available with respect to allogeneic donor characteristics such as age, weight and gender.

An investigation into the effect of operator variation was also carried out, using the CD34+ cell count of the product, and the yield of TNCs as metrics to discern whether different operators have any effect on these different metrics.

4.5.1 Overall Variation

Overall variation in the pre-freeze product – that is to say that the product prior to being cryopreserved or given to the patient – is demonstrated in the next three diagrams.

These have been illustrated in a similar manner as the literature meta-analysis, and a direct comparison has been given between the variation in the single clinical centre (CMCF) versus the variation in the 94 clinical centres (Chapter 3). Key information is represented on the Figures, with more detailed statistical information within the Tables.

Figure 4.7 is a scatter diagram showing the variation and spread of the absolute cell numbers within the pre-freeze product, in terms of CD34+ cell count and total nucleated cell count. These have been plotted on the same axis as CD34+ cells are a subset of the TNC cell count. The overall variation in absolute cell numbers is between $4.80x10^5$ cells to $4.14\ x10^9$ CD34+ cells and $2.00x10^9$ cells to $2.62\ x10^{11}$ total nucleated cells (**Table 4.2**).

Figure 4.8 is also a scatter diagram, but unlike the absolute cell count in **Figure 4.7** this demonstrates the variation in terms of cells per kilogram in the final product. This is important because it incorporates the variation due to different weights, but also because dose per kilogram is the recognisable medical metric and is how cellular therapy is currently measured and dosed. The overall variation in cells per kilogram is between $7.92x10^4$ to $7.41\ x10^7$ CD34+ cells / kg and $5.28x10^7$ to $2.87\ x10^9$ total nucleated cells / kg (**Table 4.2**). When these are measured in cells per kilogram, the range, standard deviation and variance drop by at least two orders of magnitude as weight becomes normalised, potentially indicating that weight is a source of variation and indicating it is important to report measurements in a uniform manner to allow comparison. To this end, both absolute and cells per kilogram will be reported in this Chapter.

Figure 4.9 is one of the most meaningful results from this Chapter. It overlays the data obtained from the CMCF, with each point in blue representing an individual product's cell characteristics, with the previous results shown and presented in **Chapter 3**, with each orange point representing the

median product cell count for each study and the ranges. From this diagram an earlier hypothesis (**Section 4.1**) can be addressed: the variation in a single centre is indeed significantly less than the spread of the 94 centres (4.80 x 10^6 to 4.14 x 10^9 CD34 + cells / kg within the CMCF compared to 6.0 x 10^4 to 1.10 x 10^9 CD34+ cells / kg within the Meta-analysis, **Figure 4.9**) but is still in itself significant.

The single centre has greater control over its variation in product than many of the other centres in the Meta-analysis, however there is still significant variation that may indeed be inherent to the biological starting material, if a single, highly rated clinical centre still retains a high level of variation.

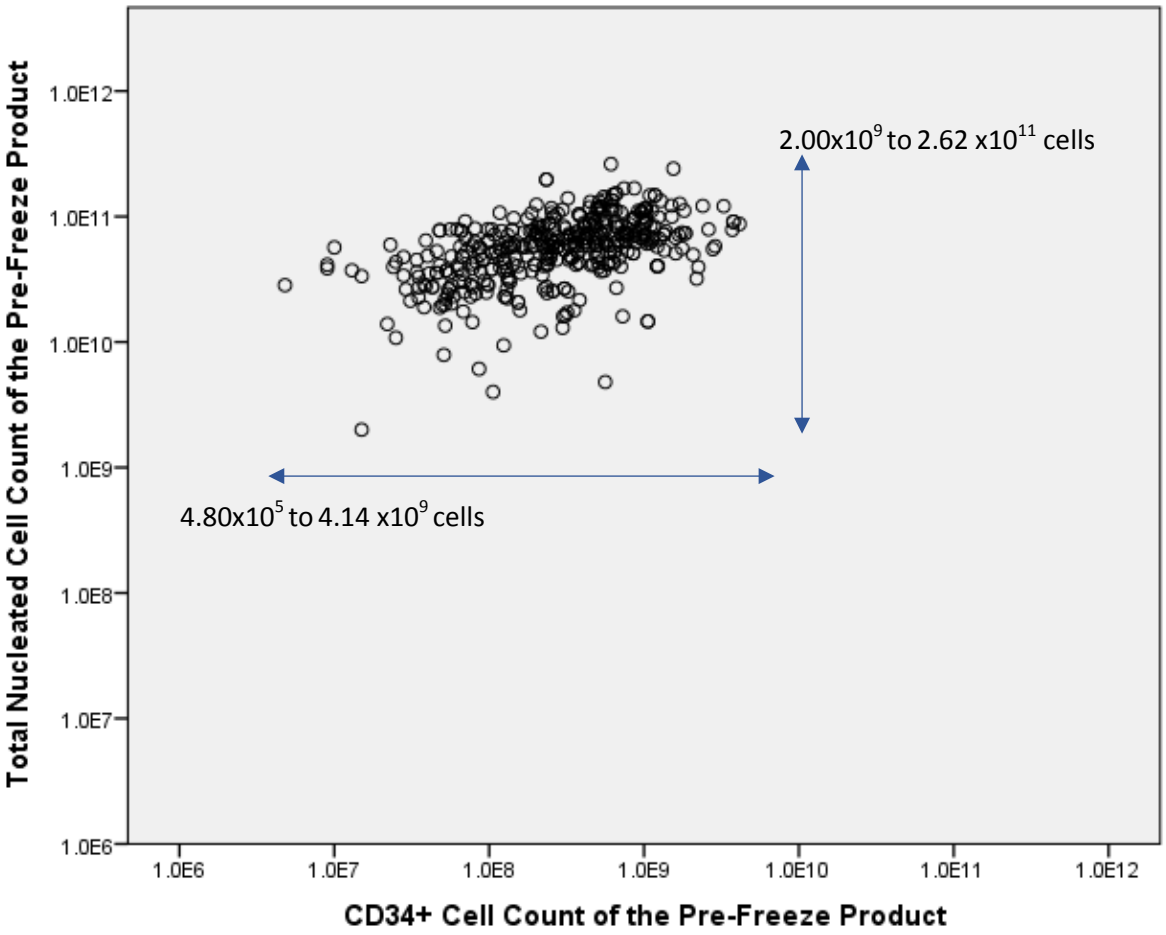


Figure 4.7: CD34+ Cell Count and TNC Count of the Pre-Freeze Product ($\log_{10}$ scale)

	Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
TNC (Product)	410	$6.45x10^{10}$	$5.94x10^{10}$	$2.00x10^{9}$	$2.62x10^{11}$	$2.60x10^{11}$	$3.56x10^{10}$	$1.28x10^{21}$
CD34 (Product)	410	$4.99x10^{8}$	$3.06x10^{8}$	$4.80x10^{6}$	$4.14x10^{9}$	$4.13x10^{9}$	$5.97x10^{8}$	$3.56x10^{17}$

Table 4.2: CD34+ Cell Count and TNC Count of the Pre-Freeze Product

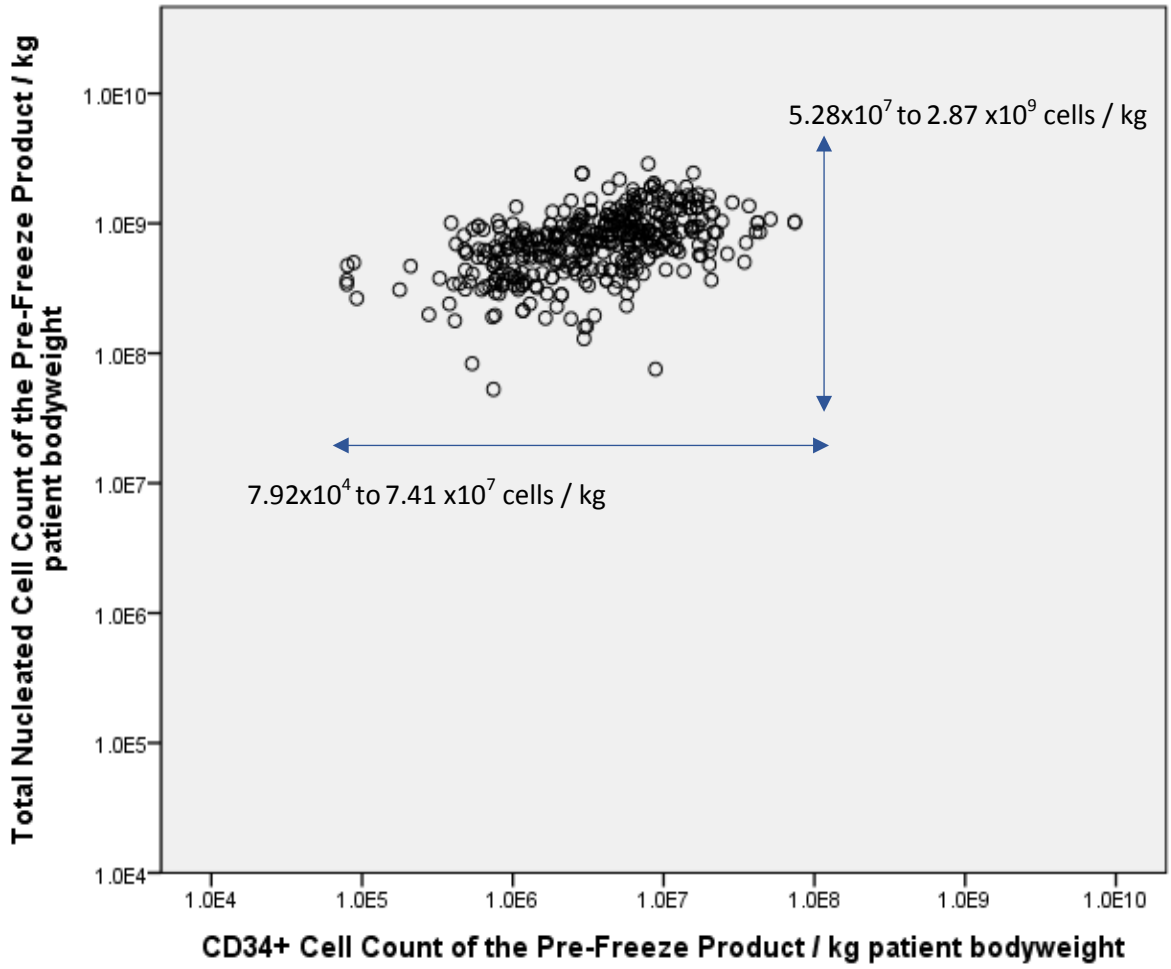


Figure 4.8: CD34+ Cell Count and TNC Count of the Pre-Freeze Product per kilogram of patient bodyweight

	Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
TNC / kg (product)	410	$8.07x10^8$	$7.42x10^8$	$5.28x10^7$	$2.87x10^9$	$2.82x10^9$	$4.29x10^8$	$1.84x10^{17}$
CD34 / kg (product)	410	$6.51x10^6$	$3.95x10^6$	$7.92x10^4$	$7.41x10^7$	$7.40x10^7$	$8.65x10^6$	$7.48x10^{13}$

Table 4.3: CD34+ Cell Count and TNC Count of the Pre-Freeze Product per kilogram of patient bodyweight

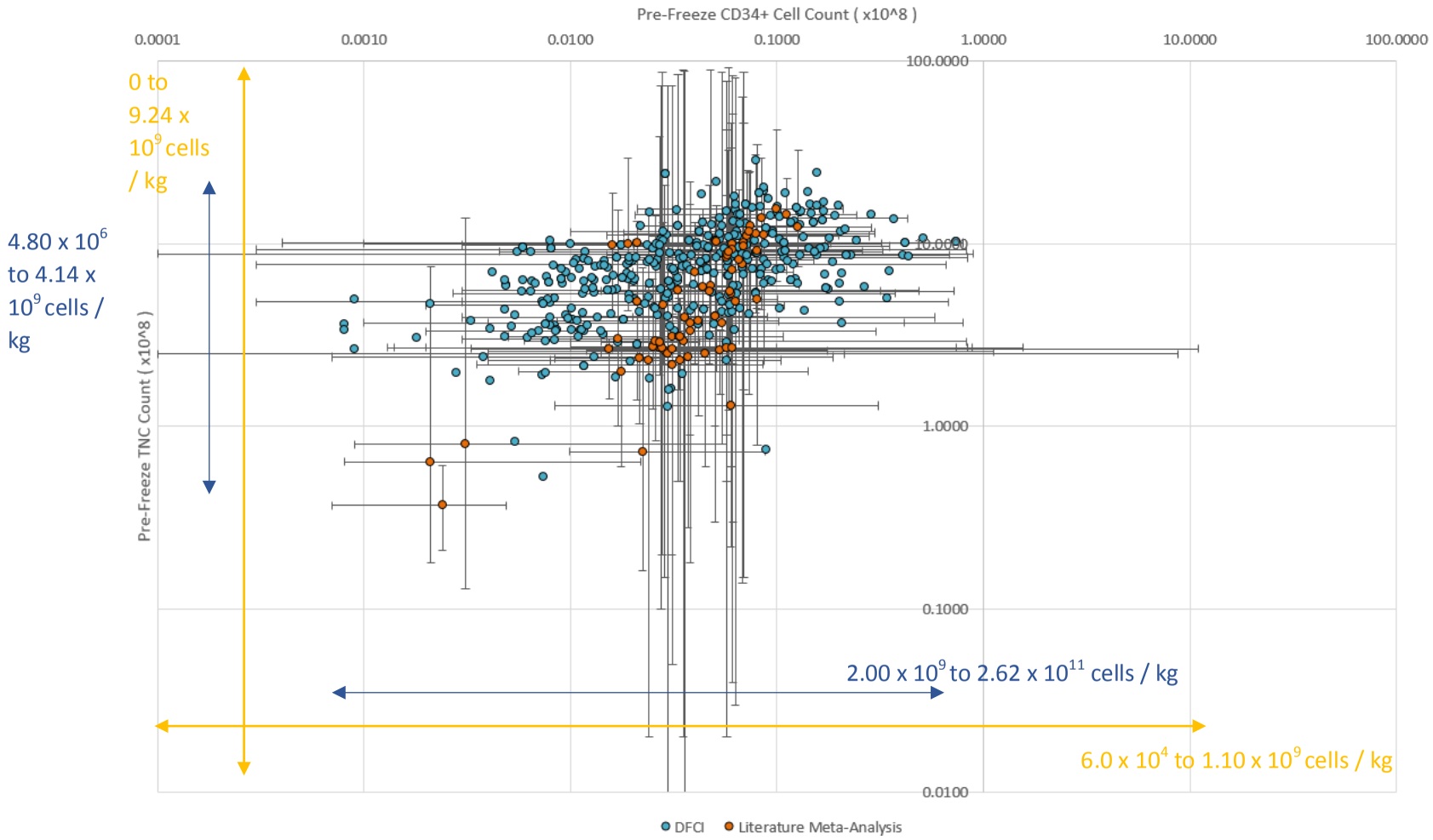


Figure 4.9: CD34+ Cell Count and TNC Count of the Pre-Freeze Product per kilogram of patient bodyweight, for the Meta-Analysis dataset in orange and the DFCI dataset in blue

4.5.2 Starting Material: Initial Volume

This metric is one of the foremost and fundamental considerations for manufacturing cell therapies, as it will affect the equipment, machinery, and in extreme cases facilities, used to produce the product. At the CMCF, initial volume refers to the volume of the apheresis product received from the hospital (either locally, or shipped from another facility), and is measured in millilitres (ml).

Firstly, variation as a function of initial post-apheresis volume was between 31.90 ml and 730.60 ml (**Figure 4.10, Table 4.4**). This is perhaps indicative of entirely different configurations, even machines, to be able to automate a process that had this amount of input variation. The standard deviation and visual inspection of **Figure 4.10** demonstrates that although, overall the variance is high, there is a clustering around the median (348.90 ml) that could be considered as two populations – this central 'expected' cluster and the outlying data points.

Figure 4.11 is a histogram of the spread of starting material volume. Conversely to visual inspection, initial volume is not normally distributed, likely due to the long tails either-side of the main peak. This is confirmed by the high kurtosis value (4.550) and by the Shapiro Wilk's test ($p > 0.05$). It is these long tails that are skewing the distribution from what would otherwise be a normal distribution, or close to, given the skewness value (0.396). Perhaps in a regular manufacturing process these extremes could be controlled for, but these are indicative of an individual patient's apheresis volume and is too valuable to discard.

In **Figure 4.12** this initial volume has been split between autologous sourced material (i.e. a patient, presumably sick) and allogeneic sourced material (i.e. an external donor, presumably healthy). Visually, autologous sourced material has a much tighter distribution than allogeneic, perhaps indicative of a single centre's procedures and techniques, compared to the 'pool' of potential centres that an allogeneic donation may have been sourced from. Statistically however, by independent samples median and Kruskal-Wallis test, the median and distribution of both autologous and allogeneic are not significantly different ($p > 0.948$ and $p > 0.979$, respectively). The spread of the data, represented by the variance, is almost five times as high in allogeneic sourced

material likely due to the lack of clustering that is apparent in the autologous material – this may be due to the lack of measured records for allogeneic, and that with sufficient numbers this may also be reflected in the allogeneic material. (Please note that as absolute cell numbers were unavailable in the Meta-analysis, cells / kg has been used)

Figure 4.13 splits **Figure 4.11** into autologous and allogeneic sourced material, and has examined the normality of each subset. By Shapiro Wilk's test both autologous and allogeneic starting volume are not normally distributed ($p > 0.05$) although visual inspection and the statistical metrics for allogeneic would suggest external donor sourced material is closer to normality than autologous sourced material. However with the large amount of spread and the long tailed edges (and a kurtosis and skew of 4.229 / 0.597 and 1.343 / 0.400 for autologous and allogeneic, respectively) it is these extremes that ensure that this metric is non-normally distributed.

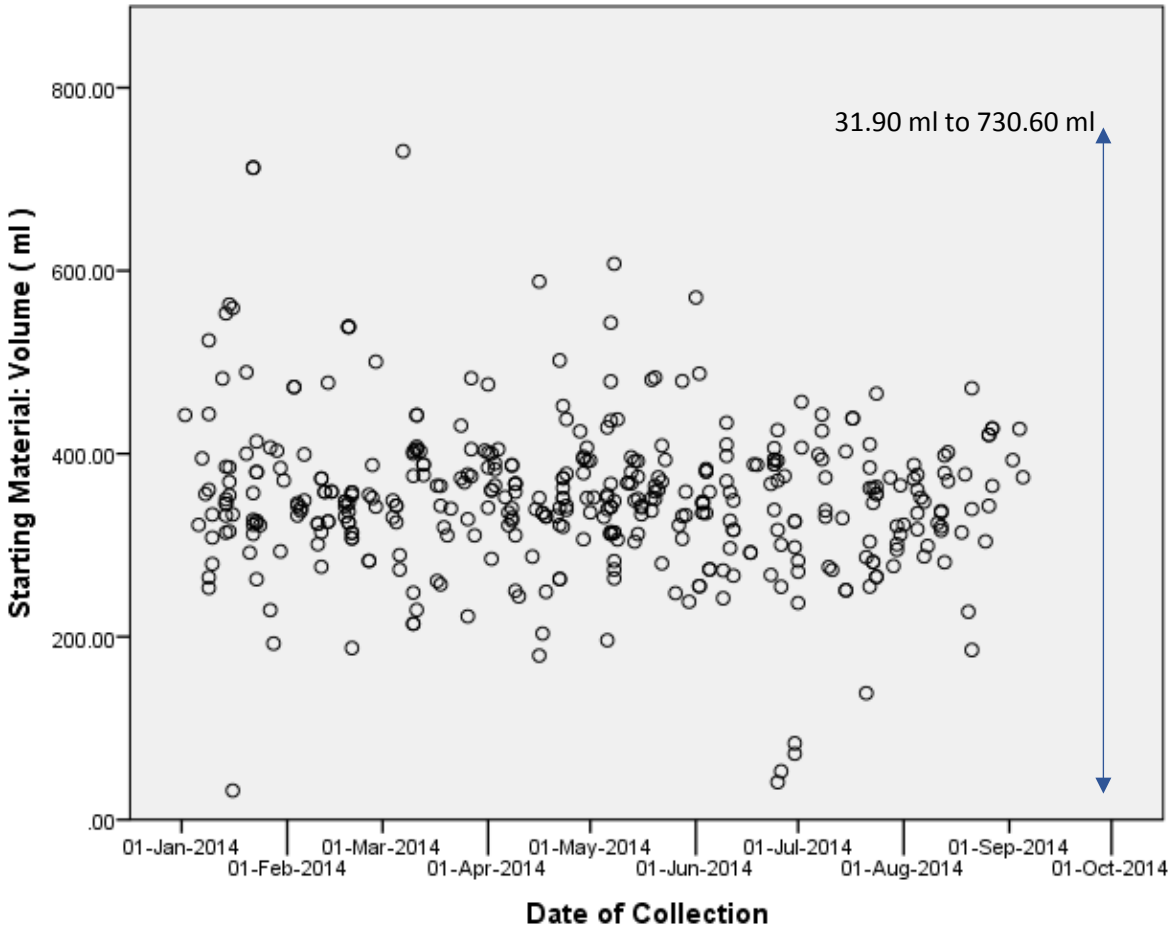


Figure 4.10: Initial Volume of the Starting Material (post-apheresis) against the date of collection

	Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
Initial Volume (ml)	406	351.78	348.90	31.90	730.60	698.70	81.28	6607.22

Table 4.4: Initial Volume of the Starting Material (post-apheresis) against the date of collection

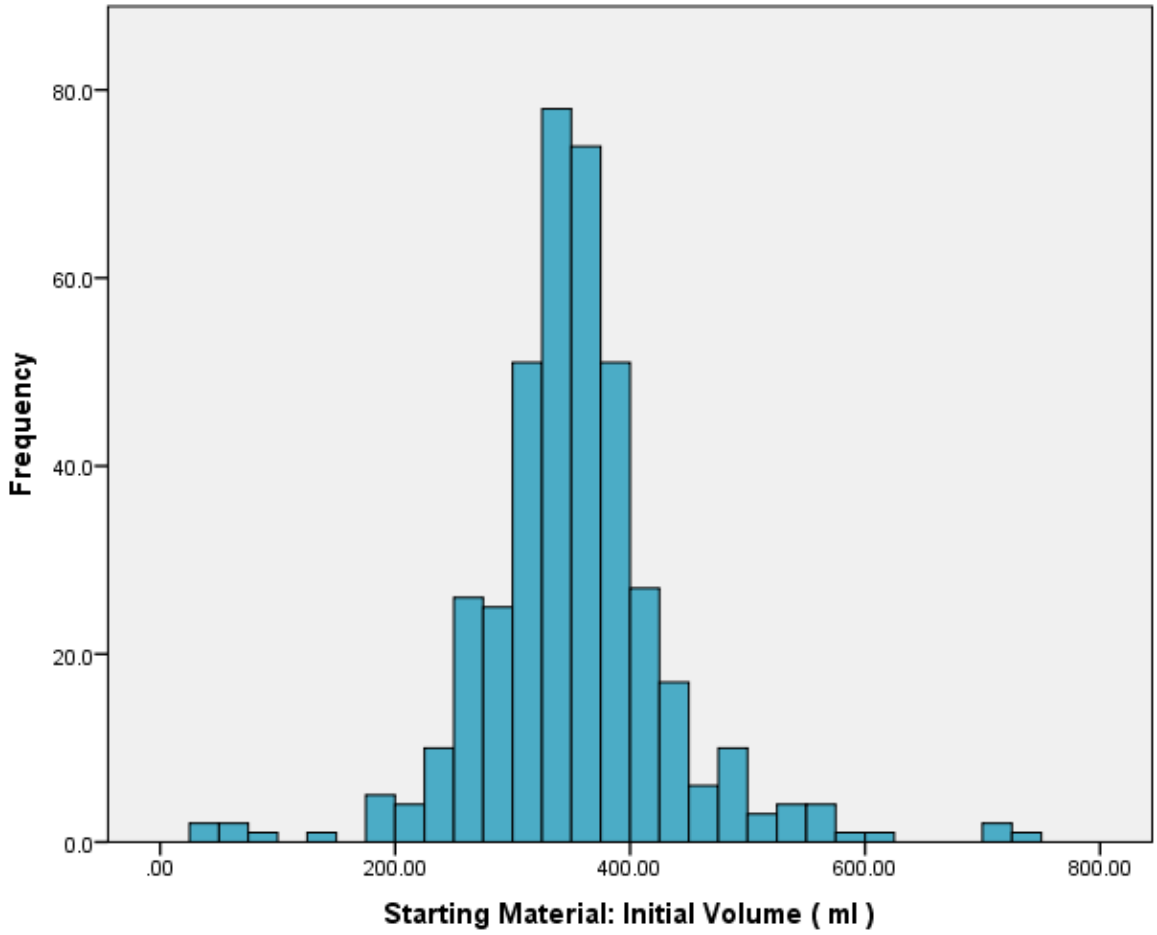


Figure 4.11: Histogram showing the spread and distribution of the initial volume of the starting material (post-apheresis)

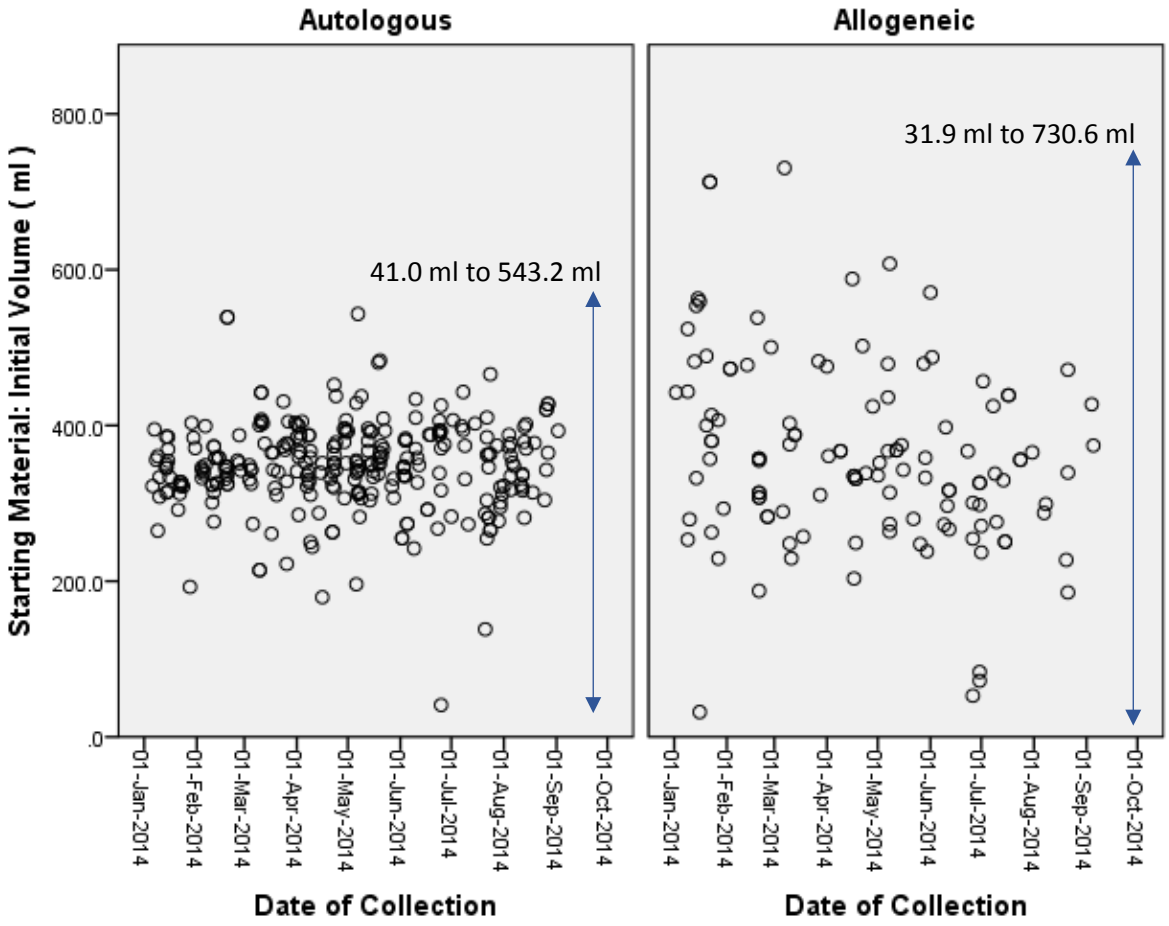


Figure 4.12: Initial Volume of the Starting Material (post-apheresis) against the date of collection, split into autologous and allogeneic sourced material

Volume (ml)	Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
Autologous	285	348.28	348.90	41.00	543.2	502.20	56.83	3230.12
Allogeneic	121	360.05	352.10	31.90	730.60	698.70	120.65	14556.58

Table 4.5: Initial Volume of the Starting Material (post-apheresis) against the date of collection, split into autologous and allogeneic sourced material

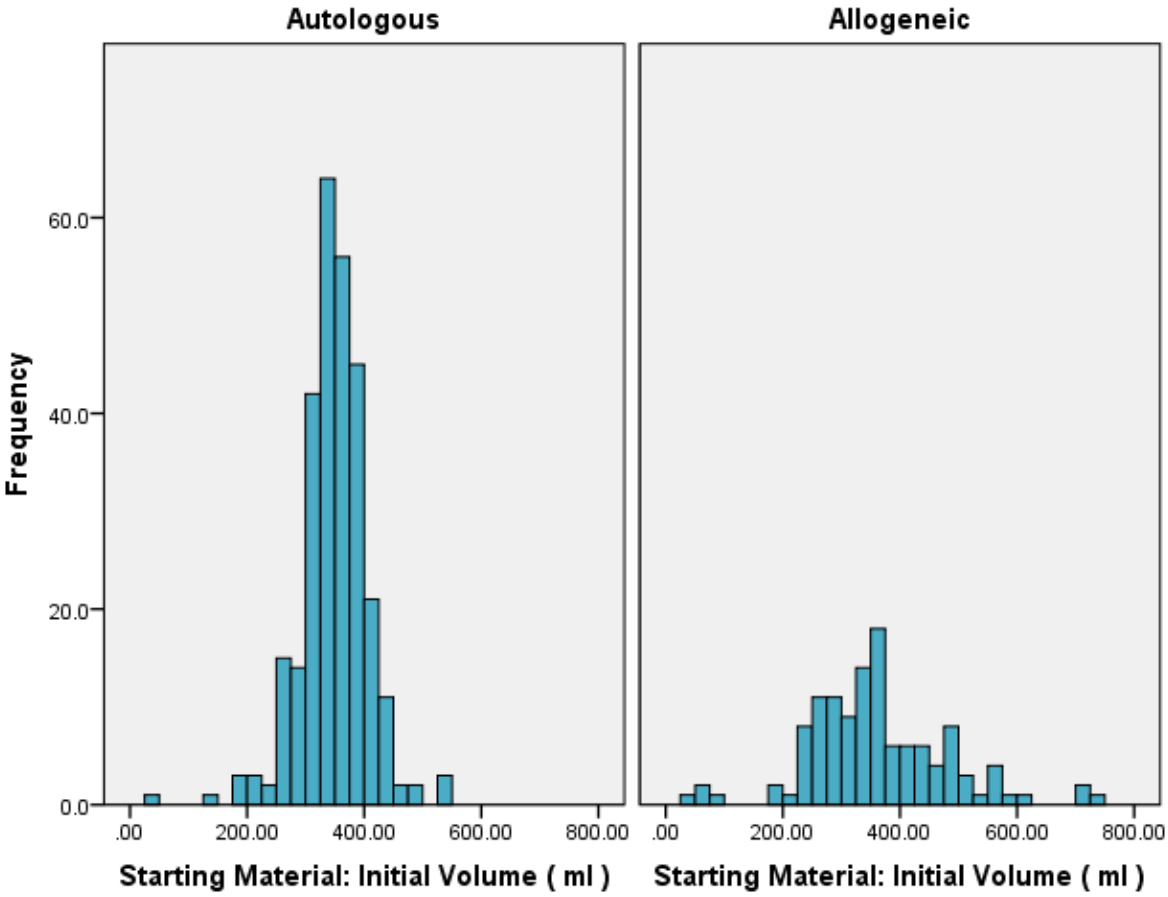


Figure 4.13: Histogram showing the spread and distribution of the initial volume of the starting material (post-apheresis) for autologous and allogeneic therapy respectively

4.5.3 Starting Material: Total Nucleated Cell Count

This metric is the least specific of the two cellular metrics used in this analysis, however it is a traditional quality metric and stem cells are a member of this cell population. Total nucleated cell count refers to the number of nucleated cells within a given sample, and is not indicative of a specific cell type. At the CMCF, initial TNC count is a measure of the number of cells within the apheresis product received from the hospital and is measured in either 'absolute' cell numbers, or cells per kg (patient weight).

This analysis has now moved on towards cellular measurements therefore figure axes are reported in scientific notation.

The spread of TNCs within the starting material, overall, is between $2.20x10^{9}$ cells and $2.70x10^{11}$ cells around the median value of $5.81x10^{9}$ cells (**Figure 4.14, Table 4.6**). This less variable from a manufacturing perspective compared to the variation in TNC count shown in the literature meta-analysis (between zero and $9.24x10^{9}$) (**Figure 4.9**), but 2 orders of magnitude in process variation would still be unmanageable.

The trend of non-normally distributed biological measurands is continued in **Figure 4.15**, but unlike initial volume which is much more central (skewness of 0.396), TNC is skewed towards the left hand side (skewness of 1.485). Both have a similar kurtosis (4.550 and 4.054), the tendency for long tails except where before in initial volume there were two long tails either side of the main distribution, where TNC is much closer to zero, there is one main, long tail that encompasses the more variant data points. Normality was tested for using Shapiro Wilk's test to confirm this visual inspection and was found to be non-parametric ($p > 0.05$).

TNC count within the starting material was further split into autologous and allogeneic sourced material in **Figure 4.16** and **Table 4.7.** From visual inspection, the variation in autologous material appears to be much higher than allogeneic, mainly due to a few 'outliers' above the $2x10^{11}$ cells mark, and there appears to be a much more central clustering. However, from a statistical

standpoint, autologous material has a much higher variance between data points and only a slighter higher standard deviation. Independent samples median and Mann-Whitney tests were used to examine the statistical differences in median and distribution and it was found that both autologous and allogeneic material has significantly different medians and distributions ($p > 0.05$) implying that these are two distinctly different subsets of the population. However, given that allogeneic only has 34 data points this may be due to an unrepresentative sample of the population compared to autologous material.

Examination of these two subsets in **Figure 4.17** appear to show a similar distribution to **Figure 4.15**, although a Shapiro Wilk's test found that allogeneic material is normally distributed ($p < 0.996$). This may be a factor of the sampling issue as mentioned above, but the skewness and kurtosis is indicative of a normal distribution (0.068 and 0.089 respectively). Autologous therapy is similarly distributed as before, with a skewness and kurtosis of 1.706 and 4.909 respectively, with the long-tailed edge that is becoming iconic of these biological measurements.

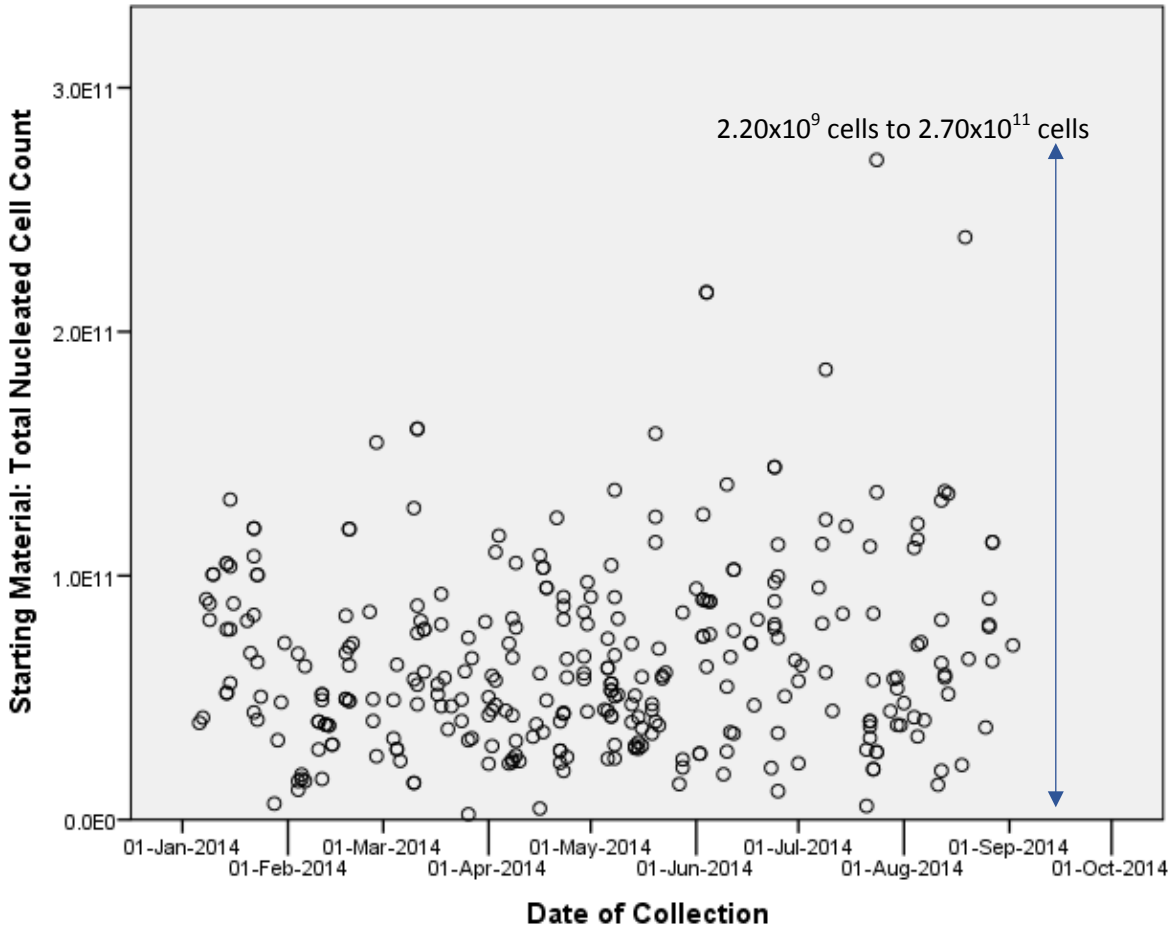


Figure 4.14: Total Nucleated Cell Count of the Starting Material (post-apheresis) against the date of collection

	Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
TNC Count (Starting Material)	319	$6.56x10^{10}$	$5.81x10^{10}$	$2.20x10^{9}$	$2.70x10^{11}$	$2.68x10^{11}$	$3.86x10^{10}$	$1.49x10^{21}$

Table 4.6: Total Nucleated Cell Count of the Starting Material (post-apheresis) against the date of collection

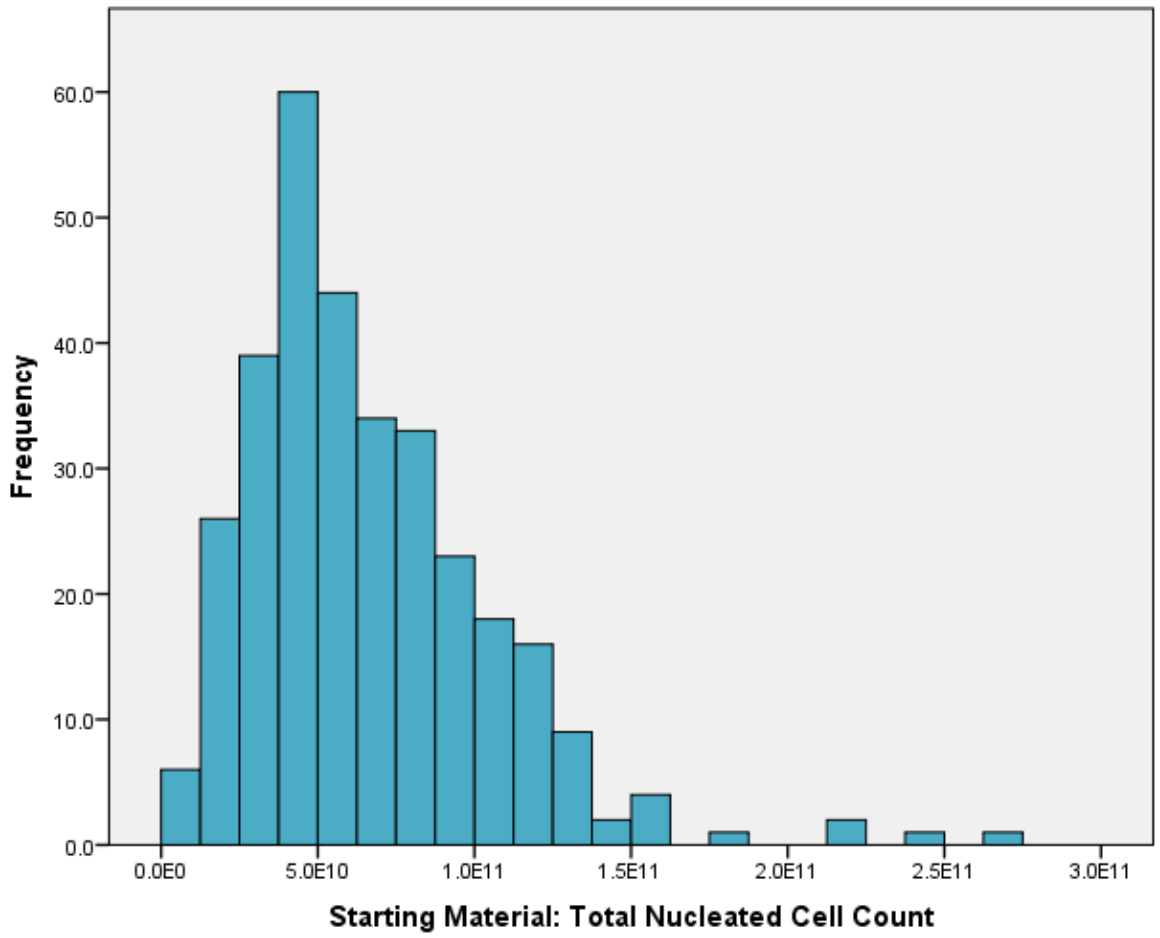


Figure 4.15: Histogram showing the spread and distribution of the total nucleated cell count of the starting material (post-apheresis)

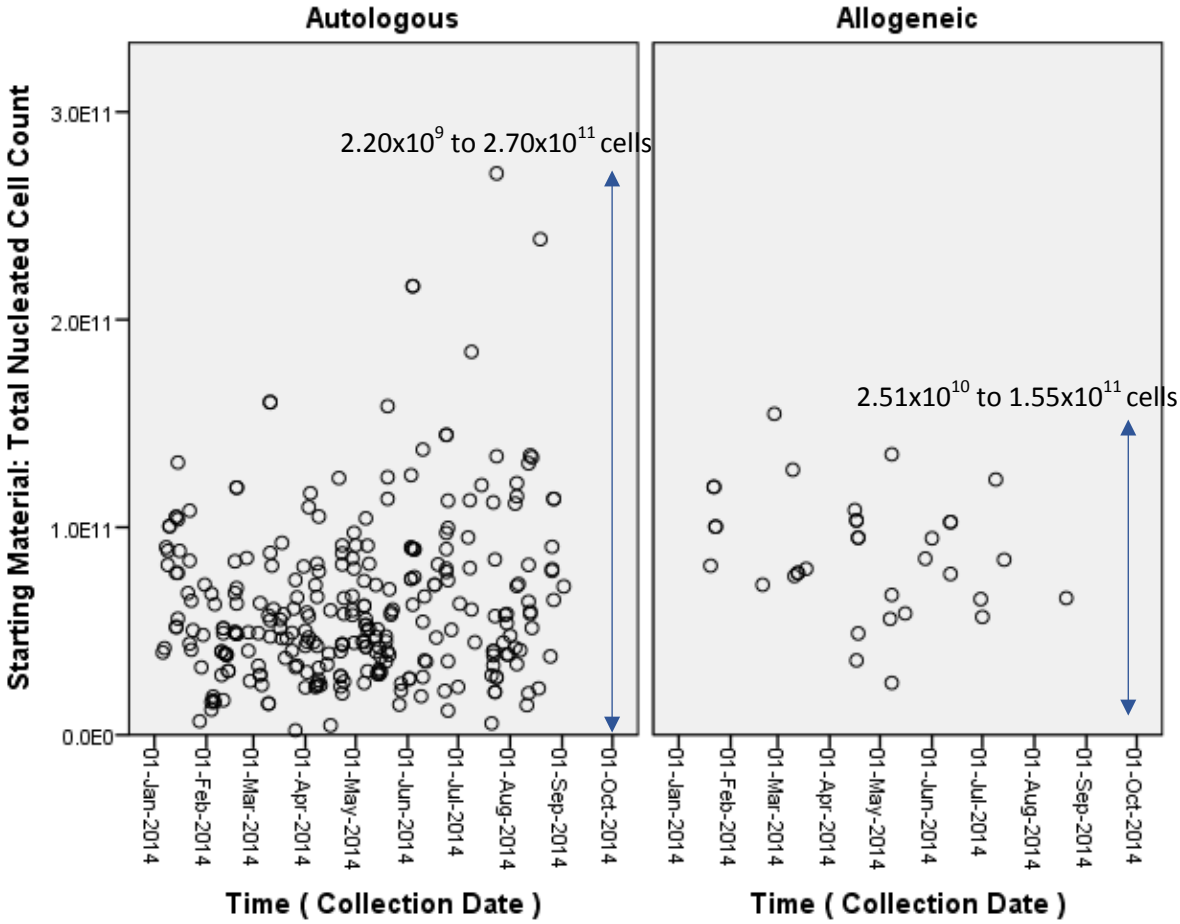


Figure 4.16: Total Nucleated Cell count of the starting material (post-apheresis) against the date of collection of autologous and allogeneic sourced material

	Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
Autologous	285	$6.30x10^{10}$	$5.38x10^{10}$	$2.20x10^{9}$	$2.70x10^{11}$	$2.68x10^{11}$	$3.88x10^{10}$	$1.51x10^{21}$
Allogeneic	34	$8.76x10^{10}$	$8.47x10^{10}$	$2.51x10^{10}$	$1.55x10^{11}$	$1.30x10^{11}$	$2.83x10^{10}$	$8.02x10^{20}$

Table 4.7: Total Nucleated Cell count of the starting material (post-apheresis) against the date of collection of autologous and allogeneic sourced material

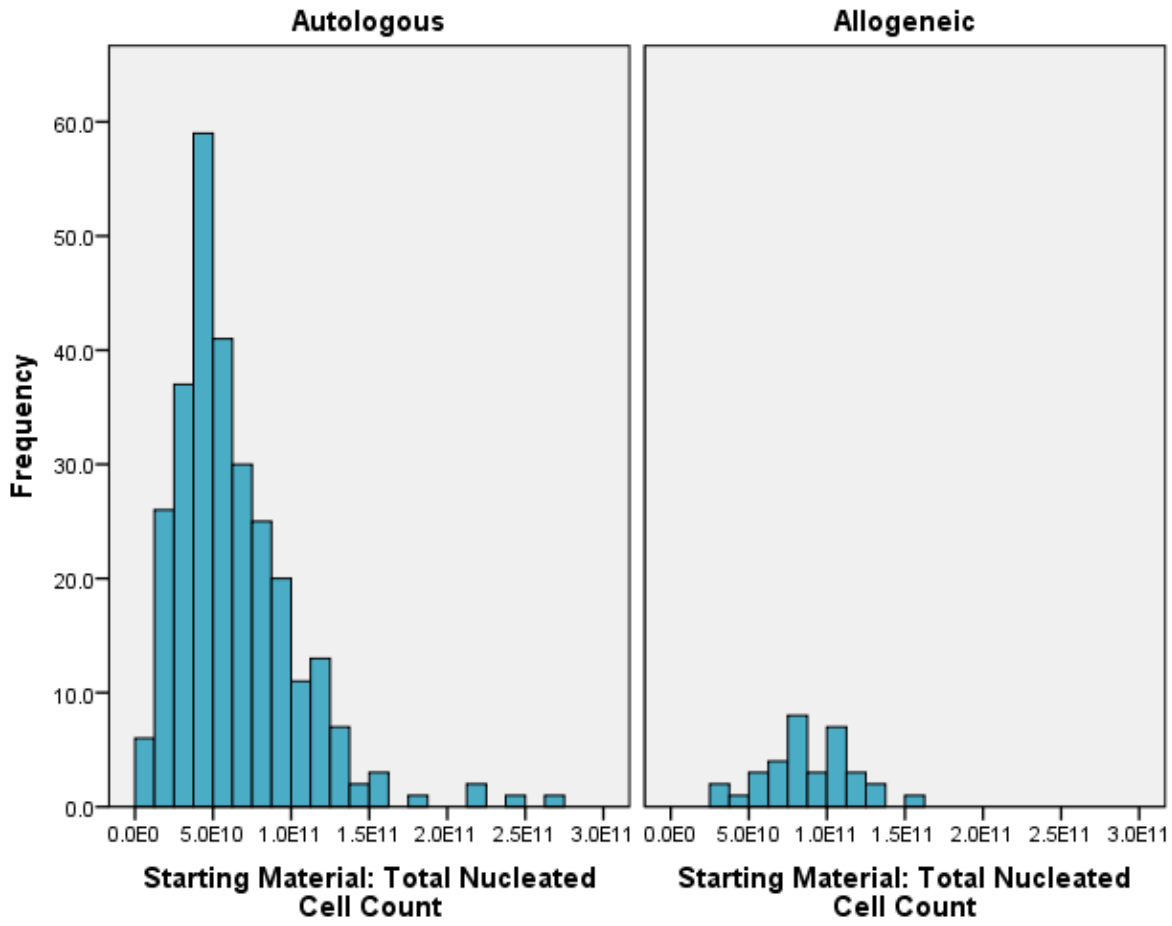


Figure 4.17: Histogram showing the spread and distribution of the total nucleated cell count of the starting material (post-apheresis) for autologous and allogeneic sourced material

4.5.4 Pre-Freeze Product: Total Nucleated Cell Count

At the CMCF pre-freeze total nucleated cell count refers to the number of nucleated cells measured in the final product, prior to cryopreservation and therapeutic application. It is also measured in absolute cell numbers, or cells per kg (patient weight). It represents the output of the overall process and therefore represents an element of the input variation and the CMCF manufacturing process, and how they can manage that variation as a manufacturing centre and how clinical practise subsequently is able to use the product.

Figure 4.18 and **Table 4.8** illustrate the spread of TNCs in the *product*. Compared to **Figure 4.14**, and the starting material, the distribution and spread is surprisingly similar, but with a slight decrease in range, standard deviation and variance which could be attributed to the process methodology. The range of TNCs is $2.00x10^9$ cells to $2.62x10^{11}$ cells. Given the consistently high yields recorded by the CMCF (see **Figure 4.41**) this is perhaps unsurprising, and may demonstrate that their process does not introduce any further variation in TNC overall (but also, does not *reduce* it).

The process has also not affected the distributions of biological metrics – **Figure 4.19** shows that similarly to **Figure 4.15**, total nucleated cell count is skewed to the left, with a long right-handed tail. Pre-Freeze TNC cell count was not normally distributed, as assessed by Shapiro-Wilk's test ($p > .05$). Kurtosis and skewness were 3.700 and 1.333 respectively. The kurtosis of TNCs post-process is moderately smaller, perhaps indicating that the process has reduced some of the extreme values by merit of the volume reduction process.

Figure 4.20 splits pre-freeze TNC count into autologous and allogeneic source. The range of cell count for autologous is between $2.00x10^9$ cells and $2.62x10^{11}$ cells, and the range of cell count for allogeneic is $7.90x10^9$ cells and $1.68x10^{11}$ cells. By visual inspection, both appear to be relatively similar to each other, apart from the clustering (likely due to having almost double the number of data points) with a similar range, standard deviation and variance. However, incorporating the number of data points an independent samples median and Mann-Whitney test determine that both the medians and distributions of autologous and allogeneic TNCs are significantly different from

each other ($p > 0.05$). This is comparable to those of the initial total nucleated count, and the process can potentially be attributed towards the mild decrease in spread and variation represented by a decrease in range, standard deviation and variance (compared with **Figure 4.16, Table 4.7**).

Given the minimal effect of the process on TNC, a similar distribution is found in the pre-freeze product as was found in the initial starting material – autologous material is non-normally distributed and allogeneic is normally distributed ($p > 0.05$, $p > 0.098$ respectively) (**Figure 4.21**). Autologous material is distributed as expected, given previous distributions, with a left hand skew and a long right hand tail, but allogeneic appears to be more centrally distributed (Kurtosis and skewness for autologous and allogeneic material were 5.424 / 1.810 and 0.593 / 0.273 respectively). This could potentially be attributed towards the significantly fewer data points for allogeneic, or an attribute specific to allogeneic sourced material, such as the storage and collection process of the donor programme for example.

Given that the dataset contains only a subset of the potential data at the CMCF, specific focus was given to the autologous sourced product characteristics. These were uniformly reported, carried out on-site, and were more likely to contain patient metrics such as age, weight and gender.

What follows is an examination of these three metrics with respect to pre-freeze total nucleated cell count and CD34+ cell count.

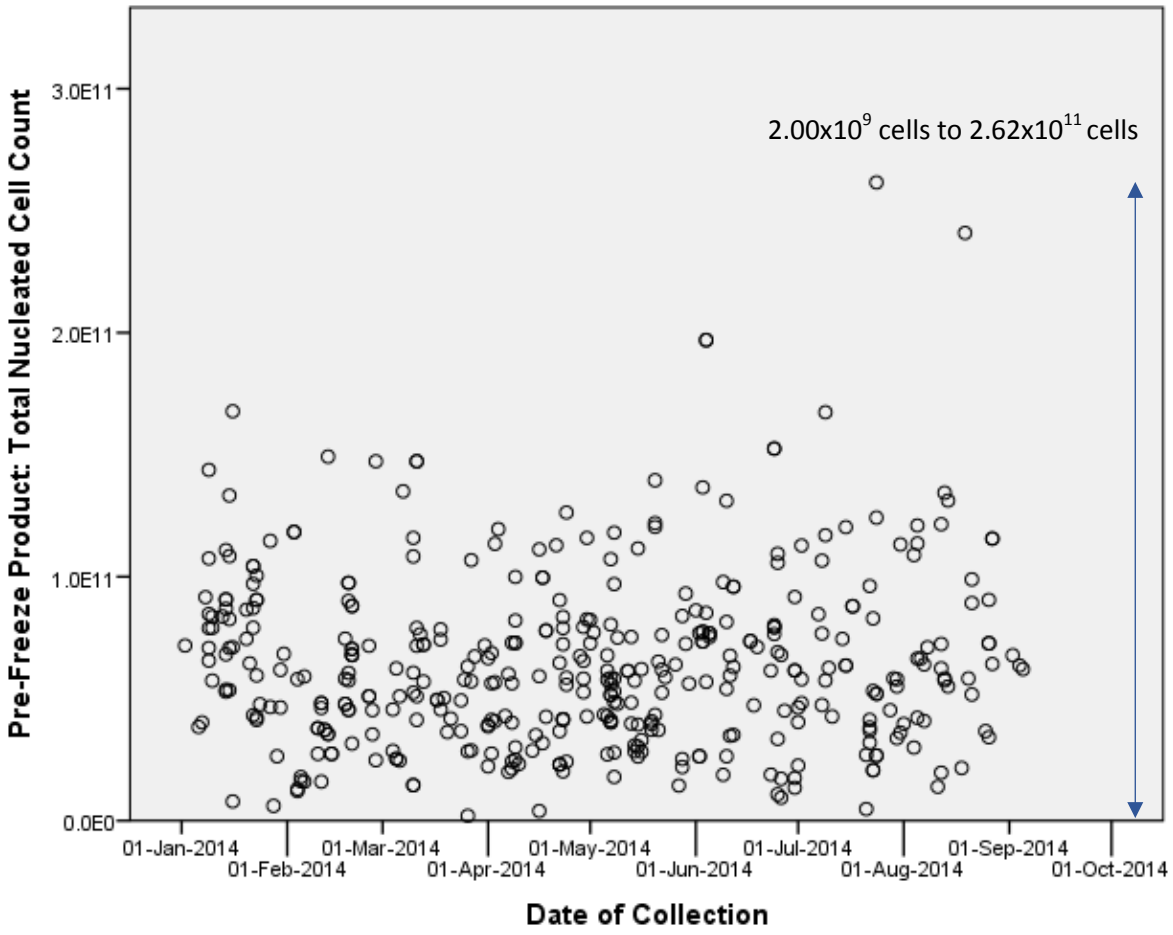


Figure 4.18: Total Nucleated Cell Count of the product (pre-freeze) against the date of collection

	Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
TNC (Product)	410	$6.45x10^{10}$	$5.94x10^{10}$	$2.00x10^{9}$	$2.62x10^{11}$	$2.60x10^{11}$	$3.57x10^{10}$	$1.28x10^{21}$

Table 4.8: Total Nucleated Cell Count of the product (pre-freeze) against the date of collection

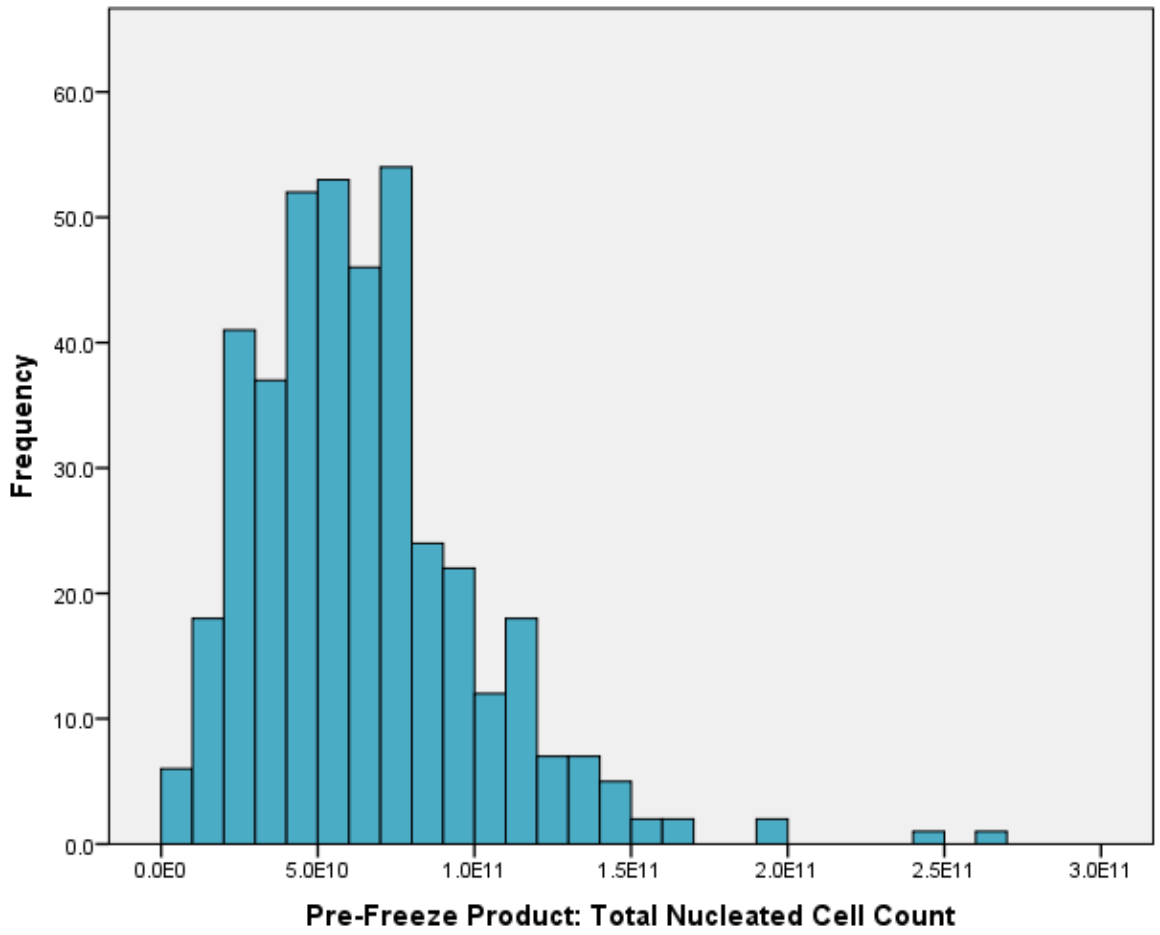


Figure 4.19: Histogram showing the spread and distribution of the total nucleated cell count of the product (pre-freeze)

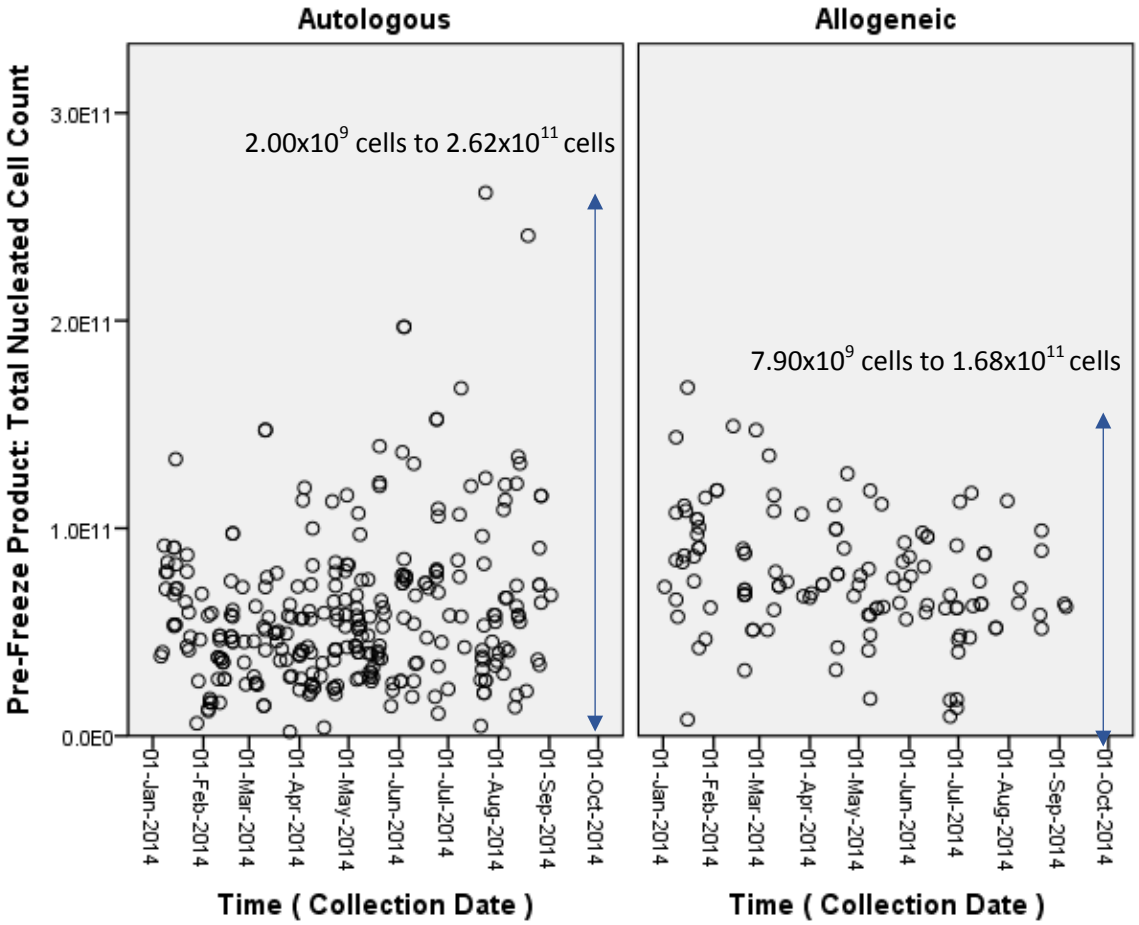


Figure 4.20: Total Nucleated Cell count of the product (pre-freeze) against the date of collection of autologous and allogeneic sourced material

	Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
Autologous	287	$5.93x10^{10}$	$5.27x10^{10}$	$2.00x10^{9}$	$2.62x10^{11}$	$2.60x10^{11}$	$3.70x10^{10}$	$1.37x10^{21}$
Allogeneic	123	$7.67x10^{10}$	$7.27x10^{10}$	$7.90x10^{9}$	$1.68x10^{11}$	$1.60x10^{11}$	$2.91x10^{10}$	$8.49x10^{21}$

Table 4.9: Total Nucleated Cell count of the product (pre-freeze) against the date of collection of autologous and allogeneic sourced material

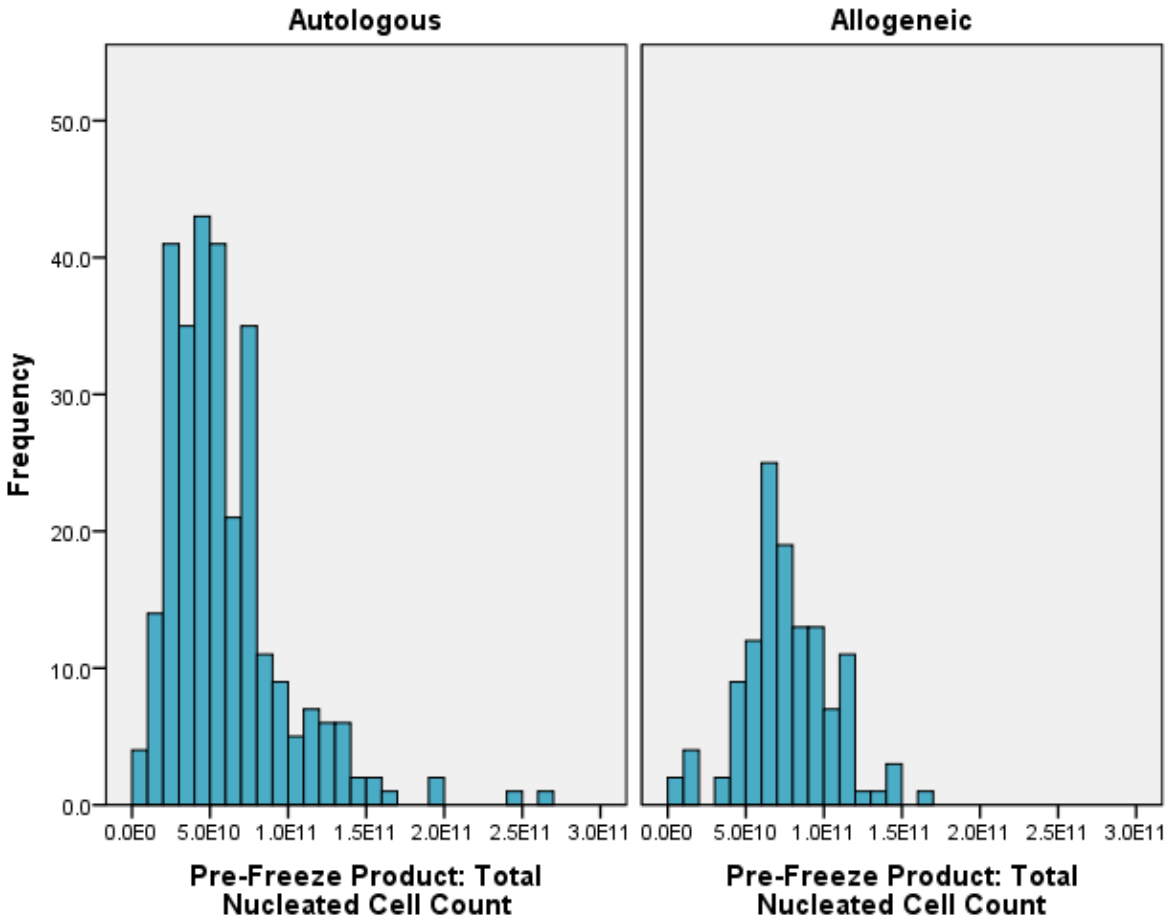


Figure 4.21: Histogram showing the spread and distribution of the total nucleated cell count of the product (pre-freeze) for autologous and allogeneic sourced material

4.5.4a Pre-Freeze Product: Total Nucleated Cell Count and Patient Weight

Patient (or donor) weight is likely to be one of the major contributors towards variation in cell numbers (and potentially quality, for bariatric [obese] patients).

Unless specified otherwise, the following analysis is based upon autologous sourced material, from a 'sick' population, where the patient's weight is the contributing factor (in allogeneic sourced material, the patient's weight would not be the correct factor, as it is the *donor's* material and therefore their weight that may have an effect).

Figure 4.22 is a scatter diagram of the pre-freeze total nucleated cell count (absolute cell numbers) against the corresponding patient's weight in kilograms. The mean and median patient weights have been indicated on the Figure, for reference. What is significant is that a given weight is not indicative of a given cell count. There are patients around the mean weight (82.3 kg) that could have between $2.8x10^{10}$ cells (for 85.0kg) and $2.0x10^{11}$ cells (for 81.20kg). A further point is that weight is not necessarily indicative of size or unhealthy size – a similar weight could represent a shorter or taller individual, a more athletic or a more obese individual - further in detail stratification of the relationship between weight and health may be crucial to understand the quality and quantity of variation in the starting material.

Figure 4.23 splits the data shown in the previous figure into 10 kg sized bins of weight, to examine the amount of variation and spread within a given weight bracket – it is therefore easier to note the aforementioned point about the lack of correlation between weight and total nucleated cell count. The greatest ranges appear to be within the lower weight category (20 kg -30 kg) and further for the categories of 80 kg -90 kg, 90kg -100kg and 110 kg -120 kg. the latter may be reflective of the wide range of body sizes and compositions that can be reflected by these weights, as mentioned before. This is also reflected by increased variance at these categories (**Table 4.10**).

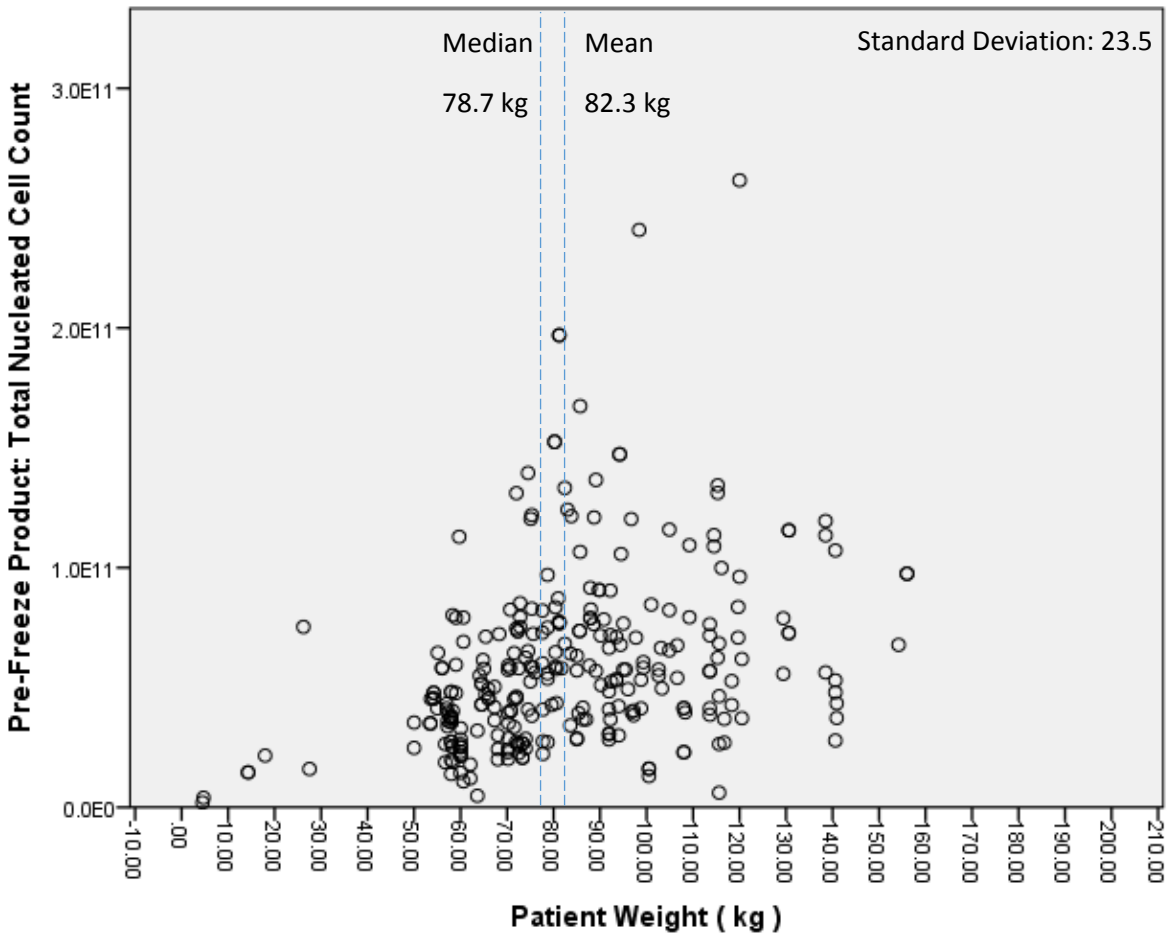


Figure 4.22: Pre-Freeze TNC Count against Patient Weight (kg)

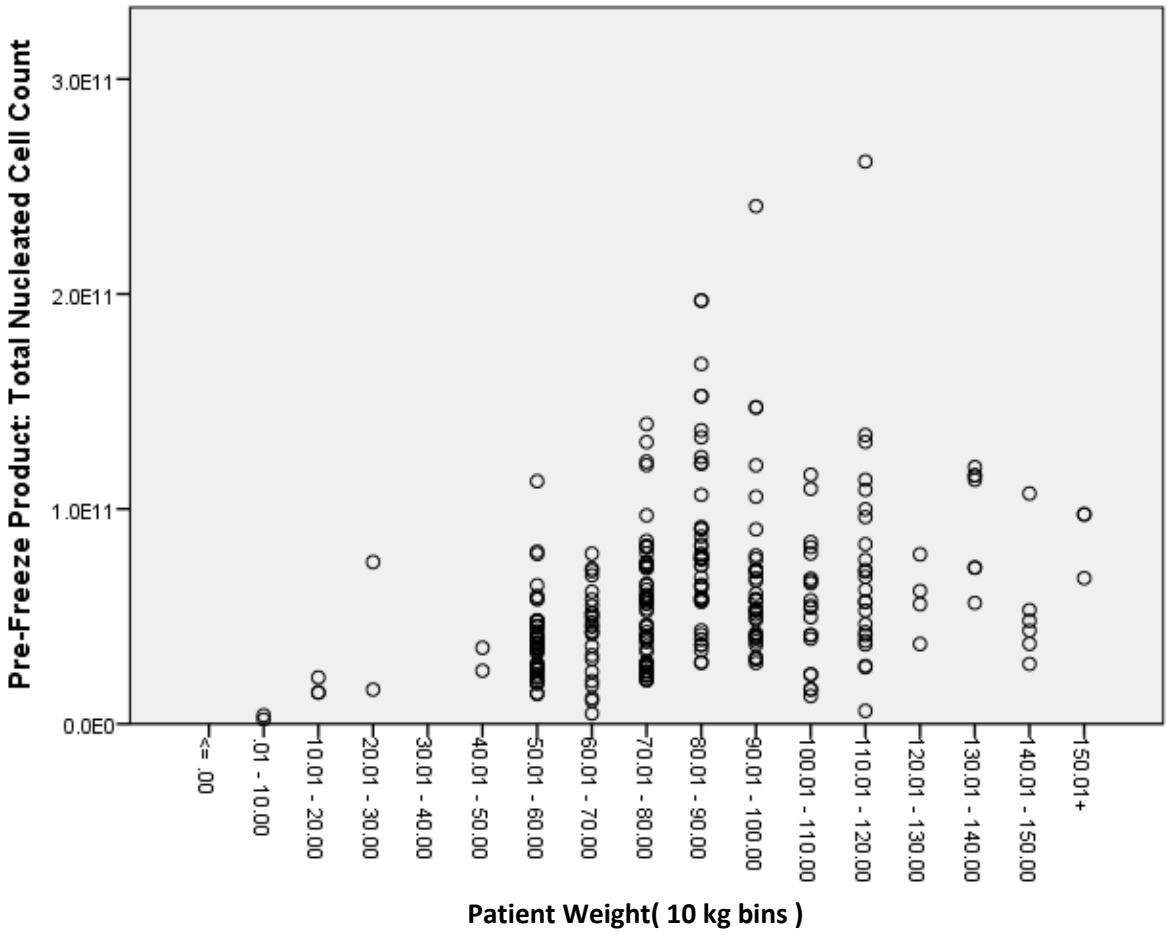


Figure 4.23: Pre-Freeze TNC Count against Patient Weight (kg) binned into 10 kg bins

		Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
Patient Weight (10kg bins)	<= .00	0	.	.	.	.	.	.	.
	.01 - 10.00	2	3.E+009	3.E+009	2.E+009	4.E+009	2.E+009	1.E+009	2.E+018
	10.01 - 20.00	3	2.E+010	1.E+010	1.E+010	2.E+010	7.E+009	4.E+009	2.E+019
	20.01 - 30.00	2	5.E+010	5.E+010	2.E+010	8.E+010	6.E+010	4.E+010	2.E+021
	30.01 - 40.00	0	.	.	.	.	.	.	.
	40.01 - 50.00	2	3.E+010	3.E+010	2.E+010	4.E+010	1.E+010	7.E+009	6.E+019
	50.01 - 60.00	48	4.E+010	4.E+010	1.E+010	1.E+011	1.E+011	2.E+010	3.E+020
	60.01 - 70.00	26	4.E+010	5.E+010	5.E+009	8.E+010	7.E+010	2.E+010	4.E+020
	70.01 - 80.00	60	5.E+010	5.E+010	2.E+010	1.E+011	1.E+011	3.E+010	8.E+020
	80.01 - 90.00	44	8.E+010	8.E+010	3.E+010	2.E+011	2.E+011	4.E+010	2.E+021
	90.01 - 100.00	36	7.E+010	6.E+010	3.E+010	2.E+011	2.E+011	4.E+010	2.E+021
	100.01 - 110.00	20	6.E+010	5.E+010	1.E+010	1.E+011	1.E+011	3.E+010	9.E+020
	110.01 - 120.00	24	8.E+010	7.E+010	6.E+009	3.E+011	3.E+011	5.E+010	3.E+021
	120.01 - 130.00	4	6.E+010	6.E+010	4.E+010	8.E+010	4.E+010	2.E+010	3.E+020
	130.01 - 140.00	7	1.E+011	1.E+011	6.E+010	1.E+011	6.E+010	3.E+010	7.E+020
	140.01 - 150.00	6	5.E+010	5.E+010	3.E+010	1.E+011	8.E+010	3.E+010	8.E+020
	150.01+	3	9.E+010	1.E+011	7.E+010	1.E+011	3.E+010	2.E+010	3.E+020

Table 4.10: Pre-Freeze TNC Count against Patient Weight (kg) binned into 10 kg bins

4.5.4b Pre-Freeze Product: Total Nucleated Cell Count and Patient Age

Between paediatric and geriatric patients, between the changes due to menopause and ageing, and the diseases and conditions that can be attributed to biological age, patient age is also likely to be a key contributor towards variation.

Unless specified otherwise, the following analysis is based upon autologous sourced material, from a 'sick' population, where the patient's age is the contributing factor. Paediatric has been defined as 18 years old or younger and geriatric has been defined as 65 years old or older. This dataset is weighted towards an older population with the majority of the dataset existing above 40 years old.

Figure 4.24 shows the absolute TNC count of a given pre-freeze product against the corresponding patient's age in years. This has been further split into three categories: paediatric, adult and geriatric, as indicated on the Figure. **Table 4.11** gives further details about the spread and distribution of these categories, with geriatric having a wider, core spread but smaller range (3.46x 10^{10} cells and 1.91 x 10^{11} cells) than adult patients' tighter spread but larger range (2.53 x 10^{11} cells and 2.57 x 10^{11} cells). Variance is similar between adult and geriatric populations (1.25 x 10^{21} and 1.20 x 10^{21}), with paediatric patients' being the most variable by any of the three metrics (2.11 x 10^{21}). Furthermore, assuming these are discrete sections of the population, by independent samples median and Kruskal-Wallis' test, they have significantly different medians (4.65 x 10^{10}, 6.59 x 10^{10}, 6.29x10^{10} for paediatric, adult and geriatric respectively) and distributions. Whilst this conclusion is to be treated with care, given the other confounding factors such as weight, this may be indicative that certain age groups may be more suitable for donation, from a manufacturing starting material perspective, which may be useful to allogeneic therapy, where donors can be selected, but indicates what variation will have to be managed for autologous therapy.

To allow for visual stratification and examination, the patient ages within **Figure 4.24** have been stratified into 5 year bins and represented as a further scatter diagram in **Figure 4.25.** The three age categories have been indicated in blue. There appear to be peaks in variance between the ages of 6-

10, 11-15, 26-30, 41-45 56-60, 61-65 and 66-70 (**Table 4.12**), potentially indicative of comparatively tumultuous biological ages that are affecting the corresponding about of TNCs collected and processed.

The aforementioned category, weight, may be a key contributor towards patient derived variation. **Figure 4.26** is a scatter diagram of binned patient age against the total nucleated cell count per kilogram of patient weight, to accommodate the variation that may be encountered as a factor of variant bodyweight. Age categories have also been added in blue.

The previous peaks in variance are now no longer apparent, with fairly consistent variance across all age groups, apart from notable decreases between the ages of 26 and 30 (**Table 4.13**). This may imply that weight introduces a significant amount of variation, and care must be taken to use the correct units of measurement when comparing, or carrying out process control measurements. The danger is that using cells / kg is a measure of the state of the donating patient, rather than the state of the product itself, and absolute cells, or cells / litre (with volume being representative of the transfer bag itself) may be more indicative of the variation within the product itself.

Given that the pre-freeze product at the CMCF was 100 ml in size, uniformly, absolute cell numbers in this case are representative of cells within a given product, of a fixed volume.

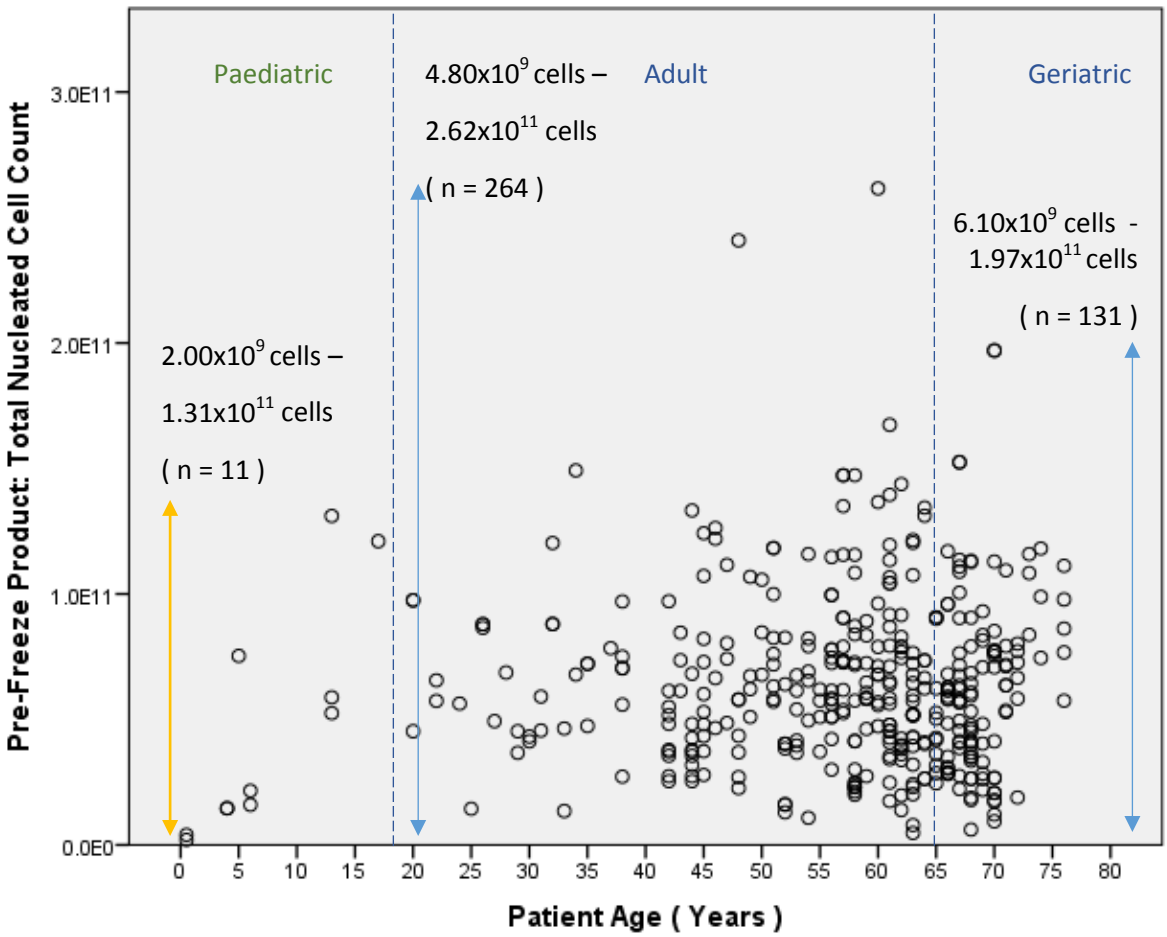


Figure 4.24: Pre-Freeze TNC Count against Patient Age (years)

Paediatric has been define as 18 years or younger, and has been indicated in orange on the diagram.

	Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
1 – 18	11	$4.65x10^{10}$	$2.16x10^{10}$	$2.00x10^{9}$	$1.31x10^{11}$	$1.29x10^{11}$	$4.59x10^{10}$	$2.11x10^{21}$
19 – 64	264	$6.59x10^{10}$	$6.04x10^{10}$	$4.80x10^{9}$	$2.62x10^{11}$	$2.57x10^{11}$	$2.53x10^{10}$	$1.25x10^{21}$
65+	131	$6.29x10^{19}$	$5.95x10^{10}$	$6.10x10^{9}$	$1.97x10^{11}$	$1.91x10^{11}$	$3.46x10^{10}$	$1.20x10^{21}$

Table 4.11: Pre-Freeze TNC Count against Patient Age (years), stratified into paediatric (≤18), adult (≥19,≤64) and geriatric (≥65).

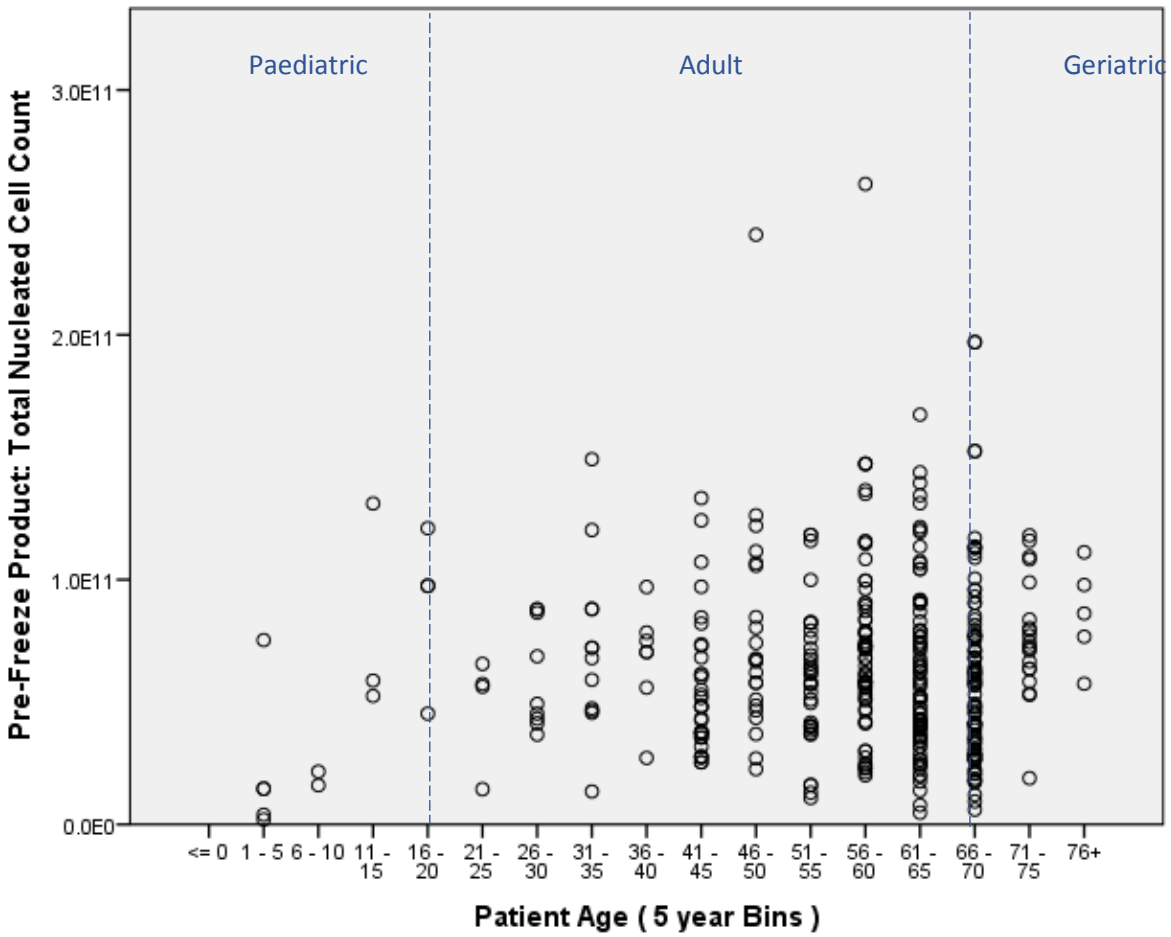


Figure 4.25: Pre-Freeze TNC Count against Patient Age (years) binned into 5 year segments

Paediatric, adult and geriatric populations have been indicated in blue

		Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
PatientAge (Binned)	<= 0	0	.	.	.	.	.	.	.
	1 - 5	5	2.E+010	1.E+010	2.E+009	8.E+010	7.E+010	3.E+010	9.E+020
	6 - 10	2	2.E+010	2.E+010	2.E+010	2.E+010	6.E+009	4.E+009	2.E+019
	11 - 15	3	8.E+010	6.E+010	5.E+010	1.E+011	8.E+010	4.E+010	2.E+021
	16 - 20	4	9.E+010	1.E+011	5.E+010	1.E+011	8.E+010	3.E+010	1.E+021
	21 - 25	2	4.E+010	4.E+010	1.E+010	6.E+010	4.E+010	3.E+010	9.E+020
	26 - 30	5	4.E+010	4.E+010	4.E+010	5.E+010	1.E+010	5.E+009	2.E+019
	31 - 35	3	8.E+010	6.E+010	5.E+010	1.E+011	7.E+010	4.E+010	2.E+021
	36 - 40	5	7.E+010	8.E+010	3.E+010	1.E+011	7.E+010	3.E+010	7.E+020
	41 - 45	27	6.E+010	5.E+010	3.E+010	1.E+011	1.E+011	3.E+010	9.E+020
	46 - 50	11	8.E+010	6.E+010	2.E+010	2.E+011	2.E+011	6.E+010	4.E+021
	51 - 55	31	5.E+010	6.E+010	1.E+010	1.E+011	1.E+011	2.E+010	6.E+020
	56 - 60	44	7.E+010	6.E+010	2.E+010	3.E+011	2.E+011	4.E+010	2.E+021
	61 - 65	65	6.E+010	5.E+010	5.E+009	2.E+011	2.E+011	3.E+010	1.E+021
	66 - 70	68	6.E+010	5.E+010	6.E+009	2.E+011	2.E+011	4.E+010	2.E+021
	71 - 75	7	7.E+010	7.E+010	2.E+010	1.E+011	9.E+010	3.E+010	8.E+020
	76+	2	7.E+010	7.E+010	6.E+010	8.E+010	2.E+010	1.E+010	2.E+020

Table 4.12: Pre-Freeze TNC Count against Patient Age (years)

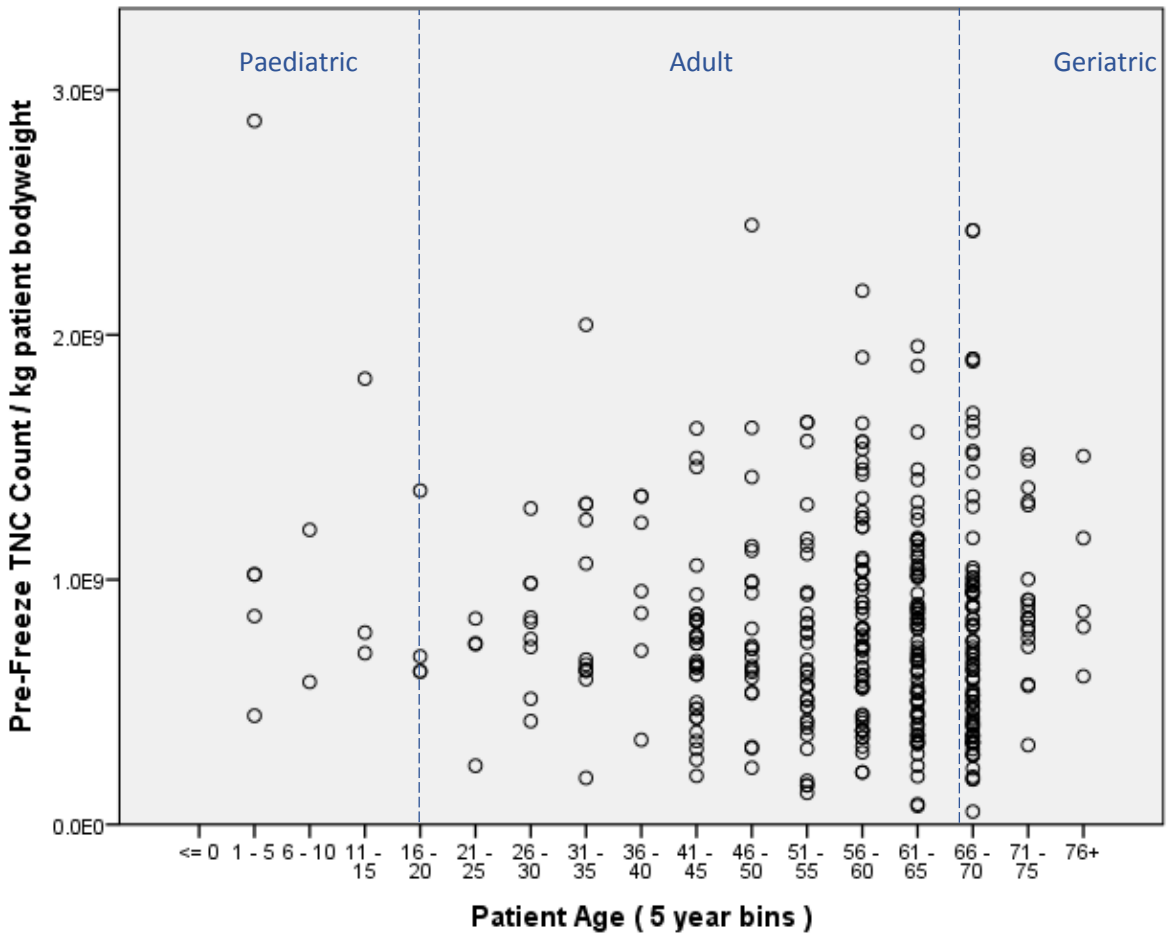


Figure 4.26: Pre-Freeze TNC Count / kg Patient bodyweight against Patient Age (years) binned into 5 year segments

		Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
PatientAge (Binned)	<= 0	0	.	.	.	.	.	.	.
	1 - 5	5	1.E+009	1.E+009	4.E+008	3.E+009	2.E+009	9.E+008	9.E+017
	6 - 10	2	9.E+008	9.E+008	6.E+008	1.E+009	6.E+008	4.E+008	2.E+017
	11 - 15	3	1.E+009	8.E+008	7.E+008	2.E+009	1.E+009	6.E+008	4.E+017
	16 - 20	4	8.E+008	7.E+008	6.E+008	1.E+009	7.E+008	4.E+008	1.E+017
	21 - 25	4	6.E+008	7.E+008	2.E+008	8.E+008	6.E+008	3.E+008	7.E+016
	26 - 30	9	8.E+008	8.E+008	4.E+008	1.E+009	9.E+008	3.E+008	7.E+016
	31 - 35	12	9.E+008	7.E+008	2.E+008	2.E+009	2.E+009	5.E+008	2.E+017
	36 - 40	7	1.E+009	1.E+009	3.E+008	1.E+009	1.E+009	4.E+008	1.E+017
	41 - 45	33	7.E+008	7.E+008	2.E+008	2.E+009	1.E+009	3.E+008	1.E+017
	46 - 50	22	9.E+008	7.E+008	2.E+008	2.E+009	2.E+009	5.E+008	2.E+017
	51 - 55	35	7.E+008	6.E+008	1.E+008	2.E+009	2.E+009	4.E+008	2.E+017
	56 - 60	67	9.E+008	8.E+008	2.E+008	2.E+009	2.E+009	4.E+008	2.E+017
	61 - 65	84	8.E+008	7.E+008	8.E+007	2.E+009	2.E+009	4.E+008	1.E+017
	66 - 70	94	8.E+008	7.E+008	5.E+007	2.E+009	2.E+009	5.E+008	2.E+017
	71 - 75	20	9.E+008	9.E+008	3.E+008	2.E+009	1.E+009	3.E+008	1.E+017
	76+	5	1.E+009	9.E+008	6.E+008	2.E+009	9.E+008	4.E+008	1.E+017

Table 4.13: Pre-Freeze TNC Count against Patient Age (years) binned into 5 year segments

4.5.4c Pre-Freeze Product: Total Nucleated Cell Count and Patient Gender

Both genders in human exhibit specific traits, and these traits may be a factor in the extent of variation as a result from using male versus female donors.

Unless specified otherwise, the following analysis is based upon autologous sourced material, from a 'sick' population, where the patient's gender is the contributing factor.

Figure 4.27 is a scatter diagram of pre-freeze total nucleated cell count against the corresponding patient's gender. Male patients appear to have considerably higher variation in terms of range compared to female patients (2.56×10^{11} cells in males compared to 1.20×10^{11} cells in females), so this data was represented further in **Figure 4.28** as a box and whisker plot.

This shows a much higher variance (1.81×10^{21} in males compared to 6.02×10^{20} in females) and a slightly higher standard deviation and range in male patients compared to female patients (a standard deviation of 4.25×10^{10} cells in males compared to 2.45×10^{10} cells in females)(**Table 4.14)**. In fact, independent sample median and Kruskal-Wallis tests consider male and female subsets' median and distribution to be significantly different ($p > 0.05$, $p > 0.05$). From this, it could be surmised that, for an as yet unknown reason, that female patients exhibit much less variation than male patients exhibit.

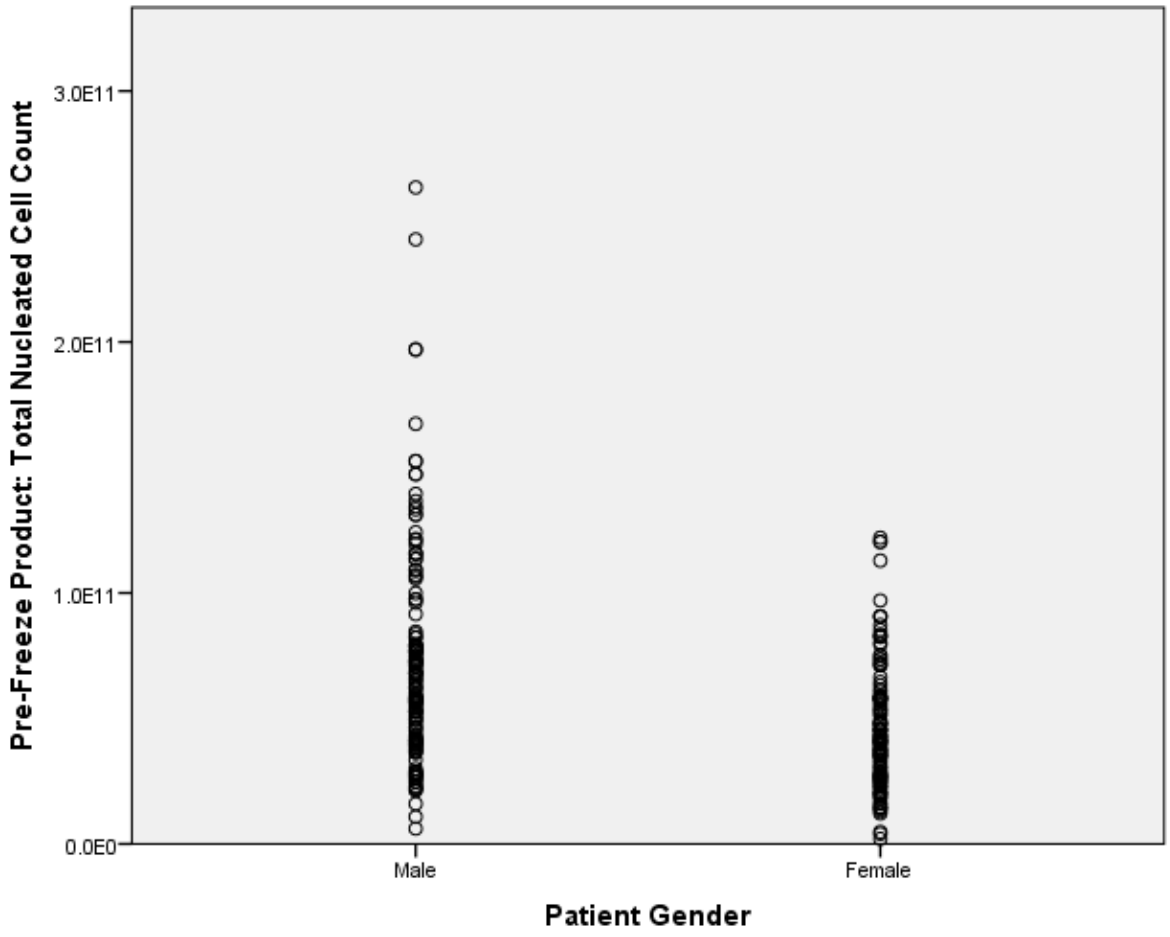


Figure 4.27: Pre-freeze TNC Count compared with Patient Gender

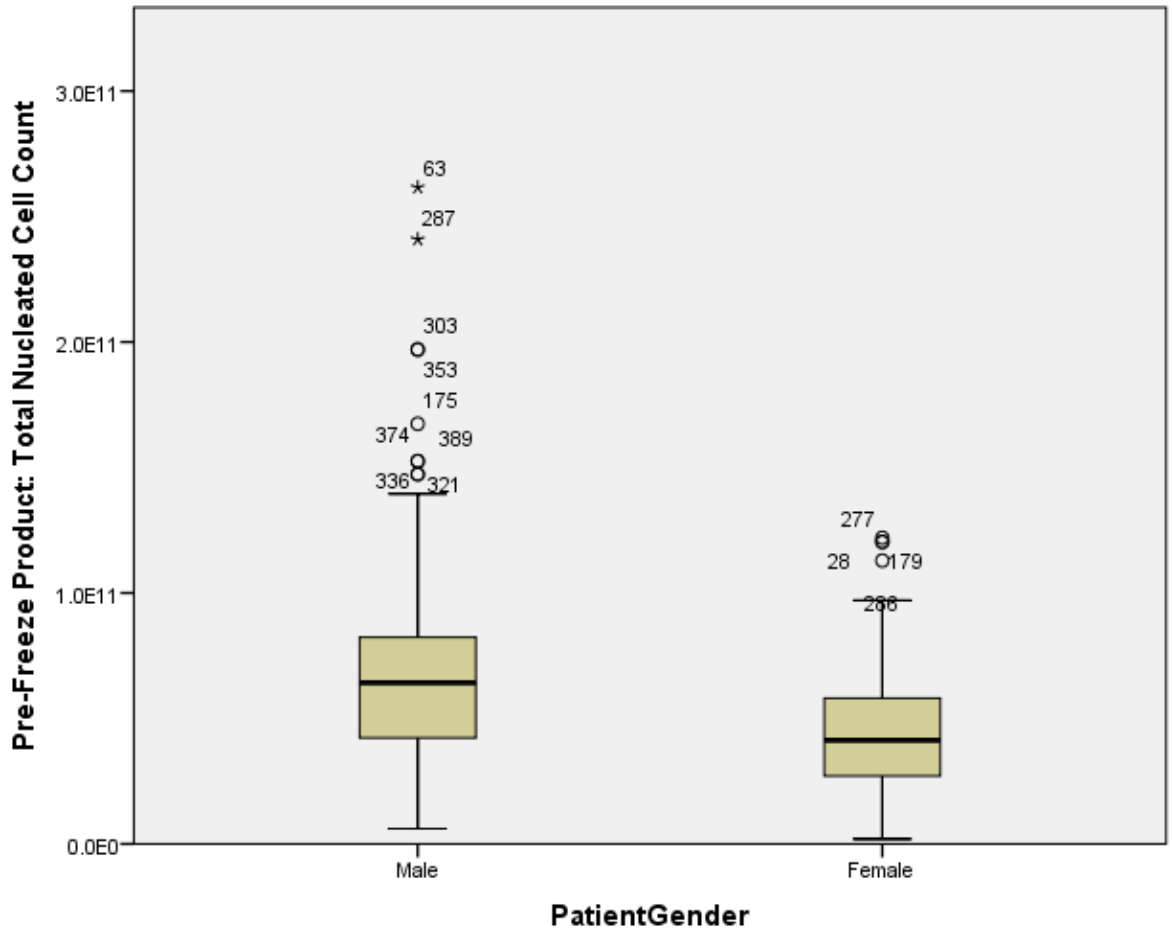


Figure 4.28: Pre-freeze TNC Count compared with Patient Gender (box and whisker plot)

	Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
Male	145	$7.22x10^{10}$	$6.42x10^{10}$	$6.10x10^{9}$	$2.62x10^{11}$	$2.56x10^{11}$	$4.25x10^{10}$	$1.81x10^{21}$
Female	135	$4.56x10^{10}$	$4.13x10^{10}$	$2.00x10^{9}$	$1.22x10^{11}$	$1.20x10^{11}$	$2.45x10^{10}$	$6.02x10^{20}$

Table 4.14: Pre-freeze TNC Count compared with Patient Gender

4.5.5 Pre-Freeze Product: CD34+ Cell Count

Pre-freeze CD34+ cell count refers to the number of cells within the final product, prior to cryopreservation and therapeutic application, that have been detected with this specific cell surface marker. This marker is indicative of HPCs, and is used as the specific measurement of cell identity and efficacy for HSCTs at the CMCF. It has been measured here in absolute cell numbers, which is normally indicative of the number of cells within 100 ml (the target product volume). It represents the specific measurement for therapeutic identity and is therefore the most important characteristic to examine with respect to biological variation.

Figure 4.29 illustrates the spread of CD34+ cells in the product. This cell population was not measured in the initial starting material, prior to the process, so a comparison cannot be drawn between CD34+ cell yields. The range of CD34+ cells for all products produced between January and October of 2014 was between $4.80x10^6$ cells and $4.14x10^9$ cells (**Table 4.15**). Given that CD34+ cells are a subset of TNCs, it is unsurprising that the range, standard deviation and variance are considerably less for CD34+ cells.

The distribution of CD34+ cells is considerably more skewed than TNCs (**Figure 4.30**). Visual inspection alone is sufficient to identify the significant skew representative of a non-normal distribution, which was confirmed by Shapiro-Wilk's test ($p > 0.05$). The skewness is twice that found in TNC (1.333 to 2.876) and three times that found in TNC (3.700 to 11.160), representing a sharp peak and a longer tail. This illustrates the earlier regulatory and therapeutic challenge of identifying the differences (if any) in therapeutic potential between those products that reside within the main peak, and those that reside in the longer tail – are these representative of different qualities or efficacies.

Figure 4.31 splits pre-freeze absolute CD34+ cell count into autologous and allogeneic source. The range of cell count for autologous is between $4.80x10^6$ cells and $4.14x10^9$ cells, and the range of cell count for allogeneic is between $4.00x10^7$ cells and $1.82x10^9$ cells. By visual inspection autologous

sourced material appears to have much higher variation, with a higher spread than allogeneic (represented in **Table 4.16** with a higher range, standard deviation and variance). This may be due to the 'sick' state of the autologous patients, compared to the relatively healthy allogeneic donors. An independent sample median and Mann-Whitney test demonstrate that these two populations' median and distribution are significantly different from one another ($p > 0.05$*, $p > 0.05$*).

Distributions of CD34+ cells are non-normally distributed regardless of source (**Figure 4.32**). Pre-Freeze TNC cell count was not normally distributed for either autologous or allogeneic sourced material as assessed by Shapiro-Wilk's test ($p > .05$, $p > 0.098$ respectively). Kurtosis and skewness for autologous sourced material were 9.733and 2.881 respectively and 0.957 and 0.873 for allogeneic sourced material respectively.

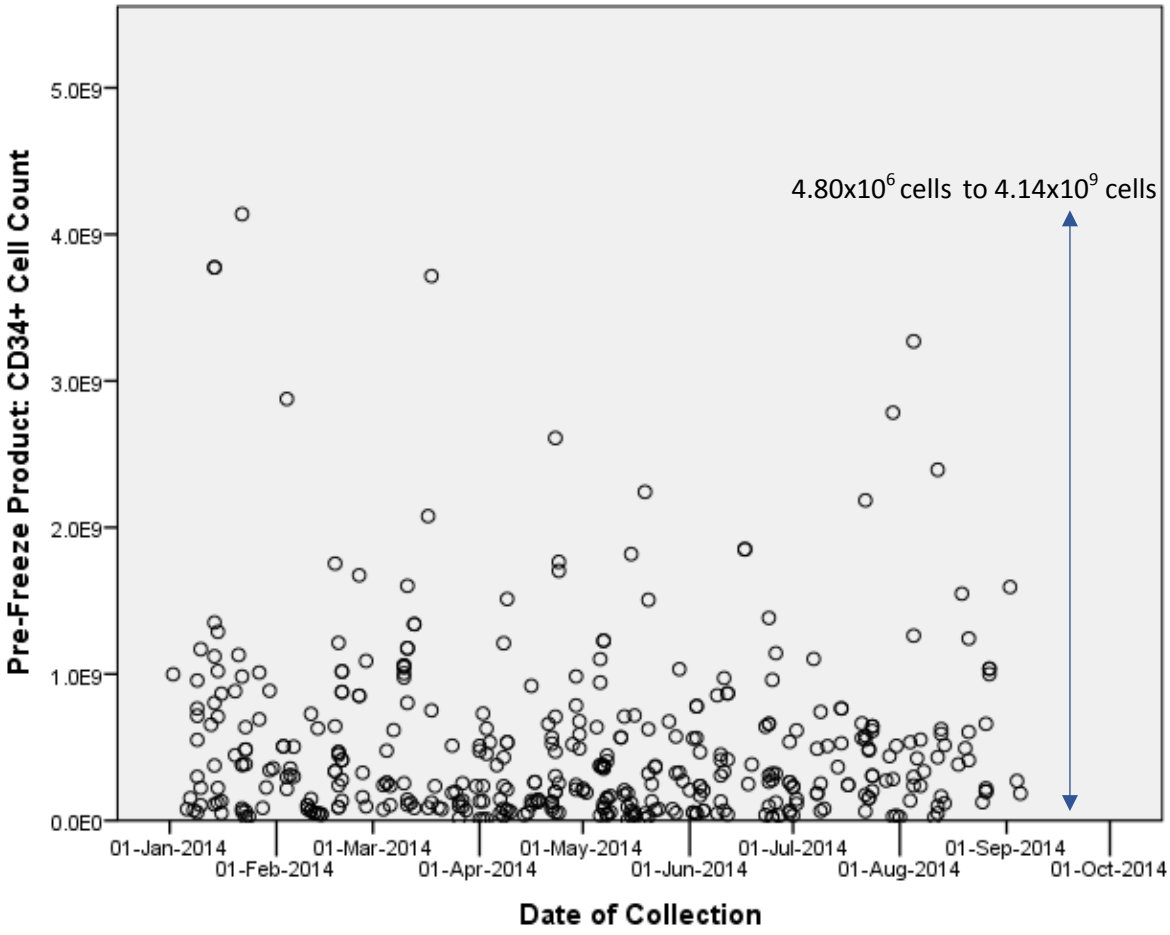


Figure 4.29: CD34+ Cell Count of the product (pre-freeze) against the date of collection

	Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
CD34 (Product)	410	$4.99x10^8$	$3.06x10^8$	$4.80x10^6$	$4.14x10^9$	$4.12x10^9$	$5.97x10^8$	$3.56x10^{17}$

Table 4.15: CD34+ Cell Count of the product (pre-freeze) against the date of collection

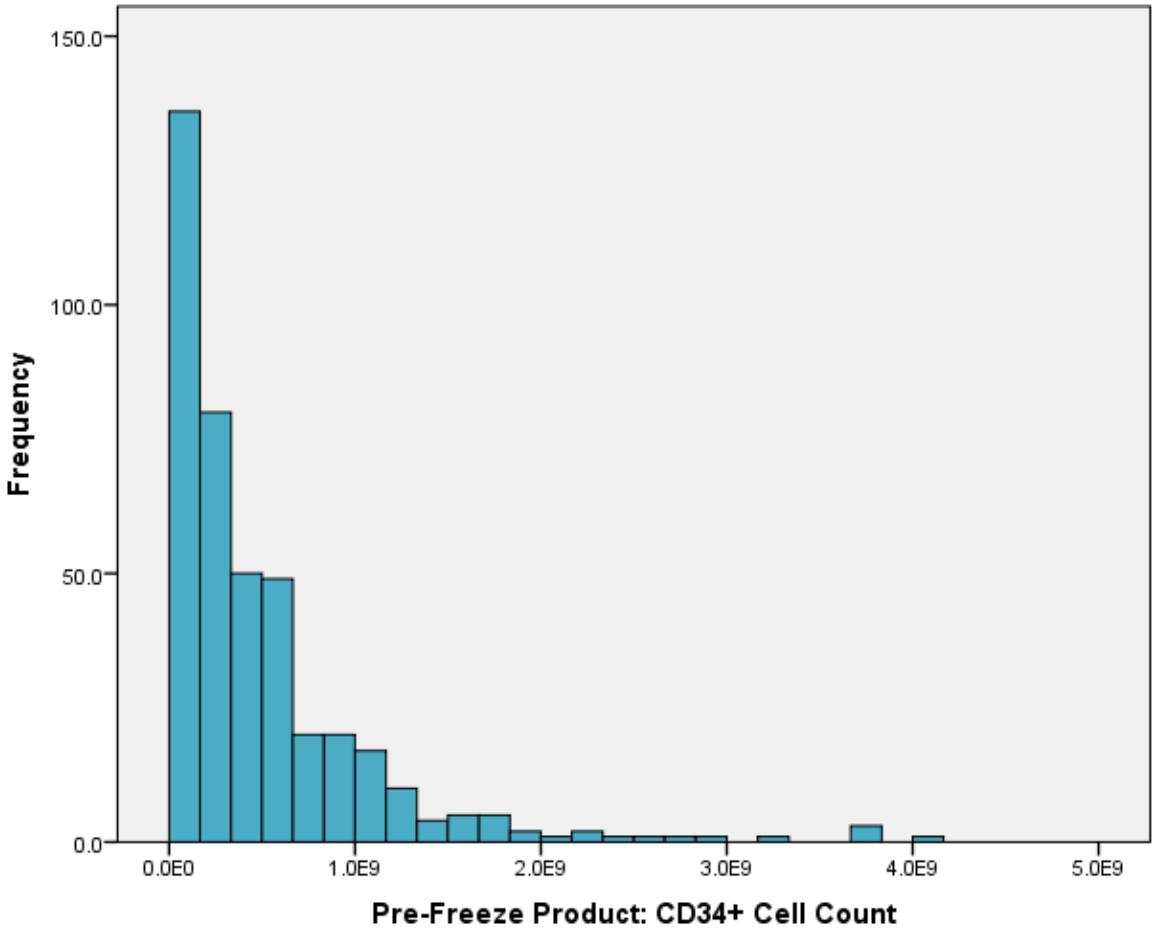


Figure 4.30: Histogram showing the spread and distribution of the CD34+ cell count of the product (pre-freeze)

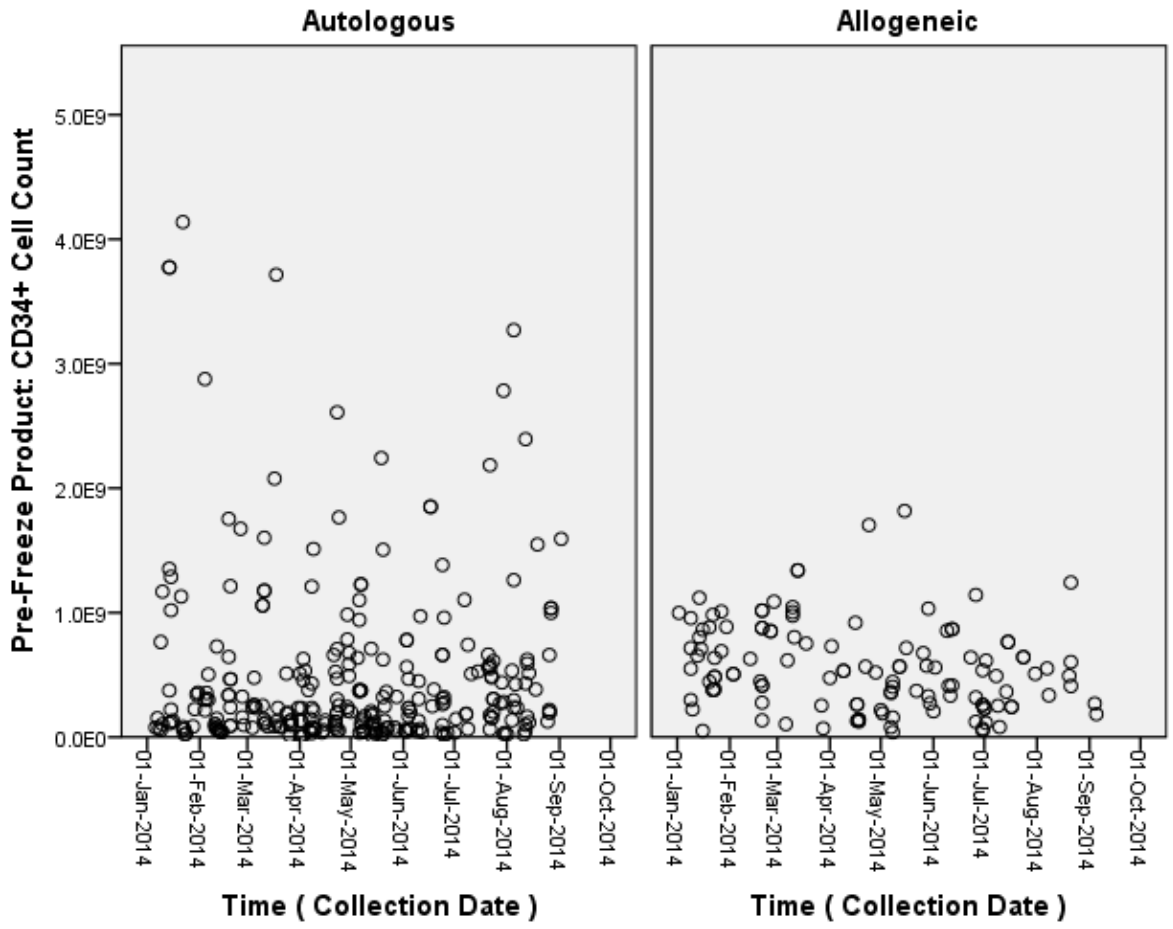


Figure 4.31: CD34+ Cell Count of the product (pre-freeze) against the date of collection by autologous and allogeneic source material

	Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
Autologous	287	$4.75x10^{8}$	$2.34x10^{8}$	$4.80x10^{6}$	$4.14x10^{9}$	$4.13x10^{9}$	$6.74x10^{8}$	$4.55x10^{17}$
Allogeneic	123	$5.55x10^{8}$	$5.06x10^{8}$	$4.00x10^{7}$	$1.82x10^{9}$	$1.78x10^{9}$	$3.50x10^{8}$	$1.23x10^{17}$

Table 4.16: CD34+ Cell Count of the product (pre-freeze) against the date of collection, split into autologous and allogeneic sourced material

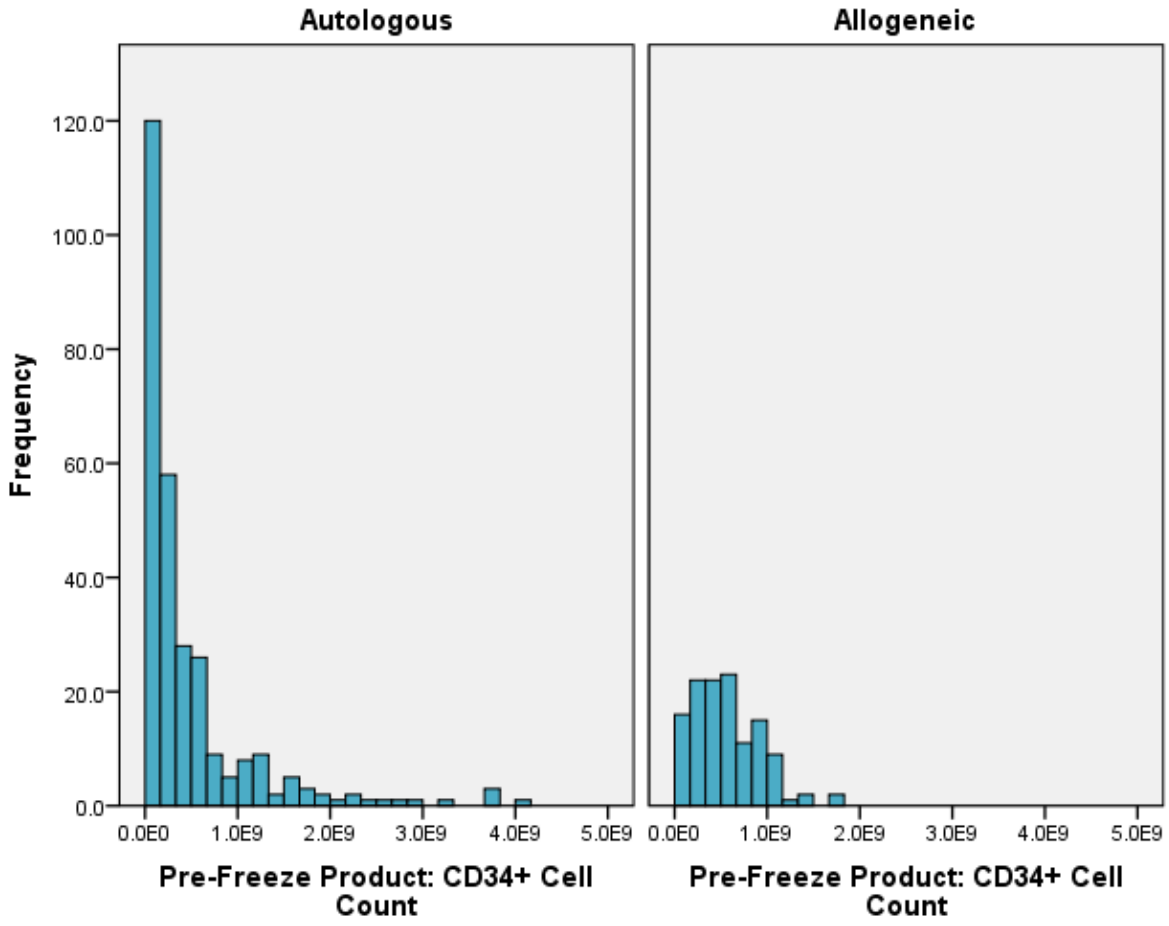


Figure 4.32: Histogram showing the spread and distribution of the CD34+ cell count of the product (pre-freeze) for autologous and allogeneic sourced material

4.5.5a Pre-Freeze Product: CD34+ Cell Count and Patient Weight

Patient (or donor) weight is likely to be one of the major contributors towards variation in cell numbers (and potentially quality, for bariatric patients). This is especially important for obese patients, whose circulatory system may not be sufficiently adapted to their size and as a result may have less circulating stem cells than an equivalent healthier individual.

Unless specified otherwise, the following analysis is based upon autologous sourced material, from a 'sick' population, where the patient's weight is the contributing factor (in allogeneic sourced material, the patient's weight would not be the correct factor, as it is the *donor's* material and therefore their weight that may have an effect).

Figure 4.33 is a scatter diagram of the pre-freeze absolute CD34+ cell count against the corresponding patient's weight in kilograms. Similarly to **Figure 4.22**, weight is not correlated with CD34+ cell count, and a patient with the mean weight (82.3 kg) may produce between zero and $4.0x10^9$ CD34+ cells.

Figure 4.34 splits the weight categories in the previous figure into 10 kg sized bins, to examine the amount of variation and spread within a given weight bracket. By visual inspection there is considerably more variation in the amount of produced CD34+ cells within the central weight categories, compared to the extremes, likely due to the myriad of body shapes and health categories these central measurements could encompass, compared to a 10 kg paediatric patient and a 150 kg+ obese patient. This could explain the particularly high standard deviations and variances attributed to the 80.01 to 90.00 kg category (1.00×10^9 cells and $9.00x10^{17}$ cells) and the 120.01 to 130.00 category (1.00×10^9 cells and 1.00×10^{18} cells) (**Table 4.17**).

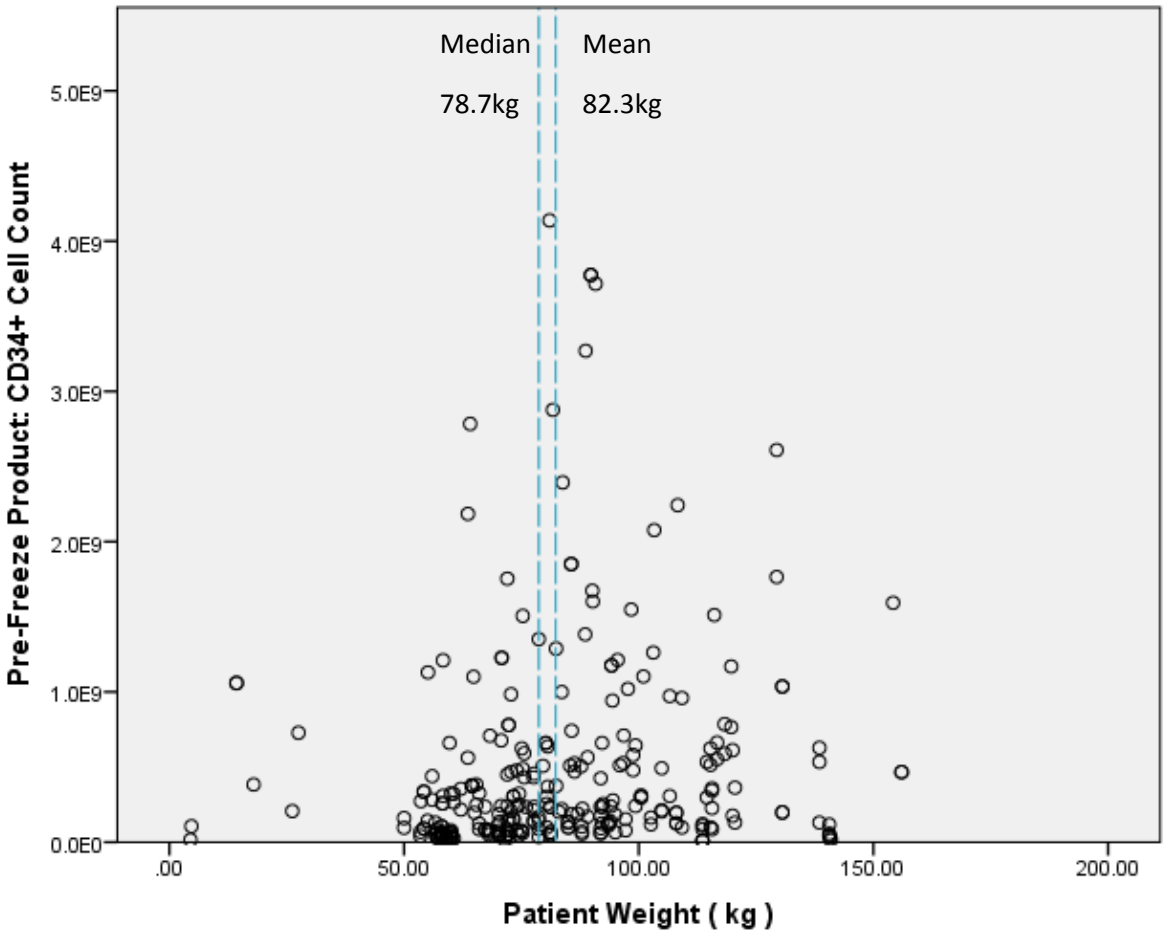


Figure 4.33: Pre-Freeze CD34+ Cell Count against Patient Weight (kg)

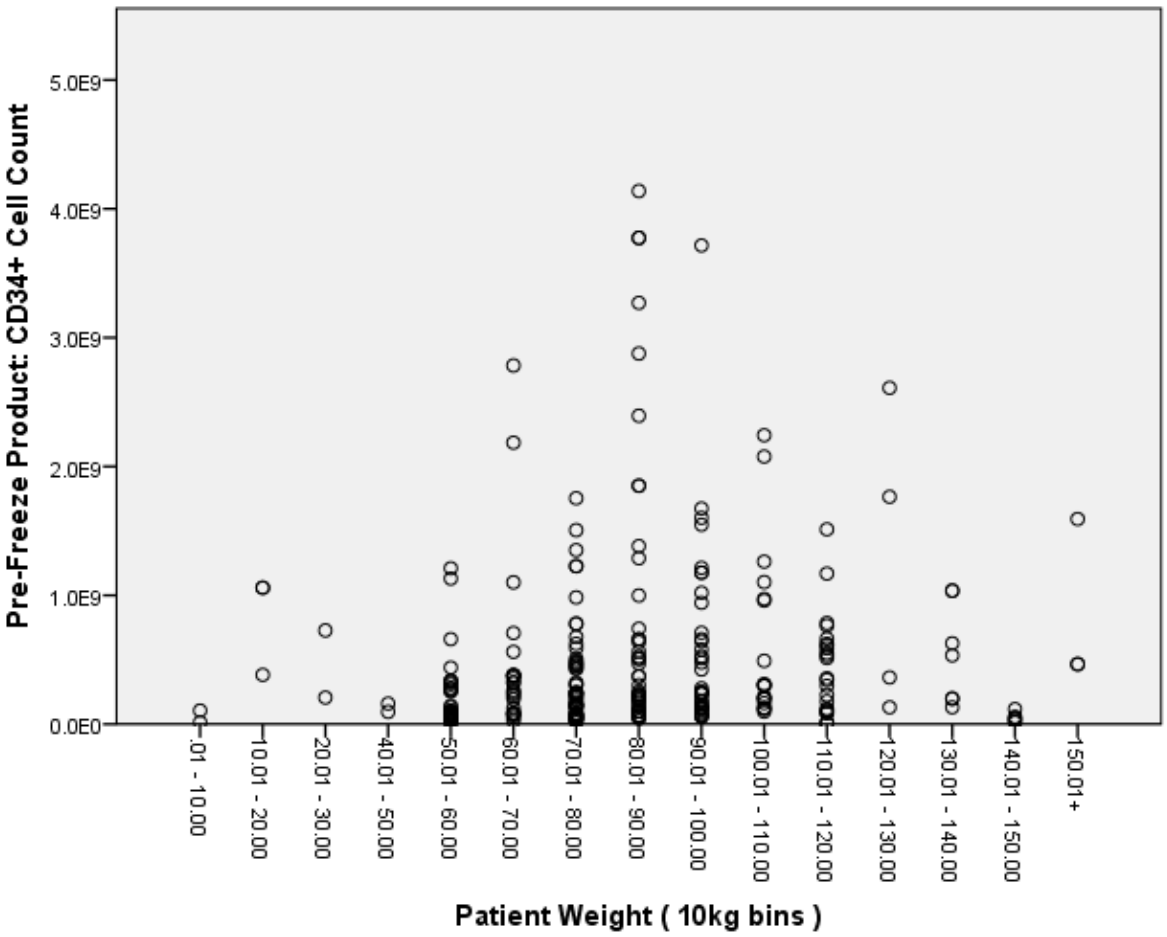


Figure 4.34: Pre-Freeze CD34+ Cell Count against Patient Weight (10 kg bins)

		Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
Patient Weight (10kg bins)	<= .00	0	.	.	.	.	.	.	.
	.01 - 10.00	2	6E+007	6E+007	2E+007	1E+008	9E+007	6E+007	4E+015
	10.01 - 20.00	3	8E+008	1E+009	4E+008	1E+009	7E+008	4E+008	2E+017
	20.01 - 30.00	2	5E+008	5E+008	2E+008	7E+008	5E+008	4E+008	1E+017
	30.01 - 40.00	0	.	.	.	.	.	.	.
	40.01 - 50.00	3	2E+008	2E+008	1E+008	4E+008	3E+008	1E+008	2E+016
	50.01 - 60.00	60	2E+008	9E+007	5E+006	1E+009	1E+009	3E+008	9E+016
	60.01 - 70.00	52	6E+008	4E+008	2E+007	3E+009	3E+009	5E+008	3E+017
	70.01 - 80.00	94	4E+008	2E+008	2E+007	2E+009	2E+009	4E+008	1E+017
	80.01 - 90.00	65	7E+008	5E+008	4E+007	4E+009	4E+009	1E+009	9E+017
	90.01 - 100.00	48	5E+008	3E+008	5E+007	4E+009	4E+009	6E+008	4E+017
	100.01 - 110.00	27	6E+008	3E+008	7E+007	2E+009	2E+009	6E+008	3E+017
	110.01 - 120.00	30	5E+008	5E+008	9E+006	2E+009	2E+009	5E+008	2E+017
	120.01 - 130.00	5	1E+009	9E+008	1E+008	3E+009	2E+009	1E+009	1E+018
	130.01 - 140.00	10	6E+008	5E+008	1E+008	1E+009	9E+008	4E+008	1E+017
	140.01 - 150.00	6	5E+007	4E+007	1E+007	1E+008	1E+008	4E+007	1E+015
	150.01+	3	8E+008	5E+008	5E+008	2E+009	1E+009	7E+008	4E+017

Table 4.17: Pre-Freeze CD34+ Cell Count against Patient Weight (10 kg bins)

4.5.5b Pre-Freeze Product: CD34+ Cell Count and Patient Age

Between paediatric and geriatric patients, between the changes due to menopause and ageing, and the diseases and conditions that can be attributed to biological age, patient age is also likely to be a key contributor towards variation.

Unless specified otherwise, the following analysis is based upon autologous sourced material, from a 'sick' population, where the patient's age is the contributing factor.

Figure 4.35 shows the absolute CD34+ cell count of a given pre-freeze product against the corresponding patient's age in years. This has been further split into three categories: paediatric, adult and geriatric, as indicated on the Figure. **Table 4.18** gives further details as to the spread and distribution within these categories. Assuming that these three age categories are discrete independent sections of the population, by independent Kruskal-Wallis' test, they have significantly different distributions ($p > 0.05$).

Figure 4.36 is a scatter diagram that stratifies patient age in five year bins against pre-freeze absolute CD34+ cell count. Unlike TNCs, variance in CD34+ is highest in only two age categories: 16 to 20 year olds (2.04×10^{18} cells) and 36 to 40 year olds (1.74×10^{18} cells), implying that it is within these age groups that the greatest amount of variation in CD34+ cell numbers is found. Given that these categories are based upon n = 5 and n = 4, this conclusion is not as statistically robust (**Table 4.19**). For a statistically meaningful examination of age groups more data is required in the younger patient categories. Furthermore, if biological state changes were more fixed, attention could be focused on these areas to determine whether these state changes – puberty or menopause for example – affect the variation in CD34+ cell count. These metrics are subjective to the individual so this may be less straightforward in practise.

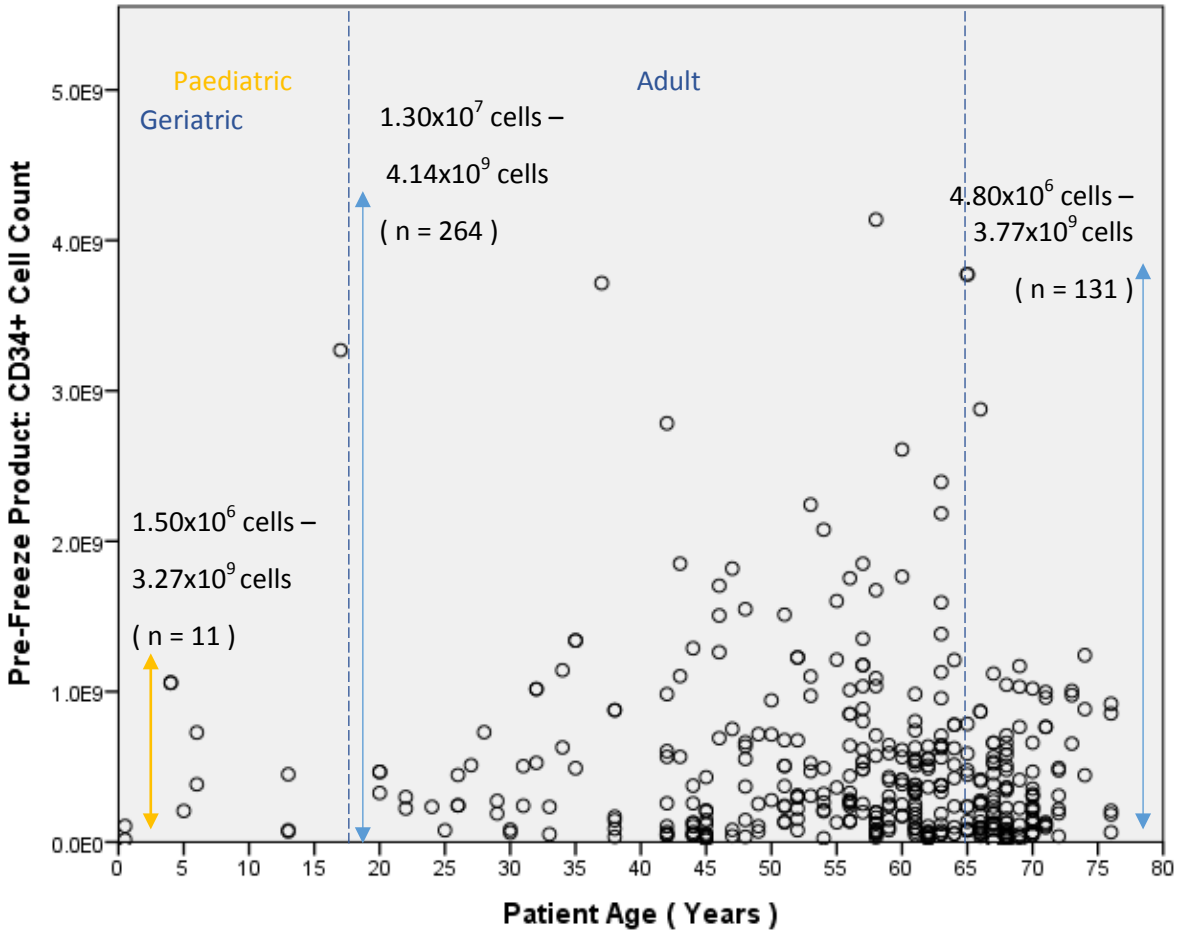


Figure 4.35: Pre-Freeze CD34+ Cell Count against Patient Age (years)

	Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
CD34+ Cells	410	$4.99x10^8$	$3.06x10^8$	$4.80x10^6$	$4.14x10^9$	$4.13x10^9$	$5.97x10^8$	$3.56x10^{17}$
1 - 18	11	$6.75x10^8$	$3.83x10^8$	$1.50x10^7$	$3.27x10^9$	$3.26x10^9$	$9.42x10^8$	$8.87x10^{17}$
19 - 64	264	$5.34x10^8$	$3.45x10^8$	$1.30x10^7$	$4.14x10^9$	$4.13x10^9$	$5.93x10^8$	$3.51x10^{17}$
65+	131	$4.10x10^8$	$2.34x10^8$	$4.80x10^6$	$3.77x10^9$	$3.77x10^9$	$5.69x10^8$	$3.23x10^{17}$

Table 4.18: Pre-Freeze CD34+ Cell Count against Patient Age (years) overall, and split into paediatric, adult and geriatric age groups

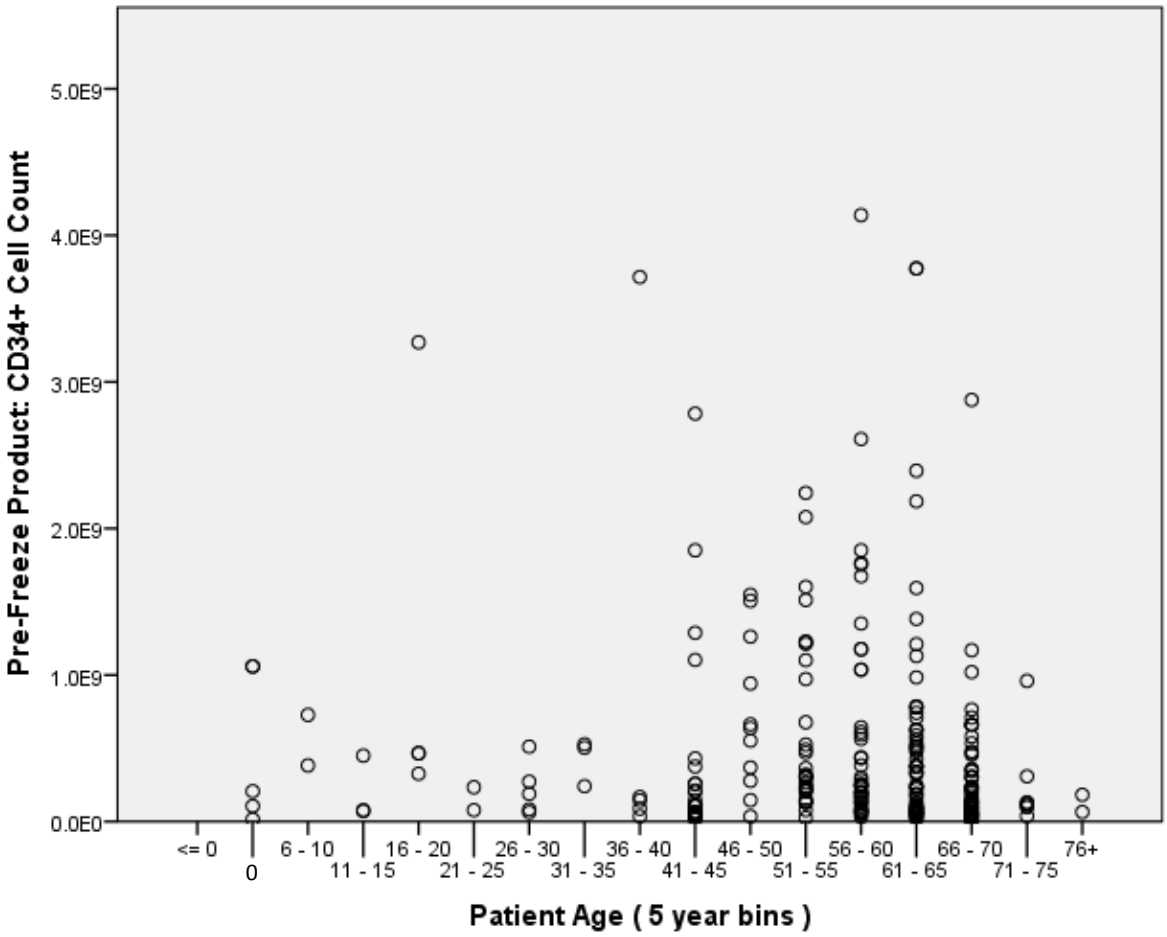


Figure 4.36: Pre-Freeze CD34+ Cell Count against Patient Age (5 year bins)

		Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
PatientAge (Binned)	<= 0	0	.	.	.	.	.	.	.
	1 - 5	5	4.89E+008	2.07E+008	1.50E+007	1.06E+009	1.04E+009	5.25E+008	2.75E+017
	6 - 10	2	5.56E+008	5.56E+008	3.83E+008	7.28E+008	3.45E+008	2.44E+008	5.95E+016
	11 - 15	3	2.00E+008	7.80E+007	7.10E+007	4.50E+008	3.79E+008	2.17E+008	4.70E+016
	16 - 20	4	1.13E+009	4.66E+008	3.26E+008	3.27E+009	2.94E+009	1.43E+009	2.04E+018
	21 - 25	4	2.08E+008	2.28E+008	7.80E+007	2.99E+008	2.21E+008	9.32E+007	8.69E+015
	26 - 30	9	3.09E+008	2.43E+008	6.30E+007	7.30E+008	6.67E+008	2.16E+008	4.66E+016
	31 - 35	12	7.11E+008	5.78E+008	5.20E+007	1.34E+009	1.29E+009	4.45E+008	1.98E+017
	36 - 40	7	8.44E+008	1.68E+008	3.50E+007	3.72E+009	3.68E+009	1.32E+009	1.74E+018
	41 - 45	33	3.88E+008	1.26E+008	1.30E+007	2.78E+009	2.77E+009	6.05E+008	3.66E+017
	46 - 50	22	6.77E+008	6.50E+008	3.50E+007	1.82E+009	1.78E+009	5.70E+008	3.25E+017
	51 - 55	35	6.09E+008	3.63E+008	2.50E+007	2.24E+009	2.22E+009	5.73E+008	3.28E+017
	56 - 60	67	5.99E+008	4.11E+008	3.10E+007	4.14E+009	4.11E+009	6.80E+008	4.62E+017
	61 - 65	84	5.16E+008	3.70E+008	2.20E+007	3.77E+009	3.75E+009	6.74E+008	4.54E+017
	66 - 70	94	3.18E+008	2.01E+008	4.80E+006	2.88E+009	2.87E+009	3.90E+008	1.52E+017
	71 - 75	20	5.44E+008	4.84E+008	3.80E+007	1.24E+009	1.20E+009	3.83E+008	1.47E+017
	76+	5	4.46E+008	2.08E+008	6.50E+007	9.19E+008	8.54E+008	4.07E+008	1.65E+017

Table 4.19 Pre-Freeze CD34+ Cell Count against Patient Age (5 year bins), absolute cell numbers

4.5.5c Pre-Freeze Product: CD34+ Cell Count and Patient Gender

Both genders exhibit specific traits, and these traits may be a factor in the extent of variation as a result from using male versus female donors.

Unless specified otherwise, the following analysis is based upon autologous sourced material, from a 'sick' population, where the patient's gender is the contributing factor.

Figure 4.37 is a scatter diagram of pre-freeze CD34+ cell count grouped according to the corresponding patient's gender. This was to identify whether there were any differences in biological variation as a function of the gender of the donor.

Contrary to the higher variation found in TNC count for male patients, variation is higher for female derived material in terms of CD34+ cell count with respect to range, standard deviation and variance. Male patients have a CD34+ range, standard deviation and variance of 3.71×10^9 cells / kg, 6.61×10^8 cells / kg and 4.37×10^{17} cells / kg respectively, compared to 4.13×10^9 cells / kg, 7.00×10^8 cells / kg and 4.89×10^{17} cells / kg for female patients (**Table 4.20**). This is due to a number of 'outliers' in the female category, circled in blue on **Figure 4.38**.

An independent samples median test considers male and female sourced material to be of equal medians ($p > 0.146$). However, the greater number of extreme values in the female sourced material, as highlighted above, means that the subsequent Kruskal-Wallis test found that the distributions are not equal for male and female derived material ($p > 0.05$).

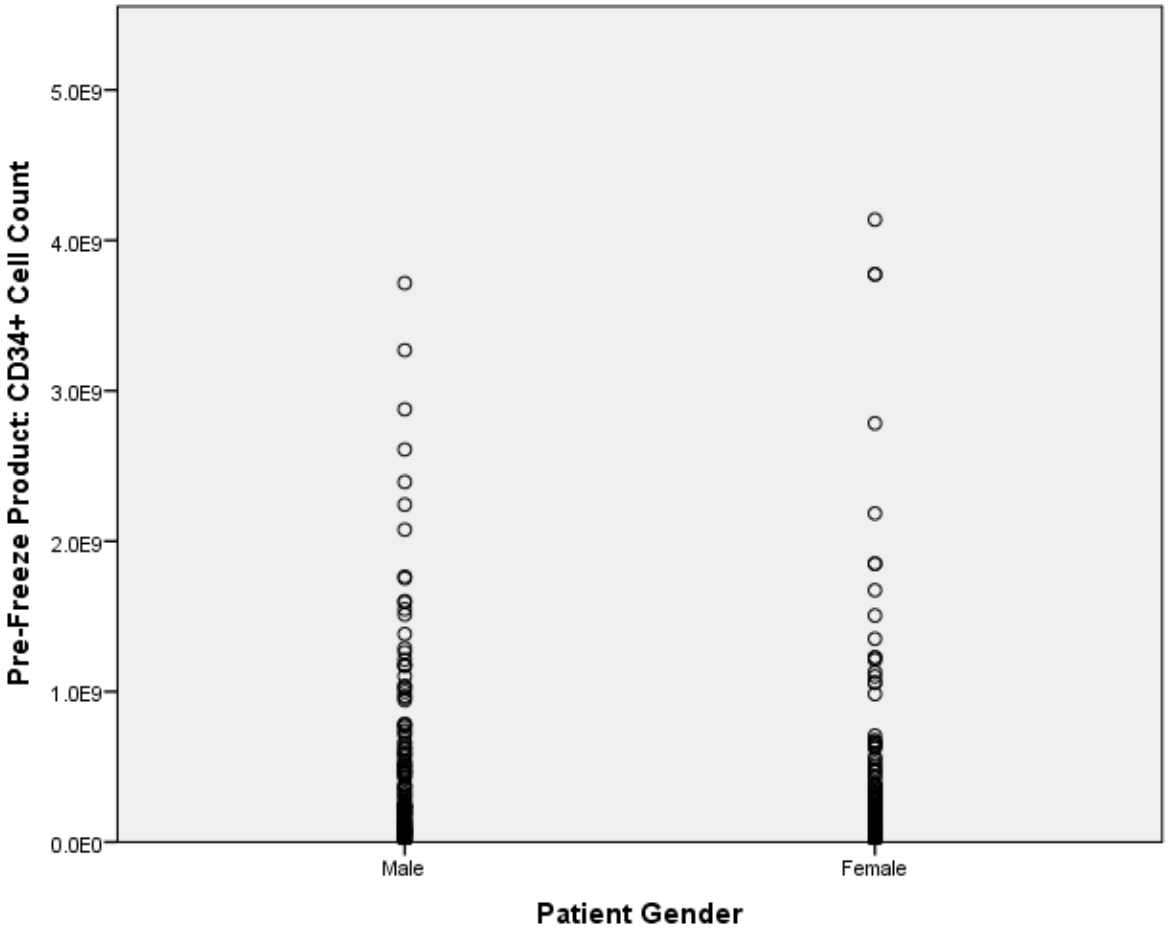


Figure 4.37: Pre-Freeze CD34+ Cell Count against Patient Gender

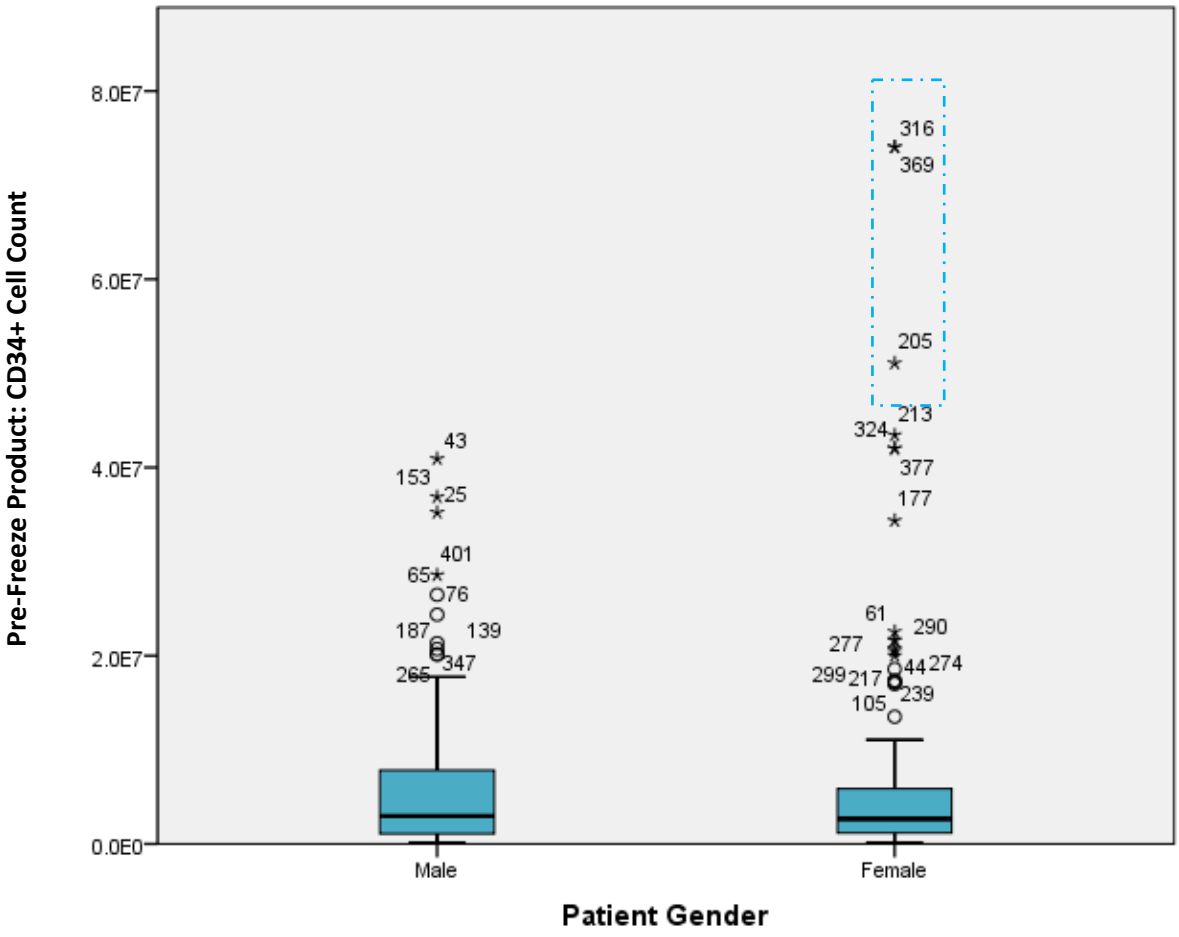


Figure 4.38: Pre-Freeze CD34+ Cell Count against Patient Gender (box and whisker plot)

Cells	Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
Male	145	$5.26x10^{8}$	$2.39x10^{8}$	$9.00x10^{6}$	$3.72x10^{9}$	$3.71x10^{9}$	$6.61x10^{8}$	$4.37x10^{17}$
Female	135	$4.36x10^{8}$	$1.96x10^{8}$	$4.80x10^{6}$	$4.14x10^{9}$	$4.13x10^{9}$	$7.00x10^{8}$	$4.89x10^{17}$

Table 4.20: Pre-Freeze CD34+ Cell Count against Patient Gender

4.5.6 Operator Variation

Technicians, in their role as operators, are the primary source of operator variation. By examining variation at the single clinical centre (characterised by the CMCF) a limited personnel pool is assumed, all of whom should be trained to a comparable level.

At the CMCF, the technician responsible for the process, for the calculations in the product and process records, and the QC analysis is recorded. Using this information, comparisons can be made between individuals to identify if any variation encountered in the product can be attributed towards an individual - and therefore surmise that operator variation is a significant challenge. This was intended to be a proof-of-concept, rather than an individual 'witchhunt' and as such, the technician's identities has been redacted. **Figure 4.39** is a comparison of the CD34+ cell count in the product grouped according to the technician recorded as having carried out the process. There were originally 9 technicians within the timeframe, but one was removed as they had only carried out 2 processes within the period. From visual inspection, it is quite difficult to discern the variation between individuals so the data was transferred to a box and whisker plot in **Figure 4.40.**

It has already been established that CD34+ cell count is non-normally distributed, so an independent-samples Kruskal-Wallis test was used to compare whether the variation can be attributed to operator variation. According to the results of this test, the distribution of CD34+ cell count were the same across the categories (technicians).

However, CD34+ cell count is perhaps not an ideal measure to discern the effects of operator variation. In the absence of a quality metric that is related to cell efficacy, TNC yield might be an appropriate examination of operator skill as it is a direct measurement of a 'before and after' state with respect to the process.

Figure 4.41 and **Table 4.22** groups technicians against the calculated TNC cell yield. A line has been drawn at 100%, to indicate whether product yields meet, or exceed 100%. Exceeding 100% would indicate more cells had been created during the process than was originally collected. Reiterating an

earlier statement, in some cases different technicians will carry out the calculations for the process records than the technician who may be carrying out the process. **Figure 4.42** is a comparison between the recorded TNC yield in the CMCF process records and a yield calculated by the author from the CMCF initial and product absolute TNC counts. The majority of these yields align with each other in a linear relationship (R^2 Linear = 0.896, y = 1.89+ 0.98x, where x is calculated yield and y is reported yield , but one point of interest is where the calculated yield exceeds 100% but the recorded yield caps at 100%. This may be reflective of the fact that more cells will not be created during the process, and to cap the yield at 100%. This may also be a result of different analytical techniques – or rather various levels of resolution at the QC stages in the initial product and the final product.

Cell yield is non-normally distributed (**Figure 4.43**, Shapiro-Wilk's test $p < 0.05$) so the independent samples median test and the independent samples Kruskal-Wallis test was used to compare the median and distributions between operators. The median and distributions were found to be the same across the categories (technicians) ($p < 0.534$ and $p < 0.609$ respectively). This could potentially mean that different operators do not have different effects on the product variation.

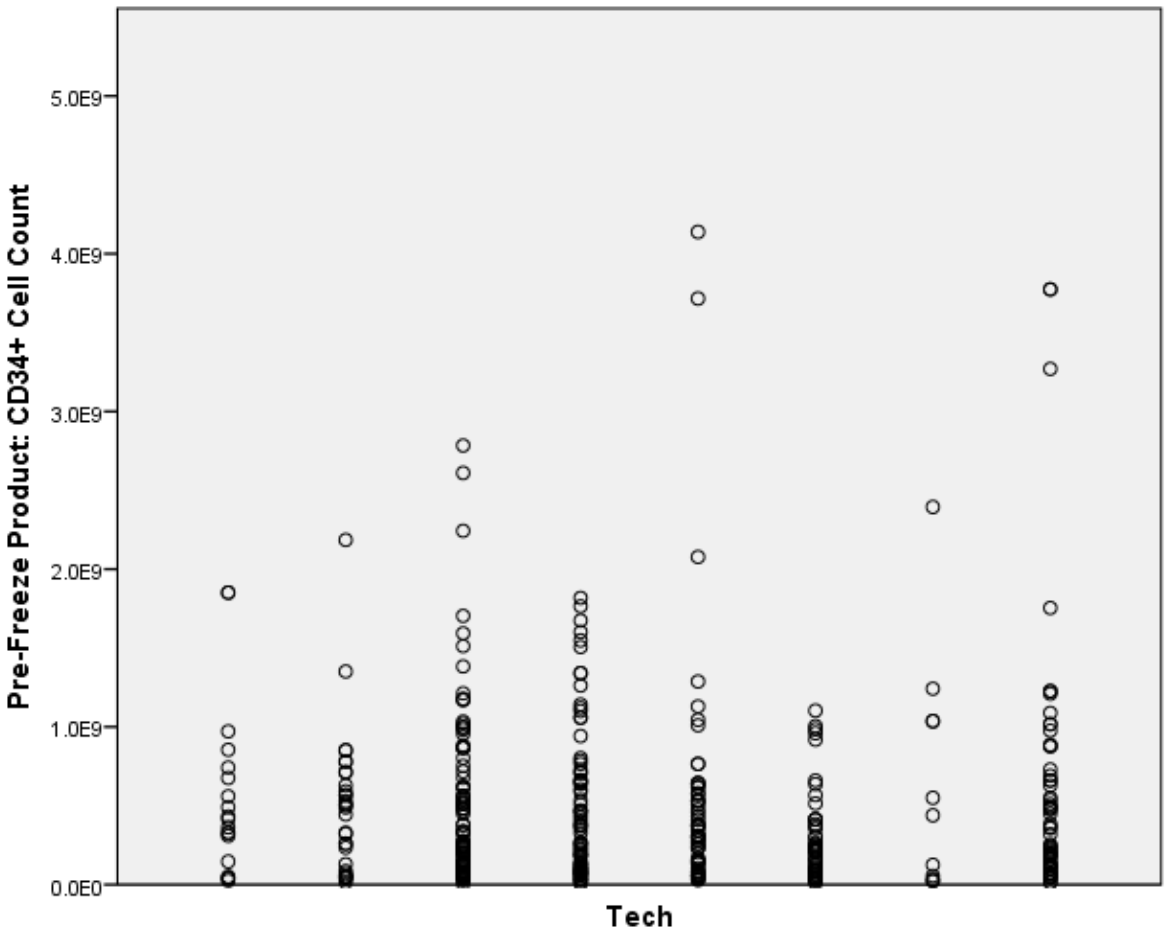


Figure 4.39: Pre-Freeze CD34+ Cell Count against Processing Technician (redacted)

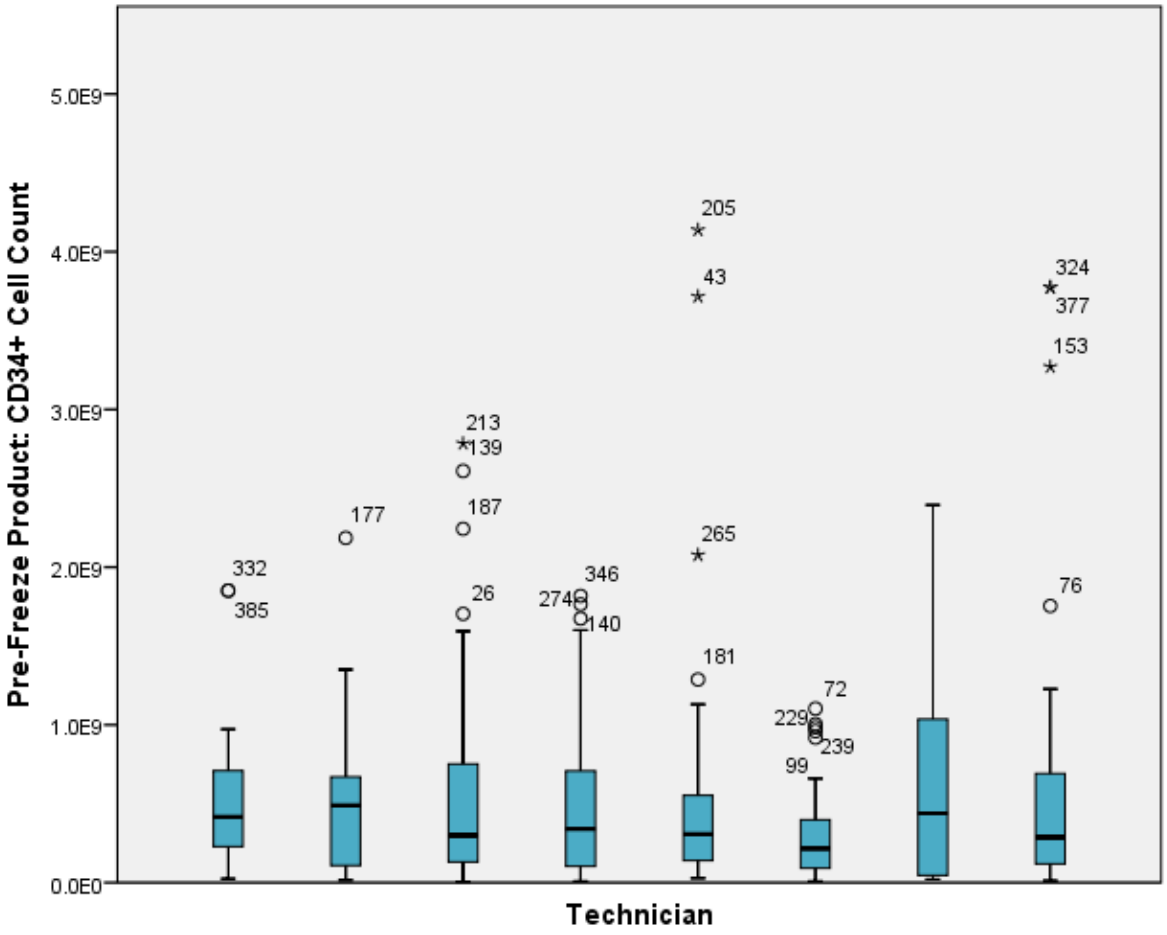


Figure 4.40: Pre-Freeze CD34+ Cell Count against Processing Technician (redacted, box and whisker plot)

			Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
Tech	[redacted]	Pre-Freeze Product: CD34+ Cell Count	13	5.58E+008	3.63E+008	2.50E+007	1.85E+009	1.83E+009	6.41E+008	4.11E+017
		Pre-Freeze Product: CD34+ Cell Count	20	4.57E+008	2.91E+008	1.50E+007	2.18E+009	2.17E+009	5.37E+008	2.88E+017
		Pre-Freeze Product: CD34+ Cell Count	72	5.05E+008	2.52E+008	4.80E+006	2.78E+009	2.78E+009	5.82E+008	3.39E+017
		Pre-Freeze Product: CD34+ Cell Count	66	4.66E+008	2.57E+008	9.00E+006	1.76E+009	1.76E+009	4.87E+008	2.37E+017
		Pre-Freeze Product: CD34+ Cell Count	49	4.81E+008	2.82E+008	2.80E+007	4.14E+009	4.11E+009	8.02E+008	6.43E+017
		Pre-Freeze Product: CD34+ Cell Count	44	2.93E+008	2.10E+008	9.00E+006	1.10E+009	1.09E+009	2.96E+008	8.76E+016
		Pre-Freeze Product: CD34+ Cell Count	2	1.47E+009	1.47E+009	6.20E+007	2.88E+009	2.82E+009	1.99E+009	3.96E+018
		Pre-Freeze Product: CD34+ Cell Count	9	5.74E+008	1.25E+008	2.20E+007	2.39E+009	2.37E+009	8.00E+008	6.41E+017
		Pre-Freeze Product: CD34+ Cell Count	43	5.94E+008	2.13E+008	1.30E+007	3.77E+009	3.76E+009	9.26E+008	8.57E+017

Table 4.21: Pre-Freeze CD34+ Cell Count against Processing Technician (redacted, box and whisker plot)

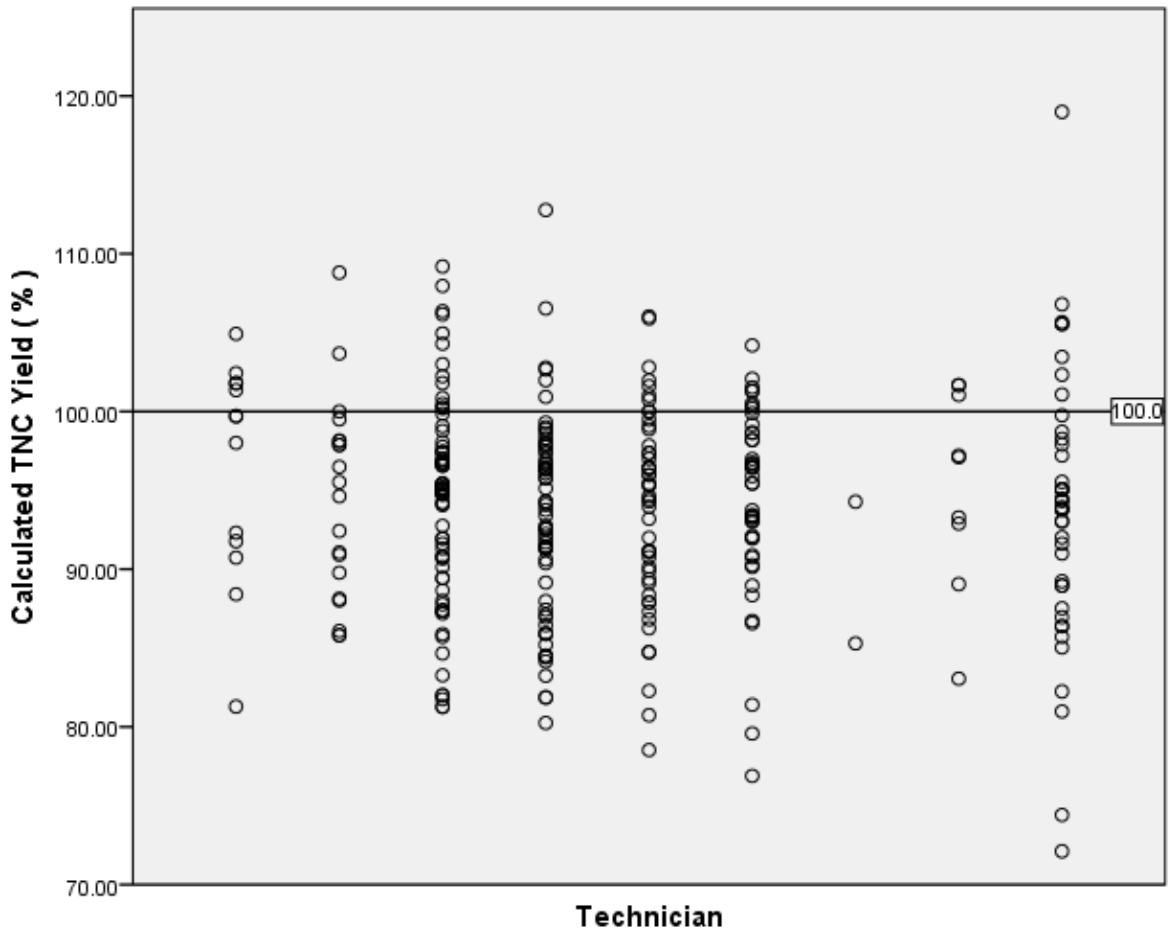


Figure 4.41: Calculated TNC Yield against Processing Technician (redacted, box and whisker plot)

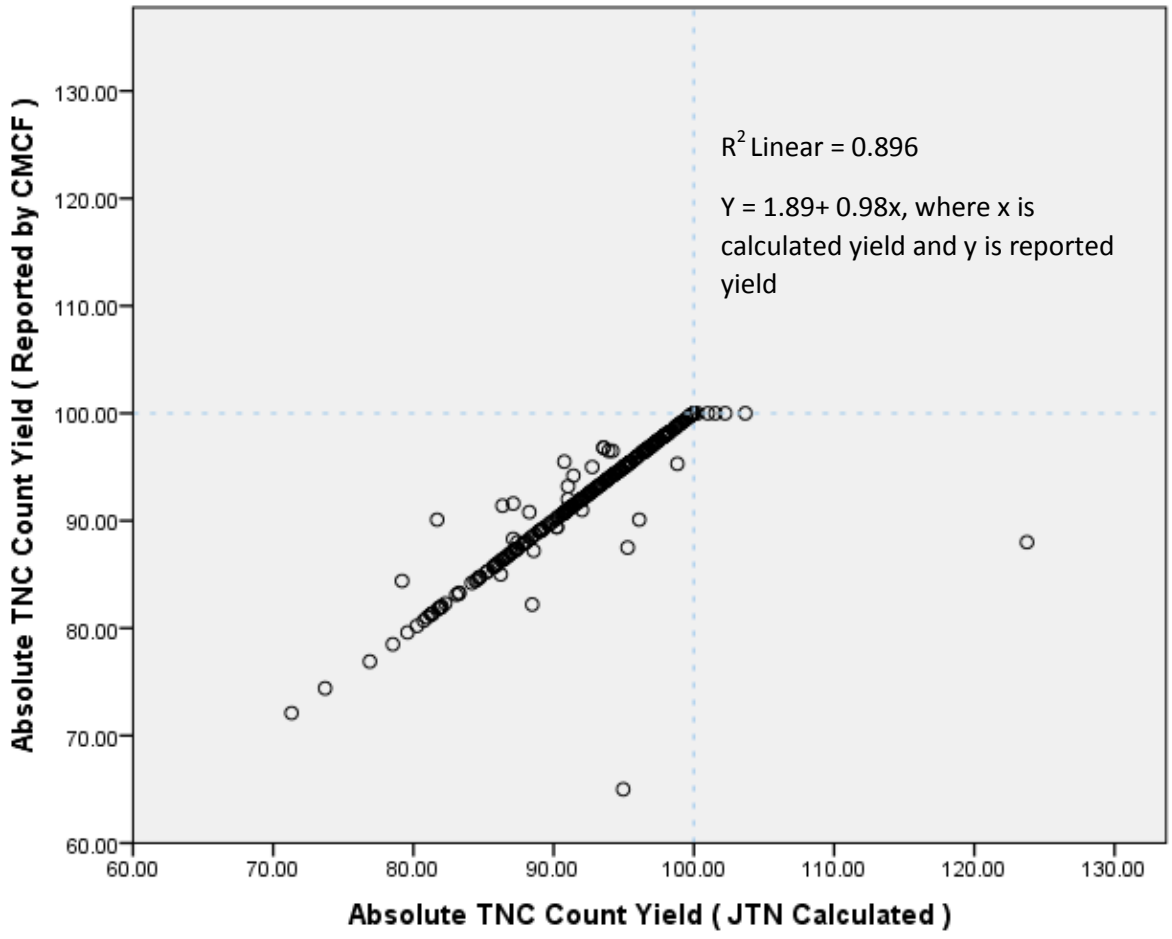


Figure 4.42: Yield of absolute TNC count, compared between recorded value and calculated value

	Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
Tech [redacted]	13	96.48	99.69	81.29	104.92	23.62	6.94	48.21
	20	94.43	95.07	85.81	108.80	22.99	6.30	39.68
	72	94.28	95.04	81.27	109.19	27.92	6.54	42.80
	66	93.18	93.57	80.25	112.77	32.52	6.29	39.60
	49	93.78	94.64	78.53	106.02	27.49	6.31	39.79
	44	94.33	95.44	76.89	104.19	27.30	5.96	35.53
	2	89.79	89.79	85.29	94.28	8.98	6.35	40.36
	9	95.22	97.09	83.05	101.67	18.62	6.32	39.97
	43	93.74	93.93	72.11	118.99	46.88	8.62	74.39

Table 4.22: Calculated TNC Yield against Processing Technician (redacted)

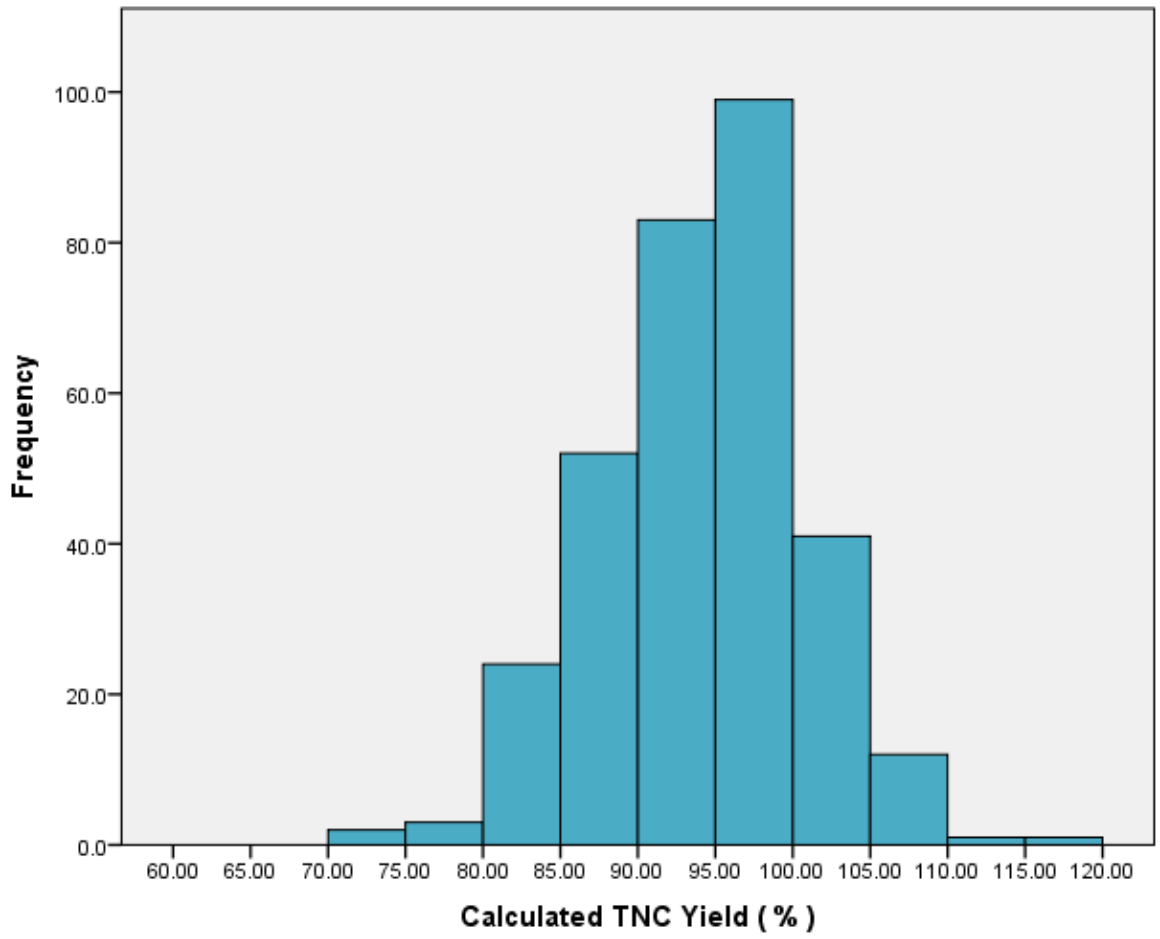


Figure 4.43: Calculated TNC Yield against Processing Technician

4.5 Single Clinical Centre Conclusions and Summary

This chapter has aimed to quantify the variation encountered in a single clinical centre, characterized by the product and patient records available at the Dana Farber Cancer Institute in Boston, USA.

A particular aim of this chapter was to reduce the number of confounding variables, such as patient age and weight, and examine the capability of a large centre with respect to the control of variation.

Biological variation in cell therapies, such as blood-based therapies, is currently accepted for two main reasons;

1. Cellular therapy such as HSCTs are delivered under a one-sided specification. This is the minimum amount determined by the overseeing physician to salve the symptoms of the primary therapeutic (bone marrow recovery in chemotherapy for example).
2. A number of cellular therapies are experimental and are offered under difficult circumstances where the alternative may mean death for the patient.

For cellular therapy to become a frontline therapy, the regulator needs to be satisfied as to long term efficacy and safety – especially in the face of approved alternatives. These therapies are produced in small numbers for relatively few patients. To become a viable frontline option, we need the capability to produce in volume. Given that i) Smart people do not scale - if the process requires a certain level of intuition and process understanding, this limits the potential employment subpopulation that can be hired from, that would be capable of manufacturing these products and, ii) hospitals do not have the space or manpower to scale up, traditionally: there will need to be a degree of mechanization or automation.

This is the primary rationale behind the investigation of biological variation. Understanding the variation that is encountered in the starting material, and the process, means an understanding of the tolerances and the specification that a machine or piece of equipment needs to be designed

around – to tolerate or control – because only by a degree of mechanization or automation can frontline cellular therapy be achieved as an option to the patient.

4.5.1 Extent of Variation in Product at a Single Site

This chapter has concluded that the variation in absolute CD34+ cell count at a single clinical centre is between $4.80x10^5$ cells and $4.14x10^9$ cells. There appears to be greater variation in autologous therapy ($4.80x10^6$ cells to $4.14x10^9$ cells) than allogeneic therapy ($4.00x10^7$ cells to $1.82x10^9$ cells) (**Figure 4.9**).

This translates to variation between $7.92x10^4$ cells / kg and $7.41x10^7$ cells / kg. Compared to the Meta-Analysis in **Chapter 3** ($6.0x10^4$ cells / kg to $1.10x10^9$ cells / kg), this is a significant reduction and an example to other clinical centres that the CMCF has particular cultures of practice, technology and biological considerations they can learn from. However, the variation is still 3 orders of magnitude and an issue that needs to be addressed.

4.5.2 Where is this variation coming from?

There are several sources of variation. Traditionally, these are split into two broad categories; common and special cause variation. Common cause variation is naturally occurring variation as a function of the process. Special cause variation is variation caused by an event of change. Determining what is common and what is special cause variation is key to process design for automation.

Within the clinical centre several sources have been identified;

- Biological
- Operator
- Process
- Measurement
- Data Storage and Handling

4.5.3 Biological

Autologous therapies are derived from unwell donors and will be dependent on the disease state of the individual, and the extent to which the disease affects the quality of the donated cells. Allogeneic therapies are derived from comparatively healthy and screened donors and may be potentially sources of healthier or higher quality cells.

Biological variation as a function of the patient and/or donor may be completely uncontrollable. Currently, with a one-sided specification based on a minimum dose required, this variation has been successfully managed with the current quality criteria and by the practice of medicine. However, for mainline cellular therapies, where these quality criteria may be different, this initial biological variation may be of greater import. Perhaps the emphasis will be on quality rather than quantity (and what do we measure as an analogue of quality ?), or perhaps a greater number of cells will be required. Whether there are specific protocols that can be followed to increase the number of isolated cells, or environmental / dietary changes that can be imposed in the donor to guarantee greater quality is unclear.

A potentially key stage in the introduction of variation, in either quantity or quality, is that of mobilisation and conditioning – something identified by the technicians at the clinical centre as the largest source of variation. These stages are either actively designed to increase the number of cells produced by the body, or as part of a prior medical intervention necessary to treat the patient's indication.

The amount of mobilisation drug used (if at all, and there are various drugs used) is dependent on the clinicians discretion as to whether it is required, based upon the number of circulating cells of note in the peripheral blood stream. In addition, the number of cells 'mobilised' will depend on the health of the individual, and will vary between individuals as a function of their own biological makeup. This is obviously a key step in determining how many cells and of what quality is available at the start of the process. Whether this is something that can be investigated, ethically and

financially, is another matter, but what may assist in reducing the variation seen is a common framework for when these therapeutics are used, how much, and in what situation. If these are applied in a predictable manner it is much easier to determine their effect on overall statistical variation.

In a potential fully automated system, this input is one of the key stages outside the GMP-in-a-box ideal situation, and must be treated with care. It will determine the volume of liquid, volume of cells and quality of cells a potential machine may have to be designed to deal with – especially as it is unethical to discard a sick patient's cells based purely on volume constraints, particularly where multiple applications and/or products may be required. This assumes a machine will not be designed for a given and fixed volume or number of cells, but be able to flexibly handle various incoming volumes and/or cells, as reflected in the case for starting materials at the CMCF.

4.5.4 Operator

Currently cellular therapies are produced manually, heavily reliant on the skill, experience and intuition of the individual operator in question. This may be currently sufficient to meet low scale demand for therapies, but greater demand will require either an increase in operators or an improved process. Clinicians are also operators in this process.

One of the challenges with passing on operator knowledge is the prevalence of intuition and interpretation of SOPs. This can make training fresh staff, or teaching existing staff new techniques difficult. A potential solution is the use of in-situ technology (tablet devices for example) that allow the operator to 'tick' off steps in the process, that simultaneously inform the laboratory manager and the clinician (potentially allowing for a product ETA). This may assist further training by allowing a visual learning tool for processes, which would allow greater visualisation and understanding of the process.

There are two types of operator, skilled and less skilled. These give rise to their own unique quirks to applying protocols. Unless specifically instructed and monitored, a skilled operator with knowledge of the process may interpret the protocol in a manner they see as 'better' – in the face of what has been agreed on an investigational new drug license (IND) for example. An unskilled operator however may skip, or rush steps in the protocol as they do not understand the import of that step.

Perhaps an annotated informal SOP or process diagram that walks the user through the stages, illustrating the reasons why, with links to supporting research would assist in this interpretation, assuming they would read them. These are not currently available at DFCI.

A further, third point of note is the attention span and concentration of the operator, regardless of skill. Fatigue will affect quality of work, and cleanroom environments are not comfortable working environments for long shift work. Furthermore the user must be mindful of the psychological effect of chronology – a protocol followed during the last hour of a shift will not be as rigidly followed, and may be rushed ('Friday feeling') compared to midday process. When the quality and efficacy of the product is so heavily reliant on the work ethic and diligence of a human operator it is in the best interests of the institute to minimise fatigue and boredom, and from this perspective mechanisation and automation have advantages.

4.5.5 Process

What isn't known is the extent to which the manual culture influences the overall variation. To meet regulatory requirements, not to mention acceptable comparability, processes need to result in a product that falls within a given specification. This will change between products, because many of these regenerative medicines are highly customised to the patient, but the process must still be capable of reliably meeting a given specification on demand.

To provide further clarity of detail of the causes of variation, more than the few hundred data points indicated here are required. This is because there needs to be statistically significant data-points in

each subset to determine causality – for example disease state, both type and severity (for autologous), donor characteristics that are indicative of health, process methodology, metrics of the initial starting material and to identify the technician responsible for each process. The latter is not to ostracise them, but to identify whether the variation as a function of the operator is not a key contributing factor.

Cryopreservation and the subsequent thawing process are another example of processes that will occur outside of the processing stages and are potentially large sources of variation, both in quality and volume. This is simply because the current methods of freezing and thawing kills cells. Particularly as thawing methods are relatively uncontrolled, using water baths, and 'crunching' up frozen samples to ease the thawing process. There are a number of equipment providers offering more controlled thawing processes, but these are expensive and proprietary solutions to simple challenges – the main challenge from the authors perspective is ensuring whatever the potential solution, it cannot compromise the product, and does not require a whole rethink of the process, as this will not be feasible, affordable or adoptable.

4.5.6 Measurement

Flow cytometry is currently the cellular measurement instrument of choice allowing measurement of cells numbers of a specific type by counting fluorescent molecules attached to characteristic cell surface markers. However this is not entirely automated, and one key stage ('gating') is entirely at the discretion of the operator. This stage essentially requires said operator to select and box subsets of cells on screen, and can vary depending on the operator. Given the number of cells that may or may not be included by moving the box a few pixels to the left or right on the screen, or the difficulty in gating certain types of cells, or our confidence in the measuring system, this begs some poignant questions as to the reliability of our measurements, and by extension the extent of variation.

This is clearly a key area of research and improvement for the field, in terms of comparability of equipment, repeatability of results given a fixed standard regardless of operator and whether they

provide the level of precision and confidence we require. Measurement is the keystone for accessing and improving biological variation.

4.5.7 Data Storage and Handling

Patient and process records are stored in various disparate databases, in different formats, that can be difficult to cross-reference. The format and standards of paperwork is known to differ between clinical centres, making comparison and large scale data mining difficult.

One way to provide confidence to the regulator of the efficacy and safety of stem cell therapy is to develop and enforce a common reporting platform for patient and process records within cellular therapy that can be shared within the medical community. Not only will this provide historical evidence to back up the establishment of regenerative medicines, but potentially allow for diagnostic and prognostic tools to be developed. These tools could predict the effectiveness of these therapies for a given patient, and indicate where established alternatives may be more efficacious (especially given the estimated costs of cellular therapies).

Chapter 5 UK Biobank

5. 1 Chapter Aims

This chapter aims to quantify the variation encountered in a general, ambulatory sub-population of the UK, represented by the participants of the UK Biobank.

Figure 5.0 is a representation of a generic HSCT process map. This chapter specifically examines the factors affecting the contribution of patient and/or donor variation, as indicated on this figure in the coloured box.

The following topics will be discussed:

Chapter Aims and Introduction to UK Biobank	5.1 and 5.2
Materials and Methods	5.3
Analysis of Results	5.4
Conclusion and Discussion	5.5
Future work with the UK Biobank	5.6
Summary	5.7

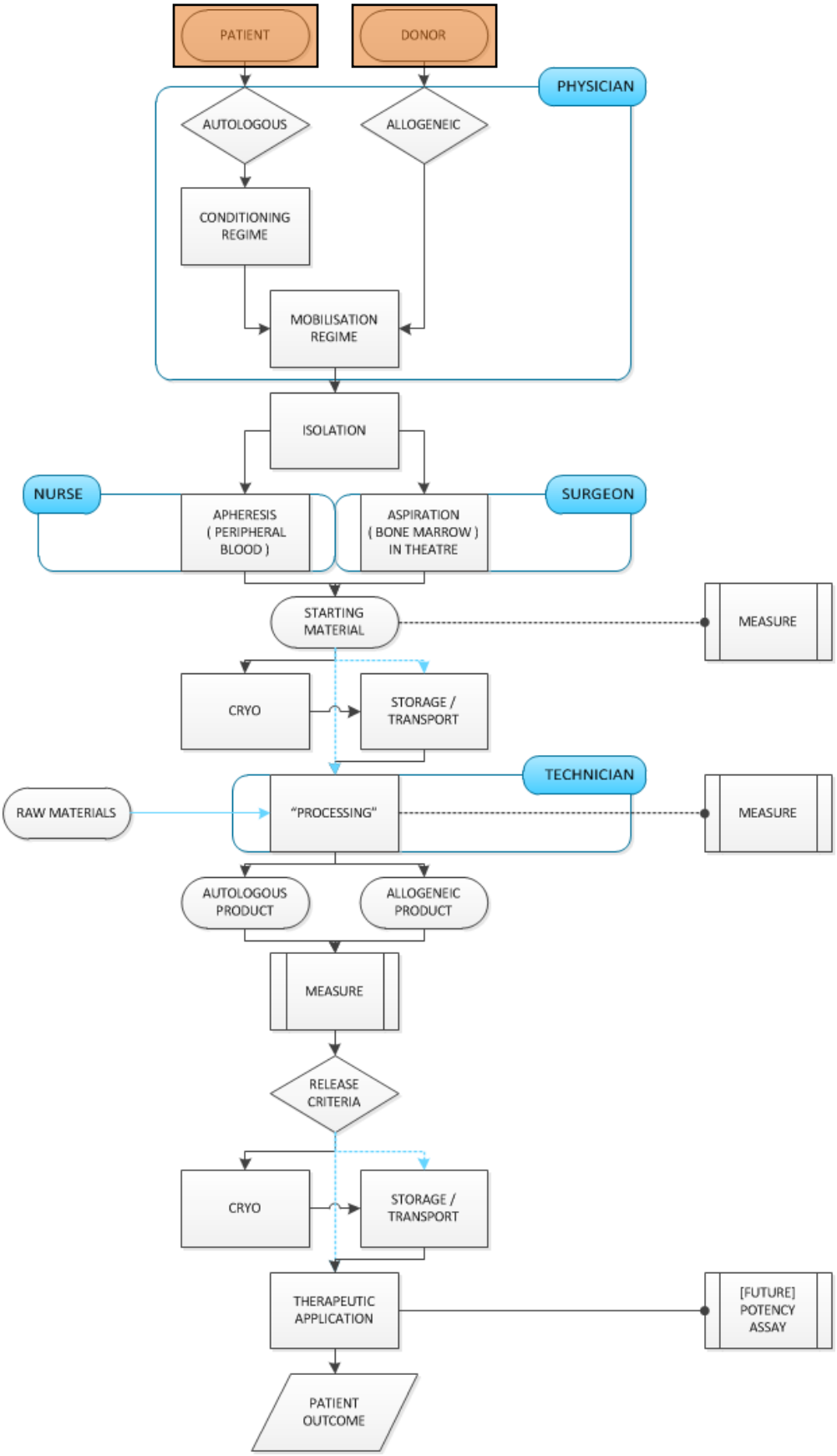


Figure 5.0: Generic Process Map indicating in orange the focus of this Chapter

5.2 Introduction to UK Biobank

UK Biobank is a unique health resource and a charity, established with the aim of improving the prevention, diagnosis and treatment of a wide range of serious and life-threatening illnesses. It consists of a databank and a biological sample bank from over 500,000 recruited individuals, between the ages of 40 and 69, with donors sampled between 2006 and 2010. This Biobank is intended to be a resource for investigating potential causes of disease development.

The large sample size allows for statistically significant investigation of possible influencing factors, such as ethnicity or diet, that may prove to be risk factor when it comes to particular diseases and conditions.

5.2.1 Analysis of Variation: Biobank

The literature meta-analysis (**Chapter 3**) demonstrated the distribution of biological variation across a notionally unhealthy baseline represented by the medical literature and across multiple centres and countries. The CMCF data (**Chapter** 4) has demonstrated the distribution of biological variation in a localised unwell population at a single centre. The aim of the analysis of the UK Biobank is to illustrate the variation inherent of a specific healthy sub-population of one country, with significant numerical power.

UK Biobank presents the opportunity to investigate further into specific patient and donor metrics that were previously unavailable, such as medical history, lifestyle specifics such as smoking and consistent physical measurement such as weight.

5.3 Materials and Methods

The UK Biobank project collected biological samples and questionnaire data from volunteers in 22 centres around the UK. The qualitative data and the biological samples were then transferred to a central processing and storage facility in Manchester.

Biological samples included urine and blood. Pre-prepared vials containing the appropriate amount of additives such as preservatives were used to extract peripheral blood using a hypodermic attachment. These containers were labelled, barcoded and sent to the central processing laboratory in temperature controlled shipping conditions. These were then centrally analysed using flow cytometry and added to the primary database. Together with the recorded physical and questionnaire results, this presents a robust platform for medical analysis and cross-comparison. This data is held by the UK Biobank and researchers can apply to investigate specific research questions, on the basis that they will publish the results and pay a small processing fee.

The application for this chapter focused specifically on the physical measurements of the donor, the questionnaire results – particularly those focused on diet and health – and the results of the whole blood test carried out on corresponding sample.

Figures 5.1 – 5.4 represent those specific measurements from the UK Biobank database required by this Chapter's application.

These results were chosen based on the experiences with the medical literature and subsequent meta-analysis, and the clinical process records at the CMCF. These results included previous medication (**Figure 5.1**) physical metrics such as diet, weight and age (**Figure 5.2**), sleep duration, ECG and pulse rates (**Figure 5.3**).

Unfortunately, UK Biobank does not currently directly measure cell markers that specifically identify stem cells such as the HSCs measured in the previous chapters. CD34+ cell counts would

have been appropriate to directly compare the relatively healthy population of the UK Biobank to the relatively unwell population at the Dana Farber Cancer Institute. UK Biobank does however record red blood cell counts, white blood cell counts and the specific subsets of white blood cells such as leukocytes and monocytes (**Figure 5.4**). As the CMCF also recorded these subsets, a comparison between the two centres could be made. Biological variation is explored and measured in this Chapter as a function of white blood cells rather than haematopoietic stem cells.

The CMCF recorded WBC count as "$x10^3$ cells / µl" and UK Biobank recorded WBC count as "$x10^9$ cells / L" so these were converted into "cells / litre" to allow for more robust and universal comparison between each other.

Access to this resource, which consisted of an online database that needed to be downloaded and un-encrypted (using a proprietary MS-DOS based software suite and an encryption key), was granted in 2014. The comma-separated variation output was then imported into IBM's SPSS 22.0 statistics package. This software was chosen due to the authors previous experience with the software, and Microsoft Excel's inability to deal with large datasets. Nominal data fields, such as gender, were originally coded as numerical identities (1 = male, 2 = female).

During December 2014, UK Biobank undertook a detailed review of their haematology data to investigate potential anomalies highlighted by researchers. This meant that this data could not be responsibly analysed until 2016. A preliminary analysis was carried out at risk to ensure the results of the UK Biobank chapter remained relevant and informed with respect to the other developing Chapters, as well as suitable methodology applied, but the subsequent results were treated with caution.

As a result, it was advised that further research that incorporated these results be postponed until the issues were resolved. Upon release, this new 2016 haematology data was combined with the older 2014 data containing participant characteristics and measurements.

The application to the UK Biobank consisted of two types of metrics that were requested; participant metrics and blood composition metrics;

- **Participant Metrics.** These measurements were either physical, or as part of the questionnaire, and were requested to determine whether these had any effect on the variation measured in the corresponding blood measurements.
- **Blood Composition Metrics:** These are characteristics specifically of the tested donor's blood content including white and red blood cell counts. These are the measurands being directly examined to quantify variation and may be affected by the physical and lifestyle metrics of the previous category

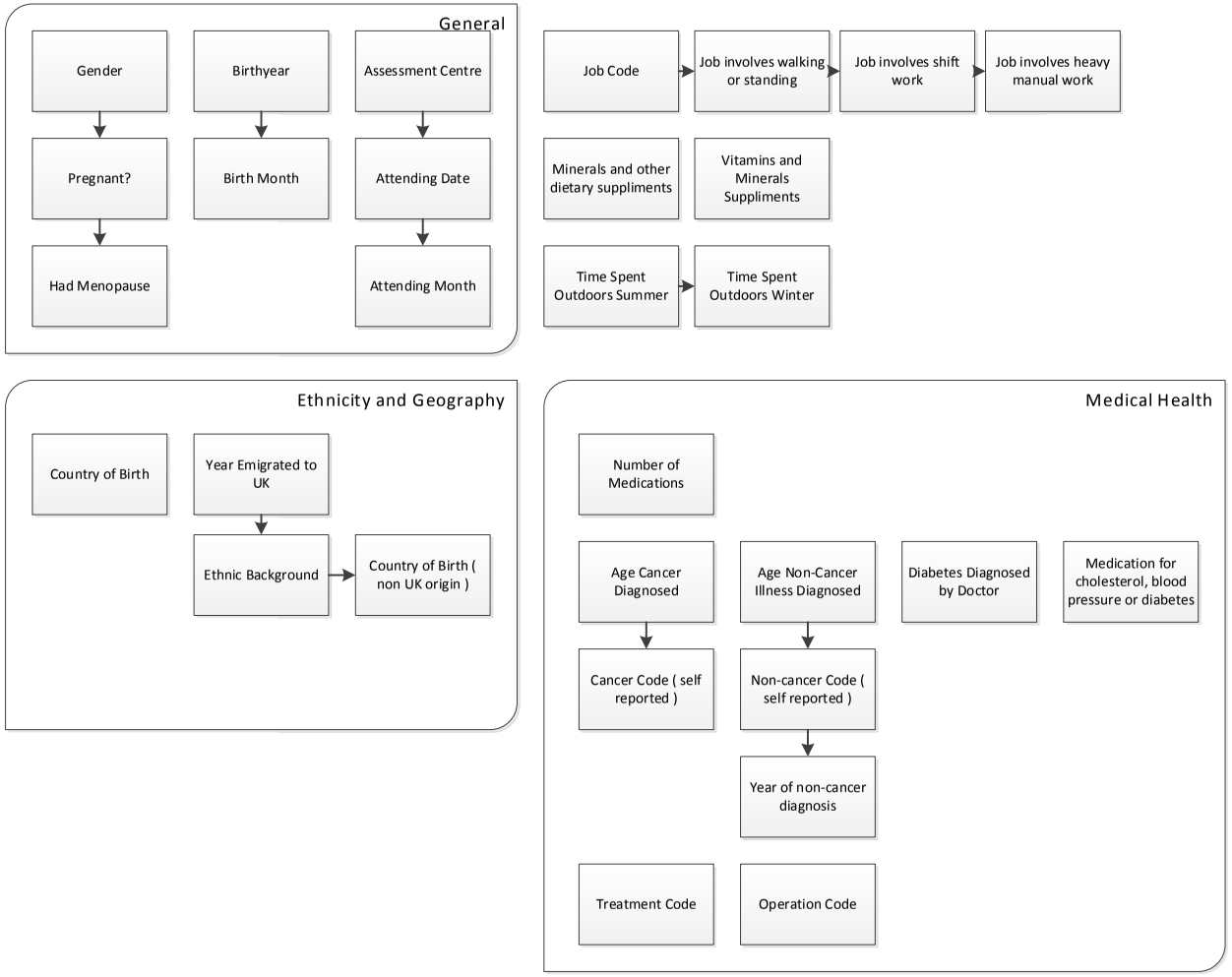


FIGURE 5.1: Participant Metrics – General, Ethnicity and Medical Health

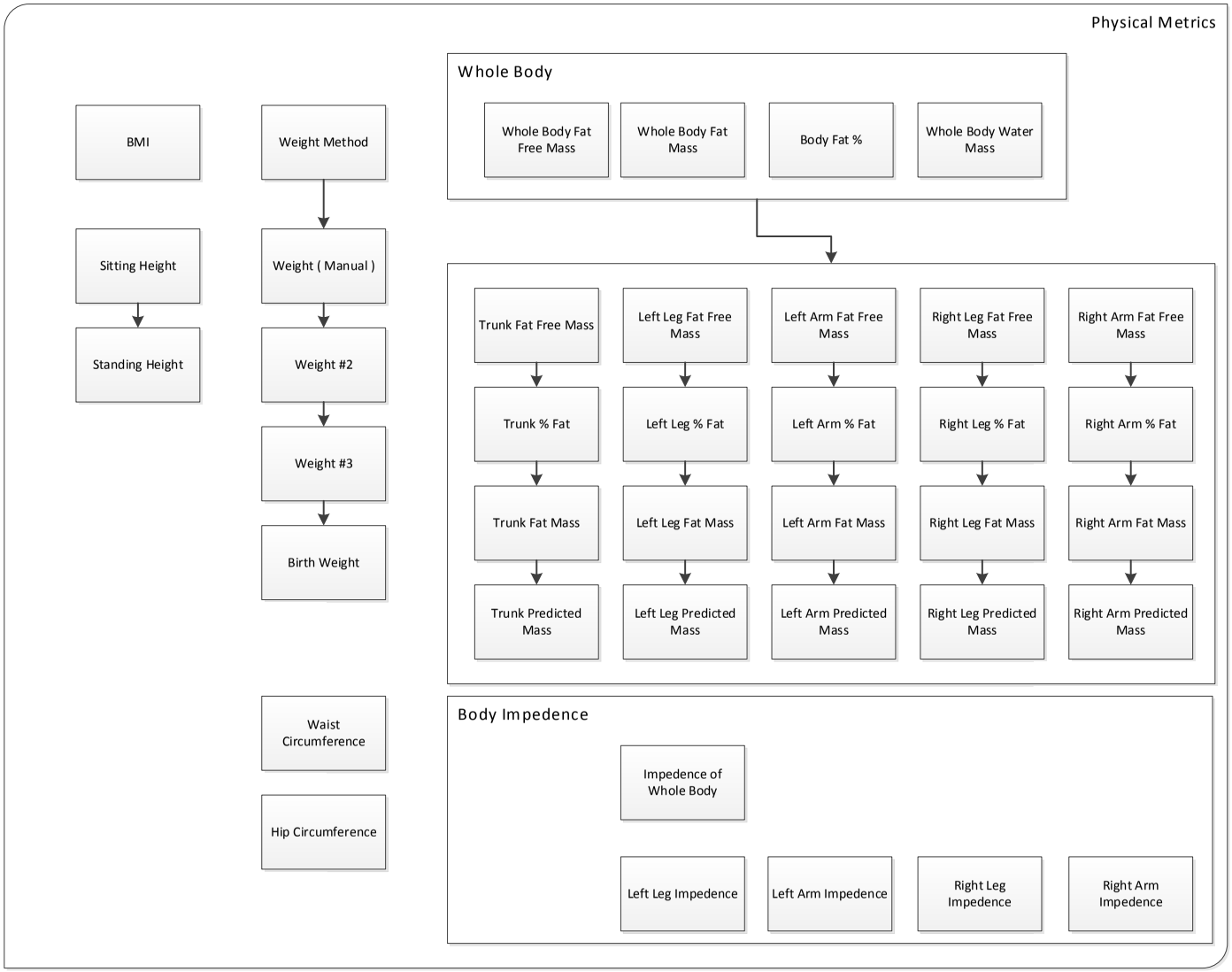


FIGURE 5.2: Participant Metrics – Physical Body Measurements

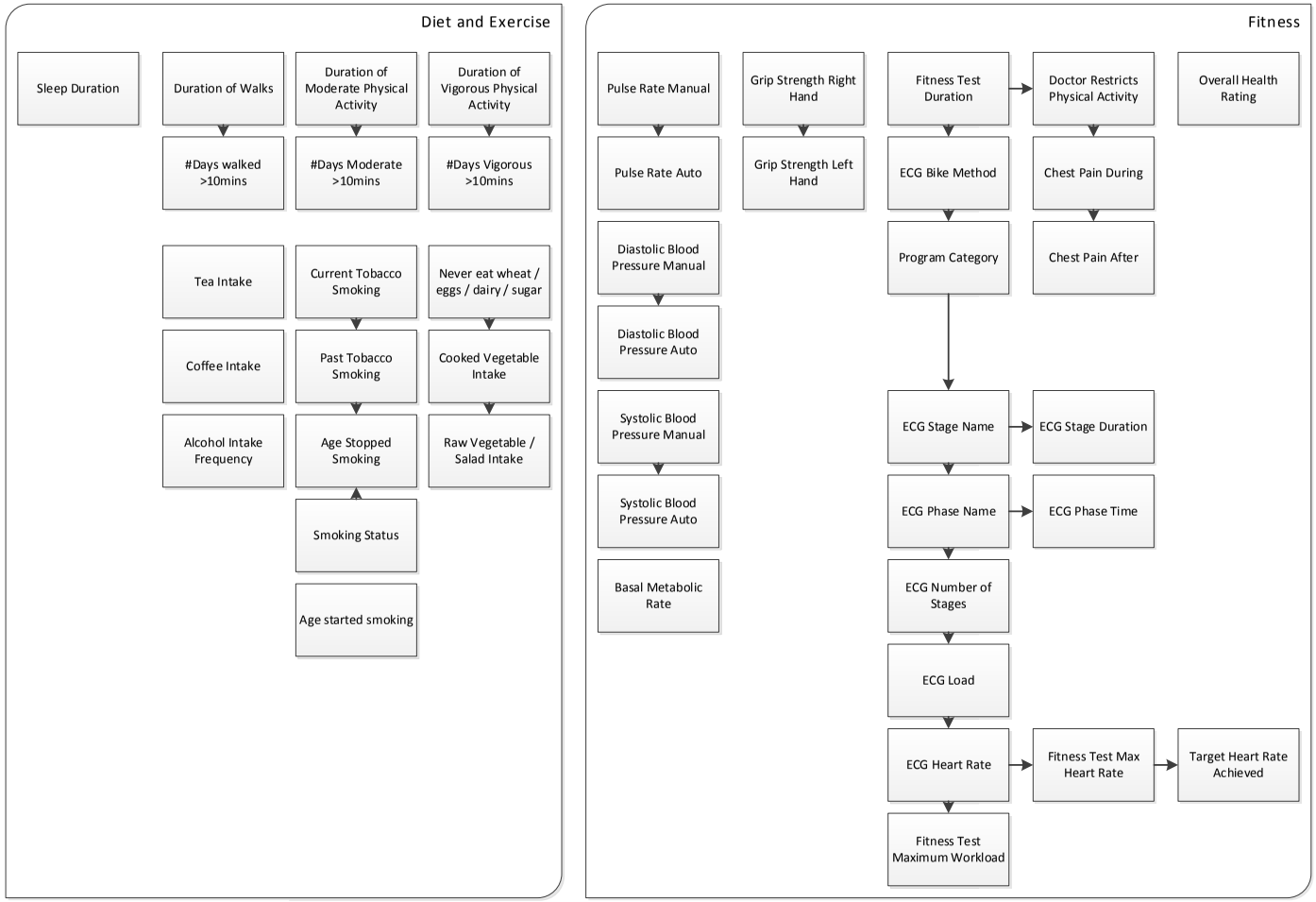


FIGURE 5.3: Participant Metrics – Diet and Exercise

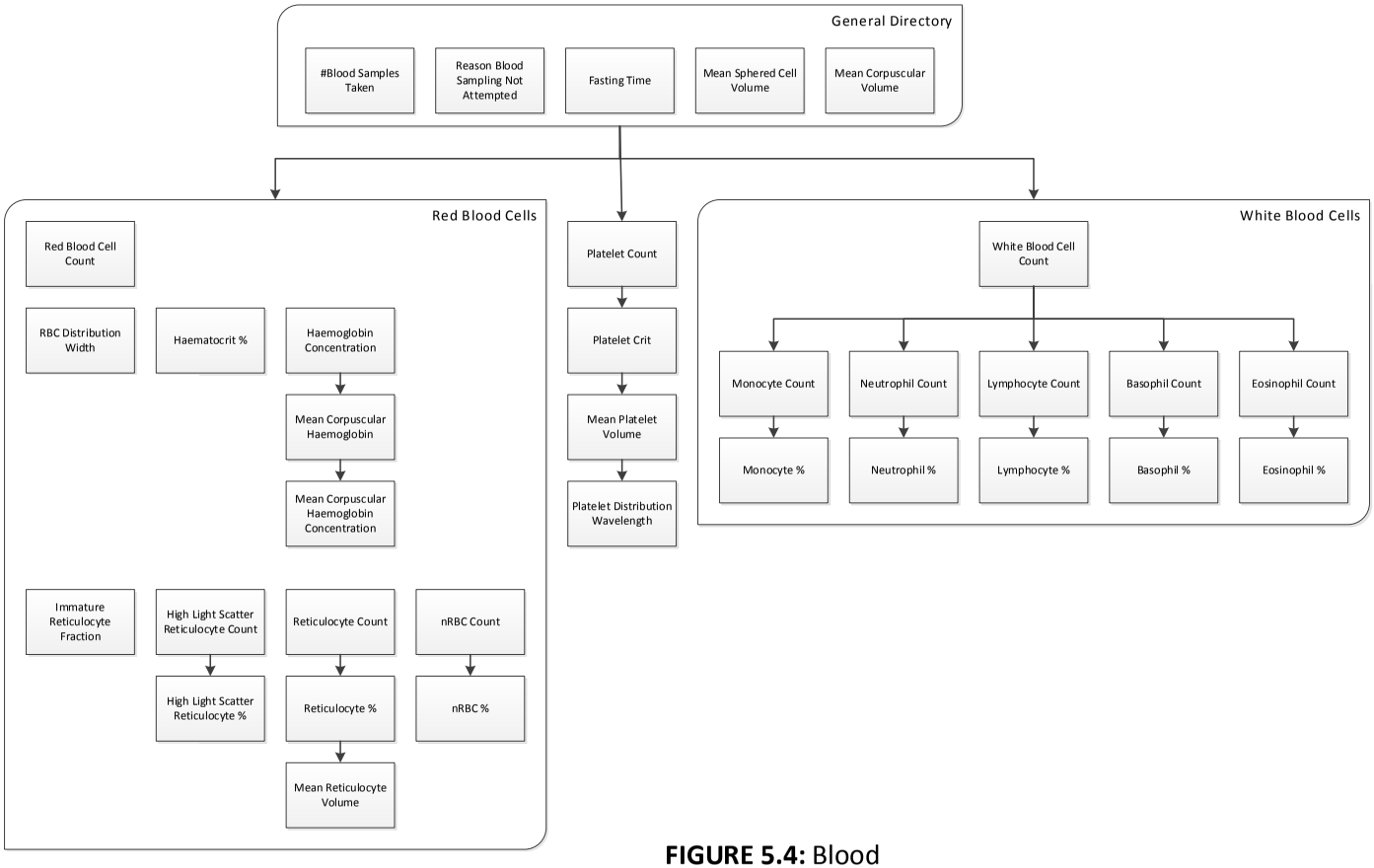


FIGURE 5.4: Blood Composition Metrics

5.3.1 UK Biobank Application - Requested Measurands

Access to the information within UK Biobank is granted as part of a detailed application process including experimental design and required variables. The following section justifies the choice of variables. (see **Figure 5.1 - 5.4**)

Pregnancy and menopause were included in this analysis because of the biological changes that come as a result of these two life phases, and whether the advent of these phases has an effect on the variation encountered as a result – this may have implications for future cellular therapy, such as using female donors over, or under, a certain age for allogeneic therapy.

Assessment centre, and attending date were included to examine the effect of geographical location on the variation and spread of biological data; is there more biological variation on the coast? If certain conditions are indicative of more or less variation or cell quality, are there greater or lesser incidences of those conditions depending on location? What effect does living in a rural area have, if any? More likely, these assessment centres represent sub-sets of the population, with particular ethnic and genetic makeups – much like the reasoning behind setting up blood donation centres in specific locations to capture more of a specific, rarer blood group or type.

Job code, and the other similar metrics shown in the top right corner of **Figure 5.1**, were included with the intention of determining whether sedentary jobs such as office work affect cell characteristics differently to more manual or active jobs.

Medication, treatment, operation code and the other metrics in the bottom right corner of **Figure 5.1** were included because of previous experience in the previous Chapters as to the effect of the mobilisation and conditioning regime. These are likely to be the most influential factors for amount and quality of cells, and were therefore included to examine their subsequent effect, if any, on biological variation. This is important because patients are likely to have had previous medical intervention, are currently on a pharmaceutical programme, or being

given treatment for a comorbidity or parallel indication, and therefore the effect of these states must be investigated as they are the likely state of health of the recipients of stem cell therapy.

Diet, included tea intake, alcohol and tobacco intake (**Figure 5.3**) were included for similar reasons as the previous metrics, as they contain chemical agents that affect body chemistry, metabolism and health, and may have a bearing on the quality of stem cells. They were included here to determine whether they have an adverse effect on biological variation.

Ethnic background was included as an analogue for the grouping due to genetics. However, there is a difference between ethnicity and race – the difference between political statement and genetic composition, where the latter is the more vital component for biological variation.

BMI and weight are fundamental physical measurements. Further physical measurements such as body fat and impedance were included as further, more detailed physical measurements.

Pulse rate and blood pressure were included, alongside overall health rating, as direct physical measurements of a person's relative health. Further health metrics such as fitness test duration and ECG were also included for this reason (**Figure 5.3**).

All available blood measurands were requested including cell counts, cell percentages and cell volumes amongst others. As there were no direct comparisons with **Chapter 2, 3 and 4** metrics (TNC and CD34+) these were all included as Dana Farber included these cell counts and percentages and would provide a direct comparison of where the 'unwell' population corresponds to the 'healthy' population, in respect to data spread and variation.

5.3.2 Statistical Analysis of UK Biobank Data

Unless stated otherwise, white blood cell count is measured as cells per litre. Absolute cell numbers and cells per kilogram were unavailable in the UK Biobank database.

Graphical representations including biological measurements such as white blood cells were plotted on a logarithmic scale to allow for visual inspection of spread and distribution that would otherwise be difficult on a linear scale.

Pareto charts, typically used to identify the key 20% of a population that delivers 80% of the observable effect, were used to describe the certain characteristics of the Biobank dataset – in particular health state and ethnicity. These are favoured as they are useful visual tools for examining the contents of a dataset, and have been used in previous chapters to positive effect. These were created within SPSS 22.0 by using it's 'Quality Control' test entitled 'Pareto Chart'.

The spread and distribution of biological measurements such as white blood cells were represented graphically as scatter charts and box and whisker charts. Box and whisker plots were used to demonstrate the core statistical data and the outliers, the spread of the core data versus the extremes, and provide a more accessible format for graphical comparison between groups, such as age or weight. However, box and whisker plots do not handle extensive statistical outliers well, so scatter diagrams have been included. Scatter charts were used to illustrate the spread of biological measurements, and plot the cell count of each individual product, representing all the data.

In the same manner as the CMCF database, distributions of cell metrics from the UK Biobank were analysed for normality, including graphical representation as a histogram. These biological measurements were also found to be non-normal and consequentially similar non-parametric statistical tools to those used in **Chapter 4** to compare median and distribution of the data were used: Pearson product-moment correlation co-efficient was used to measure linear correlation

the Mann-Whitney test for two independent groups (e.g gender) and the Kruskal-Wallis test for multiple independent groups (e.g. weight groups) (see **Section 4.4.2**).

5.4 Analysis of Results

A summary of the contents of the UK Biobank database can be found in **Table 5.0**. The greater resolution of the UK Biobank (n = 502,664) has allowed analysis of participant / patient specific characteristics such as lifestyle medical history and other physical metrics (e.g. weight) that were previously impossible.

Blood specific measurements such as white blood cell counts were recorded at three time intervals: initial testing, repeat testing and imaging. Analysis within this chapter is based upon the counts recorded at initial testing, as this time period contains the largest proportion of the dataset.

		Valid N	Minimum	Maximum	Mean	Median
Unique ID		502,664	-	-	-	-
Gender	Male	229,182	-	-	-	-
	Female	273,467	-	-	-	-
Birth Year		502,649	1934	1971	1952	1950
Age		502,649	45	82	64.46	66.00
Weight (kg)		499,874	30.0	197.7	78.1	76.4
BMI		499,543	12.12	74.68	27.43	26.74

TABLE 5.0: Summary of Participants within UK Biobank

5.4.1 Overall Variation as a function of White Blood Cell Count / Litre

To date, UK Biobank does not measure total nucleated cell count or CD34+ cell count, the measures of cell identity used in **Chapter 2**, **Chapter 3** and **Chapter 4.** However, UK Biobank's haematology data includes a complete blood count, which was also included in the CMCF's database in **Chapter 4.** Blood cells such as white blood cells are generated from HPCs and are morphologically difficult to discern from HPCs, so white blood cell count was used as an analogue in this Chapter to examine biological variation in the relatively healthy population of UK Biobank. As this count also exists in the CMCF's database, a comparison can also be made between the relatively healthy population of UK Biobank and the relatively sick members of the CMCF's database.

White blood cells were reported as "$x10^9$ cells / litre" and as such, due to a lack of corresponding fields, an accurate measure of absolute cells count or cell count per kilogram of patient bodyweight (such as those found in previous chapters, or reported in the literature) was unavailable. Appropriate conversions were made within both UK Biobank and CMCF data so that white blood cell count was reported in corresponding units (cells / litre).

Two participants of the UK Biobank were recorded as having a white blood cell of zero. Disregarding the likelihood of these being errors, it is unlikely an equivalent biological process will continue if its starting material has zero functional therapeutic cells, and as a result these two participants have been removed from all subsequent analysis.

Figure 5.5 is a scatter plot of white blood cell count per litre per individual measured, measured against unique participant ID to illustrate the spread and distribution of this biological metric. There are clear groupings between the regular expected spread, and outliers outside of this regular spread. This is reminiscent of Parnaby's manufacturing process descriptions of 'regular runners', 'repeaters and 'strangers' [207]. The former two refers to product types the manufacturing facility is regularly exposed to and prepared for, whilst the latter represent

dramatic changes in process. On **Figure 5.5** this is represented by the central band, where the majority of the donor characteristics lie – the band of variation that a theoretical processing machine, with this starting material as its input, would be required to regularly handle. The samples outside of this band represent starting material the machine would not be prepared to handle, and could require adaptation or even a different process.

If this graph represents the biological variation of a potential starting material then it represents the variation a particular process or automated system would have to manage or control – approximately between $2.4x10^9$ cells / litre and $1.6x10^{10}$ cells / litre regularly (as indicated by 'runners and repeaters' marked on **Figure 5.5**) and between $7.00x10^7$ cells / litre and $3.90x10^{11}$ cells / litre irregularly (as indicated by 'strangers and aliens' variation marked on **Figure 5.5)** (**Table 5.1**).

This is a variation of four orders of magnitude around the median ($6.65x10^9$ cells / litre), a similar degree of variation as to what has been found in prior Chapters, but for total nucleated cell count.

Figure 5.6 compares the white blood cell count distributions of the UK Biobank dataset and the previously described CMCF dataset from the Dana Farber Cancer Institute. The CMCF dataset is the comparatively 'unwell' population, and has a higher average white blood cell count than the heathier population from UK Biobank, in terms of mean, median and spread of the data (**Table 5.1**). The variance of the CMCF white blood cell count is also much higher, indicating the variation in cell count between any given individual is much higher for the unwell, CMCF population – this is potentially a result of the extreme state the CMCF patients are in.

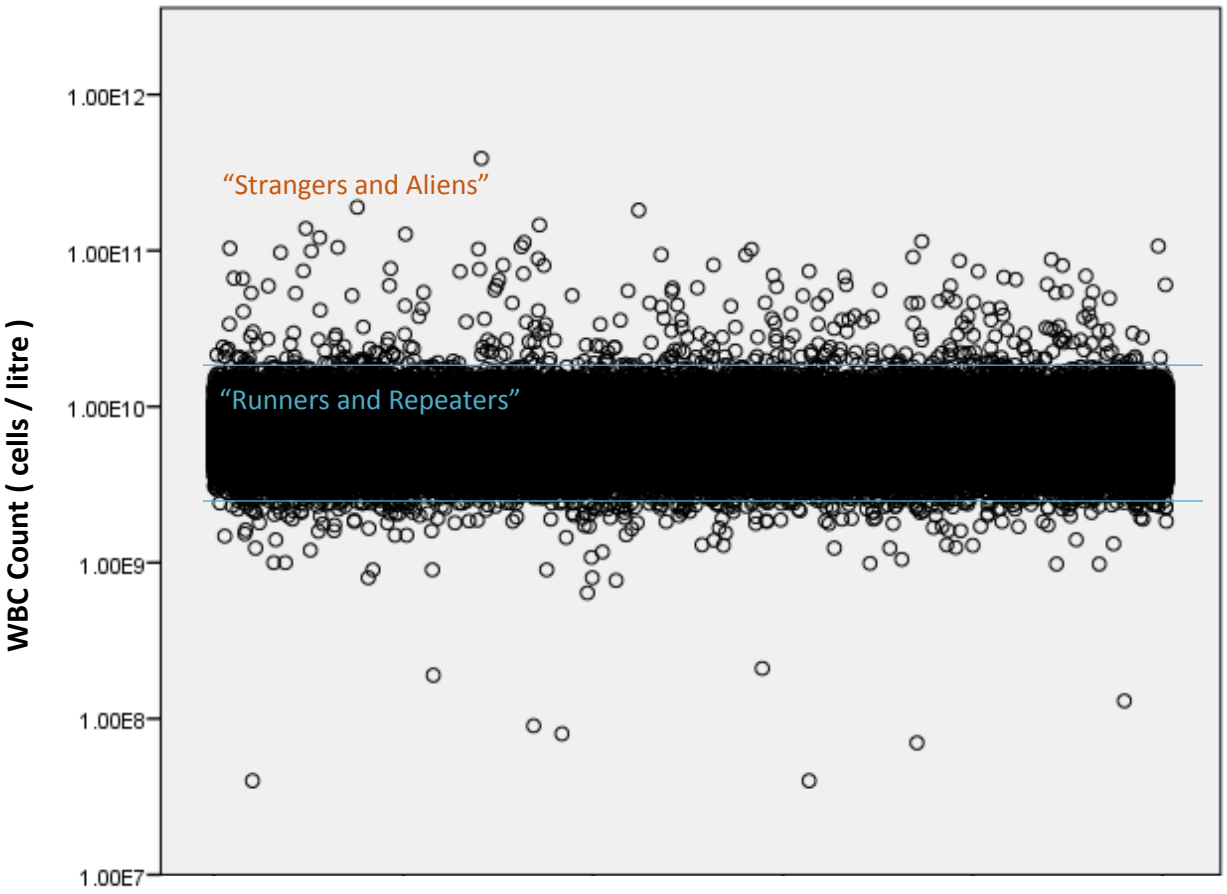


FIGURE 5.5: Scatter plot demonstrating the spread of white blood cells per litre measured per individual participant

Parnaby's runners, repeaters, strangers and aliens have been indicated

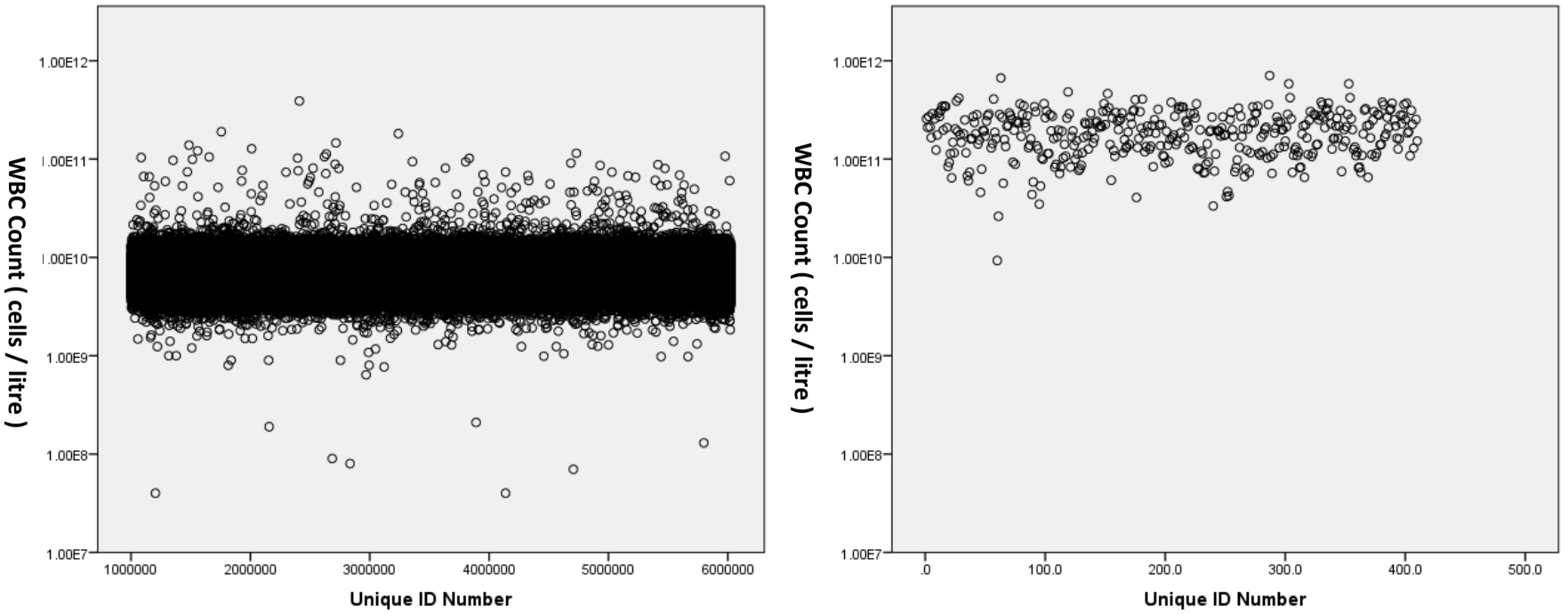


FIGURE 5.6: Comparison Scatter diagram of WBC Count / Litre for UK Biobank (left) and DFCI (right) datasets

	Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
WBC Count	478,290	$6.89x10^{9}$	$6.65x10^{9}$	$4.00x10^{7}$	$3.90x10^{11}$	$3.90x10^{11}$	$2.12x10^{9}$	$4.51x10^{18}$

	Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
WBC Count	282	$1.69x10^{11}$	$1.50x10^{11}$	$9.91x10^{9}$	$3.77x10^{11}$	$3.67x10^{11}$	$8.37x10^{10}$	$7.01x10^{21}$

TABLE 5.1: Comparison of WBC Count / L overall between UK Biobank and CMCF data.

UK Biobank data: Top, blue

CMCF data: Bottom, orange

24,372 entries from UK Biobank containing missing data – no white blood cell reading for example. At least 1,583 participants of those entries could not be measured for various reasons (e.g. they declined, equipment failure). 2 entries were removed as the white blood cell count equalled zero, and would substantially skew the range of reported variation. This brought the overall total to 478,290 instead of the 502, 664 shown in **Table 5.0**.

5.4.2 Variation as a function of Participant Age

UK Biobank had a mandate to recruit a particular demographic - 45 to 69 year olds. As a result, the population of UK Biobank is weighted towards a more mature population than the Meta-analysis (**Chapter 3**) or CMCF (**Chapter 4**) datasets, which included paediatric patients and donors.

Due to the increase in life expectancy and the subsequent growth of the ageing population, this demographic is important, particular as age related diseases and dysfunctions may be a key target for regenerative medicine.

White blood cell count per litre relative to age in years has been plotted in **Figure 5.7.** Age appears to have an insignificant effect on the variation of WBCs, but there is a marked increase in the number of extreme values from 60 years old and onwards. This particular spike in 'outliers' and the subsequent increase in the spread of cell counts may be a result of the diverse ways in which people age relative to one another. This is especially noticeable in **Figure 5.8**, where age has been grouped into five year intervals, and plotted against WBC count / litre. The mean and core values represented by the standard deviation within the box of the box and whisker plot remains relatively constant across age groups, but there is a dramatic increase in outliers as the patient's age increases. This may be due to the increase likelihood of the effects of ageing, or age-related diseases – which is of particular important with the current ageing population being a likely target for future regenerative medicines.

Table 5.2 represents the white blood cell count corresponding to these age groups, for both the UK Biobank data, and for the CMCF data, to allow comparison. The median cell count remains relatively comparable, with an increasing range in cell count as the participants age increased – this appears to be the case for the CMCF data although there are almost no outliers – which may be a product of the much smaller dataset (**Figure 5.9)**. It is noticeable that the significant participant numbers in the UK Biobank allow the dataset to settle irrespective of influencing

factors, whereas the CMCF is a small enough dataset to be strongly influenced, and this is reflected in the box and whisker plot in **Figure 5.9**. There is significantly less data in the CMCF dataset, and the danger is that unlike UK Biobank which has enough data to be a representative sample, the small, restricted sample of the CMCF dataset hasn't settled around the average and is much more likely to be skewed by extreme values.

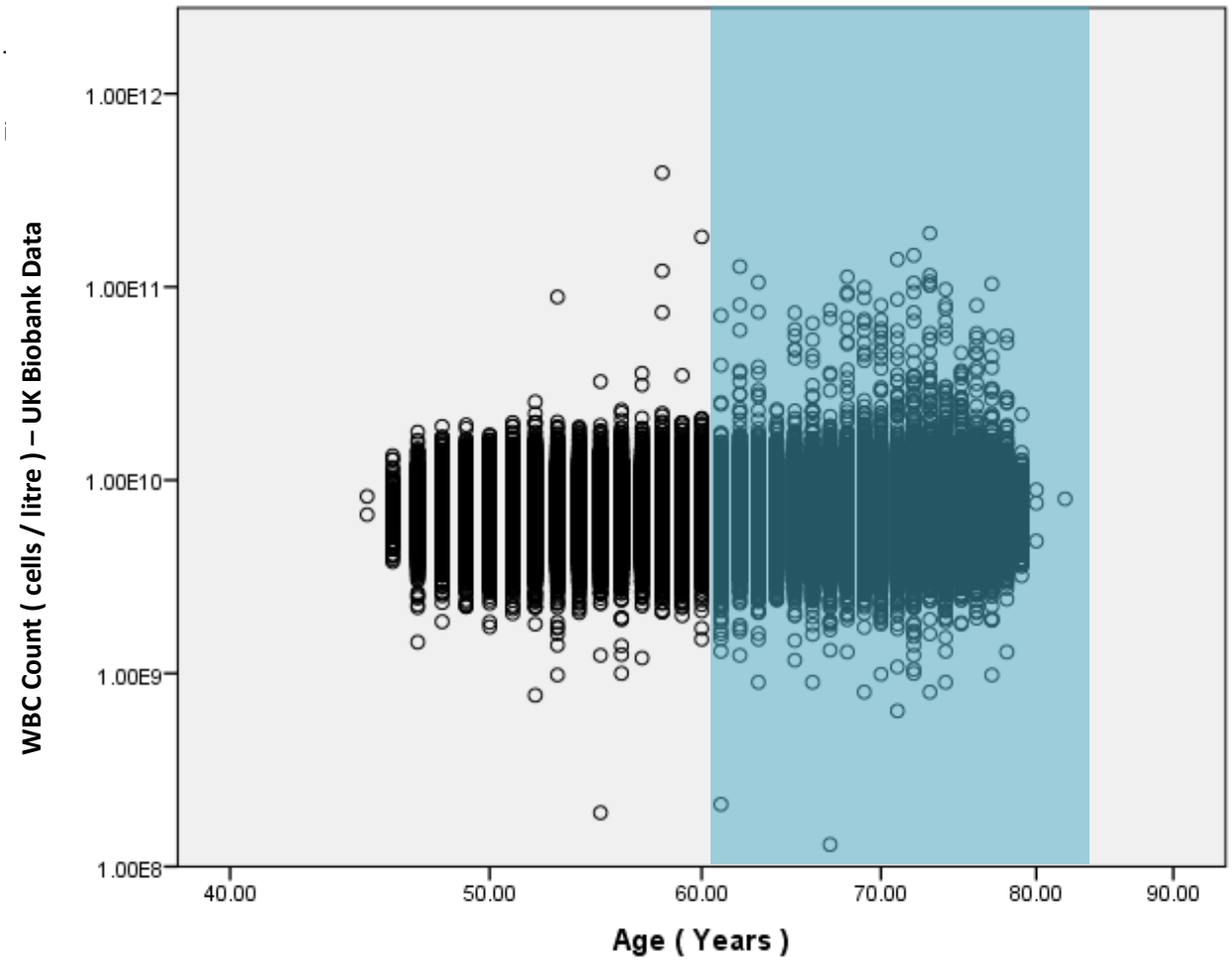


FIGURE 5.7: Scatter plot demonstrating the spread of WBC count per litre measured per individual participant, against that participants age in years

The **blue** section indicates the dramatic increase in outliers mentioned in the text.

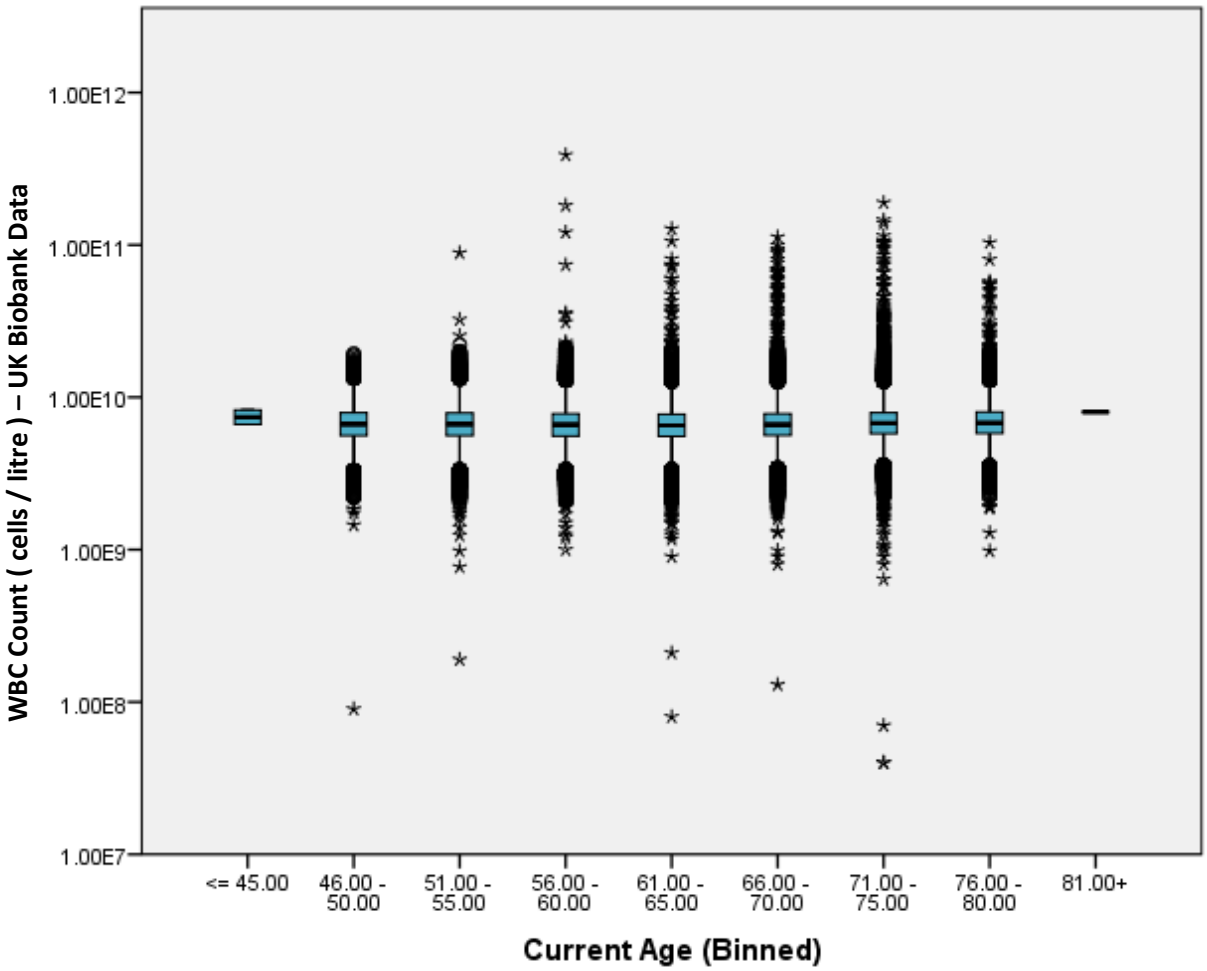


FIGURE 5.8: Box and whisker plot demonstrating the spread of WBC count per litre measured per individual participant, against that participants age in years (binned into five year categories)

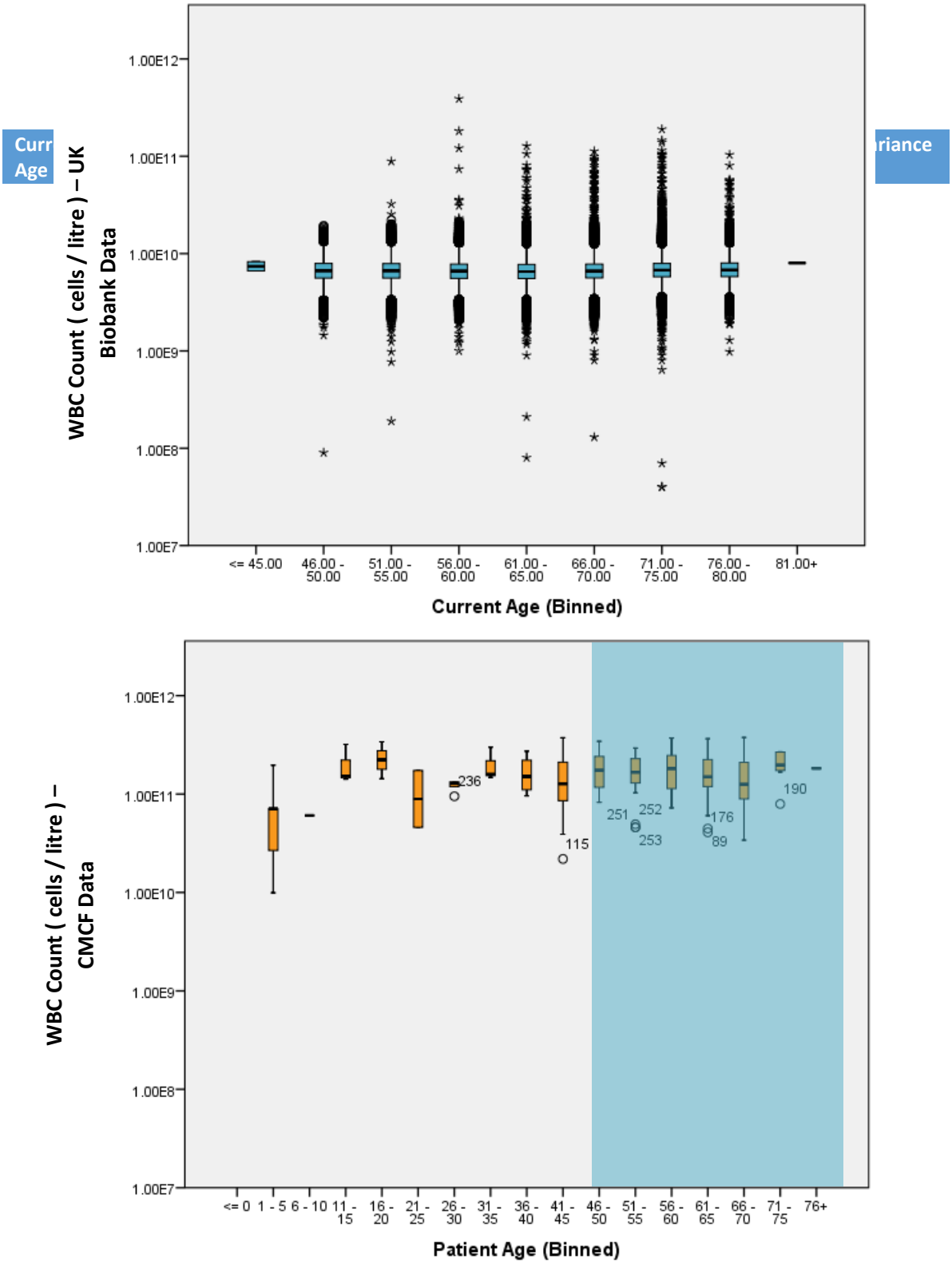


FIGURE 5.9: Stratified box-plot of WBC count per litre into binned age groups (5 years)

UK Biobank data: Top, blue

CMCF data: Bottom, orange

Note the more varied spread of data in the CMCF chart, and where there is insufficient data for a box plot (age 6 to 10 for example). The **blue** section indicates where the age groups of UK Biobank are, within the CMCF age ranges.

<45	2	$7.44x10^{9}$	$7.44x10^{9}$	$6.63x10^{9}$	$8.24x10^{9}$	$1.61x10^{9}$	$1.14x10^{9}$	$1.30x10^{18}$
46 – 50	26,942	$6.89x10^{9}$	$6.67x10^{9}$	$9.00x10^{7}$	$1.93x10^{10}$	$1.92x10^{10}$	$1.83x10^{9}$	$3.35x10^{18}$
51 – 55	59,631	$6.88x10^{9}$	$6.65x10^{9}$	$1.90x10^{8}$	$8.87x10^{10}$	$8.85x10^{10}$	$1.88x10^{9}$	$3.53x10^{18}$
56 – 60	68,698	$6.83x10^{9}$	$6.60x10^{9}$	$1.00x10^{9}$	$3.90x10^{11}$	$3.89x10^{10}$	$2.48x10^{9}$	$6.13x10^{18}$
61 – 65	80,089	$6.78x10^{9}$	$6.55x10^{9}$	$8.00x10^{7}$	$1.28x10^{11}$	$1.27x10^{11}$	$1.98x10^{9}$	$3.91x10^{18}$
66 – 70	111,857	$6.85x10^{9}$	$6.60x10^{9}$	$1.30x10^{8}$	$1.13x10^{11}$	$1.13x10^{11}$	$2.02x10^{9}$	$4.09x10^{18}$
71 – 75	98,530	$7.00x10^{9}$	$6.77x10^{9}$	$4.00x10^{7}$	$1.90x10^{11}$	$1.89x10^{11}$	$2.30x10^{9}$	$5.29x10^{18}$
76 – 80	32,525	$7.04x10^{9}$	$6.80x10^{9}$	$9.80x10^{8}$	$1.04x10^{11}$	$1.02x10^{11}$	$2.06x10^{9}$	$4.24x10^{18}$
81+	1	$8.00x10^{9}$	$8.00x10^{9}$	$8.00x10^{9}$	$8.00x10^{9}$	0	-	-

Patient Age	Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
<0	0	-	-	-	-	-	-	-
1 – 5	5	$7.50x10^{10}$	$7.10x10^{10}$	$9.91x10^{9}$	$1.96x10^{11}$	$1.86x10^{11}$	$7.30x10^{10}$	$5.33x10^{21}$
6 – 10	1	$6.06x10^{10}$	$6.06x10^{10}$	-	-	-	-	-
11 – 15	3	$2.05x10^{11}$	$1.53x10^{11}$	$1.42x10^{11}$	$3.20x10^{11}$	$1.78x10^{11}$	$9.98x10^{10}$	$9.96 x10^{21}$
16 – 20	4	$2.32x10^{11}$	$2.23x10^{11}$	$1.43x10^{11}$	$3.40x10^{11}$	$1.96x10^{11}$	$8.09x10^{10}$	$6.55x10^{21}$
21 – 25	2	$1.10x10^{11}$	$1.10x10^{11}$	$4.58x10^{10}$	$1.73x10^{11}$	$1.27x10^{11}$	$9.01x10^{10}$	$8.13x10^{21}$
26 – 30	5	$1.22x10^{11}$	$1.28x10^{11}$	$9.50x10^{10}$	$1.34x10^{11}$	$3.91x10^{10}$	$1.61x10^{10}$	$2.61x10^{20}$
31 – 35	3	$2.02x10^{11}$	$1.59x10^{11}$	$1.48x10^{10}$	$2.99x10^{11}$	$1.51x10^{11}$	$8.41x10^{10}$	$7.07x10^{21}$
36 – 40	4	$1.69x10^{11}$	$1.53x10^{11}$	$9.57x10^{10}$	$2.72x10^{11}$	$1.77x10^{11}$	$7.72x10^{10}$	$5.96x10^{21}$
41 – 45	27	$1.58x10^{11}$	$1.27x10^{11}$	$2.19x10^{10}$	$3.73x10^{11}$	$3.51x10^{11}$	$9.08x10^{10}$	$8.25x10^{21}$
46 – 50	11	$1.89x10^{11}$	$1.75x10^{11}$	$8.26x10^{10}$	$3.43x10^{11}$	$2.61x10^{11}$	$9.09x10^{10}$	$8.26x10^{21}$
51 – 55	30	$1.73x10^{11}$	$1.67x10^{11}$	$4.58x10^{10}$	$2.92x10^{11}$	$2.48x10^{11}$	$6.91x10^{10}$	$4.78x10^{21}$
56 – 60	43	$1.88x10^{11}$	$1.82x10^{11}$	$7.23x10^{10}$	$3.71x10^{11}$	$2.99x10^{11}$	$8.54x10^{10}$	$7.29x10^{21}$
61 – 65	64	$1.72x10^{11}$	$1.50x10^{11}$	$4.08x10^{10}$	$3.64x10^{11}$	$3.24x10^{11}$	$7.90x10^{10}$	$6.25x10^{21}$
66 – 70	68	$1.60x10^{11}$	$1.26x10^{11}$	$3.40x10^{10}$	$3.77x10^{11}$	$3.42x10^{11}$	$9.11x10^{10}$	$8.31x10^{21}$
71 – 75	7	$2.04x10^{11}$	$1.97x10^{11}$	$7.92x10^{10}$	$2.68x10^{11}$	$1.89x10^{11}$	$7.03x10^{10}$	$4.94x10^{21}$
76+	2	$1.82x10^{11}$	$1.82x10^{11}$	$1.82x10^{11}$	$1.82x10^{11}$	$6.80x10^{8}$	$4.81x10^{10}$	$2.31x10^{17}$

TABLE 5.2: WBC per litre for UK Biobank participants stratified into binned age groups of 5 years

UK Biobank data: Top, blue

CMCF data: Bottom, orange

5.4.3 Variation as a function of Participant Gender

UK Biobank contains 229,182 male participants and 273,467 female participants (as of April 2016).

The range of white blood cell counts for male participants is between $8.00x10^{7}$ to $1.90x10^{11}$ cells per litre, and for male participants is between $4.00x10^{7}$ and $3.90x10^{11}$ cells per litre.

White blood cell count per litre relative to gender has been plotted in **Figure 5.10**. White blood cell count per litre relative to gender, for both UK Biobank and CMCF datasets has been plotted in **Figure 5.11.**

The median WBC per litre is higher for male participants than female patients, for both UKB and CMCF (**Table 5.3**). However, this may be due to the male population of UKB having a larger average weight than the female population (84.3 kg for men and 69.1 kg for women) and therefore a greater number of white blood cells per person on average.

There is an increased range (and therefore overall variation from a manufacturing perspective as a raw material) for female participants over male participants, although by visual inspection of the box and whisker plot this may be due to a small number of extreme cases. In fact, when a random smaller sample of the UKB population was taken (20%) the mean and median was the same, but the range, standard deviation and variance was higher for male participants. This demonstrates how sensitive these tests are to the extreme values / outliers and how dependent the spread of the data becomes due to these 'outliers'. As a result it becomes difficult to compare extreme ranges between UKB and the CMCF as anything other than a complete dataset. It also identifies that a complete picture of the variation at the CMCF may be difficult to obtain with such a small sample of the population (as taking a sample of UKB gives a completely different perspective on the variation, due to the dependent nature on these random, individual cases at the extremes). For either the whole population or the 20% randomized sample

however, both the median and the distributions are significantly different between genders ($p < 0.05$) indicating that genders are distinct subpopulations with respect of white blood cells, and that variation may be different between the two. For the whole population of UKB, the variance between participants is higher for female participants – meaning the product to product variation in terms of cell number will be higher – but the range of male participant's white blood cell counts are higher – meaning the process will have to accommodate a wider spread of white blood cell counts for male sourced starting material over female sourced material. If a similar result is found for HSCs and other stem cells this indicates that the gender of the donor will have

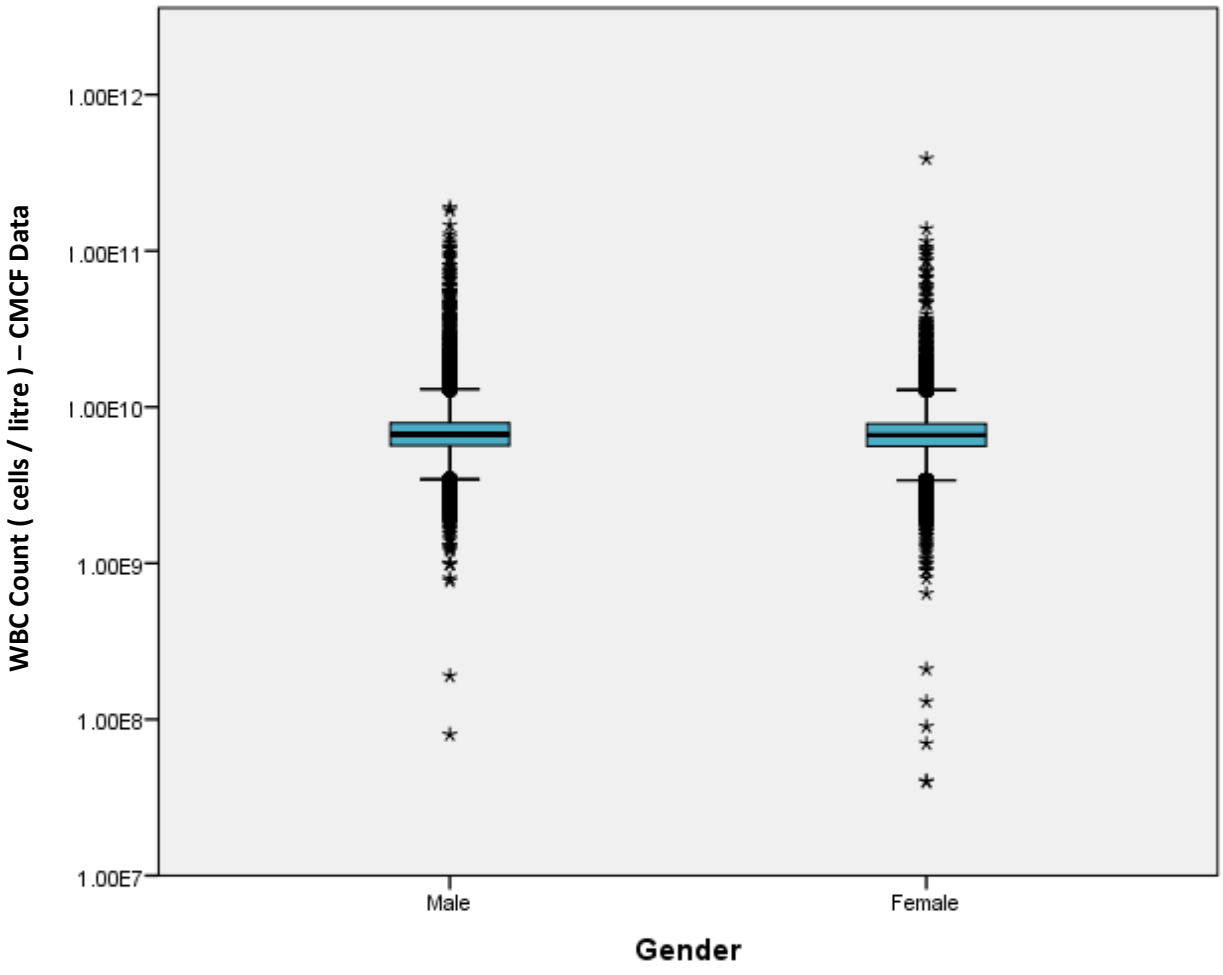


FIGURE 5.10: Box and Whisker plot of UK Biobank white blood cell count per litre per individual against their respective gender.

significant effects on manufacturing process design.

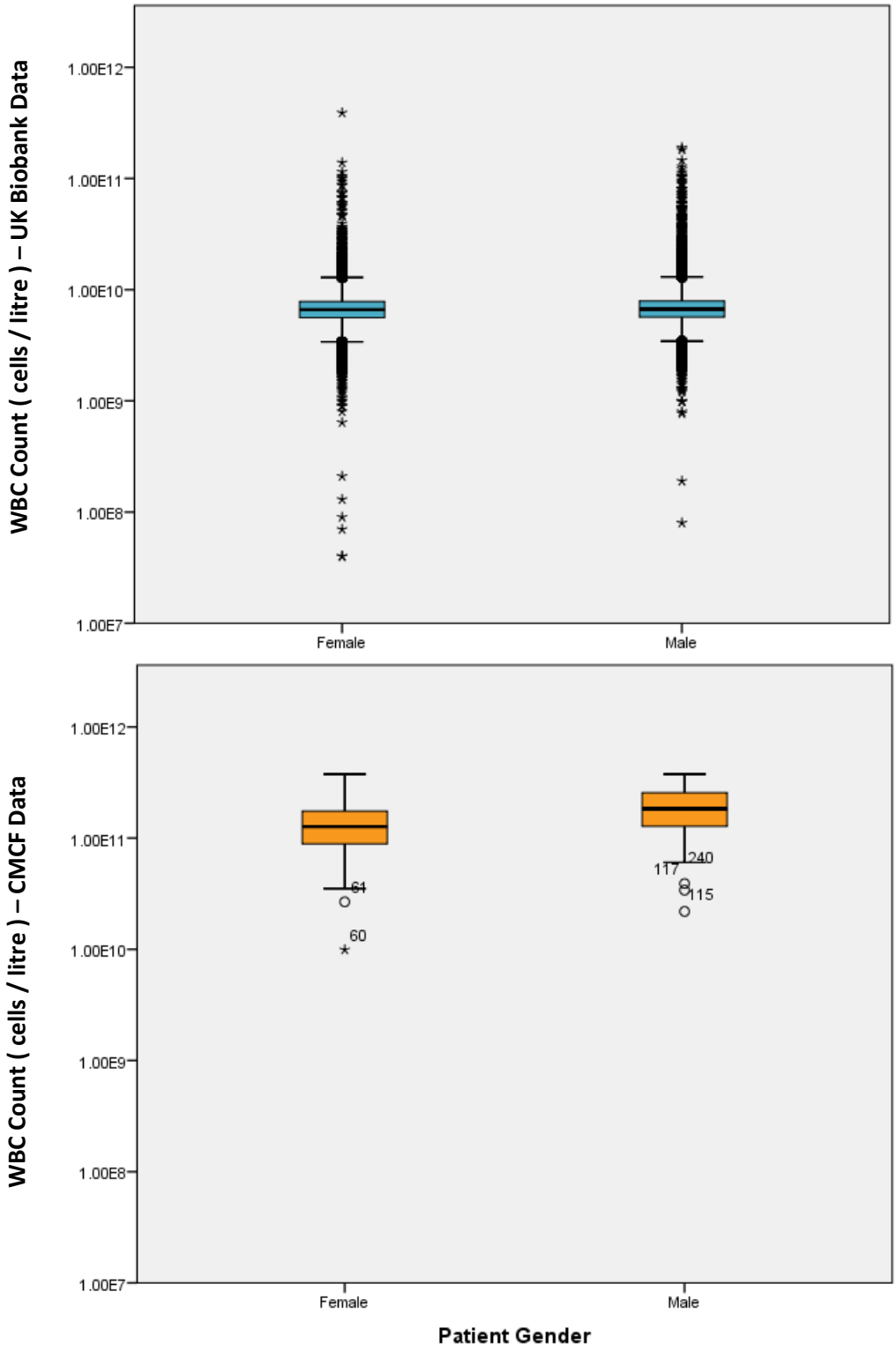


FIGURE 5.11: Stratified box-plot of WBC Count / L into gender

UK Biobank data: Top, blue

CMCF data: Bottom, orange

	Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
Male	219,317	6.92×10^9	6.69×10^9	8.00×10^7	1.90×10^{11}	1.89×10^{11}	2.19×10^9	4.81×10^{18}
Female	258,958	6.86×10^9	6.62×10^9	4.00×10^7	3.90×10^{11}	3.90×10^{11}	2.06×10^9	4.25×10^{18}

	Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
Male	144	1.93×10^{11}	1.83×10^{11}	2.19×10^{10}	3.76×10^{11}	3.54×10^{11}	8.59×10^{10}	7.38×10^{21}
Female	134	1.43×10^{11}	1.27×10^{11}	9.91×10^{9}	3.77×10^{11}	3.67×10^{11}	7.42×10^{10}	5.50×10^{21}

TABLE 5.3: WBC per litre for UK Biobank participants stratified into gender

UK Biobank data: Top, blue

CMCF data: Bottom, orange

As before, the difference between the number of male and female participants of UK Biobank shown here and those shown in **Table 5.0** are missing data points where participants have not given a white blood cell count, or one has not been recorded. So although 229,182 male and 273,467 female participants were recruited, white blood cell count was only recorded for 219,317 male and 258,958 female donors.

5.4.4 Variation as a function of Participant Weight

Weight is a key physical metric for determining biological measurements such as blood. As a participant's physical mass increases, so will the volume of blood required to supply the body with nutrients. Whether the circulatory system of a given participant can cope with this volume of blood and physical mass is another matter (obesity and physical fitness). Weight is a commonly used metric in medicine, used to determine pharmaceutical dose for example.

The average weight of a given participant in the UK Biobank is 78.1 kg (mean) and 76.4 kg (median) (n = 499,874) (**Table 5.4**).

Table 5.5 shows the white blood cell count for binned weight categories, for both UKB and CMCF datasets. **Figure 5.12** is a box and whisker plot of white blood cell count / litre against corresponding weight, where weight has been categorised into 10 kilogram bins. **Figure 5.13** is a box and whisker plot of white blood cell count / litre against weight for both UKB and CMCF datasets. The blue section identifies comparable weights, and that the CMCF contains paediatric or very low weight patients that UK Biobank doesn't.

Differences between weight categories was determined by independent samples median test and independent sample Kruskal-Wallis test. In both the overall population and a representative, randomly chosen sample of the population (20%) these groups are significantly different in terms of both median white blood cell count and distribution of cell counts ($p < 0.05$ for both). This is unsurprising as the median cell count increases with participant weight.

This is also the case for CMCF data ($p < 0.05$ for both median and distribution).

The spread of the data does not appear to be correlated to weight. Variation and spread in the data tends to tail off towards the heavier weights; this is likely because of the variety of body types that can be encapsulated within lighter weights (shorter and heavier versus taller and thinner for example) compared to the heavier weights which are likely to be more limiting in

body shape (and therefore variation in the amount of blood required to supply) (**Figure 5.12** and **Figure 5.13**).

This effect may well be dependent on the significant clustering around the median value, where most the data occurs, and further work may benefit from a full spectrum of weight categories with an equal and representative number of cases.

Figure 5.12 is particular is interesting for a variation and outliers perspective, as the median white blood cell count appears to increase with patient size, and the interquartile ranges appear to be comparable between weight categories, but the outliers and the far outliers (the circles and crosses) have considerable impact on the expected spread of white blood cell counts for a given starting material for each category.

For a given weight category, such as the modal category 70.1 kg to 80.0 kg, the number of 'outliers' can be calculated. For data points to be considered outliers in the box and whisker plot, they need to be outside of 1.5 times the interquartile range (the width of the 'box') above the 25^{th} quartile or the 75^{th} quartile (the brackets of the box and whisker plot). As a result, for the 70.1 kg to 80.0 kg category there are 1,257 outliers, which account for 1.05% of the total category. Without these outliers the range of cell count within this category is between $2.29x10^{9}$ cells / litre and $1.12x10^{10}$ cells / litre (as opposed to $4.00x10^{7}$ cells / litre to $1.07x10^{11}$ cells / litre including these outliers). If it could be identified whether there are factors that contribute to these 'outliers' or whether they are part of the naturally occurring biological variation is an important result as it would determine whether a manufacturing process can work within the boundaries of a box and whisker plot, or whether these outliers must be considered. These outliers are a small proportion of the overall population and cannot be easily discounted (these are 1,257 people) but they have a strong influence on the extreme values of the data which is the specific focus of this research.

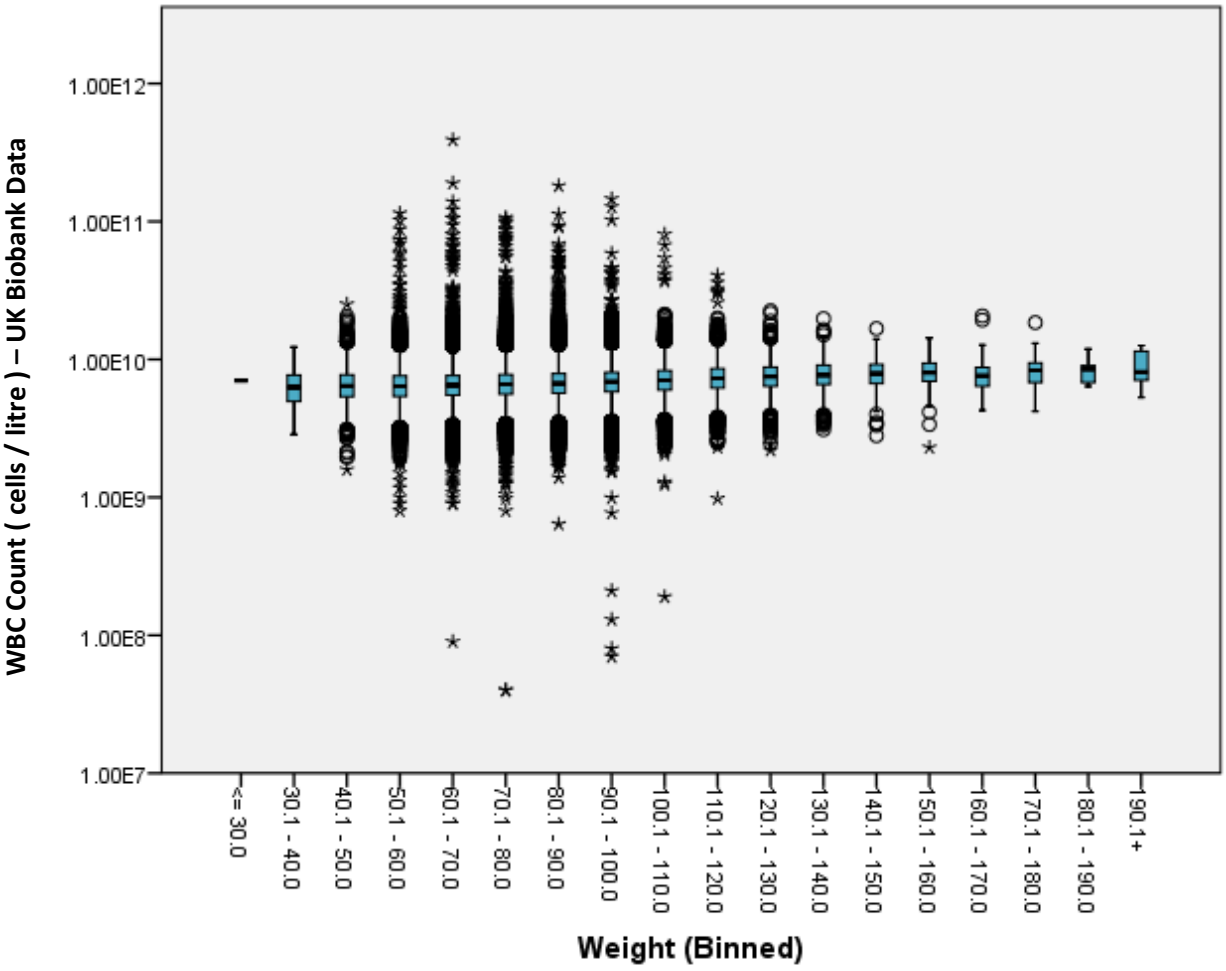


FIGURE 5.12: Box and Whisker plot of calculated WBC count per litre per individual plotted against their respective weight in kilograms (binned into 10kg intervals)

	Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
Weight (kg)	499,874	78.1	76.4	30.0	197.7	167.7	15.9	254.3

TABLE 5.4: Summary of Participant Weight (kg)

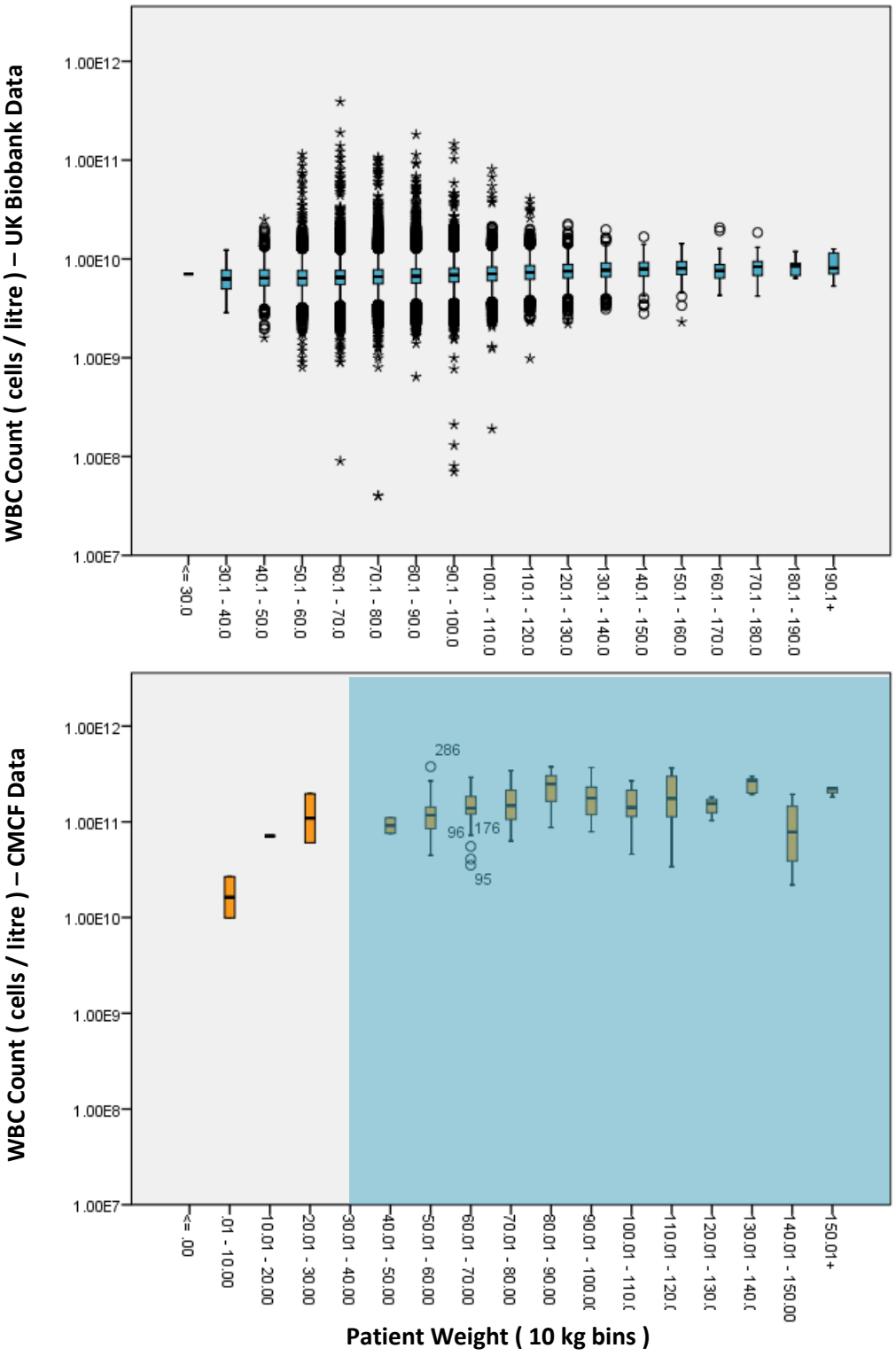


FIGURE 5.13: Stratified box-plot of WBC Count / L into binned weight groups (10 kg)

UK Biobank data: Top, blue

CMCF data: Bottom, orange

The **blue** section indicates the corresponding UK biobank weight range.

Weight (kg)	Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
<30	1	$7.03x10^{9}$	$7.03x10^{9}$	$7.03x10^{9}$	$7.03x10^{9}$	0	-	-
30.1 – 40.0	111	$6.50x10^{9}$	$6.28x10^{9}$	$2.86x10^{9}$	$1.23x10^{10}$	$9.46x10^{9}$	$1.90x10^{9}$	$3.62x10^{18}$
40.1 – 50.0	5,461	$6.71x10^{9}$	$6.42x10^{9}$	$1.59x10^{9}$	$2.52x10^{10}$	$2.36x10^{10}$	$1.94x10^{9}$	$3.77x10^{18}$
50.1 – 60.0	48,773	$6.64x10^{9}$	$6.40x10^{9}$	$8.00x10^{8}$	$1.25x10^{11}$	$1.14x10^{11}$	$2.05x10^{9}$	$4.21x10^{18}$
60.1 – 70.0	106,876	$6.72x10^{9}$	$6.49x10^{9}$	$9.00x10^{7}$	$3.90x10^{11}$	$3.90x10^{11}$	$2.42x10^{9}$	$5.84x10^{18}$
70.1 – 80.0	119,627	$6.82x10^{9}$	$6.60x10^{9}$	$4.00x10^{7}$	$1.07x10^{11}$	$1.07x10^{11}$	$1.98x10^{9}$	$3.93x10^{18}$
80.1 – 90.0	97,825	$6.94x10^{9}$	$6.70x10^{9}$	$6.40x10^{8}$	$1.81x10^{11}$	$1.81x10^{11}$	$2.04x10^{9}$	$4.15x10^{18}$
90.1 – 100.0	55,698	$7.08x10^{9}$	$6.87x10^{9}$	$7.00x10^{7}$	$1.46x10^{11}$	$1.46x10^{11}$	$2.06x10^{9}$	$4.23x10^{18}$
100.1 – 110.0	25,480	$7.27x10^{9}$	$7.06x10^{9}$	$1.90x10^{8}$	$8.08x10^{10}$	$8.06x10^{10}$	$1.97x10^{9}$	$3.89x10^{18}$
110.1 – 120.0	10,166	$7.50x10^{9}$	$7.30x10^{9}$	$9.80x10^{8}$	$4.06x10^{10}$	$3.97x10^{10}$	$1.96x10^{9}$	$3.82x10^{18}$
120.1 – 130.0	4,018	$7.69x10^{9}$	$7.50x10^{9}$	$2.20x10^{9}$	$2.25x10^{10}$	$2.03x10^{10}$	$1.91x10^{9}$	$3.63x10^{18}$
130.1 – 140.0	1,538	$7.93x10^{9}$	$7.71x10^{9}$	$3.10x10^{9}$	$1.97x10^{10}$	$1.66x10^{10}$	$1.97x10^{9}$	$3.89x10^{18}$
140.1 – 150.0	658	$7.98x10^{9}$	$7.90x10^{9}$	$2.80x10^{9}$	$1.67x10^{10}$	$1.39x10^{10}$	$1.82x10^{9}$	$3.32x10^{18}$
150.1 – 160.0	253	$8.23x10^{9}$	$8.01x10^{9}$	$2.30x10^{9}$	$1.43x10^{10}$	$1.20x10^{10}$	$1.89x10^{9}$	$3.56x10^{18}$
160.1 – 170.0	113	$7.91x10^{9}$	$7.61x10^{9}$	$4.28x10^{9}$	$2.07x10^{10}$	$1.64x10^{10}$	$2.47x10^{9}$	$6.12x10^{18}$
170.1 – 180.0	58	$8.31x10^{9}$	$8.33x10^{9}$	$4.20x10^{9}$	$1.85x10^{10}$	$1.43x10^{10}$	$2.29x10^{9}$	$5.23x10^{18}$
180.1 – 190.0	13	$8.34x10^{9}$	$8.51x10^{9}$	$6.40x10^{9}$	$1.19x10^{10}$	$5.50x10^{9}$	$1.66x10^{9}$	$2.76x10^{18}$
190.1+	7	$9.03x10^{9}$	$8.10x10^{9}$	$5.20x10^{9}$	$1.26x10^{10}$	$7.30x10^{9}$	$2.86x10^{9}$	$8.16x10^{18}$

Weight (kg)	Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
<0	0	-	-	-	-	-	-	-
.01 – 10.00	2	$1.83x10^{10}$	$1.83x10^{10}$	$9.91x10^{9}$	$2.67x10^{10}$	$1.68x10^{10}$	$1.19x10^{10}$	$1.41x10^{20}$
10.01 -20.00	2	$7.10x10^{10}$	$7.10x10^{10}$	$7.10x10^{10}$	$7.10x10^{10}$	-	-	-
20.01 – 30.00	2	$1.28x10^{11}$	$1.28x10^{11}$	$6.06x10^{10}$	$1.96x10^{11}$	$1.36x10^{11}$	$9.61x10^{10}$	$9.23x10^{21}$
30.01 – 40.00	0	-	-	-	-	-	-	-
40.01 – 50.00	2	$9.30x10^{10}$	$9.30x10^{10}$	$7.60x10^{10}$	$1.10x10^{11}$	$3.41x10^{10}$	$2.41x10^{10}$	$5.82x10^{20}$
50.01 – 60.00	48	$1.27x10^{11}$	$1.17x10^{11}$	$4.46x10^{10}$	$3.77x10^{11}$	$3.32x10^{11}$	$6.20x10^{10}$	$3.84x10^{21}$
60.01 – 70.00	26	$1.47x10^{11}$	$1.39x10^{11}$	$3.53x10^{10}$	$2.92x10^{11}$	$2.57x10^{11}$	$6.67x10^{10}$	$4.45x10^{21}$
70.01 – 80.00	59	$1.62x10^{11}$	$1.48x10^{11}$	$6.30x10^{10}$	$3.43x10^{11}$	$2.80x10^{11}$	$7.21x10^{10}$	$5.19x10^{21}$
80.01 – 90.00	41	$2.35x10^{11}$	$2.48x10^{11}$	$8.73x10^{10}$	$3.76x10^{11}$	$2.89x10^{11}$	$8.70x10^{10}$	$7.57x10^{21}$
90.01 – 100.00	36	$1.85x10^{11}$	$1.77x10^{11}$	$7.88x10^{10}$	$3.70x10^{11}$	$2.91x10^{11}$	$7.95x10^{10}$	$6.32x10^{21}$
100.01 – 110.00	20	$1.56x10^{11}$	$1.41x10^{11}$	$4.58x10^{10}$	$2.68x10^{11}$	$2.22x10^{11}$	$6.82x10^{10}$	$4.65x10^{21}$
110.01 – 120.00	24	$1.99x10^{11}$	$1.76x10^{11}$	$3.40x10^{10}$	$3.64x10^{11}$	$3.30x10^{11}$	$1.03x10^{11}$	$1.06x10^{22}$
120.01 – 130.00	4	$1.49x10^{11}$	$1.56x10^{11}$	$1.03x10^{11}$	$1.92x10^{11}$	$7.85x10^{10}$	$3.33x10^{10}$	$1.11x10^{21}$
130.01 – 140.00	7	$2.45x10^{11}$	$2.69x10^{11}$	$1.92x10^{11}$	$2.96x10^{11}$	$1.04x10^{11}$	$4.62x10^{10}$	$2.13x10^{21}$
140.01 – 150.00	6	$9.27x10^{10}$	$7.83x10^{10}$	$2.19x10^{10}$	$1.94x10^{11}$	$1.72x10^{11}$	$6.53x10^{10}$	$4.27x10^{21}$
150.01+	3	$2.09x10^{11}$	$2.23x10^{11}$	$1.82x10^{11}$	$2.23x10^{11}$	$4.13x10^{10}$	$2.38x10^{10}$	$5.68x10^{20}$

TABLE 5.5: WBC / L for UK Biobank participants stratified into binned weight groups of 10 kg

UK Biobank data: Top, blue

CMCF data: Bottom, orange

5.4.5 Variation as a function of Geography (Assessment Centre)

Figure 5.14 is a map of the United Kingdom, indicating the locations of the assessment centres used in the UK Biobank study, with the central processing facility in Manchester highlighted in blue.

Assessment centre and attending date were included in the original proposal to examine whether biological variation as a function of white blood cells was affected by the geographical location of different subpopulations (**Table 5.6**) – are coastal towns less variable than industrial cities for example. These different sub-populations are likely to contain localised ratios of particular social class or ethnic groups – which is one of the reasons why blood banks are set up in particular areas of the UK.

Figure 5.15 is a box and whisker plot illustrating the spread of white blood cells per litre between separate locations categorised by assessment centre. According to independent samples median test (H (20) = 1,893.425, $p < .05$) and independent samples Kruskal-Wallis test (H (20) = 2,702.357, $p < .05$), the median cell count and distributions of cell counts are significantly different between centres, so it is reasonable to assume there is a geographically based reason for this difference.

It stands to reason that there is a cause for these differences in median or spread of cell counts as a function of geographical location. This could be local environmental conditions such as quality of life or air or water quality – although air quality is unlikely to vary enough between cities in the UK to influence white blood cell count as it might if you compared the effect of air quality in London versus Beijing when compared.

An alternative reason could be procedural differences at the collection centres, or the transit time and methodology – although UKB has put considerable effort into ensuring the equivalency of process and protocols between centres, and analyses samples in a centralised location.

Comparing the variation between geographical centres (**Figure 5.15**) allows for further illustration of the compounding effect of patients with 'extreme' cell counts. Taking the most populated category (Leeds, n = 44,215) as an example, and by counting those values that exceed 1.5 times the interquartile range outside of the 25^{th} and 75^{th} quartile, 5.5% (2,330) of this sub-population could be identified as outliers. Without these outliers the range of cell counts within the Leeds catchment area would be between $3.45x10^{9}$ cells / litre and $9.90x10^{9}$ cells / litre but including these outliers (but excluding the one zero value) the range of cell counts is between $8.00x10^{8}$ cells / litre and $8.68x10^{10}$ cells / litre. The question remains whether these 'outliers' are part of the natural background variation, the common cause variation, or whether these 'outliers' are as a result of a particular influence or factor (special cause variation) and in which case, this factor or factors will need to be identified. Understanding these outliers will be critical for understanding the behaviour of the starting material and the process.

FIGURE 5.14: Geographical Locations of the UK Biobank assessment centres (red) with the central processing facility in Manchester highlighted in blue. (*Image courtesy of Google Maps under Fair Use Policy*)

		Count
UK Biobank Assessment Centre	Stockport (Pilot)	3,798
	Manchester	13,941
	Oxford	14,063
	Cardiff	17,884
	Glasgow	18,653
	Edinburgh	17,202
	Stoke	19,440
	Reading	29,422
	Bury	28,321
	Newcastle	37,009
	Leeds	44,215
	Bristol	43,017
	Barts	12,584
	Nottingham	33,883
	Sheffield	30,397
	Liverpool	32,825
	Middlesborough	21,289
	Houndslow	28,880
	Croydon	27,388
	Birmingham	25,506
	Swansea	2,283
	Wrexham	649
	Cheadle (Revisit)	0

TABLE 5.6: Participants per UK Biobank Assessment Centres

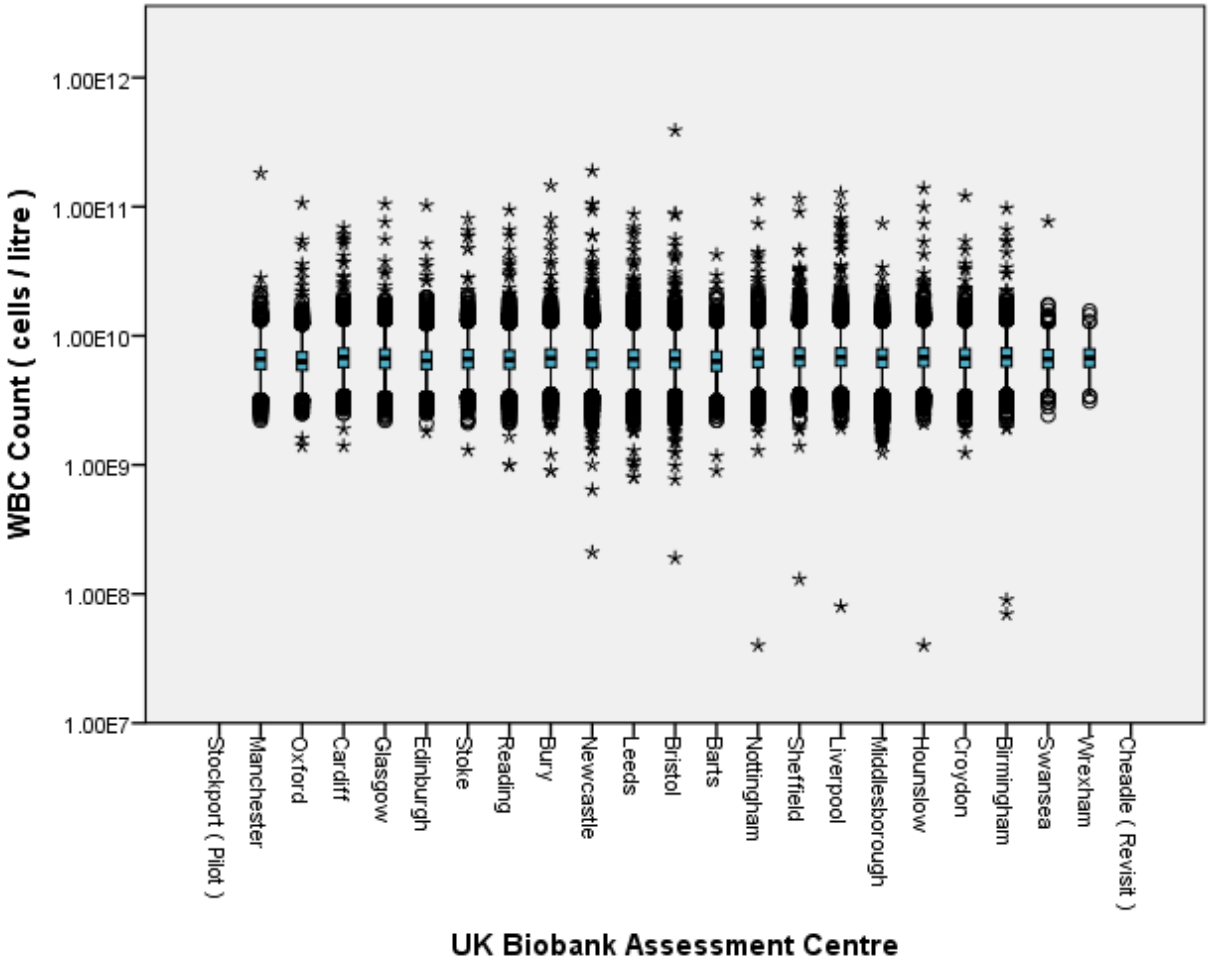


FIGURE 5.15: Box and Whisker plot of WBC count per litre per individual, against the location of the assessment centre they attended

5.4.6 Variation as a function of Female Specific Measurands

Menopause can be defined by a decrease in hormone production that results in the cessation of menstrual periods and being unable to conceive children. Considering the change in body chemistry, particularly hormones, it is reasonable to explore the effects of this change on white blood cell counts and any subsequent variation between those who have passed the climacteric and those who have not (**Table 5.7**).

Table 5.8 compares the white blood cell counts per litre for each participant against whether or not this change has happened – for over 60% of the female population in this sample this has happened.

Figure 5.16 is a box and whisker plot of white blood cell count per litre for each participant grouped into the categories in **Table 5.7**: yes, no, not sure (hysterectomy), not sure (other) and prefer not to answer. An independent-samples median test and an independent samples Kruskal-Wallis test determined that these groups are significantly different from one another with respect to median WBC / L ($H(4) = 865.633$, $p < .05$) and WBC distribution ($H(4) = 1{,}225.230$, $p < .05$).

Median and mean white blood cell count per litre is higher in those participants who have not passed the climacteric, but the variance between cell counts appears to be higher for those who have. These differences are perhaps not dramatic enough to warrant donor / patient stratification according to menopause status.

Another potential variable is pregnancy: again, a female specific measurand but has the potential to influence cell numbers and quality (**Table 5.9**).

Table 5.10 compares the white blood cell counts per litre for each participant, against whether they are pregnant or not (or not sure). An extremely small proportion of the population were pregnant at the time of assessment (0.05%, n = 150).

An independent-samples median test and an independent samples Kruskal-Wallis test determined that these groups are significantly different from one another with respect to median WBC / L ($H(2) = 62.261$, $p < .05$) and WBC distribution ($H(2) = 124.762$, $p < .05$). Pairwise comparisons were performed using Dunn's (1964) procedure with a Bonferroni correction for multiple comparisons. Adjusted p-values are presented. This post hoc analysis revealed statistically significant differences in white blood cell count per litre between all groups except no / unsure ($p = 0.615$) when comparing median cell count. All other comparisons between groups were statistically significantly different ($p < 0.05$). Given this limited data pool, it appears that this state change has an effect on both the median (8.70×10^9 cells / litre for pregnant and 6.62×10^9 cells / litre for not pregnant) and the distribution of white blood cells. The variance appears to be similar, but with a much larger range of cell counts for participants who were not pregnant (3.90×10^{11} cells / litre) compared to those who were (9.78×10^9 cells / litre).

The range of white blood cells per litre is between 4.00×10^7 cells / L and 3.90×10^{11} cells / litre for participants who are not pregnant and between 4.52×10^9 cells / L and 1.43×10^{10} cells / litre for pregnant participants. Without outliers (14 for pregnant, 14,088 for non-pregnant) this is reduced to 5.32×10^9 cells / litre to 1.23×10^{10} cells / litre for non-pregnant and 3.38×10^9 cells / litre to 1.00×10^{10} cells / litre for pregnant participants.

		Count
Menopause	Yes	165,441
	No	64,097
	Not sure – had a hysterectomy	31,187
	Not sure – other	11,734
	Prefer not to answer	539

TABLE 5.7: Menopause Status

Menopause	Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
Prefer not to answer	485	$7.28x10^{9}$	$7.00x10^{9}$	$2.95x10^{9}$	$2.22x10^{10}$	$1.93x10^{10}$	$2.19x10^{9}$	$4.80x10^{18}$
No	60,605	$7.02x10^{9}$	$6.80x10^{9}$	$9.00x10^{7}$	$2.54x10^{10}$	$2.53x10^{10}$	$1.83x10^{9}$	$3.35x10^{18}$
Yes	156,926	$6.76x10^{9}$	$6.53x10^{9}$	0	$1.39x10^{11}$	$1.39x10^{11}$	$1.96x10^{9}$	$3.84x10^{18}$
Not sure – had a hysterectomy	29,587	$6.98x10^{9}$	$6.76x10^{9}$	$1.08x10^{9}$	$4.66x10^{10}$	$4.55x10^{10}$	$1.86x10^{9}$	$3.46x10^{18}$
Not sure - other	11,074	$6.95x10^{9}$	$6.68x10^{9}$	$1.30x10^{9}$	$3.90x10^{11}$	$3.88x10^{11}$	$4.10x10^{9}$	$1.68x10^{18}$

TABLE 5.8: White Blood Cell Count against Menopause Status

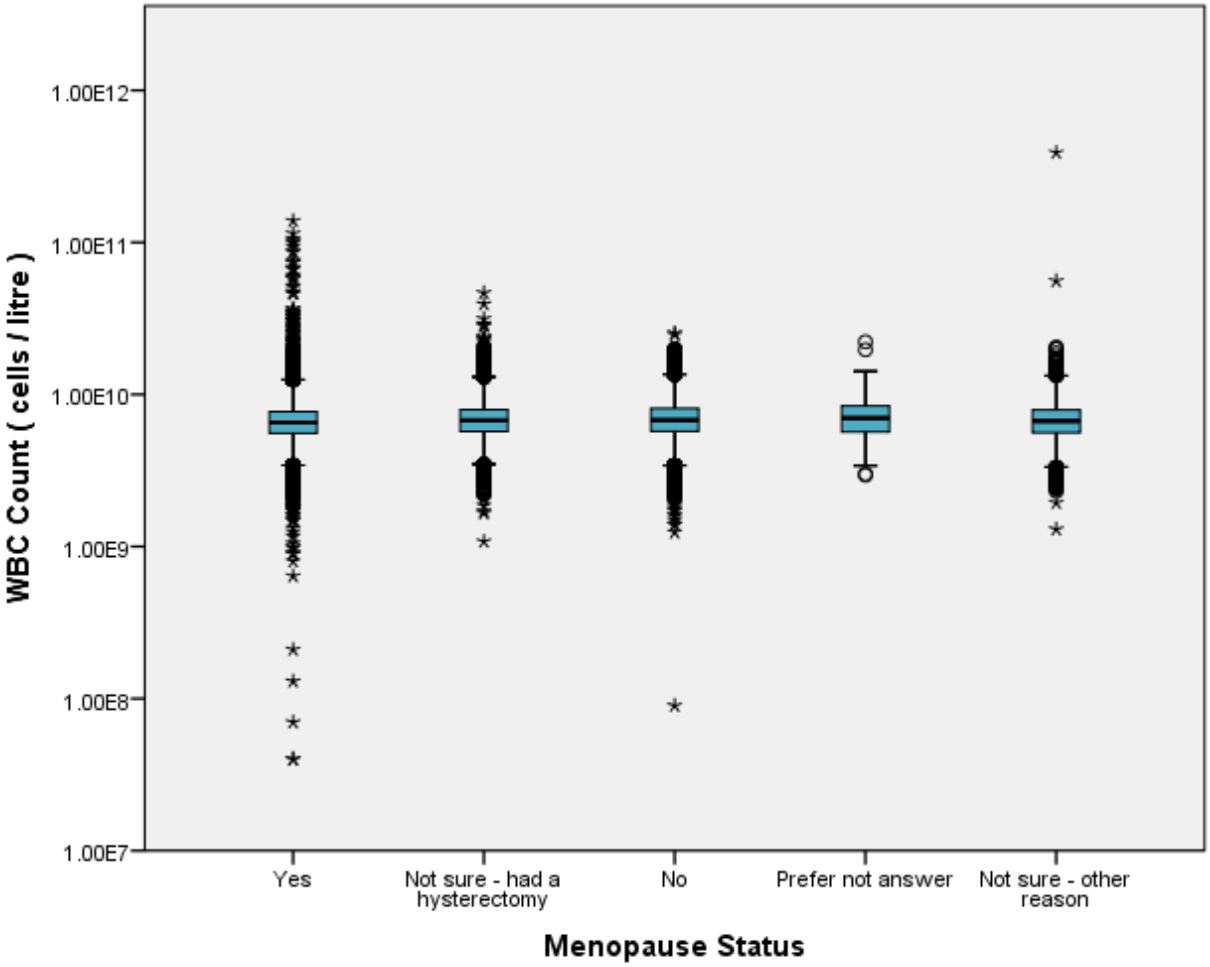


FIGURE 5.16: Box and whisker plot comparing whether female participants have had menopause with the white blood cell count per litre pre individual

		Count
Pregnant	Yes	150
	No	272,259
	Unsure	222

TABLE 5.9: Distribution of participants who were pregnant

Pregnant	Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
No	258,261	$6.85x10^{9}$	$6.62x10^{9}$	$4.00x10^{7}$	$3.90x10^{11}$	$3.90x10^{11}$	$2.06x10^{9}$	$4.25x10^{18}$
Yes	137	$8.78x10^{9}$	$8.70x10^{9}$	$4.52x10^{9}$	$1.43x10^{10}$	$9.78x10^{9}$	$2.09x10^{9}$	$4.36x101^{8}$
Unsure	208	$7.20x10^{9}$	$7.00x10^{9}$	$3.40x10^{9}$	$1.57x10^{10}$	$1.23x10^{10}$	$1.99x10^{9}$	$3.98x10^{18}$

TABLE 5.10: White Blood Cell Count compared against Pregnancy

5.4.7 Variation as a function of Smoking

Smoking is known to decrease the level of circulating CD34+ progenitor cells in young women [208], an increased risk factor for haematopoietic stem cell transplantation [209] and a reduction in overall survival rate in allogeneic stem cell transplantation, to name a few, therefore whether or not the patient and/or donor smokes is a key factor in the quality and quantity of stem cells provided, and whether or not they have the desired clinical result. The problem is measuring cell count is not indicative of cell quality, which is a likely result of smoking.

226,096 participants of the UK Biobank either currently smoke or have previously smoked, compared to 273,616 participants who have never smoked (**Table 5.11**).

Table 5.12 illustrates the statistics surrounding white blood cell count per litre against smokers and non-smokers. An independent sample median test ($H(3) = 13{,}382.399$, $p < 0.05$) and an independent sample Kruskal-Wallis test ($H(3) = 21{,}599.988$, $p < 0.05$) have shown that the median and distribution of these groups are significantly different from one another. Pairwise comparison showed that each group was significantly different from one another ($p < .05$) In fact, the median and mean white blood cell counts increases between participants who have never smoked, to those who have previously smoked, to current smokers, indicating that smoking increases the number of circulated white blood cells – not necessarily a good thing considering their role within the body (6.48×10^9[never smoked], 6.64×10^9 [previous smoker], 7.88×10^9 [current smoker]). Furthermore, the variance – the spread between data-points – also increases from participants who have never smoked to those that have, suggesting that the variation in white blood cell count is much higher for those who smoke against those who do not (3.66×10^{18} [never smoked], 4.95×10^{18} [previous smoker], 5.66×10^{18} [current smoker]).

Figure 5.17 is a box and whisker plot of white blood cell count per litre for each participant, grouped into whether they have previously smoked, currently smoke, or have never smoked.

The category including those who have previously smoked appears to be the most variable in terms of range (likely due to two extreme values at the lower scale) - this is may be representative of the highly variable states that 'previous smoker' could fall into, in terms of when they stopped smoking and how many they used to smoke, and for how long.

		Count
Smoking Status	Current	52,991
	Previous	173,105
	Never	273,616
	Prefer not to answer	2,059

TABLE 5.11: Distribution of participants who smoked

Smoking	Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
Prefer not to answer	1,927	$7.14x10^{9}$	$6.86x10^{9}$	$2.89x10^{9}$	$7.36x10^{10}$	$7.08x10^{10}$	$2.43x10^{9}$	$5.91x10^{18}$
Never	260,398	$6.67x10^{9}$	$6.48x10^{9}$	$4.00x10^{7}$	$1.39x10^{11}$	$1.39x10^{11}$	$1.91x10^{9}$	$3.66x10^{18}$
Previous	165,305	$6.85x10^{9}$	$6.64x10^{9}$	$7.00x10^{7}$	$3.90x10^{11}$	$3.90x10^{11}$	$2.22x10^{9}$	$4.95x10^{18}$
Current	50,161	$8.10x10^{9}$	$7.88x10^{9}$	$9.00x10^{8}$	$1.81x10^{11}$	$1.81x10^{11}$	$2.38x10^{9}$	$5.66x10^{18}$

TABLE 5.12: WBC Count / L compared with smoking status

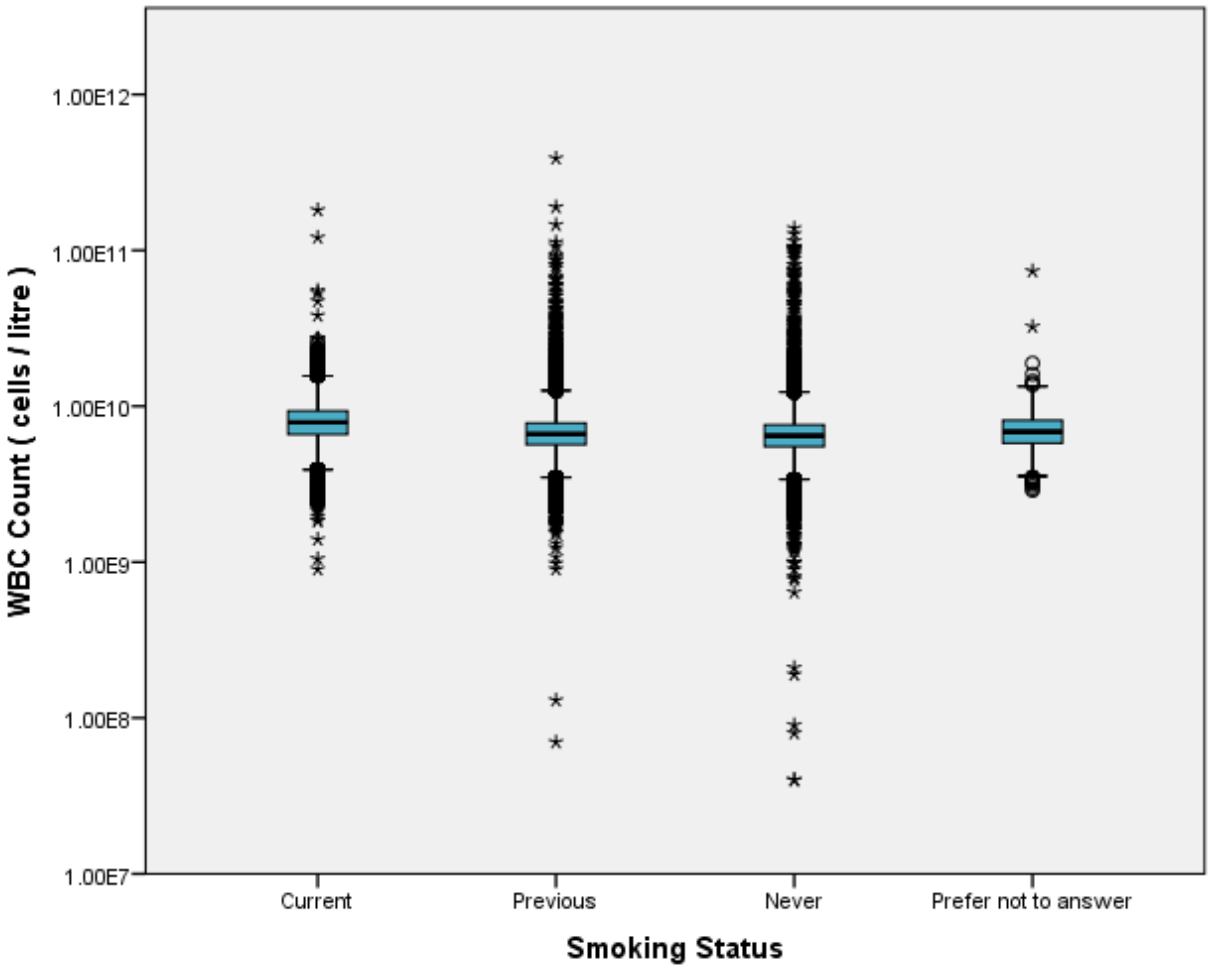


FIGURE 5.17: Box and whisker plot comparing the smoking status of participants against their corresponding white blood cell count per litre

5.4.8 Variation as a function of Alcohol

Alcohol consumption is prevalent in Western culture. In the UKB, only 8% of the population completely abstains from alcohol (**Table 5.13**). Recent research [210] has suggested that bone marrow stem cells are sensitive to by-products of alcohol and can cause permanent DNA damage, so it is important to identify whether this alcohol consumption is affecting the amount and variation of cell counts, and subsequent changes in cell quality.

Table 5.13 compares the frequency of alcohol consumption against corresponding participants white blood cell counts per litre. **Figure 5.18** is a box and whisker plot comparing the frequency of alcohol consumption against corresponding participants white blood cell counts per litre.

Alcohol consumption frequency is not a quantitative measure of alcohol consumption, and is as a result of a participant's opinion when answering the questionnaire – what constitutes consumption in units of alcohol and how does this differ between individuals? As such it is unlikely to highlight particular effects of alcohol consumption unless the effect was particularly significant.

Alcohol Frequency	Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
Prefer not to answer	549	$7.19x10^{9}$	$6.81 x10^{9}$	$1.59x10^{9}$	$1.90x10^{10}$	$1.74x10^{10}$	$2.11x10^{9}$	$4.46x10^{18}$
Daily / Almost daily	97,312	$6.77x10^{9}$	$6.55 x10^{9}$	$7.70x10^{8}$	$1.90x10^{11}$	$1.89x10^{11}$	$2.01x10^{9}$	$4.04x10^{18}$
3-4 / week	110,352	$6.73x10^{9}$	$6.50 x10^{9}$	$8.00x10^{7}$	$1.81x10^{11}$	$1.81x10^{11}$	$2.08x10^{9}$	$4.32x10^{18}$
1-2 / week	123,302	$6.88x10^{9}$	$6.66 x10^{9}$	$9.00x10^{7}$	$1.46x10^{11}$	$1.46x10^{11}$	$1.94x10^{9}$	$3.76x10^{18}$
1-3 / month	53,045	$7.01x10^{9}$	$6.78 x10^{9}$	$9.00x10^{7}$	$1.05x10^{11}$	$1.05x10^{11}$	$2.06x10^{9}$	$4.26x10^{18}$
Special occasion only	54,858	$7.12x10^{9}$	$6.90 x10^{9}$	$4.00x10^{7}$	$1.13x10^{11}$	$1.13x10^{11}$	$2.15x10^{9}$	$4.61x10^{18}$
Never	38,355	$7.12x10^{9}$	$6.86 x10^{9}$	$9.00x10^{8}$	$3.90x10^{11}$	$3.89x10^{11}$	$2.93x10^{9}$	$8.60x10^{18}$

TABLE 5.13: WBC Count / L compared with alcohol intake frequency

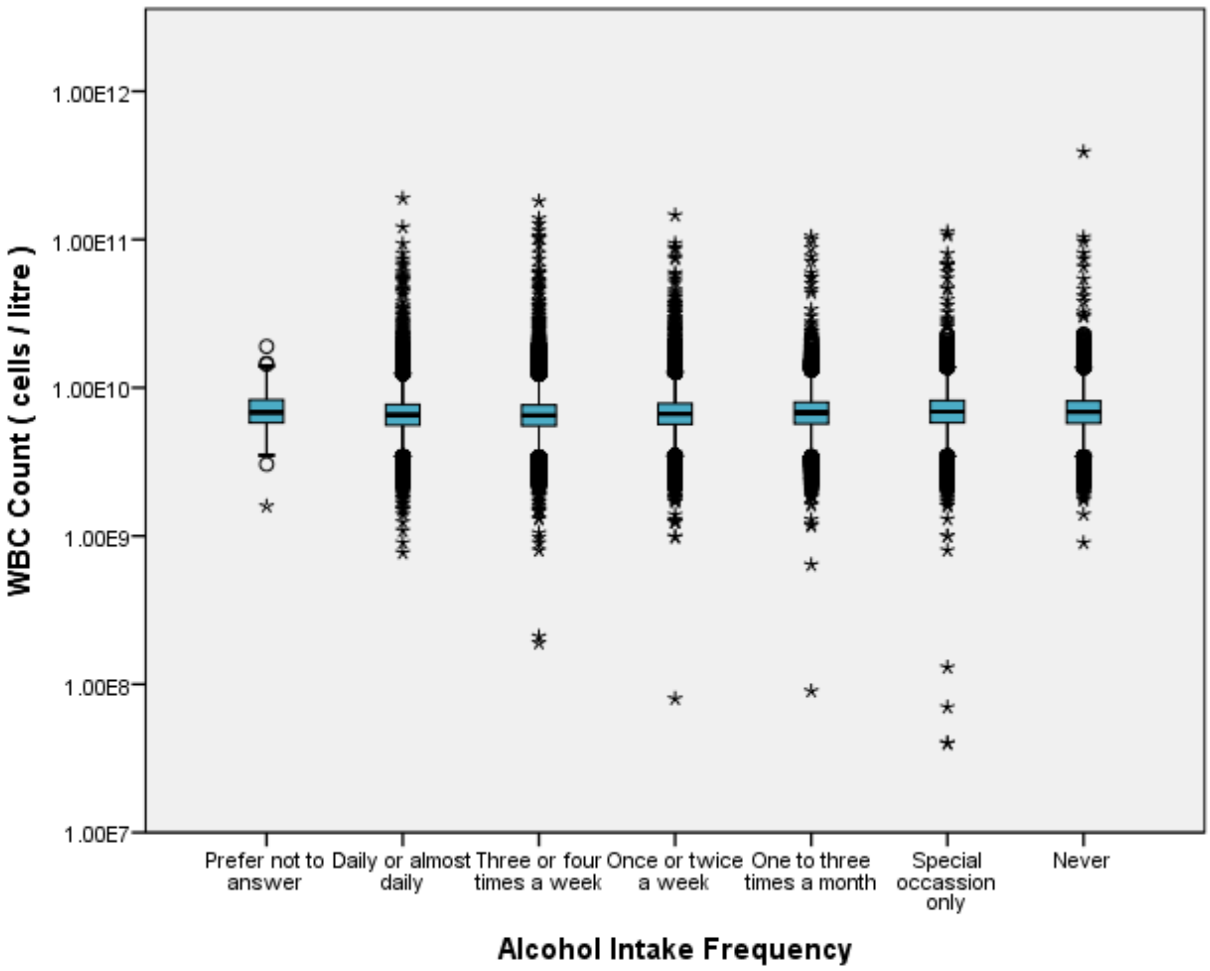


FIGURE 5.18: Box and whisker plot comparing the alcohol intake frequency of participants against their corresponding white blood cell count per litre

5.4.9 Variation as a function of Sleep

Sleep is a necessary part of life and a key factor in physical and mental wellbeing – lack of sleep can be detrimental to mental health and increases long term risk of heart disease, increased blood pressure and diabetes. In fact, in mice, sleep deprivation reduces the ability of donor HSCs to engraft and reconstitute in an irradiated 'patient' by more than 50% [211]. Additionally, human patients undergoing HSCT suffer from reduced and irregular sleep patterns [212][213] . As a result, quality and quantity of sleep before transplantation may be a key factor in both the functional ability of donated cells, but the variation and quantity too.

Table 5.14 compares the white blood cell count per litre of participants against their corresponding, self-declared sleep duration in hours. Unfortunately, there is not anything clear cut to support the above hypothesis, bar a slight reduction in both median and variance around the 7 hours category. Categories beyond the 16 hours per day do not have enough statistical power (i.e. they are usually single cases) but inspire curiosity as to the circumstances of the individual that has 23 hours of sleep per day and whether this is a clerical error. The bulk of the participants have between five and nine hours of sleep per night.

Figure 5.19 is a box and whisker plot of white blood cell count per litre against the corresponding participant's duration of daily sleep in hours. The clustering of data-points between five and nine hours can be clearly seen, as well as a sharp increase in the number of data-points declared as statistical outliers.

Duration of sleep	Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
<1 / Day	0	-	-	-	-	-	-	-
Prefer not to answer	347	$7.43x10^{9}$	$7.00 x10^{9}$	$1.59x10^{9}$	$8.02x10^{10}$	$7.86x10^{10}$	$4.46x10^{9}$	$1.99x10^{19}$
Do not know	2,786	$7.24x10^{9}$	$6.97x10^{9}$	$2.30x10^{9}$	$1.04x10^{11}$	$1.01x10^{11}$	$2.66x10^{9}$	$7.10x10^{18}$
1	32	$7.24x10^{9}$	$7.08x10^{9}$	$3.78x10^{9}$	$1.27x10^{10}$	$8.92x10^{9}$	$1.87x10^{9}$	$3.48x10^{18}$
2	162	$7.70x10^{9}$	$7.48x10^{9}$	$3.15x10^{9}$	$2.23x10^{10}$	$1.92x10^{10}$	$2.46x10^{9}$	$6.03x10^{18}$
3	772	$7.33x10^{9}$	$7.00x10^{9}$	$2.70x10^{9}$	$1.92x10^{10}$	$1.65x10^{10}$	$2.06x10^{9}$	$4.25x10^{18}$
4	4,349	$7.22x10^{9}$	$6.98x10^{9}$	$2.50x10^{9}$	$2.52x10^{10}$	$2.27x10^{10}$	$2.04x10^{9}$	$4.16x10^{18}$
5	20,727	$7.03x10^{9}$	$6.80x10^{9}$	$7.00x10^{7}$	$5.59x10^{10}$	$5.58x10^{10}$	$1.99x10^{9}$	$3.95x10^{18}$
6	91,204	$6.90x10^{9}$	$6.68x10^{9}$	$9.00x10^{8}$	$1.28x10^{11}$	$1.27x10^{11}$	$2.05x10^{9}$	$4.21x10^{18}$
7	183,552	$6.79x10^{9}$	$6.57x10^{9}$	$8.00x10^{7}$	$1.90x10^{11}$	$1.89x10^{11}$	$2.05x10^{9}$	$4.19x10^{18}$
8	137,499	$6.89x10^{9}$	$6.67x10^{9}$	$4.00x10^{7}$	$3.90x10^{11}$	$3.90x10^{11}$	$2.24x10^{9}$	$5.01x10^{18}$
9	27,651	$7.06x10^{9}$	$6.82x10^{9}$	$4.00x10^{7}$	$1.05x10^{11}$	$1.05x10^{11}$	$2.14x10^{9}$	$4.57x10^{18}$
10	6,698	$7.30x10^{9}$	$7.06x10^{9}$	$8.00x10^{8}$	$2.98x10^{10}$	$2.90x10^{10}$	$1.98x10^{9}$	$3.93x10^{18}$
11	639	$7.51x10^{9}$	$7.15x10^{9}$	$1.90x10^{9}$	$8.62x10^{10}$	$8.43x10^{10}$	$3.80x10^{9}$	$1.44x10^{19}$
12	1,087	$7.61x10^{9}$	$7.30x10^{9}$	$2.20x10^{9}$	$8.08x10^{10}$	$7.86x10^{10}$	$3.07x10^{9}$	$9.39x10^{18}$
13	70	$7.24x10^{9}$	$6.91x10^{9}$	$4.41x10^{9}$	$1.81x10^{10}$	$1.37x10^{10}$	$2.21x10^{9}$	$4.88x10^{18}$
14	91	$7.65x10^{9}$	$7.47x10^{9}$	$3.37x10^{9}$	$1.41x10^{10}$	$1.07x10^{10}$	$1.86x10^{9}$	$3.47x10^{18}$
15	48	$7.46x10^{9}$	$7.24x10^{9}$	$3.73x10^{9}$	$1.56x10^{10}$	$1.18x10^{10}$	$2.25x10^{9}$	$5.05x10^{18}$
16	42	$7.82x10^{9}$	$7.61x10^{9}$	$4.08x10^{9}$	$1.36x10^{10}$	$9.52x10^{9}$	$2.17x10^{9}$	$4.71x10^{18}$
17	1	$4.10x10^{9}$	$4.10x10^{9}$	$4.10x10^{9}$	$4.10x10^{9}$	0	-	-
18	10	$6.80x10^{9}$	$6.74x10^{9}$	$5.34x10^{9}$	$8.22x10^{9}$	$2.88x10^{9}$	$9.83x10^{9}$	$9.65x10^{17}$
19	2	$9.55x10^{9}$	$9.55x10^{9}$	$5.60x10^{9}$	$1.35x10^{10}$	$7.90x10^{9}$	$5.59x10^{9}$	$3.12x10^{19}$
20	7	$7.26x10^{9}$	$7.70x10^{9}$	$4.00x10^{9}$	$9.88x10^{9}$	$5.88x10^{9}$	$1.80x10^{9}$	$3.25x10^{18}$
21	1	$6.40x10^{9}$	$6.40x10^{9}$	$6.40x10^{9}$	$6.40x10^{9}$	0	-	-
22	1	$3.61x10^{9}$	$3.61x10^{9}$	$3.61x10^{9}$	$3.61x10^{9}$	0	-	-
23	1	$9.24x10^{9}$	$9.24x10^{9}$	$9.24x10^{9}$	$9.24x10^{9}$	0	-	-

TABLE 5.14: WBC count / L against daily sleep duration

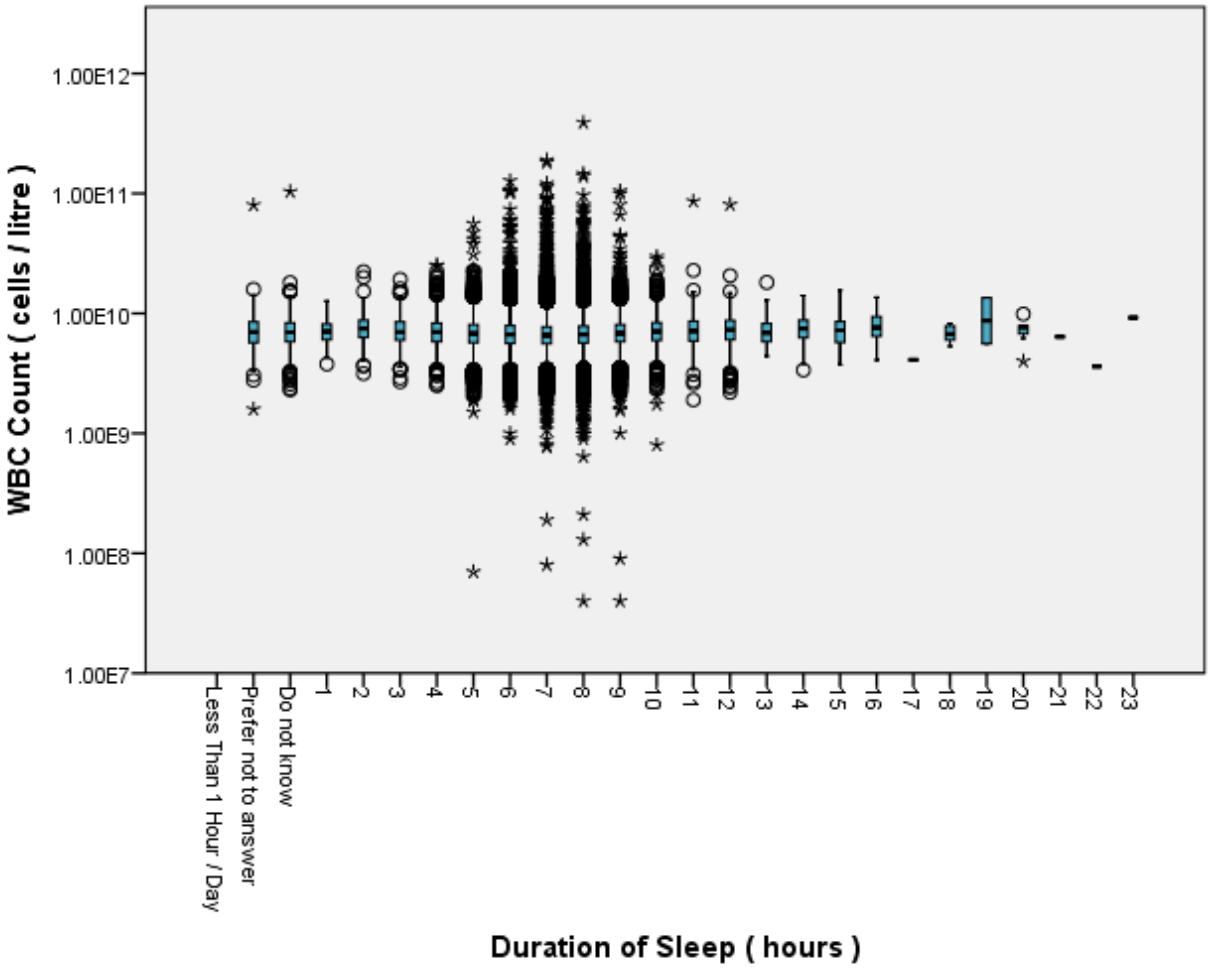


FIGURE 5.19: WBC Count / L against recorded daily sleep duration (hours)

5.4.10 Variation as a function of Health

This is a qualitative measure of health as determined by the participant themselves, in answer to the question – "In general, how would you rate your health".

Figure 5.20 is a Pareto chart of the results of this question. The most common health state is 'good', then 'fair', then 'excellent', and finally 'poor'. This is a subjective measure of health, but gives an indication of the average health of the population. Overall health rating is not a quantitative measure of health, and is as a result of a participant's opinion when answering the questionnaire, not a medical professional. Additionally, a study like UK Biobank is likely to attract those who are more health conscious and may have a biased opinion as to what constitutes good or excellent health – either in their favour or against it. More objective measures may include physical measurements such as blood pressure, fitness and mental health.

The median cell count ($H(5) = 8{,}422.004$, $p < 0.05$) and the distribution ($H(5) = 12{,}523.480$, $p < 0.05$) of cell counts between categories were found to be significantly different, apart from the categories of excellent / prefer not to answer (p = 0.200), good / prefer not to answer (p = 1.000), do not know / prefer not to answer(p = 0.375), fair / prefer not to answer (p = 0.252) and fair / do not know (p = 1.000) for median cell count and good /prefer not to answer (p = 1.000) and fair / do not know (p = 1.000) for distributions. As a result, median and distributions can be compared between those other categories.

Figure 5.21 is a box and whisker plot illustrating the white blood cell count per litre per individual against their overall health rating. The median and variances in cell count rises from 'excellent' down to 'poor' health, although it is important to bear in mind the non-quantitative nature of this measure of health. That said, comparing the two extremes, there is a noted increases in the variation between data-points, an increase in median cell count and standard deviation (illustrating that the central spread of data is wider) and a slight increase in range (although as already identified, range can be strongly influenced by singular extreme values) (**Table 5.15**).

	Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
Prefer not to answer	326	$6.94x10^{9}$	$6.60x10^{9}$	$1.24x10^{9}$	$1.51x10^{10}$	$1.38x10^{10}$	$2.10x10^{9}$	$4.39x10^{18}$
Do not know	2,043	$7.25x10^{9}$	$7.00x10^{9}$	$2.22x10^{9}$	$2.91x10^{10}$	$2.69x10^{10}$	$2.16x10^{9}$	$4.67x10^{18}$
Excellent	78,382	$6.48x10^{9}$	$6.30x10^{9}$	$9.00x10^{7}$	$1.02x10^{11}$	$1.02x10^{11}$	$1.71x10^{9}$	$2.93x10^{18}$
Good	275,802	$6.79x10^{9}$	$6.60x10^{9}$	$4.00x10^{7}$	$1.90x10^{11}$	$1.89x10^{11}$	$1.91x10^{9}$	$3.64x10^{18}$
Fair	99,895	$7.28x10^{9}$	$7.02x10^{9}$	$7.00x10^{7}$	$3.90x10^{11}$	$3.90x10^{11}$	$2.64x10^{9}$	$6.96x10^{18}$
Poor	21,132	$7.76x10^{9}$	$7.44x10^{9}$	$1.00x10^{9}$	$1.13x10^{11}$	$1.12x10^{11}$	$2.78x10^{9}$	$7.73x10^{18}$

TABLE 5.15: WBC count / L against overall health rating

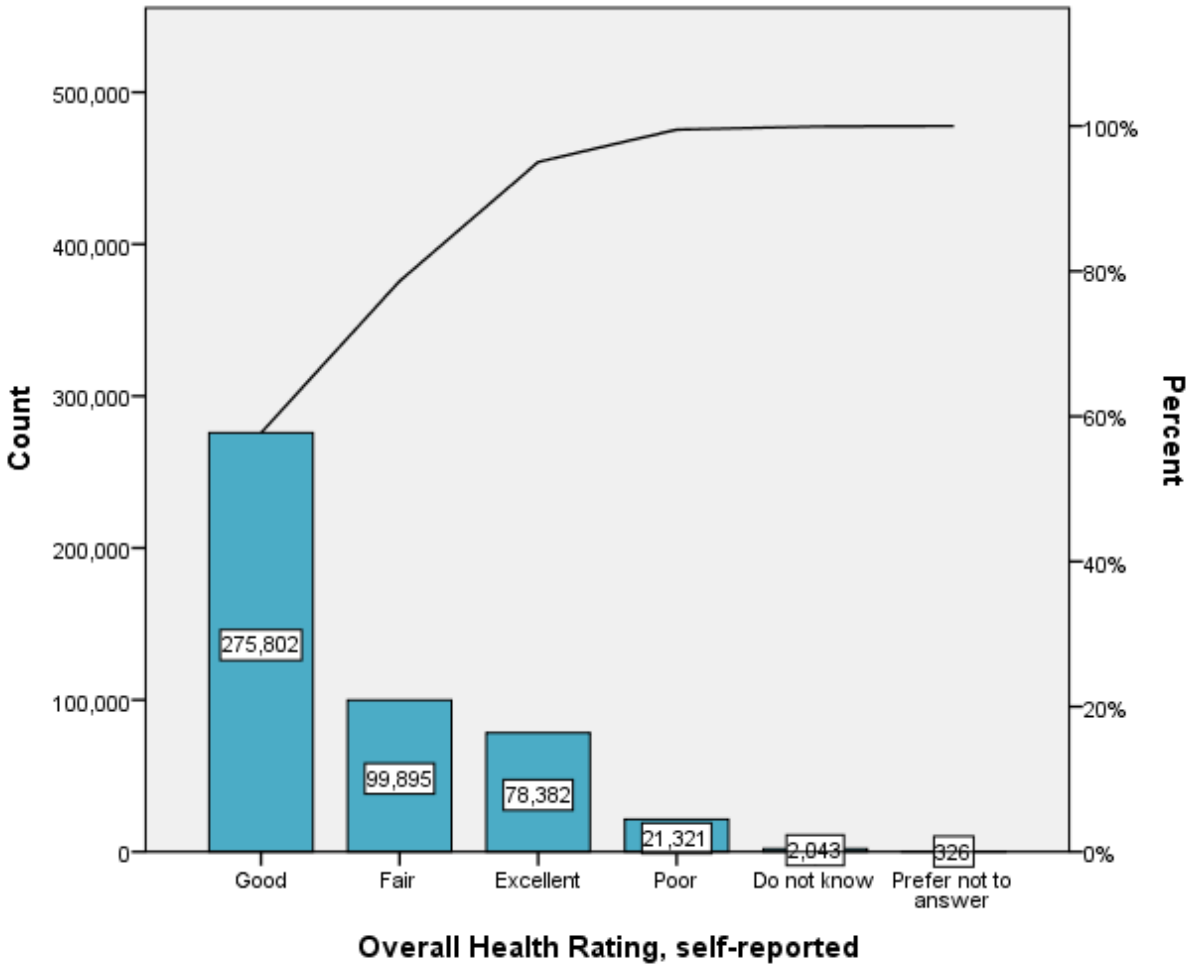


FIGURE 5.20: Pareto Chart of Participant Health Rating

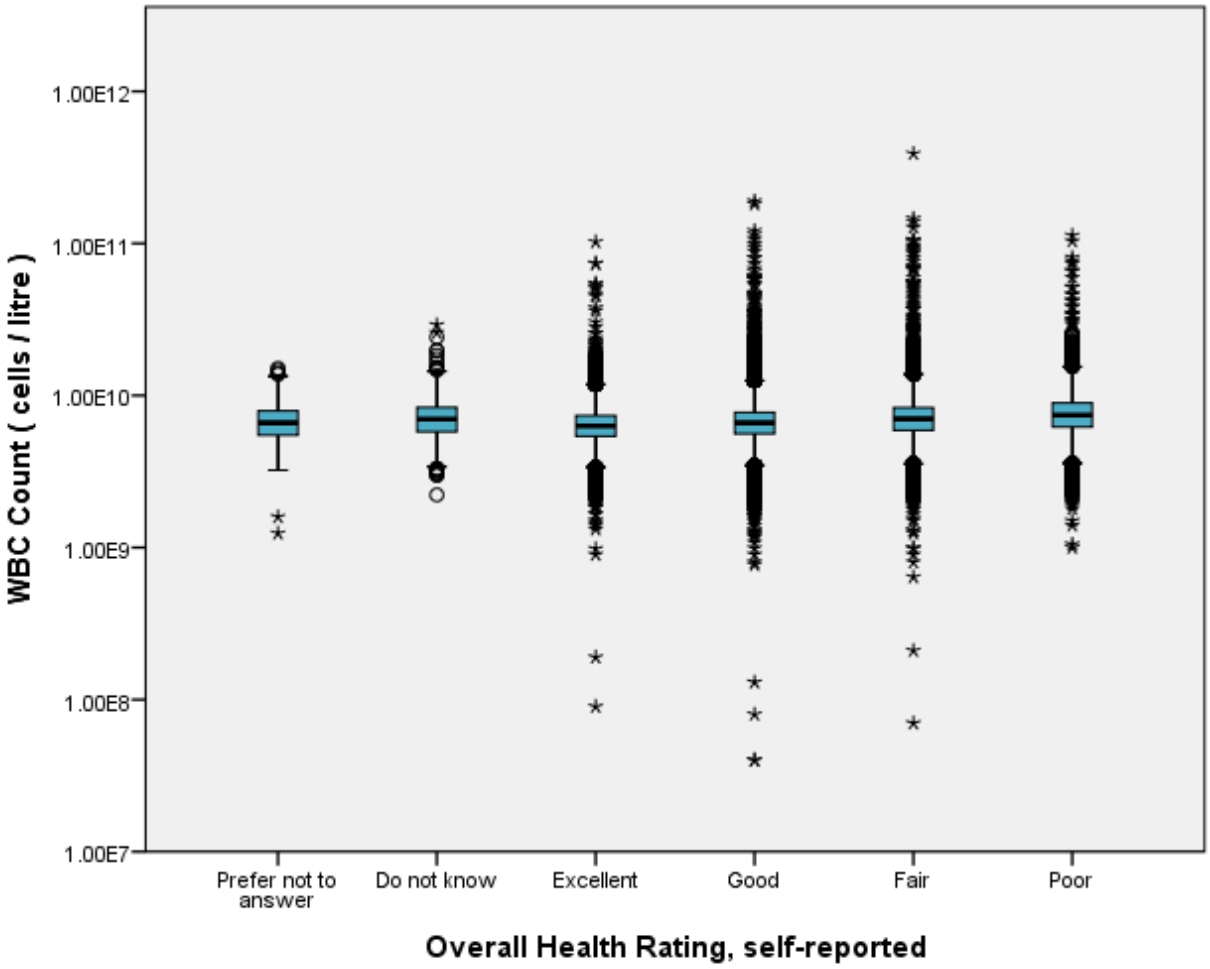


FIGURE 5.21: A box and whisker plot of overall health rating and corresponding WBC count / L per individual

5.4.11 Variation as a function of Ethnicity

Ethnicity, from a biological variation perspective is a complicated measure. The overwhelming majority of the UK Biobank identify as being 'British' (**Table 5.16** n = 442,72), but from a genetic and environmental perspective – the reason ethnicity would be examined as a cause of variation – being British is not indicative of a genetic sub-group, but rather a geographical allegiance. To fully understand ethnicity, it must be as a result of particular ethnic groups' genetic disposition – the traits likely to influence the comparative quality and/or quantity of cells, and if there are any unique quirks to a specific genetic group. As a result, even if British was identified as originating from English, Scottish, Irish or Welsh descent, genetically these groups will originate from very particular genetic origins – Anglo Saxon, or Germanic tribes for example.

As a result, it is difficult at this level to identify differences in ethnic group as a result of these questionnaires.

Figure 5.22 is a box and whisker plot of average white blood cell count against ethnic background. The 'British' category has been removed, for the reasons discussed above. Future work should involve studying a large sample of different ethnic groups, from different continents, to clearly define whether there is any genetic segregation in terms of stem cell numbers of efficacy.

		Count
Ethnic Background	British	442,702
	Any other white background	16,342
	Irish	13,215
	Indian	5,951
	Other ethnic group	4,560
	Caribbean	4,520
	African	3,396
	Pakistani	1,837
	Any other Asian background	1,815
	Prefer not to answer	1,663
	Chinese	1,574
	Any other mixed background	1,033
	White and Asian	831
	White and Black Caribbean	620
	White	571
	White and Black African	425
	Bangladeshi	236
	Do not know	217
	Any other black background	123
	Mixed	49
	Asian or Asian British	43
	Black or Black British	27
	TABLE 5.16: Ethnic Background	

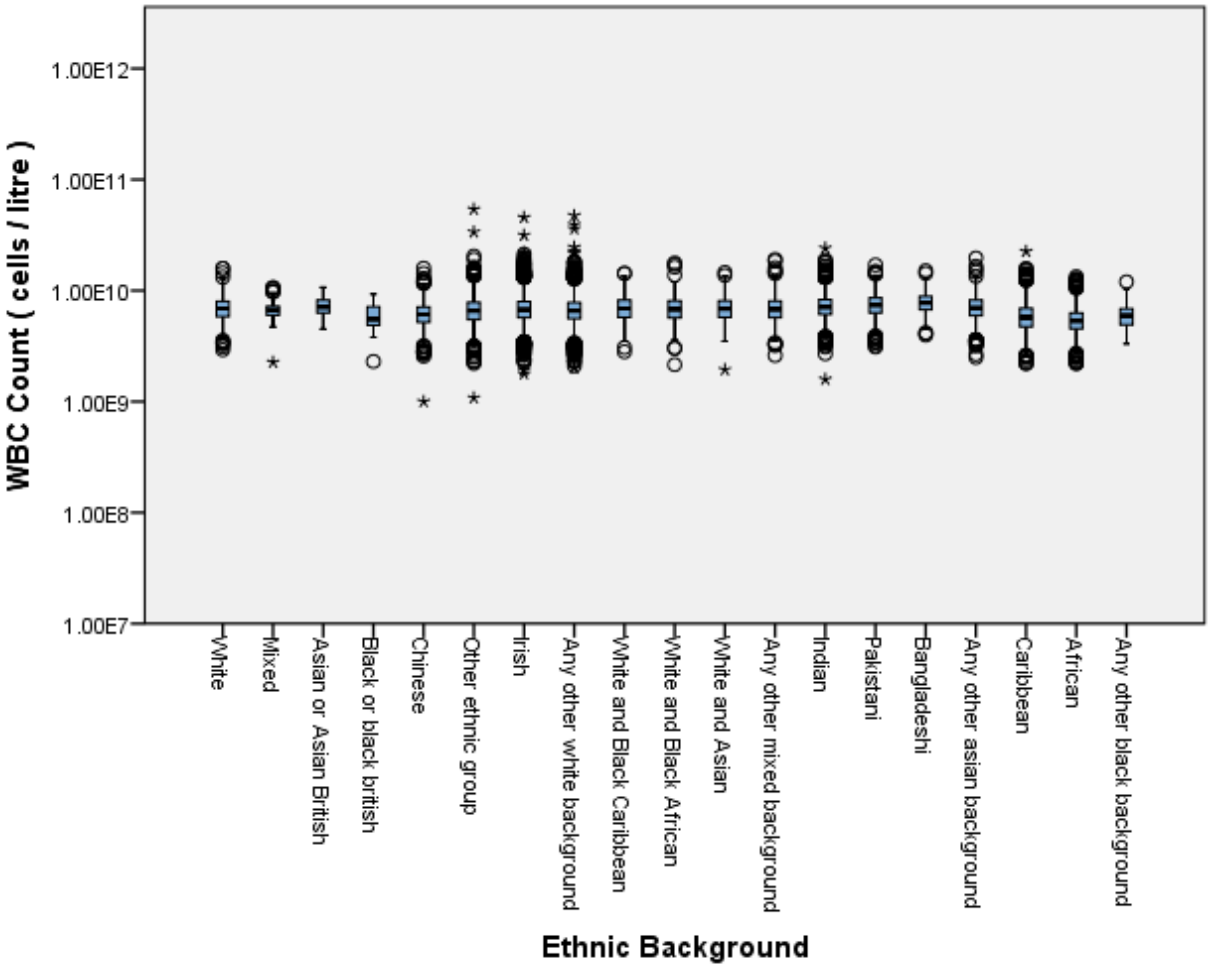


FIGURE 5.22 A box and whisker plot of declared ethnic background and corresponding WBC Count per litre per individual

5.4.12 'Unhealthy' Biobank Participants

The participants of the UK Biobank survey are notionally healthy, a baseline of the UK adult population – however there are participants who have had or have serious illness such as cancer so it is important to stratify these participants and compare the relatively healthy population with that of the sick population. This is particularly interesting in conjuncture with the CMCF dataset which was almost exclusively a sick population.

The following section compares the white blood cell counts and distributions of UK Biobank participants who either have or haven't had cancer. The aim of this is to identify whether there is a significant difference between a healthy and a sick population that has been processed and analysed in a comparable manner.

In this example, 'healthy' participants have been identified as those who have declared being cancer-free, and 'un-healthy' participants have been identified as those who have declared on the questionnaire that they have had or have cancer, in some form or another (**Table 5.17**).

Figure 5.23 is a comparative scatter diagram of 'healthy' and 'un-healthy' UKB participants with respect to cancer. Variation as a function of both range and variance between individuals is higher for those individuals who have had cancer compared to those who have not. There is also a much wider spread of data points around the mean for those who have had cancer compared to those who have not.

This is contrary to what was shown earlier, where the relatively healthy UKB population had a higher variation than the relatively unwell population at the Dana Farber Cancer Institute. This is likely due to the wide variety of results covered by 'has or had cancer' in terms of type, severity, longevity, treatment type and whether the patient is in remission or not, compared to a comparatively stable, ambulatory population.

This example does not take into account, however, the differences between cancer patients – when did the illness occur, what is its severity and what treatment has been applied, are they in remission – this example has assumed that exposure at some level to a malignancy will have a noticeable effect on biological variation as a function of white blood cells. The more interesting investigation would be the variation as a function of cell quality and function with respect to cancer stage and type – something that is not possible with this dataset, but would be a more appropriate measure of variation (it is unlikely cancer will affect numbers of cells further than an overall increase as a result of immune response, or decrease as a result of radiotherapy for example).

	Valid N	Mean	Median	Minimum	Maximum	Range	Standard Deviation	Variance
Has / Has Had Cancer	39,275	$6.99x10^{9}$	$6.58x10^{9}$	$1.00x10^{9}$	$3.90x10^{11}$	$3.90x10^{11}$	$4.24x10^{9}$	$1.80x10^{19}$
Has not Had Cancer	439,015	$6.88x10^{9}$	$6.66x10^{9}$	$4.00x10^{7}$	$1.04x10^{11}$	$1.04x10^{11}$	$1.82x10^{9}$	$3.30x10^{18}$

TABLE 5.17: WBC count / L for UK Biobank participants that have had or have cancer versus those who have not

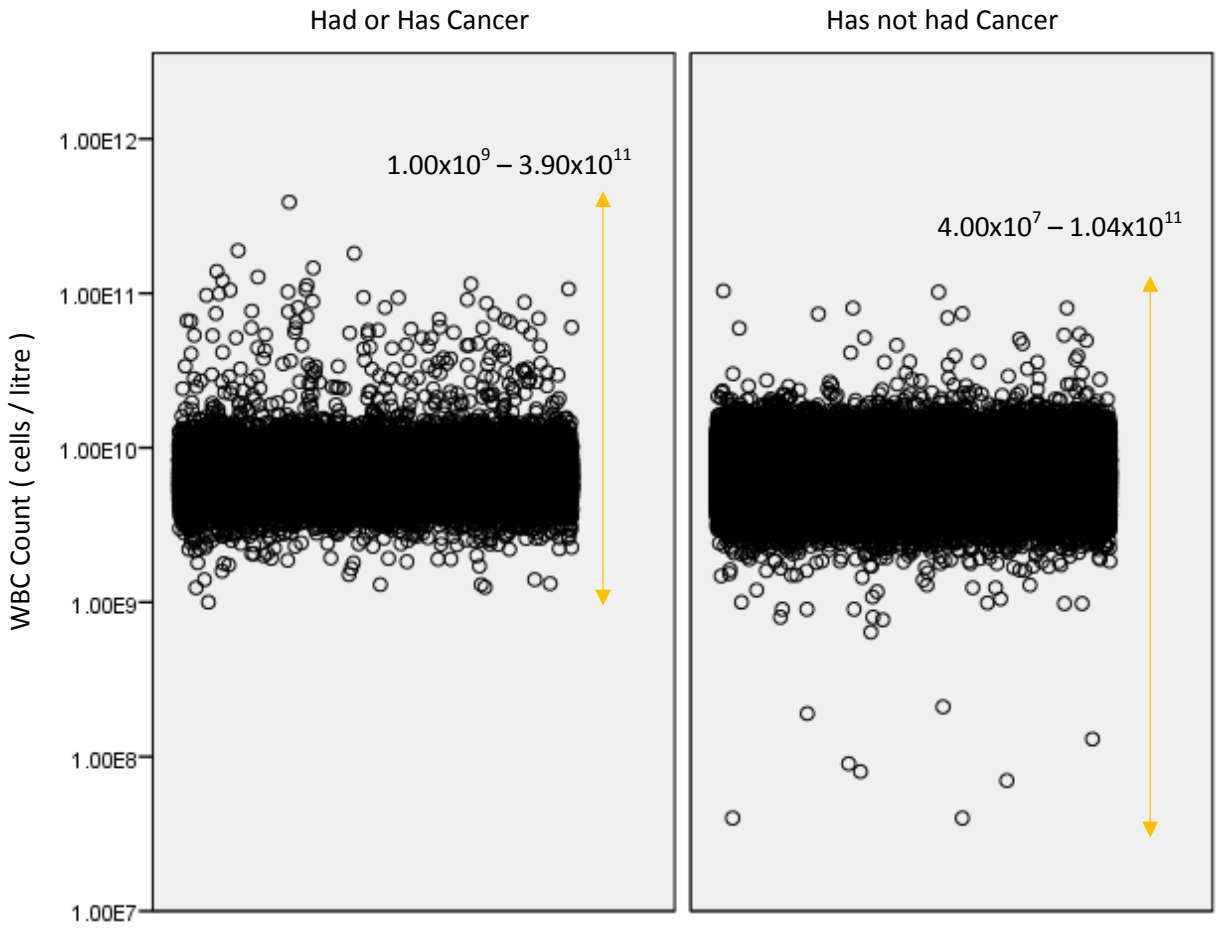


FIGURE 5.23: Scatter diagram of WBC count / L for UK Biobank participants that have had or have cancer versus those who have not

5.5 Conclusion and Discussion

The aim of this chapter was to quantify the variation encountered in a general, healthy population, characterised by the UK Biobank.

This variation is that of a statistically robust, healthy representation of the population to augment the global 'unwell' population characterised by the literature meta-analysis and the localised 'unwell' population characterised by the Dana Farber database.

The variation in **white blood cells** / litre can be between 7.00 x 10^7 cells / litre and 3.90 x 10^{11} cells / litre – **four orders of magnitude.**

As a representation of a healthy population, UK Biobank is an opportunity to study the baseline population in the UK for overall biological variation and potential influencing factors. This may inform future allogeneic cell sources and the design of stem cell processing and automation. It may also contribute to our understanding of the differences between the cell state for a healthy donor compared to an unwell patient.

Both UK Biobank WBC and TNC have similar ranges (range = 3.90 x 10^{11} and 3.86 x 10^{11}), similar distributions around the mean (standard deviation = 2.12 x 10^9 and 2.10 x 10^9), and similar data spread (variance = 4.51 x 10^{18} / 4.42 x 10^{18}).

The range of WBC count / litre between Biobank and DFCI are almost identical (3.90 x 10^{11} cells / litre and 3.67x10^{11} cells / litre respectively, **Table 5.1**), however the spread of the data around the mean is considerably higher in the DFCI dataset, and the distribution of the data (variance) is also much higher – indicating that the 'unwell' population at DFCI is much more variable than the relatively healthy population of the Biobank.

The implication is that whilst healthy and unhealthy populations appear to have similar ranges, at least for white blood cells and total nucleated cells, unhealthy populations (DFCI) appear to be more spread out from the mean value, and therefore more greatly variable from case to case.

Presumably as a result of the disparate indications, their severity and subsequent influence on quality and quantity of cells.

Databases made available in **Chapters 3** and **4** have been unable to examine the effect of patient influences such as physical and lifestyle metrics, because the data has either been unavailable in the public domain or protected by patient confidentiality. The UK Biobank data has allowed some light to be shed on these potential sources of variation that have previously been unexplored.

The influences examined in this chapter (smoking, alcohol, sleep, ethnicity, age, weight, gender and geography) all appear to have very mild influences on variation, implying that the actual mechanisms that influence variation are less likely to be a handful of key metrics, but a multiplicative effect based upon many different variables. However, other potentially influential metrics such as disease state, medication and diet have not been examined and these have the potential to be greatly influential.

- Gender appears to have some small effect on the amount of variation in white blood cells and total nucleated cells encountered.
- Increasing age appears to increase the amount of variation in white blood cells and total nucleated cells encountered.
- Increasing weight appears to decrease the amount of variation in WBCs and TNCs.

Assessment centre, analogous of geographical area, appears to have a statistically significant effect on WBC count. This may represent the differences between built up, industrial, country and coastal regions in terms of lifestyle and air quality for example.

Ethnicity is not representative of genetic makeup within the UK population as citizens from various genetic backgrounds identify as British, and therefore this metric cannot be used to stratify the dataset appropriately. A more genetic based stratification would be more appropriate in this instance.

However, the UK Biobank has a distinct lack of direct data corresponding the stem cell quantity or potential quality, such as cells positive for the CD34 marker, and as a result data from this Chapter has acted in a more illustrative and informative role, rather than a direct role for HSCT.

5.5.1 Outliers

A significant observation from the UK Biobank data is the considerable number of "outliers". As mentioned in earlier Chapters outliers in traditional manufacturing would be discard material or our-of-specification products however in cellular therapy they cannot be discarded. Similarly, to assess variation, these outliers are representative of an individual's state of health, and cannot be removed to smoothen the statistical distribution. Understanding these outliers will be critical for understanding the behaviour of the starting material and the process.

5.5.2 Sampling

One important observation as a result of this Chapter has been the sampling used for the UK Biobank. For the purposes of variation analysis, the assumption is that this is a representative sample of the UK population, and it is likely that this is the case. However, these studies were carried out on a volunteer / targeted basis and has the potential to influence the population recruited as a result – those of a particular mindset that may be reflective of their lifestyle, or health.

UK Biobank is a sample of the population as a whole; the question is whether it is representative of the population. Specifically, for this use of the data, is variation found in this population representative of the variation found in the recipients of the treatment?

5.5.3 Big Data

This dataset provided the opportunity to examine biological measurements on a large, statistically robust scale, with a wide and detailed variety of variables. This statistical power has allowed much more detailed analysis of patient variables than was previously possible and as such highlights the importance of big data analysis for biological systems. Unlike pharmaceutical drugs, with one particular (designed) mode of action by a typically inert and quantifiable chemical, biological therapies such as stem cells are highly complex, living materials with multiple potential modes of action and interactions and a capability to change and grow *in situ*. Big data such as Biobank allows for enough statistical power to examine the myriad of potentially influential variables for example, Elsevier's genetic data mining (https://pharma.elsevier.com/pharma-rd/using-text-mining-to-find-treatments-for-rare-diseases/).

DFCI demonstrated that biological and donor characteristic data is available, but not in the public domain. This will be a similar case in other key transplant centres, although in the US these are likely to be discouraged from data sharing due to commercial interests. The NHS is in prime position, however, to make use of a large amount of patient metrics and corresponding stem cell metrics to begin to build up a database of evidence to support both the efficacy of cell therapy, and the potential processing pitfalls such as biological variation. However, this data could not be held by a commercial entity due to the sensitivity and privacy of patient information, and the potential scandals surrounding profit based data mining of private information – for example see the NHS Big Data scandal in 2014 [214] [215]and more recently the Google Deepmind privacy breach in May 2016 [216]. Therefore, it is important for not-for-profit charity organisations such as UK Biobank to hold this information.

This chapter has demonstrated the amount and distribution of variation in an ambulatory, statistically robust population that has significant outliers.

5.6 Future Work with UK Biobank

Datasets such as the UK Biobank could be a key contributor to our understanding of biological process control issues. Other large datasets such as those held by national health services or large pharmaceutical companies could also be critical. Datasets such as UK Biobank have the potential to examine a wide variety of patient and processing metrics that may not be available in the public domain.

A potential future project would involve an international collaboration of cellular therapy processing, that stores predetermined metrics such as process data and donor characteristics to build up a large dataset of biological variation and biological processing, which the community can build upon and speed up the adoption and manufacture of cellular therapy.

In the more immediate future, the UK Biobank would benefit from further analysis of specific lifestyle metrics and its effect on white blood cell counts – such as diet or exercise. A question that needs to be asked is whether variation as a function of white blood cell numbers is applicable to examining the variation of cellular therapy, specifically blood. Further important factors are medications, supplements and lifestyle as a function of jobs and activeness.

A further point of research would be to examine the difference in genetic markers of donors, and the corresponding cellular metrics. This would warrant examination of variation as a function of cell numbers (through cell surface markers) and cell quality (likely as a result of 7AAD staining of the correct cell population. Although whether cells are alive or dead, as a result of the 7AAD staining, is a measure of live or dead, rather than the ability of the cell to provide a therapeutic effect).

With a large number of variables such as those found in UK Biobank, the NHS, or the EBMT records, more intelligent statistical tools will be required – such as dimension reduction of

variables or regression analysis – that can investigate the influence of factors as a *whole* rather than individually.

An interesting future point could be the investigation of analogues for stem cells that can be used, that are cheaper and faster than the current systems. With the increase in demand these technologies will decrease in cost as a function of supply and demand, but if a cheaper alternative could be discovered, such as a chemical or particular physical measurement, that is a predictable analogue of a type of cell, it would be much easier, faster and cheaper for real time monitoring and quality control tests that would contribute to the improvement of the individual product and the whole product process as a whole.

To investigate some of the mysteries highlighted in this Chapter, an EPSRC DTC mini-project was designed to investigate certain other factors, specifically the influence of weight on white blood cell count variation. This project was on-going at the time of writing and may form part of further publication in the future.

5.7 Summary

Biological variation as a function of white blood cell count in the healthy population represented by UK biobank, can be up to four orders of magnitude around the median.
Gender appears to have negligible effect on this variation.
Increasing donor age appears to mildly increase this variation.
Increasing weight appears to mildly decrease this variation.
Lifestyle factors such as smoking, alcohol and geographical location appear to have small statistically significant effects on variation
Big datasets such as UK Biobank may be important in establishing mode of action and public / investor confidence in cellular therapy
Variation in the UK Biobank is lower than that found in the unwell population of the CMCF – however the variation in the 'unhealthy' sub-population is higher than that of the 'healthy' sub-population (as a function of cancer incidence).

Chapter 6: Conclusions and Areas of Future Work

6.0 Thesis Aims

For cellular therapies to become a frontline option for healthcare providers, the regulator, clinician and patient need to be satisfied as to the therapy's long term efficacy and safety – especially when these therapies are in competition with existing, proven alternatives.

Cellular therapies are currently produced in small batches, for relatively few patients, in a predominantly clinical setting. This method of production does not have the capability to produce in the volume that may be required to meet future demand. Given the limited space, budget, and manpower of our clinical centres, there will be a necessity for a degree of mechanisation and/or automation.

Before the work presented in this thesis, the extent and range of variation within cellular therapy was anecdotal. Understanding the variation that is encountered in the starting material, and during the process, will lead to an understanding of the limits and the specification that a machine or a process needs to be designed around – either to tolerate or control.

This research has presented an analysis of the range, distribution and sources of biological variation for a representative exemplar, based upon an in-depth study of clinical datasets and medical literature which will inform manufacturing and product development within the cell therapy field. This is a key stage in understanding the process – being able to manufacture a fully defined product at a consistent standard and/or designing new manufacturing technologies that meet, or inform, regulatory requirements.

6.1 Summary of the Thesis and its Conclusions

This thesis has:

- Reviewed the current research into biological and stem cell therapy related variation, and identified key data sources for further analysis such as the EBMT, NHSBT, UK Biobank and the medical literature.
- Used a number of identified data sources to quantify biological variation, using a blood-based stem cell therapy exemplar, both overall, and as a function of allogeneic and autologous donors
- Examined variation from a global perspective and a single centre perspective, by comparing the medical literature with the data collected from the Dana Farber Cancer Institute, at a statistically valuable scale.
- Examined variation from a "healthy" and "sick" donor perspective, by comparing the "sick" sub-populations provided by the Dana Farber Cancer Institute, and the medical literature, against the "healthy" sub-population provided by the UK Biobank, at a statistically valuable scale
- Identified several potential sources of variation, a number of which are a result of biological systems and will present new and unique challenges, and a number of which are more traditional in nature and have the potential to be addressed through application of traditional manufacturing tools and approaches.
- Through the use of public forums, it has been identified that biological variation is a universal concern for the field, but this has not yet been formally and publicly discussed in depth. The author believes this may be because of the vulnerability that may come from identified such a commercial weakness to competitors or regulators.

Table 6.0 illustrates the significant novel contributions of the thesis, whether this work has been published or presented elsewhere, and where this novelty can be found within the thesis. **Table 6.1** summarises the biological variation quantified by this research, stratified into transplant type and information source.

		Published/ Presented	Novelty	Chapter
Sources of Variation				
	Patient/ donor characteristics	Presented	Novel	2 – 5
	Measurement variation	Presented	Novel	2 – 4
	Operator variation	Presented	Novel	2 – 4
	Process variation	Presented	Novel	2 – 4
	Practice of medicine	Presented	Novel	2 – 5
Spread of Variation				
	Total nucleated cell count	Published	Sign. Novelty	3, 4
	CD34+ cell count	Published	Sign. Novelty	3, 4
	White blood cell count	Presented	Novel	5
	Apheresis volume	Presented	Sign. Novelty	4
	Outliers	Presented	Sign. Novelty	3, 4, 5
	Distributions	Presented	Sign. Novelty	3, 4, 5

Table 6.0: Summary of Thesis Novelty

Source	Population	Transplant Type	N	CD34+ cell count/ kg	WBC count / L	Orders of Magnitude
Medical literature	Unhealthy, global	Autologous	110	$6.00x10^4$ to $3.00x10^8$		4
Medical literature	Unhealthy, global	Allogeneic	188	$1.00x10^3$ to $1.21x10^9$		6
UK Biobank	Ambulatory, UK/ national	Autologous	478,290		$4.00x10^7$ to $3.90x10^{11}$	4
CMCF/ DFCI	Unhealthy, single site	Autologous	287	$4.80x10^6$ to $4.14x10^9$		3
CMCF/ DFCI	Unhealthy, single site	Autologous	282		$9.91x10^9$ to $3.77x10^{11}$	2
CMCF/ DFCI	Unhealthy, single site	Allogeneic	123	$4.00x10^7$ to $1.82x10^9$		2

Figure 6.1: Summary of Biological Variation in HSCT, quantified during this research

6.2 Future Work

This work has identified several priority research questions.

- Variation has been quantified in terms of cell numbers, but now requires quantification in terms of cell quality, and for this to be related to patient outcome. Given the number of separate, commercially competing entities, and the small scale of clinical trials, a large scale collaborative effort will be required to facilitate statistically significant results.
- How do we measure cell efficacy and quality?
- How much variation is acceptable without negatively affecting patient outcome or safety?
- What is the difference in terms of cell quality and function between cells from paediatric and adult donors, and will the variation have to be approached differently depending on which group is used?
- What is the interaction between patient/ donor weight, and biological variation in terms of numbers and quality?
- What is the effect of biological variation as a function of genetic differences? This is a key question, in particular for gene therapy. Can specific quality metrics of the donor be quantified in terms of specific genetic structures and what effect does variation in genetic stricture have in practical terms as a function of viable cell numbers, and patient outcome?
- What is the effect of patient indication and disease severity on stem cell quality, particularly in autologous therapy?
- What is the effect of lifestyle measurements such as diet on stem cell quantity and quality – particularly during the lead up to stem cell donation – and is there a regime that could be followed to ensure higher collection quality?
- Standardisation is required for apheresis procedure, cryopreservation and thawing, stem cell measurement in terms of flow cytometry, and whether there are any cheaper measurement analogues, that are representative of stem cell quality
- What statistical tools are appropriate for analysing non-normal biological data – do we require new tools, or can we adapt the more traditional engineering tools? What will be the standard – transformation of data, or tools specifically designed for these datasets? How are outliers going to be dealt with when many statistical tools are reliant on outliers being removed?

- How does the product behave from a therapeutic point of view at the centre of the distribution versus the trailing edges and the extremes? Is the therapeutic and safety profile different? This will be of critical importance to the regulators of stem cell therapies.

Four general but critical areas of research will now be discussed. Increased understanding in these areas is key to further progression in this field.

6.2.1 Patient Variation/ Weight

Donor and patient weight is a readily available, key physical measurement tied into the practice of medicine, used to tailor treatment plans and drug regimens and traditionally used to measure medication dose.

Weight is currently used in regenerative medicine in a similar fashion to determine dose, however defining dose is more than a simple correlation between size and the number or quality of cells. This may be due to the difference in body health between patients of a given weight – such as a weight due to muscle versus weight due to fat content. Although cell quality has not been measured directly because of changing weight, being heavier because of healthy or unhealthy reasons may have implications for cardiovascular strain and blood chemistry that may affect stem cell quality, and therefore warrants further research.

This is an important, topical question given the rise of obesity, in the Western world in particular, and the respective availability and price of unhealthy versus healthy food.

Furthermore, lighter patients may fall into the paediatric patient category – a sub-population that was unfortunately not strongly represented in any of the databases used in this thesis' research. Paediatric patients have a different biological situation than adult patients and it is important that these are treated and explored as separate biological states (which is why comparisons will need to be made between cell number and quality of adult and paediatric patients). This will be of particular importance in gene therapy, where this is the ideal treatment age to diagnose and treat various malignant indications before they develop.

Weight will also influence the volume of circulating blood and the number of cells as a result. It has been identified that there is not a simple relationship between the two, but differing weights of patients will yield different volumes of starting material, in addition to differing volumes not being indicative of particular numbers of cells. Exploration of the effect of varying weight with respect to biological variation as a function of cell number and cell quality is a key area of research and will require collaboration between sports scientists, utilising their knowledge of physical health, and clinical researchers.

6.2.2 Relationships between components of blood

Compared to pharmaceutical drugs, stem cell therapy has a particular challenge due to the nature of the therapeutic product which is more sensitive than pharmaceutical drugs, is constantly in a state of flux and is extremely complex – possibly beyond our current understanding. Stem cell measurements are approximate, and any new developments in biological metrology that can improve this resolution or sensitivity, will take time to build up a supporting evidence base. Furthermore, the specific mode of action of cellular therapies is unclear – is the patient benefit a result of direct engraftment, or due to the release of cell signalling molecules due to their presence?

Total nucleated cells and CD34+ cells are not directly correlated, as previously illustrated using cord blood by May Win Naing [217]. Both of these variables vary significantly, to a degree that would be unmanageable in a traditional manufacturing process. Therefore, one of the key research directions should be exploration of the interaction between cell populations and quality, in particular what biological factors affect the production, number and quality of stem cell populations, and if there are any analogues for these factors that we can measure in an easier, or cheaper manner. This would facilitate a faster turn-around time for quality control testing, which is a current concern in a fast-paced clinical environment with time sensitive products.

Additionally, a more consistent approach to measurement would allow for greater confidence in the clinical effect of these new therapies, both from the perspective of the healthcare provider, and the regulator.

Measurement of the stem cell content in a given product is time consuming, and has the potential to be influenced by the operator and the chosen methodology, so it is imperative that we as a community improve our understanding of how to appropriately measure the starting material during the process, intermediates at individual process steps and within the final product, in an accurate and comparative manner. This will facilitate comparison between academic research, clinical trials and patient outcomes, identify potential side effects and improve process design within the manufacturing and development community.

Because of its complexity, the work in this thesis has chosen to not determine or take account of the contribution of variation in the performance of the measurement system to either the variation in individual process steps or the overall process. Given that the therapeutic products we are concerned with are based upon stem cells and that their characterisation is still an active area of research dependent on a better understanding of the performance of these products in the clinic, this highlights the critical requirement for work in measurement system analysis for core characterisation techniques.

Exploration of these issues will require collaboration between the haematology, developmental biology and metrology fields – those who are familiar with the interaction between blood components and blood chemistry and can stratify and quantity the mechanisms for stem cell production, and how they can be measured (both in ideal circumstances such as clear media, and realistic circumstances such as ‘dirty’ media and inflamed biological systems).

6.2.3 The interface with the practice of medicine

One of the key conclusions from this thesis has been that although there are several traditional sources of variation, such as process control and operator variation, there remains significant

variation that can be attributed towards the biological system and one of the more potential influential factors is the use of conditioning and mobilisation regimes.

These interventions are both determined exclusively by the individual healthcare providers approach and practice of medicine, and will vary depending on the centre and individual. Both regimes dramatically change the state if the donor's cell populations, and potentially their cell quality, and as a result increasing the evidence base of the relationship between these regimes and the quality of donated stem cells is a crucial next step in establishing their effect on patient outcome.

This will require close collaboration with the individual clinical fields and understanding and working around the practice of medicine as it expertly balances risk and patient benefit.

6.2.4 Manufacturing control

The motivation behind this thesis is to scrutinize the current state of play within the biological therapy world from a manufacturing perspective – the key objective being how to facilitate repeatable and comparable manufacture at scale that a cellular therapy 'big win' will require, and begin to predict and model these processes in the face of biological variation.

During traditional manufacturing, there is a focus on two key measurements of the product, its mean and range. This thesis has focused on establishing the range of the product, but in fact has identified the presence and importance of a third measurement – the outliers. Usually, these are removed statistically during data analysis, or physically discarded as out of specification products, but in the case of biological therapeutics these products are a limited and precious resource that is both expensive and immoral to waste or discard. As a result, it is critical that we identify the cause of these extremes and whether they are truly outliers or extreme patient states we will have to work with.

There is significant variation not just in cell number, but physical volume of starting material. Within this research this variation could be between 30 ml and 700 ml, and these are indicative of

completely different scales of physical manipulation and storage, particularly when automation is concerned. Additionally, a given volume is not representative of a given number of cells, and smaller volumes may contain higher numbers of key cell populations. This is particularly concerning when this research has identified that several clinical centres triage and discard donations away based on weight or physical volume, and that they may be trading high quality donations in favour of higher volumes. This implies that more appropriate analogues of quality are required.

The non-normally skewed distribution of much biological data is also an important consideration, such as how the product behaves from an efficacy and safety perspective at the trailing edges. The regulator and the clinician will need to be reassured as to whether the product works the same way and has the same effect regardless of its position in this distribution – is the product less safe, less efficacious or less viable at its extremes? A focused effort is required on examining current statistical tools such as six sigma, and how we can apply them to address non-normally distributed data from a processing perspective, or whether we need to design new tools that are specific to biological manufacturing.

This issue will require large scale collaboration between multiple organisations and stakeholders, as it will underpin biological manufacturing moving forward. Engineering and biology will need to reach an appropriate compromise on the level of acceptable variation, and this will require an investigation of the relationship between a given level of variation and the resultant patient outcome. For ethical reasons, this is highly likely to be a retroactive study of historical data.

6.3 High Priority Research Areas to Understand and Control Variation

This research has identified several potential sources of variation and areas of research that will be required to understand biological variation, and therefore process control, and large-scale manufacturing for biological therapies.

The generic process map for HSCT (see **Figure 6.2**) will now be used to identify sources of variation and triage them according to research priority – low, medium and high. This aims to support the

allocation of the limited resources available to the community and its key stakeholders, in both time and manpower.

A foremost and critical observation is that biological variation does not need to be eliminated, just controlled sufficiently such that it does not negatively affect patient outcome, or safety. Removing all variation or addressing all the sources may not be feasible, or possible, especially given the urgency of which these therapies are needed.

- **Patient/ Donor [LOW]:** This may be impractical to control as there will be a background level of variation as a function of differences in ethnicity, age, gender, lifestyle, etc. Developers are encouraged to work around this variation, as there are other areas of significant improvement that will have more immediate, and tangible effects
- **Autologous [LOW]:** Patients cannot be stratified according to quality metrics, so variation as a function of an individual's state of health may have to be accepted and worked around
- **Allogeneic [LOW]**: Donors could be stratified according to quality metrics, but this is dependent on appropriate and representative markers of therapeutic effectiveness. This would require a large and robust clinical dataset, so developers are encouraged to work around this variation in the first instance.
- **Genetics [HIGH]**: Genetic variation was not addressed in this thesis and will require a high level of basis science research in the academic and clinical setting – this is a traditional academic research question.
- **Conditioning [HIGH]**: An understanding of how previous therapeutics and interventions, prior to stem cell therapy, affect the quality of isolated cells and its subsequent clinical effectiveness is required. This would require a large scale, robust, clinical dataset and a systematic and rigorous investigation.
- **Mobilisation [HIGH]**: A wide range of pharmaceuticals and regimes exist, and their application varies between centre, clinician and patient. This step has the potential to influence quality and quantity of cells within the process starting material for a cell therapy product. This would require a large scale,

robust, clinical dataset and a systematic and rigorous investigation and a parallel and rigorous manufacturing science investigation.

- **Isolation [LOW]**: Isolation is already supported by a large and experienced industry as part of the supply chain.
- **Measurement [HIGH]**: Appropriate measures of cell identity, phenotype, and function are required. These will inform the generation of key quality metrics that currently do not exist.
    - **Potency Assays**: Specifications for cell therapy products will require a large scale collaborative effort between developers, clinicians, regulators and academic researchers and is critical to the future of cell therapy products. Potency assays will also allow for examination of whether the current, pharmaceutical-based, approach to dosing for cell therapies is appropriate for biological medicines
    - **Measuring key characteristics**:
        - **Cell Counting**: Are our tools for cell counting fast and reliable enough for the future of cell therapy? This is a question for manufacturing scientists working alongside key measurement authorities such as NIBSC, NIST and LGC.
        - **Cell surface markers**: The use of gating in flow cytometry has the potential to introduce significant measurement variation, is dependent on the operator's skill and judgement (which may not be transferable) and can vary depending on who is carrying out the measurement. In addition, the identification of quality associated cell surface markers may allow for generation of a quality-based reference artefact that can be used to benchmark future measurements. This is a large-scale question for multiple authorities: clinical and academic researchers with guidance and advice from measurement authorities such as NIBSC, NIST and LGC.
        - **Statistical tools**: New tools are required to handle non-normally distributed data, or the application of existing tools. The question needs to be raised as to whether traditional manufacturing and process statistical tools are appropriate for biological therapies, and to what extent will they need to be redesigned or adapted to deal with the highly variable and sensitive therapeutic agents. This will require a high

level of cerebral research from mathematicians in tandem with processing and manufacturing scientists.

    - **Standardisation**: This is the first step in traceability of measurement, and will need equivalency between national and international centres. This is reliant on the justification of a given measurement system based on statistically robust evidence, and the co-operation between regulators in key influential states such as the USA, EU and Asian markets.

- **Raw Material [LOW]**: Raw materials are subject to existing pharmaceutical guidance and regulations regarding process control and variation, as part of the supply chain.
- **Cryopreservation [MEDIUM]**: Basic investigative research is required to determine whether cryopreservation and subsequent thawing effects the therapeutic function of a cell therapy product. This includes whether the thawing process affects the cell's phenotype or efficacy, and whether any resultant apoptosis or cell death brings the number of cells in the dose below the safety or efficacy threshold. Cryopreservation and thawing protocols require standardisation – particularly thawing which can include massaging bags of ice crystals. This will require academic investigation coupled with clinical researchers.
- **Processing [MEDIUM]**: Cell therapy products are currently produced via manual cell culture, which is subjective to the operator and limited in scale. Automation may be required, but is dependent on appropriate measures of quality and a sufficient understanding of the process to replicate safely. Care must be taken to avoid replication of operator bias or error. This is a supply chain issue, currently locked by a problematic business case, and could be unlocked, or encouraged by demonstrating true comparability between machines. This will require a collaboration between academic research, alongside the supply chain manufacturers and standards organisations such as LGC and NIBSC.
- **Technicians [MEDIUM]**: A better understanding of the process, or an alternative way of representing SOPs and protocols may improve replication and reduce errors in the current manual processing setting – one of the key observations from the clinical centre was how difficult new protocols were to learn and copy from others due to personal alternations of SOPs.

- **Practice of Medicine [MEDIUM]:** We need to understand exactly how new therapies are being used in the clinic, especially when this differs from intended use. Developers will have to work around this practice of medicine, but it is important that we understand how these therapies are being used. Alongside appropriate measures of quality, this is critical to understanding dosing of cell therapy products and raises questions about liability in the event of adverse effects (by using a product in a different way as was intended by the manufacturer, and by the regulations that have been established). By understanding this relationship, developers can work around this requirement to personalise therapy, and build it into the development process. It may not be feasible to remove variation as a function of clinical practice due to this highly-personalised nature of healthcare reflecting the needs of individual patients. The medical regulator will have to work closely with the product/ process regulator, alongside the developer, to achieve this.

6.4 Summary

The extent of variation has been established, in terms of cell numbers, for existing blood-based stem cell therapy. This variation is currently accepted under the practice of medicine, as it can be controlled in the clinic by “pooling” products together to meet minimum collection criteria, and by reducing the volume of larger products.

More advanced therapeutics are likely to be complicated by the presence of an expansion and growth stage in the process, although this may compensate for pooling and volume reduction by being able to grow specific numbers of cells – assuming that the product does not become contaminated by this step, or more critically for cellular therapy, does not proliferate into an unwanted, or unsafe cell type/ line.

Autologous therapies will remain reliant on patient specific factors, but allogeneic therapies may be able to source donor material, in terms of quality and quantity, based on critical quality attributes. The next stage in the investigation of variation is the correlation between variation and product quality, which will require a cost-effective and representative quality metric.

If variation can be related to a quantifiable measure of quality, then the amount of acceptable variation with respect to patient outcome can be established, and a manufacturing process designed accordingly. Currently, the amount of variation can be up to six orders of magnitude around the median, so it is important to establish how much variation is acceptable without affecting patient safety, or product efficacy.

Clinical centres such as the Dana Farber Cancer Institute or the UK National Health Service holds information about products and patients in multiple databases – patient records, process records, outcome registries – and they may be difficult to access and compare without proper ethical approval and security measures. Unlocking and utilising these databases is important.

New and astounding advances in biological medicines have the potential to revolutionise healthcare, but before this can be a reality, we must prove their long-term safety and efficacy, their cost-effectiveness and demonstrate the capability to produce them at a sufficient scale. This cannot be achieved without addressing questions of biological variation, appropriate measurement and statistical tools, and an understanding of the interface between engineers, scientists and clinicians. This will require a collaborative framework between industrial and commercially driven stakeholders, working in the interests of patients, aligning together to address dramatically unmet clinical needs and creating the future of medicine.

7.0 References

[1] C. Mason and P. Dunnill, “A brief definition of regenerative medicine,” Regenerative Medicine, vol. 3, no. 1. pp. 1–5, 2008.

[2] M. J. Haller, H. Viener, C. Wasserfall, T. Brusko, M. A. Atkinson, and D. A. Schatz, “Autologous umbilical cord blood infusion for type 1 diabetes,” Experimental Hematology, vol. 36, no. 6, pp. 710–715, 2008.

[3] J. Ringe, C. Kaps, G.-R. Burmester, and M. Sittinger, “Stem cells for regenerative medicine: advances in the engineering of tissues and organs,” Naturwissenschaften (The Science of Nature), vol. 89, no. 8, pp. 338–51, 2002.

[4] J. Jansen, J. M. Thompson, M. J. Dugan, P. Nolan, M. C. Wiemann, R. Birhiray, P. J. Henslee-Downey, and L. P. Akard, “Peripheral blood progenitor cell transplantation,” Therapeutic Apheresis and Dialysis, vol. 6, no. 1, pp. 5–14, 2002.

[5] O. Lindvall and Z. Kokaia, “Stem cells for the treatment of neurological disorders,” Nature, vol. 441, no. 7097, pp. 1094–1096, 2006.

[6] D. J. Williams, “Overcoming manufacturing and scale-up challenges.,” Regenerative Medicine, vol. 6, no. 6 Supplement, pp. 67–9, 2011.

[7] D. J. Williams, R. J. Thomas, P. C. Hourd, A. Chandra, E. Ratcliffe, Y. Liu, E. A. Rayment, and J. R. Archer, “Precision manufacturing for clinical-quality regenerative medicines,” Philosophical Transactions of the Royal Society A, vol. 370, no. 1973, pp. 3924–3949, 2012.

[8] O. Militsakh, T. Day, J. Hornig, E. Lentsch, J. Skoner, M. B. Gillespie, A. Sharma, B. Neville, Z. Rumboldt, R. James, J. Goddard, K. Shikata, Y. Doi, E. M. Rosen, I. D. Goldberg, S. Fan, L. Kong, J. J. Lu, A. Chlenski, S. L. Cohn, F. Hlubek, C.-N. Qian, B. T. Teh, A. Batistatou, K. Charalabopoulos, C. Christensen, R. Reddel, I. M. C. Ploeg, B. B. R. Kroon, O. E. Nieweg, M. C. Rijk, O. Winqvist, M. Thörn, C. Zwick, M. Pfreundschuh, B. Knudsen, J. Lucas, P. Nelson, N. D. Kashikar, P. K. Datta, A. F. Hottinger, A. Hormigo, E. Knobler, R. Knobler, J. H. McCarty, A. Bardia, C. L. Loprinzi, R. Buettner, R. Jove, R. Garcia, D. A. Frank, T. Nakatsura, O. Witt, K. Yoshida, P.-L. Chou, G. J. Tsay, T. Takeuchi, M. Furihata, F. M. Robertson, T. E. Werbowetski-Ogilvie, R. F. Maestro, M. S. Sadr, S. Dennler, P. Dijke, S. Gadgeel, K.-H. Thierauch, K. W. Yip, F.-F. Liu, A. Cano, M. A. Neto, T. M. Rünger, M. C. Frühwald, L. Trakhtenbrot, J. Mi, J. M. Larner, J. L. Hamlin, N. S. Radin, T. L. Lo, G. R. Guy, W. A. Yeudall, S. J. Parsons, F. Fan, L. C. Platanias, M. Mcgahren-Murray, K. Keyomarsi, J. Kos, G. M. Seigel, M. R. Alison, E. Hiyama, A. G. Renehan, C. S. Potten, D. G. Tang, P. Foster, K. Lin, C. Bergelt, Z. Ronai, E. Cukierman, M. Aumercier, M. Hodsdon, E. Lolis, A. Mehta, F.-L. Chung, H. Glatt, A. Oberthür, P. Warnat, S. Aranda, Z. Culig, B. H. Chung, Y.-B. Shin, M. Kim, W. M. U. Grevenstein, C. H. J. Eijck, S. G. Baker, B. S. Kramer, C. Mahotka, M. Carbone, H. Yang, S. C. Mueller, P. J. Coopman, D. Bruijn, A. G. Kessel, and J. M. George, “Stem Cell Plasticity,” in Encyclopedia of Cancer, Berlin, Springer Berlin Heidelberg, 2011, pp. 3511–3513.

[9] L. B. Jorde and S. P. Wooding, “Genetic variation, classification and ‘race,” Nature Genetics, vol. 36, no. 11 Supplement, pp. S28–S33, 2004.

[10] L. Provost and C. Norman, “Variation through the Ages,” Quality Progress, Special Issue, pp. 39–44, 1990.

[11] J. Byrnes, “7 Tactics To Reduce Variation in Clinical Practice,” Healthcare Financial Management, vol. 66, no. 4, pp. 146-148, 2012.

[12] K. M. Nordström, M. O. Närhi, and A. P. J. Vepsäläinen, “Services for distribution of tissue engineering products and therapies,” International Journal of Productivity and Performance Management, vol. 58, no. 1, pp. 11–28, 2009.

[13] B.-H. Mevik, E. M. Faergestad, M. R. Ellekjaer, and T. Naes, “Using Raw Material Measurements in Robust Process Optimization,” Chemometrics & Intelligent Laboratory Systems 55, pp. 133–145, 2001.

[14] Y. Liu, P. Hourd, A. Chandra, and D. J. Williams, “Human cell culture process capability: a comparison of manual and automated production,”Journal of Tissue Engineering and Regenerative Medicine, vol. 4, no. 1, pp. 45–54, 2010.

[15] P. Martin, “The Market Opportunity,” in Building A Viable Regenerative Medicine Industry - A Guide for Stakeholders, 2010, pp. 13–20.

[16] L. Li and T. Xie, “Stem cell niche: structure and function.,” Annual Review Cell and Developmental Biology, vol. 21, pp. 605–31, 2005.

[17] D. T. Scadden, “The stem-cell niche as an entity of action,” Nature, vol. 441, no. 7097, pp. 1075–1079, 2006.

[18] B. Ohlstein, T. Kai, E. Decotto, and A. Spradling, “The stem cell niche: theme and variations.,” Current Opinion in Cell Biology, vol. 16, no. 6, pp. 693–699, 2004.

[19] J. Jansen, J. Thompson, M. Dugan, P. Nolan, M. Wiemann, R. Birhiray, P. Henslee-Downey, and L. Akard, “Peripheral blood progenitor cell transplantation,” Therapeutic Apheresis and Dialysis, vol. 6, no. 1, pp. 5–14, 2002.

[20] E. Thomas, H. J. Lochte, W. Lu, and J. Ferrebee, “Intravenous Infusion of Bone Marrow in Patients Receiving Radiation and Chemotherapy,” New England Journal of Medicine, vol. 257, no. 11, pp. 491–496, 1957.

[21] J. van Rood, “The detection of transplantation antigens in leukocytes.,” Seminars in Hematology, vol. 5, no. 2, pp. 187–214, 1968.

[22] A. Gratwohl, J. Passweg, H. Baldomero, and J. Hermans, “Special report: blood and marrow transplantation activity in Europe 1997,” Bone Marrow Transplantation, vol. 24, no. 3, pp. 231–245, 1999.

[23] “Regulation (EC) No 1394/2007 of the European Parliament and of the Council of 13 November 2007 on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004,” Official Journal of the European Union, no. 324, pp. 121–137, 2007.

[24] Investigational new drug application, Title 21, Chapter 1, Subchapter D, Part 312, Code of Federal Regulations (2003).

[25] Z. Ivanovic, “Hematopoietic stem cells in research and clinical applications: The ‘CD34 issue’,” World Journal of Stem Cells, vol. 2, no. 2, pp. 18–23, 2010.
[26] P. Hourd, A. Chandra, N. Medcalf, and D. J. Williams, “Regulatory challenges for the manufacture and scale-out of autologous cell therapies,” in StemBook, pp. 1–13.

[27] A. Bernard and L. Boumsell, “[Human leukocyte differentiation antigens].,” La Presse Médicale, vol. 13, no. 38, pp. 2311–2316, 1984.

[28] M. K. Mamidi, S. Dutta, R. Bhonde, A. K. Das, and R. Pal, “Allogeneic and autologous mode of stem cell transplantation in regenerative medicine: Which way to go?,” Medical Hypotheses, vol. 83, no. 6, pp. 787–791, 2014.

[29] A. Gratwohl, “Theoretical and practical issues of autologous versus allogeneic stem cell transplantation in multiple sclerosis,” Cell, vol. 2, no. 6, pp. 6–8, 2011.

[30] S. C. Juneja, S. Viswanathan, M. Ganguly, and C. Veillette, “A simplified method for the aspiration of bone marrow from patients undergoing hip and knee Joint replacement for isolating mesenchymal stem cells and in vitro chondrogenesis,” Bone Marrow Research, vol. 2016, pp. 1–18, 2016.

[31] C. Okafor, D. M. Ward, M. H. Mokrzycki, R. Weinstein, P. Clark, and R. A. Balogun, “Introduction and overview of therapeutic apheresis,” Journal of Clinical Apheresis, vol. 25, no. 5, pp. 240–249, 2010.

[32] Stem Cell Trialists’ Collaborative Group, “Allogeneic peripheral blood stem-cell compared with bone marrow transplantation in the management of hematologic malignancies: an individual patient data meta-analysis of nine randomized trials,” Journal of Clinical Oncology, vol. 23, no. 22, pp. 5074–5087, 2005.

[33] D. Gallardo, R. De La Cámara, J. B. Nieto, I. Espigado, A. Iriondo, A. Jiménez-Velasco, C. Vallejo, C. Martín, D. Caballero, S. Brunet, D. Serrano, C. Solano, J. M. Ribera, J. De La Rubia, and E. Carreras, “Is mobilized peripheral blood comparable with bone marrow as a source of hematopoietic stem cells for allogeneic transplantation from HLA-identical sibling donors? A case-control study,” Haematologica, vol. 94, no. 9, pp. 1282–1288, 2009.

[34] I. Sirinoglu Demiriz, E. Tekgunduz, and F. Altuntas, “What Is the Most Appropriate Source for Hematopoietic Stem Cell Transplantation? Peripheral Stem Cell/Bone Marrow/Cord Blood,” Bone Marrow Research, vol. 2012, pp. 1–5, 2012.

[35] D. Stamatovic, B. Balint, L. Tukic, M. Elez, O. Tarabar, M. Todorovic, G. Ostojic, Z. Tatomirovic, M. Ljubenov, S. Marjanovic, and M. Malesevic, “Impact of stem cell source on allogeneic stem cell transplantation outcome in hematological malignancies,” Vojnosanitetski Pregled, vol. 68, no. 12, pp. 1026–1032, 2011.

[36] N. Reddy, M. Vemuri, and R. Pallu, “Isolation of stem cells from human umbilical cord blood.,” Methods in Molecular Biology, vol. 407, pp. 149–163, 2007.

[37] M. Eapen, J. Le Rademacher, J. H. Antin, R. E. Champlin, J. Carreras, J. Fay, J. R. Passweg, J. Tolar, M. M. Horowitz, J. C. W. Marsh, and H. J. Deeg, “Effect of stem cell source on outcomes after unrelated donor transplantation in severe aplastic anemia,” Blood, vol. 118, no. 9, pp. 2618–2621, 2011.

[38] D. M. Lathers, E. Lubbers, N. M. Beal, M. A. Wright, and M. R. Young, “Cultures derived from peripheral blood CD34+ progenitor cells of head and neck cancer patients and from cord blood are functionally different.,” Human Immunology, vol. 60, no. 12, pp. 1207–1215, 1999.

[39] N. A. P. Franken, H. M. Rodermond, J. Stap, J. Haveman, and C. van Bree, “Clonogenic assay of cells in vitro.,” Nature Protocols, vol. 1, no. 5, pp. 2315–2319, 2006.

[40] W. Strober, “Trypan blue exclusion test of cell viability.,” Current Protocols in Immunology, vol. Appendix 3: Appendix 3B, 2001.

[41] N. C. L. Zembruski, V. Stache, W. E. Haefeli, and J. Weiss, “7-Aminoactinomycin D for apoptosis staining in flow cytometry.,” Analytical Biochemistry, vol. 429, no. 1, pp. 79–81, 2012.

[42] L. Fina, H. V Molgaard, D. Robertson, N. J. Bradley, P. Monaghan, D. Delia, D. R. Sutherland, M. A. Baker, and M. F. Greaves, “Expression of the CD34 gene in vascular endothelial cells.,” Blood, vol. 75, no. 12, pp. 2417–2426, 1990.

[43] W. E. Beschorner, C. I. Civin, and L. C. Strauss, “Localization of hematopoietic progenitor cells in tissue with the anti-My-10 monoclonal antibody.,” American Journal of Pathology, vol. 119, no. 1, pp. 1–4, 1985.

[44] S. M. Watt, K. Karhi, K. Gatter, A. J. Furley, F. E. Katz, L. E. Healy, L. J. Altass, N. J. Bradley, D. R. Sutherland, and R. Levinsky, “Distribution and epitope analysis of the cell membrane glycoprotein (HPCA-1) associated with human hemopoietic progenitor cells.,” Leukemia, vol. 1, no. 5, pp. 417–426, 1987.

[45] E. A. Sankey, L. More, and A. P. Dhillon, “QBEnd/10: a new immunostain for the routine diagnosis of Kaposi’s sarcoma.,” Journal of Pathology, vol. 161, no. 3, pp. 267–271, 1990.

[46] R. O. Schlingemann, F. J. Rietveld, R. M. de Waal, N. J. Bradley, A. I. Skene, A. J. Davies, M. F. Greaves, J. Denekamp, and D. J. Ruiter, “Leukocyte antigen CD34 is expressed by a subset of cultured endothelial cells and on endothelial abluminal microprocesses in the tumor stroma.,” Laboratory Investigation, vol. 62, no. 6, pp. 690–696, 1990.

[47] C. Hicks, R. Wong, a Manoharan, and Y. L. Kwan, “Viable CD34+/CD133+ blood progenitor cell dose as a predictor of haematopoietic engraftment in multiple myeloma patients undergoing autologous peripheral blood stem cell transplantation.,” Annals of Hematology, vol. 86, no. 8, pp. 591–598, 2007.

[48] P. Salmikangas, M. Menezes-Ferreira, I. Reischl, A. Tsiftsoglou, J. Kyselovic, J. J. Borg, S. Ruiz, E. Flory, J.-H. Trouvin, P. Celis, J. Ancans, M. Timon, G. Pante, D. Sladowski, M. Lipnik-Stangelj, and C. K. Schneider, “Manufacturing, characterization and control of cell-based medicinal products: challenging paradigms toward commercial use.,” Regenerative Medicine, vol. 10, no. 1, pp. 65–78, 2015.

[49] J. Basu and J. W. Ludlow, “Cell-based therapeutic products: potency assay development and application.,” Regenerative Medicine, vol. 9, no. 4, pp. 497–512, 2014.

[50] Centre for Biologics Evaluation and Research, “Guidance for industry: potency tests for cellular and gene therapy products,” 2011.

[51] C. a. Bravery, J. Carmen, T. Fong, W. Oprea, K. H. Hoogendoorn, J. Woda, S. R. Burger, J. a. Rowley, M. L. Bonyhadi, and W. Van'T Hof, "Potency assay development for cellular therapy products: An ISCT* review of the requirements and experiences in the industry," Cytotherapy, vol. 15, no. 1, pp. 9–19, 2013.

[52] I. Mills, "Draft Chapter 2 for SI Brochure, following redefinitions of the base units," 2010.
[53] L. Ährlund-Richter, M. De Luca, D. R. Marshak, M. Munsie, A. Veiga, and M. Rao, "Isolation and Production of Cells Suitable for Human Therapy: Challenges Ahead," Cell Stem Cell, vol. 4, no. 1, pp. 20–26, 2009.

[54] Y. Liu, P. Hourd, A. Chandra, and D. Williams, "Human Cell Culture Process Capability: a comparison of manual and automated production," Journal of Tissue Engineering and Regenerative Medicine, vol. 4, no. 1, pp. 45–54, 2010.

[55] S. J. Pocock, D. Ashby, a G. Shaper, M. Walker, and P. M. Broughton, "Diurnal variations in serum biochemical and haematological measurements.," Journal of Clinical Pathology, vol. 42, no. 2, pp. 172–179, 1989.

[56] M. Maes, K. Mommen, D. Hendrickx, D. Peeters, P. D'Hondt, R. Ranjan, F. De Meyer, and S. Scharpé, "Components of biological variation, including seasonality, in blood concentrations of TSH, TT3, FT4, PRL, cortisol and testosterone in healthy volunteers.," Clinical Endocrinology, vol. 46, no. 5, pp. 587–598,1997.

[57] M. W. Maijenburg, M. Kleijer, K. Vermeul, E. P. J. Mul, F. P. J. Van Alphen, C. E. Van Der Schoot, and C. Voermans, "The composition of the mesenchymal stromal cell compartment in human bone marrow changes during development and aging.," Haematologica, vol. 97, no. 2, pp. 179–183, 2012.

[58] R. H. Kao, C. C. Li, C. K. Shaw, T. F. Wang, S. C. Chu, S. H. Chen, C. Y. Yao, K. P. Huang, and Y. F. Wu, "Correlation between characteristics of unrelated bone marrow donor and cell density of total nucleated cell in bone marrow harvest," International Journal of Hematology, vol. 89, no. 2pp. 227–230, 2009.

[59] B. J. Bain, "Ethnic and sex differences in the total and differential white cell count and platelet count.," Journal of Clinical Pathology, vol. 49, no. 8, pp. 664–666, 1996.

[60] A. K. Lindahl, "Variation in clinical practice - an impetus for change and improvement.," European Journal of Vascular and Endovascular Surgery, vol. 42, no. 5, pp. 596–597, 2011.

[61] R. Gauld, J. Horwitt, S. Williams, and A. B. Cohen, "What Strategies do US hospitals employ to reduce unwarranted clinical practice variations?," American Journal of Medical Quality, vol. 26, no. 2, pp. 120–126, 2011.

[62] P. a Poole-Wilson, "Global differences in the outcome of heart failure: implications for clinical practice.," Journal of the American College of Cardiology, vol. 52, no. 20, pp. 1649–1651, 2008.

[63] J. K. Karp and K. E. King, "International variation in volunteer whole blood donor eligibility criteria.," Transfusion, vol. 50, no. 2, pp. 507–513, 2010.

[64] M. Ali Erkurt, I. Berber, I. Kuku, E. Kaya, and I. Nizam, “Donor Selection in Allogeneic Stem Cell Transplantation,” American Journal of Clinical Medical Research, vol. 2, no. 1, pp. 32–35, 2014.

[65] H. R. Lee, J. S. Park, S. Shin, E. Y. Roh, J. H. Yoon, K. S. Han, B. J. Kim, R. W. Storms, and N. J. Chao, “Increased numbers of total nucleated and CD34+ cells in blood group O cord blood: An analysis of neonatal innate factors in the Korean population,” Transfusion, vol. 52, no. 1, pp. 76–81, 2012.

[66] M. A. Shammas, “Telomeres, lifestyle, cancer, and aging,” Current Opinion in clinical Nutrition and Metabolic Care, vol. 14, no. 1, pp. 28–34, 2011.

[67] J. de La Rubia, M. a Díaz, A. Verdeguer, M. J. Pascual, C. Arbona, R. Arrieta, S. Brunet, J. Bargay, C. Martínez, I. Espigado, D. Serrano, A. Alegre, F. de Arriba, J. de La Serna, C. Zamora, L. Benlloch, and M. a Sanz, “Donor age-related differences in PBPC mobilization with rHuG–CSF,” Transfusion, vol. 41, no. 2, pp. 201–205, 2001.

[68] C. Arber, A. Gratwohl, A. Tichelli, J. Halter, M. Stern, and A. Rovo, “Graft source determines human hematopoietic progenitor distribution pattern within the CD34 þ compartment,” Bone Marrow Transplantation, vol. 46, pp. 650–658, 2011.

[69] J. De Rubia, C. Arbona, F. De Arriba, C. Can, C. Zamora, and M. A. Dı, “Transplantation and cellular engineering,” Tranfusion, vol. 42, pp. 4–9, 2002.

[70] R. M. McCann, D. R. Marsh, A. Horner, and S. A. Clarke, “Body mass index is more predictive of progenitor number in bone marrow stromal cell population than age in men: expanding the predictors of the progenitor compartment.,” Tissue Engineering Part A, vol. 16, no. 3, pp. 889–896, 2010.

[71] W. Dc, P. Grigg, A. W. Roberts, H. Raunow, S. Houghton, J. E. Layton, A. W. Boyd, K. M. Mcgrath, and D. Maher, “Optimizing dose and scheduling of filgrastim (granulocyte colony-stimulating factor) for mobilization and collection of peripheral blood progenitor cells in normal volunteers,” Blood, vol. 86, no. 12, pp. 4437–4445, 2013.

[72] C. Arbona, F. Prosper, I. Benet, F. Mena, C. Solano, and J. Garcia-Conde, “Comparison between once a day vs twice a day G-CSF for mobilization of peripheral blood progenitor cells (PBPC) in normal donors for allogeneic PBPC transplantation.,” Bone Marrow Transplantation, vol. 22, no. 1, pp. 39–45, 1998.

[73] A. Stolzing, E. Jones, D. McGonagle, and A. Scutt, “Age-related changes in human bone marrow-derived mesenchymal stem cells: consequences for cell therapies.,” Mechanisms of Ageing and Development, vol. 129, no. 3, pp. 163–173, 2008.

[74] P. Anderlini, D. Przepiorka, C. Seong, T. Smith, Y. Huh, J. Lauppe, R. Champlin, and M. Korbling, “Factors affecting mobilization of CD34+ cells in normal donors treated with filgrastim,” Transfusion, vol. 37, pp. 507–512, 1997.

[75] S. J. Ings, C. Balsa, D. Leverett, S. Mackinnon, D. C. Linch, and M. J. Watts, “Peripheral blood stem cell yield in 400 normal donors mobilised with granulocyte colony-stimulating factor (G-CSF): impact of age, sex, donor weight and type of G-CSF used.,” British Journal of Haematology, vol. 134, no. 5, pp. 517–525, 2006.

[76] R. H. Jan, S. H. Wen, M. H. Shyr, and B. L. Chiang, “Impact of maternal and neonatal factors on CD34+ cell count, total nucleated cells, and volume of cord blood,” Pediatric Transplantation, vol. 12, no. 8, pp. 868–873, 2008.

[77] M. A. Baxter, R. F. Wynn, S. N. Jowitt, J. E. Wraith, and L. J. Fairbairn, “Study of Telomere Length Reveals Rapid Aging of Human Marrow Stromal Cells following In Vitro Expansion,” Stem Cell, vol. 22, no. 5, pp. 675–682, 2004.

[78] J. Domen, K. Gandy, and J. Dalal, “Emerging uses for pediatric hematopoietic stem cells.,” Pediatric Research, vol. 71, no. 4 (pt 2), pp. 411–417, 2012.

[79] W. P. T. James, “The epidemiology of obesity: the size of the problem.,” Journal of International Medicine, vol. 263, no. 4, pp. 336–352, 2008.

[80] I. Keser, E. Suyani, S. Z. Aki, and A. Gulsan Turkoz Sucak, “The positive impact of regular exercise program on stem cell mobilization prior to autologous stem cell transplantation,” Transfusion and Apheresis Science, vol. 49, no. 2, pp. 302–306, 2013.

[81] M. N. Shalaby, M. Saad, S. Akar, M. A. A. Reda, and A. Shalgham, “The Role of Aerobic and Anaerobic Training Programs on CD34+ Stem Cells and Chosen Physiological Variables.,” Journal of Human Kinetics, vol. 35, pp. 69–79, 2012.

[82] C. Zhang, X.-H. Chen, X. Zhang, L. Gao, P.-Y. Kong, X.-G. Peng, a-H. Sun, Y. Gong, D.-F. Zeng, and Q.-Y. Wang, “Stem cell collection in unmanipulated HLA-haploidentical/mismatched related transplantation with combined granulocyte-colony stimulating factor-mobilised blood and bone marrow for patients with haematologic malignancies: the impact of donor characteristic,” Transfusion Medicine, vol. 20, no. 3, pp. 169–77, 2010.

[83] J. Müller-Ehmsen, D. Braun, T. Schneider, R. Pfister, N. Worm, K. Wielckens, C. Scheid, P. Frommolt, and M. Flesch, “Decreased number of circulating progenitor cells in obesity: beneficial effects of weight reduction.,” European Heart Journal, vol. 29, no. 12, pp. 1560–1568, 2008.

[84] M. Hadjibabaie, H. Tabeefar, K. Alimoghaddam, M. Iravani, K. Eslami, H. Honarmand, M. R. Javadi, F. Khatami, A. Ashouri, and A. Ghavamzadeh, “The relationship between body mass index and outcomes in leukemic patients undergoing allogeneic hematopoietic stem cell transplantation,” Clinical Transplantation, vol. 26, no. 1, pp. 149–155, 2012.

[85] D. T. Vogl, T. Wang, W. S. Pérez, E. A. Stadtmauer, D. F. Heitjan, H. M. Lazarus, R. A. Kyle, R. Kamble, D. Weisdorf, V. Roy, J. Gibson, K. Ballen, L. Holmberg, A. Bashey, P. L. McCarthy, C. Freytes, D. Maharaj, A. Maiolino, D. Vesole, and P. Hari, “Effect of obesity on outcomes after autologous hematopoietic stem cell transplantation for multiple myeloma.,” Biology of Blood and Marrow Transplantation, vol. 17, no. 12, pp. 1765–1774, 2011.

[86] S. a Al-Sweedan, L. Musalam, and B. Obeidat, “Factors predicting the hematopoietic stem cells content of the umbilical cord blood.,” Transfusion and Apheresis Science, vol. 48, no. 2, pp. 247–252, 2013.

[87] W. C. Strohsnitter, T. M. Savarese, H. P. Low, D. P. Chelmow, P. Lagiou, M. Lambe, K. Edmiston, Q. Liu, I. Baik, K. L. Noller, H. O. Adami, D. Trichopoulos, and C. C. Hsieh, “Correlation of umbilical cord blood haematopoietic stem and progenitor cell levels with birth weight: implications for a prenatal influence on cancer risk,” British Journal of Cancer, vol. 98, no. 3, pp. 660–663, 2008.

[88] P. Aroviita, K. Teramo, V. Hiilesmaa, P. Westman, and R. Kekoma, “Birthweight of full-term infants is associated with cord blood CD34 ‡ cell concentration,” Acta Paediatrica, vol. 93, pp. 1323–1329, 2004.

[89] G. Zamora-Ortiz, R. Morales-Floranes, S. Velázquez-Sánchez-de-Cima, A. García-Villaseñor, and G. Ruiz-Argüelles, “Gender as a prognostic factor for overall survival in patients auto or allografted employing the Mexican Method: A single institution experience,” Rev Hematologica Mex, vol. 14, no. 3, pp. 120–123, 2013.

[90] S. Morishita, K. Kaida, S. Yamauchi, T. Wakasugi, S. Yoshihara, K. Taniguchi, S. Ishii, K. Ikegame, N. Kodama, H. Ogawa, and K. Domen, “Gender differences in health-related quality of life, physical function and psychological status among patients in the early phase following allogeneic haematopoietic stem cell transplantation.,” Psycho-Oncology., vol. 22, no. 5, pp. 1159–1166, 2013.

[91] S. Vasu, S. F. Leitman, J. F. Tisdale, M. M. Hsieh, R. W. Childs, a J. Barrett, D. H. Fowler, M. R. Bishop, E. M. Kang, H. L. Malech, C. E. Dunbar, H. M. Khuu, R. Wesley, Y. Y. Yau, and C. D. Bolan, “Donor demographic and laboratory predictors of allogeneic peripheral blood stem cell mobilization in an ethnically diverse population.,” Blood, vol. 112, no. 5, pp. 2092–2100, 2008.

[92] M. S. Cairo, E. L. Wagner, J. Fraser, G. Cohen, C. Van De Ven, S. L. Carter, N. A. Kernan, and J. Kurtzberg, “Characterization of banked umbilical cord blood hematopoietic progenitor cells and lymphocyte subsets and correlation with ethnicity, birth weight, sex, and type of delivery: a Cord Blood Transplantation (COBLT) Study report,” Transfusion, vol. 45, pp. 856–866, 2005.

[93] K. K. Ballen, J. Kurtzberg, T. A. Lane, B. R. Lindgren, J. P. Miller, D. Nagan, B. Newman, N. Rupp, and N. R. Haley, “Racial diversity with high nucleated cell counts and CD34 counts achieved in a national network of cord blood banks,” Biology of Blood and Marrow Transplantation, vol. 10, no. 4, pp. 269–275, 2004.

[94] J. W. Hsu, J. R. Wingard, B. R. Logan, P. Chitphakdithai, G. Akpek, P. Anderlini, A. S. Artz, C. Bredeson, S. Goldstein, G. Hale, P. Hematti, S. Joshi, R. T. Kamble, H. M. Lazarus, P. V. O’Donnell, M. A. Pulsipher, B. N. Savani, R. M. Schears, B. E. Shaw, and D. L. Confer, “Race and Ethnicity Influences Collection of Granulocyte Colony-Stimulating Factor-Mobilized Peripheral Blood Progenitor Cells from Unrelated Donors, a Center for International Blood and Marrow Transplant Research Analysis,” Biology of Blood and Marrow Transplantation, vol. 21, no. 1, pp. 165–171, 2015.

[95] N. Majhail, S. Nayyar, M. Burton Santibañez, E. Murphy, and E. Denzen, “Racial disparities in hematopoietic cell transplantation in the United States,” Bone Marrow Transplantation, vol. 47, no. 11, 2012.

[96] S. Fruehauf, R. Haas, W. J. Zeller, and W. Hunstein, “CD34 selection for purging in multiple myeloma and analysis of CD34+ B cell precursors.,” Stem Cells, vol. 12, no. 1, pp. 95–102, 1994.

[97] D. R. Sutherland, A. Keating, R. Nayar, S. Anania, and A. K. Stewart, “Sensitive detection and enumeration of CD34+ cells in peripheral and cord blood by flow cytometry.,” Experimental Hematology, vol. 22, no. 10, pp. 1003–1010, 1994.

[98] S.-Q. Ji, H.-R. Chen, H.-X. Wang, H.-M. Yan, S.-P. Pan, and C.-Q. Xun, “Comparison of outcome of allogeneic bone marrow transplantation with and without granulocyte colony-stimulating factor (lenograstim) donor-marrow priming in patients with chronic myelogenous leukemia,” Biology of Blood and Marrow Transplantation, vol. 8, no. 5, pp. 261–267, 2002.

[99] F. Lefrère, L. Mauge, D. Réa, J. A. Ribeil, L. Dal Cortivo, A. C. Brignier, C. Aoun, J. Larghéro, M. Cavazzana-Calvo, and J. M. Micléa, “A specific time course for mobilization of peripheral blood CD34+ cells after plerixafor injection in very poor mobilizer patients: Impact on the timing of the apheresis procedure,” Transfusion, vol. 53, no. 3, pp. 564–569, 2013.

[100] S. Sivgin, E. Karakus, L. Kaynar, F. Kurnaz, C. Pala, M. Keklik, G. Zararsiz, M. Solmaz, B. Eser, M. Cetin, and A. Unal, “The comparison of Filgrastim (Neupogen®), biosimilar filgrastim (Leucostim®) and Lenograstim (Granocyte®) as a first line peripheral blood stem cell mobilization strategy in autologous hematopoieitic stem cell transplantation: A single center experience,” Transfusion and Apheresis Science, pp. 0–5, 2013.

[101] J. Manko, A. Walter-Croneck, D. Jawniak, N. Grzasko, M. Gorska-Kosicka, M. Cioch, and A. Dmoszynska, “A clinical comparison of the efficacy and safety of biosimilar G-CSF and originator G-CSF in haematopoietic stem cell mobilization,” Pharmacology Reports, vol. 66, no. 2, pp. 239–242, 2014.

[102] B. J. Bolwell, M. Goormastic, T. Yanssens, R. Dannley, P. Baucco, and A. Fishleder, “Comparison of G-CSF with GM-CSF for mobilizing peripheral blood progenitor cells and for enhancing marrow recovery after autologous bone marrow transplant.,” Bone Marrow Transplant., vol. 14, no. 6, pp. 913–918, 1994.

[103] R. Meisel, H.-J. Laws, S. Balzer, B. Bernbeck, C. Kramm, S. Schönberger, K. Sinha, A. Tröger, M. Schmitz, J. Fischer, U. Göbel, J. Enczmann, and D. Dilloo, “Comparable long-term survival after bone marrow versus peripheral blood progenitor cell transplantation from matched unrelated donors in children with hematologic malignancies.,” Biology of Blood and Marrow Transplantation, vol. 13, no. 11, pp. 1338–1345, 2007.

[104] G. Fischmeister, M. Kurz, O. A. Haas, M. Micksche, P. Buchinger, D. Printz, G. Ressmann, T. Stroebel, C. Peters, G. Fritsch, and H. Gadner, “G-CSF versus GM-CSF for stimulation of peripheral blood progenitor cells (PBPC) and leukocytes in healthy volunteers: comparison of efficacy and tolerability.,” Annals of Hematology, vol. 78, no. 3, pp. 117–123, 1999.

[105] N. Kröger, H. Renges, W. Krüger, K. Gutensohn, C. Löliger, I. Carrero, L. Cortes, and A. R. Zander, “A randomized comparison of once versus twice daily recombinant human granulocyte colony-stimulating factor (filgrastim) for stem cell mobilization in healthy donors for allogeneic transplantation.,” British Journal Haematology, vol. 111, no. 3, pp. 761–765, 2000.

[106] L. Eidenschink, G. Dizerega, K. Rodgers, M. Bartlett, D. A. Wells, and M. R. Loken, “Basal levels of CD34 positive cells in peripheral blood differ between individuals and are stable for 18 months,” Cytometry Part B: Clinical Cytometry, vol. 82 B, no. 1, pp. 18–25, 2012.

[107] C. Huang, W. Li, W. Wu, Q. Chen, Y. Guo, Y. Zhang, D. Xu, and W. Cui, “Intra-day and inter-day biological variations of peripheral blood lymphocytes,” Clinica Chimica Acta, vol. 438, pp. 166–170, 2014.

[108] Y.-C. Hung, J. Bauer, P. Horsley, M. Waterhouse, J. Bashford, and E. Isenring, “Changes in nutritional status, body composition, quality of life, and physical activity levels of cancer patients undergoing autologous peripheral blood stem cell transplantation.,” Support. Care Cancer, vol. 21, no. 6, pp. 1579–1586, 2013.

[109] L. Jiang, S. Malik, M. Litzow, D. Gastineau, I. Micallef, V. Roy, L. Solberg, and A. C. Zubair, “Hematopoietic stem cells from poor and good mobilizers are qualitatively equivalent,” Transfusion, vol. 52, no. 3, pp. 542–548, 2012.

[110] Z. Ivanovic, M. Kovacevic-Filipovic, M. Jeanne, L. Ardilouze, A. Bertot, M. Szyporta, F. Hermitte, X. Lafarge, P. Duchez, M. Vlaski, N. Milpied, M. Pavlovic, V. Praloran, and J. M. Boiron, “CD34+ cells obtained from ‘good mobilizers’ are more activated and exhibit lower ex vivo expansion efficiency than their counterparts from ‘poor mobilizers,’” Transfusion, vol. 50, no. 1, pp. 120–127, 2010.

[111] E. Fennema, A. Renard, A. Leusink, C. van Blitterswijk, and J. de Boer, “The Effect of Bone Marrow Aspiration Strategy on the Yield and Quality of Human Mesenchymal Stem Cells,” Acta Orthopaedica, vol. 80, no. 5, pp. 618–21, 2009.

[112] D. Batinić, M. Marusić, Z. Pavletić, V. Bogdanić, B. Uzarević, D. Nemet, and B. Labar, “Relationship between differing volumes of bone marrow aspirates and their cellular composition,” Bone Marrow Transplantation, vol. 6, no. 2, pp. 103–107, 1990.

[113] P. Anversa, J. Kajstura, and A. Leri, “Circulating progenitor cells: search for an identity,” Circul ation, vol. 110, no. 20, pp. 3158–60, 2004.

[114] G. F. Muschler, C. Boehm, and K. Easley, “Aspiration to obtain osteoblast progenitor cells from human bone marrow: the influence of aspiration volume,” Journal of Bone and Joint Surgery, vol. 79, no. 11, pp. 1699–1709, 1997.

[115] M. Tol, A. R. Akar, S. Durdu, E. Ayyildiz, and O. Ilhan, “Comparison of different needle diameters and flow rates on bone marrow mononuclear stem cell viability: an ex vivo experimental study,” Cytotherapy, vol. 10, no. 1, pp. 98–99, 2008.

[116] D. F. Stroncek, P. Jin, E. Wang, and B. Jett, “Potency analysis of cellular therapies: the emerging role of molecular assays,” Journal of Translational Medicine, vol. 5, p. 24, 2007.

[117] J. F. Abrahamsen, R. Smaaland, R. B. Sothern, and O. D. Laerum, “Variation in cell yield and proliferative activity of positive selected human CD34+ bone marrow cells along the circadian time scale.,” European Journal of Haematology, vol. 60, no. 1, pp. 7–15, 1998.

[118] C. D. Ford, J. Greenwood, A. Strupp, and C. M. Lehman, “Effects on Collection Efficiency and Efficacy,” Transfusion, vol. 42, no. 7, pp. 904–911, 2002.

[119] L. Dubrovsky, E. C. C. Wong, E. Perez-Albuerne, B. Loechelt, N. Kamani, J. Sande, K. Mintz, W. Paul, N. L. C. Luban, B. R. Rood, and T. Fry, “CD34+ collection efficiency as a function of blood volumes processed in pediatric autologous peripheral blood stem cell collection,” Journal of Clinical Apheresis, vol. 26, no. 3, pp. 131–137, 2011.

[120] S. Flommersfeld, T. Bakchoul, G. Bein, A. Wachtel, C. Loechelt, and U. J. Sachs, “A single center comparison between three different apheresis systems for autologous and allogeneic stem cell collections,” Transfusion and Apheresis Science, vol. 49, no. 3, pp. 428–433, 2013.

[121] B. Cherqaoui, N. Rouel, A. Auvrignon, A.-S. Defachelles, F. Deméocq, J. Kanold, and E. Merlin, “Peripheral blood stem cell collection in low-weight children: retrospective comparison of two apheresis devices.,” Transfusion, vol. 54, pp. 1371–1378, 2013.

[122] S. Brauninger, H. Bialleck, K. Thorausch, T. Felt, E. Seifried, and H. Bonig, “Allogeneic donor peripheral blood ‘stem cell’ apheresis: Prospective comparison of two apheresis systems,” Transfusion, vol. 52, no. 5, pp. 1137–1145, 2012.
[123] K. W. Sung, H. W. Chueh, N. H. Lee, D. H. Kim, S. H. Lee, K. H. Yoo, H. H. Koo, E. S. Kang, and D. W. Kim, “Optimal time to start peripheral blood stem cell collection in children with high-risk solid tumors,” Journal of Korean Medical Science, vol. 29, no. 1, pp. 110–116, 2014.

[124] E. Derenzini, V. Stefoni, R. Maglie, B. Casadei, C. Pellegrini, A. Broccoli, G. Stefani, S. Fanti, M. R. Motta, R. Narducci, L. Argnani, and P. L. Zinzani, “Collection of Hematopoietic Stem Cells after Previous Radioimmunotherapy is Feasible and Does Not Impair Engraftment after Autologous Stem Cell Transplantation inFollicular Lymphoma,” Biology of Blood and Marrow Transplantation, vol. 19, no. 12, pp. 1695–1701, 2013.

[125] W. Zeller, I. Hesse, M. Dürken, M. Stockschläder, W. Krüger, S. O. Peters, T. A. Walter, C. Greenshaw, H. Kabisch, and A. R. Zander, “Increasing the yield of harvested bone marrow cells by raising room temperature during marrow collection.,” Experimental Hematology, vol. 23, no. 14, pp. 1527–1529, 1995.

[126] J. Vives, I. Oliver-Vila, and A. Pla, “Quality compliance in the shift from cell transplantation to cell therapy in non-pharma environments,” Cytotherapy, vol. 17, no. 8. Pp. 1009-1014, 2015

[127] E. Ratcliffe, R. J. Thomas, and D. J. Williams, “Current understanding and challenges in bioprocessing of stem cell-based therapies for regenerative medicine,” British Medical Bulletin, vol. 100, no. 1, pp. 137–155, 2011.

[128] H. Moore, “The medium is the message.,” Nature Biotechnology, vol. 24, no. 2, pp. 160–161, 2006.

[129] I. Hirosawa and J. Yoneda, “Subjective mood variations of student nurses during clinical practice: a pilot study,” Stress Medicine, vol. 14, pp. 49–55, 1998.

[130] H. Lannert, T. Able, S. Becker, M. Sommer, M. Braun, P. Stadtherr, and a D. Ho, “Optimizing BM harvesting from normal adult donors.,” Bone Marrow Transplantation, vol. 42, no. 7, pp. 443–447, 2008.

[131] M. Bevans, K. Tierney, C. Bruch, M. Burgunder, K. Castro, R. Ford, M. Miller, S. Rome, and K. Schmit-Pokorny, “Hematopoietic Stem Cell Transplantation Nursing: A Practise Variation Study,” Oncology Nursing. Forum, vol. 36, no. 6, pp. 317–325, 2012.

[132] F. Frassoni, M. Labopin, R. Powles, J.-Y. Mary, W. Arcese, A. Bacigalupo, D. Bunjes, E. Gluckman, T. Ruutu, U. W. Schaefer, J. Sierra, J. P. Vernant, R. Willemze, T. de Witte, and N. C. G. Transplantation, “Effect of centre on outcome of bone-marrow transplantation for acute myeloid leukaemia,” Lancet, vol. 355, no. 9213, pp. 1393–1398, 2000.

[133] R. Archer and D. J. Williams, “Why tissue engineering needs process engineering.,” Nature Biotechnology, vol. 23, no. 11, pp. 1353–1355, 2005.

[134] H. M. Blommestein, S. G. R. Verelst, P. C. Huijgens, N. M. a Blijlevens, J. J. Cornelissen, and C. a Uyl-de Groot, “Real-world costs of autologous and allogeneic stem cell transplantations for haematological diseases: a multicentre study.,” Annals of Hematology, vol. 91, no. 12, pp. 1945–1952, 2012.

[135] N. S. Majhail, L.-W. Mau, E. M. Denzen, and T. J. Arneson, “Costs of Autologous and Allogeneic Hematopoietic Cell Transplantation in the United States: A Study Using a Large National Private Claims Database,” Bone Marrow Transplantation, vol. 48, no. 2, pp. 294–300, 2013.

[136] J. Gardner, A. Faulkner, A. Mahalatchimy, and A. Webster, “Are there specific translational challenges in regenerative medicine? Lessons from other fields,” Regenerative Medicine, vol. 10, no. 7, pp. 885–895, 2015.

[137] J. Passweg, H. Baldomero, M. Stern, M. Bargetzi, M. Ghielmini, K. Leibundgut, M. Duchosal, U. Hess, R. Seger, E. Buhrfeind, U. Schanz, and A. Gratwohl, “Haematopoietic stem cell transplantation in Switzerland: A comprehensive quality control report on centre effect,” Swiss Medical Weekly, vol. 140, no. 23–24, pp. 326–334, 2010.

[138] I. C. Marschner, “Regional differences in multinational clinical trials: anticipating chance variation.,” Clinical Trials, vol. 7, no. 2, pp. 147–56, 2010.

[139] R. J. Mentz, J. Kaski, G. Dan, and S. Goldstein, “Implications of geographical variation on clinical outcomes of cardiovascular trials,” American Heart Journal, vol. 164, no. 3, pp. 303–312, 2012.

[140] J. W. Gratama, J. Kraan, W. Levering, D. R. Van Bockstaele, G. T. Rijkers, and C. E. Van der Schoot, “Analysis of variation in results of CD34+ hematopoietic progenitor cell enumeration in a multicenter study.,” Cytometry, vol. 30, no. 3, pp. 109–17, 1997.

[141] J. Y. Seo, H.-J. Huh, H.-K. Park, H.-K. Choung, D.-W. Kim, H. H. Koo, K. W. Sung, and E.-S. Kang, “Evaluation of overnight storage conditions for autologous peripheral blood stem cell products: comparison of three different conditions.,” Vox Sanguinus, vol. 103, no. 2, pp. 150–8, 2012.

[142] R. Parody, D. Caballero, F. J. Márquez-Malaver, L. Vázquez, R. Saldaña, M. D. Madrigal, C. Calderón, E. Carrillo, L. Lopez-Corral, I. Espigado, M. Carmona, O. López-Villar, and J. A. Pérez-Simón, “To freeze or not to freeze peripheral blood stem cells prior to allogeneic transplantation from matched related donors,” European Journal of Haematology, vol. 91, no. 5, pp. 448–455, 2013.

[143] D. H. Kim, N. Jamal, R. Saragosa, D. Loach, J. Wright, V. Gupta, J. Kuruvilla, J. H. Lipton, M. Minden, and H. A. Messner, “Similar Outcomes of Cryopreserved Allogeneic Peripheral Stem Cell Transplants (PBSCT) Compared to Fresh Allografts,” Biology of Blood and Marrow Transplantation, vol. 13, no. 10, pp. 1233–1243, 2007.

[144] J. M. Winter, P. Jacobson, B. Bullough, A. P. Christensen, M. Boyer, and J.-A. Reems, “Long-term effects of cryopreservation on clinically prepared hematopoietic progenitor cell products.,” Cytotherapy, vol. 16, no. 7, pp. 965–75, 2014.

[145] L. J. Fry, S. Q. Giner, S. G. Gomez, M. Green, S. Anderson, J. Horder, S. McArdle, R. Rees, and J. A. Madrigal, “Avoiding room temperature storage and delayed cryopreservation provide better postthaw potency in hematopoietic progenitor cell grafts,” Transfusion, vol. 53, no. 8, pp. 1834–1842, 2013.

[146] K. Bieback, S. Kern, H. Klüter, and H. Eichler, “Critical parameters for the isolation of mesenchymal stem cells from umbilical cord blood.,” Stem Cells, vol. 22, no. 4, pp. 625–634, 2004.

[147] A. Donmez, F. Ozsan, B. Arik, N. Ozkayin, S. çagirgan, S. Mir, F. Vural, and M. Tombuloglu, “The clearance time of infused hematopoietic stem cell from the blood circulation,” Transfus. Apher. Science, vol. 48, no. 2, pp. 235–239, 2013.

[148] D. B. Kim-Shapiro, J. Lee, and M. T. Gladwin, “Storage lesion: role of red blood cell breakdown.,” Transfusion, vol. 51, no. 4, pp. 844–851, 2011.

[149] D. Allan, T. Petraszko, H. Elmoazzen, and S. Smith, “A Review of Factors Influencing the Banking of Collected Umbilical Cord Blood Units Cord Blood Transplantation : Implications,” Stem Cells International, pp. 1–7, 2013.

[150] H. Buchan, “Gaps between best evidence and practice: causes for concern.,” Medical Journal Austalia, vol. 180, no. 6 Suppl, pp. S48-9, 2004.

[151] A. Lindhal, “Variation in Clinical Practise - an Impetus for Change and Improvement,” European Journal of Vascular and Endovascular Surgery, vol. 42, no. 5, pp. 596–597, 2011.

[152] J. B. Mckinlay, C. L. Link, K. M. Freund, L. D. Marceau, A. B. O. Donnell, and K. L. Lutfey, “Sources of Variation in Physician Adherence with Clinical Guidelines : Results from a Factorial Experiment,” Journal of General Internal Medicine, vol. 22, no.3 pp. 289–296, 2007.

[153] European Commission, “Understanding and influencing blood prescription. A market research report prepared by Eureka Strategic Research for the Clinical Excellence Commission and the National Blood Authority,” 2007.

[154] F. R. Loberiza, D. S. Serna, M. M. Horowitz, and J. D. Rizzo, “Transplant center characteristics and clinical outcomes after hematopoietic stem cell transplantation: what do we know?,” Bone Marrow Transplantation, vol. 31, no. 6, pp. 417–21, 2003.

[155] P. J. Kennedy, C. M. Leathley, and C. F. Hughes, “Clinical practice variation,” Medical Journal of Australia, vol. 193, no. 8, pp. 97–99, 2010.

[156] M. Panella, S. Marchisio, and F. Di Stanislao, “Reducing clinical variations with clinical pathways: do pathways work?,” International Journal of Quality Health Care, vol. 15, no. 6, pp. 509–21, 2003.

[157] A. Merry, J. Weller, and S. Mitchell, “Teamwork, Communication, Formula-One Racing and the Outcomes of Cardiac Surgery,” Journal of Extra-corporeal Technology, vol. 46, pp. 7–14, 2014.

[158] S. Singhal, R. Powles, S. Kulkarni, J. Treleaven, B. Sirohi, B. Millar, V. Shepherd, R. Saso, a Rowland, S. Long, S. Cabral, C. Horton, and J. Mehta, “Comparison of marrow and blood cell yields from the same donors in a double-blind, randomized study of allogeneic marrow vs blood stem cell transplantation.,” Bone Marrow Transplantation, vol. 25, no. 5, pp. 501–505, 2000.

[159] M. Dimitrios, E. Read, M. Cottler-fox, D. Couriel, J. Molldrem, C. Carter, M. Yu, C. Dunbar, and J. Barrett, “CD34+ cell dose predicts survival, posttransplant morbidity, and rate of hematologic recovery after allogeneic marrow transplants for hematologic malignancies,” Blood, vol. 88, no. 8, pp. 3223–3229, 1996.

[160] N. H. Collins, A. P. Gee, A. G. Durett, F. Kan, M.-J. Zhang, R. E. Champlin, D. Confer, M. Eapen, A. Howard, R. King, M. J. Laughlin, R. J. Plante, M. Setterholm, S. Spellman, C. Keever-Taylor, J. E. Wagner, and D. J. Weisdorf, “The effect of the composition of unrelated donor bone marrow and peripheral blood progenitor cell grafts on transplantation outcomes.,” Biology of blood and marrow Transplantation, vol. 16, no. 2, pp. 253–262, 2010.

[161] H. Bittencourt, V. Rocha, S. Chevret, G. Socie, H. Espérou, A. Devergie, L. Dal Cortivo, J. Marolleau, F. Garnier, P. Ribaud, and E. Gluckman, “Association of CD34 cell dose with hematopoietic recovery, infections and other outcomes after HLA-indentical sibling bone marrow transplantation,” Blood, vol. 99, no. 8, pp. 2726–2733, 2002.

[162] S. Singhal, R. Powles, J. Treleaven, S. Kulkarni, B. Sirohi, C. Horton, B. Millar, V. Shepherd, D. Tait, R. Saso, A. Rowland, S. Long, and J. Mehta, “A low CD34 cell dose results in higher mortality and poorer survival after blood or marrow stem cell transplantation from HLA-identical siblings : should 2 × 10 6 CD34 cells / kg be considered the minimum threshold ?,” Bone Marrow Transplantation, vol.26, no.5, pp. 489–496, 2000.

[163] A. P. Jillella and C. Ustun, “What is the optimum number of CD34+ peripheral blood stem cells for an autologous transplant?,” Stem Cells Development, vol. 13, pp. 598–606, 2004.

[164] J. R. Passweg, M.-J. Zhang, V. Rocha, F. Kan, R. E. Champlin, L. M. Isola, A. P. Gee, J. Gibson, M. J. Laughlin, H. M. Lazarus, A. Loren, D. I. Marks, A. Gratwohl, and M. Eapen, “Donor Characteristics Affecting Graft Failure, Graft-versus-Host Disease, and Survival after Unrelated Donor Transplantation with Reduced-Intensity Conditioning for Hematologic Malignancies.,” Biology of Blood and Marrow Transplantation, vol. 17, no. 12, pp. 1869–73, 2011.

[165] V. Gupta, M. Eapen, R. Brazauskas, J. Carreras, M. Aljurf, R. P. Gale, G. A. Hale, O. Ilhan, J. R. Passweg, O. Ringdén, M. Sabloff, H. Schrezenmeier, G. Socié, and J. C. W. Marsh, “Impact of age on outcomes after bone marrow transplantation for acquired aplastic anemia using HLA-matched sibling donors.,” Haematologica, vol. 95, no. 12, pp. 2119–2125, 2010.

[166] C. Kollman, “Donor characteristics as risk factors in recipients after transplantation of bone marrow from unrelated donors: the effect of donor age,” Blood, vol. 98, no. 7, pp. 2043–2051, 2001.

[167] C. Ustün, O. Arslan, M. Beksaç, H. Koç, G. Gürman, T. Ozçelik, B. Yilmaz, O. Ilhan, H. Akan, M. Ozcan, T. Demirer, a Uysal, N. Konuk, M. Arat, I. Dilek, H. Celebi, and H. S. Coskun, “A retrospective comparison of allogeneic peripheral blood stem cell and bone marrow transplantation results from a single center: a focus on the incidence of graft-vs.-host disease and relapse.,” Biology of Blood and Marrow Transplantation, vol. 5, no. 1, pp. 28–35, 1999.

[168] K. K. Ballen, J. Hicks, B. Dharan, D. Ambruso, K. Anderson, C. Bianco, L. Bemiller, W. Dickey, R. Lottenberg, M. O. Neill, M. Popovsky, D. Skerrett, A. N. Registry, C. R. Interna-, and B. E. T. Al, “Racial and ethnic composition of volunteer cord blood donors: comparison with volunteer unrelated marrow donors,” Transfusion, vol. 42, no. October, pp. 1279–1284, 2002.

[169] E. M. Richa, R. Kunnavakkam, L. a Godley, J. Kline, O. Odenike, R. a Larson, V. Nguyen, W. Stock, A. Wickrema, K. Van Besien, and A. S. Artz, “Influence of related donor age on outcomes after peripheral blood stem cell transplantation.,” Cytotherapy, vol. 14, no. 6, pp. 707–15, 2012.

[170] G. Gahrton, S. Iacobelli, J. Apperley, G. Bandini, B. Björkstrand, J. Bladé, J. M. Boiron, M. Cavo, J. Cornelissen, P. Corradini, N. Kröger, P. Ljungman, M. Michallet, N. H. Russell, D. Samson, a Schattenberg, B. Sirohi, L. F. Verdonck, L. Volin, a Zander, and D. Niederwieser, “The impact of donor gender on outcome of allogeneic hematopoietic stem cell transplantation for multiple myeloma: reduced relapse risk in female to male transplants.,” Bone Marrow Transplantation, vol. 35, no. 6, pp. 609–617, 2005.

[171] W. Thomson, “Electrical Units of Measurement,” in Popular Lectures and Addresses, W. Thomson, Ed. London: Macmillan and Co, 1889, p. 73.

[172] E. J. Escalante, “Quality and productivity improvement: a study of variation and defects in manufacturing,” Quality Engineering, vol. 11, no. 3, pp. 427–442, 1999.

[173] S. Steiner, B. Abraham, and J. MacKay, “Understanding Process Capability Indices.” [Online]. Available: http://www.stats.uwaterloo.ca/~shsteine/papers/cap.pdf. [Accessed: 29-Jul-2015].

[174] D. Montgomery, Introduction to Statistical Quality Control, 5th Edition, New York: John Wiley & Sons, 2004.

[175] P. Hourd, P. Ginty, A. Chandra, and D. J. Williams, “Manufacturing models permitting roll out/scale out of clinically led autologous cell therapies: Regulatory and scientific challenges for comparability,” Cytotherapy, vol. 16, no. 8, pp. 1033–1047, 2014.

[176] European Council, “Directive 2001/83/EC of the European parliament and of the council of 6 November 2001 on the community code relating to medicinal products for human use,” Official Journal of the European Union, no. 311, p. 67, 2001.

[177] P. Chevallier, N. Robillard, M. Illiaquer, J. Esbelin, M. Mohty, C. Bodin-Bressollette, T. Guillaume, V. Stocco, F. Auffray, S. Derenne, L. Planche, M. C. Bene, and B. M. Imbert-Marcille, “Characterization of various blood and graft sources: A prospective series,” Transfusion, vol. 53, no. 9, pp. 2020–2026, 2013.

[178] D. Moher, A. Liberati, J. Tetzlaff, and D. G. Altman, “Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement.,” PLoS Med., vol. 6, no. 7, 2009.

[179] A. Buthmann, “Dealing with Non-normal Data: Strategies and Tools,” iSixSigma [Online] Available at: https://www.isixsigma.com/tools-templates/normality/dealing-non-normal-data-strategies-and-tools/ [Accessed 21/09/16]

[180] S. S. SHAPIRO and M. B. WILK, “An analysis of variance test for normality (complete samples),” Biometrika, vol. 52, no. 3–4, pp. 591–611, 1965.

[181] K. Ishikawa, Introduction to Quality Control, 1st Edition, Tokyo: 3A Corp, 1990.

[182] T. Gilovich, R. Vallone, and A. Tversky, “The hot hand in basketball: On the misperception of random sequences,” Cognitive Psychology, vol. 17, no. 3, pp. 295–314, 1985.

[183] "China issues rules banning dishonesty in science publishing," Phys.org [Online] Available at: http://phys.org/news/2015-12-china-issues-dishonesty-science-publishing.html [Accessed 21/09/16]

[184] E. Gerstner, "Chinese science comes of age," Research Information, 2015 [Online] Available at: https://www.researchinformation.info/feature/chinese-science-comes-age [Accessed 21/09/16]

[185] European Medicines Agency, Committee for Medicinal Products for Human Use (CHMP) Guideline of Conduct of Pharmacovigilance for Medicines used by the Paediatric Population. 2007. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003764.pdf [Accessed 21/09/16]

[186] "The United Nations Convention on the Rights of the Child," United Nations, General Assembly Resolution 44/25 of 20th November 1989. [Online] Available at: http://www.unicef.org.uk/Documents/Publication-pdfs/UNCRC_PRESS200910web.pdf [Accessed 21/09/16]

[187] Department of Health, "Core Standards," National Service Framework for Children, Young People and Maternity Services, 2004 [Online] Available at: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/199952/National_Service_Framework_for_Children_Young_People_and_Maternity_Services_-_Core_Standards.pdf [Accessed 21/09/16]

[188] Children (Scotland) Act 1995, Chapter 3. [Online] Available at: http://www.legislation.gov.uk/ukpga/1995/36/pdfs/ukpga_19950036_en.pdf [Accessed 21/09/16]

[189] A. D. Auerbach, C. S. Landefeld, and K. G. Shojania, "The tension between needing to improve care and knowing how to do it.," New England Journal of Medicine, vol. 357, pp. 608–613, 2007.

[190] R. K. Hopman and J. F. DiPersio, "Advances in stem cell mobilization," Blood Reviews, vol. 28, no. 1. pp. 31–40, 2014.

[191] M. Marshall, P. Pronovost, and M. Dixon-Woods, "Promotion of improvement as a science," Lancet, vol. 381, no. 9864, pp. 419–421, 2013.

[192] US News, "Best Hospitals for Adult Cancer," US News and World Report. [Online]. Available: http://health.usnews.com/best-hospitals/rankings/cancer. [Accessed: 21/09/16].

[193] J. Barry, J. Hyllner, G. Stacey, C. J. Taylor, and M. Turner, "Setting Up a Haplobank: Issues and Solutions," Current Stem Cell Reports, vol. 1, no. 2, pp. 110–117, 2015.

[194] U. Dührsen, J. L. Villeval, J. Boyd, G. Kannourakis, G. Morstyn, and D. Metcalf, "Effects of recombinant human granulocyte colony-stimulating factor on hematopoietic progenitor cells in cancer patients.," Blood, vol. 72, no. 6, pp. 2074–2081, 1988.

[195] H. K. Duong, B. N. Savani, E. Copelan, S. Devine, L. J. Costa, J. R. Wingard, P. Shaughnessy, N. Majhail, M.-A. Perales, C. S. Cutler, W. Bensinger, M. R. Litzow, M. Mohty, R. E. Champlin, H. Leather, S. Giralt, and P. A. Carpenter, "Peripheral blood progenitor cell mobilization for autologous and allogeneic hematopoietic cell transplantation: guidelines from the American Society for Blood and Marrow Transplantation.," Biology of Blood and Marrow Transplantation, vol. 20, no. 9, pp. 1262–73, 2014.

[196] "BD Stem Cell Enumeration Kit." BD Biosciences [Online]. Available at: https://www.bdbiosciences.com/documents/BD_StemCell_EnumerationKit_Datasheet.pdf. [Accessed 21/09/16]

[197] M. Brown and C. Wittwer, "Flow Cytometry: Principles and Clinical Applications in Hematology," Clinical Chemistry, vol. 46, no. 8, pp. 1221–1229, 2000.

[198] "How to Apply for a CLIA Certificate, Including International Laboratories," Centres for Medicare and Medicaid Services. [Online]. Available at: https://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/How_to_Apply_for_a_CLIA_Certificate_International_Laboratories.html [Accessed: 07-Jan-2016].

[199] NIST/Sematech, "e-Handbook of Statistical Methods," in Engineering Statistics Handbook, 2012. [Online] Available at: http://www.itl.nist.gov/div898/handbook/pmc/section1/pmc16.htm [Accessed 21/09/16]

[200] J. H. McDonald, Handbook of Biological Statistics, 3rd edition, Baltimore, Maryland: Sparky House Publishing, 2014.

[201] J. D. Hayes, "Understanding Process Capability Indices," Auburn Technical Assistance Centre, 2010. [Online]. Available: http://virtual.auburnworks.org/profiles/blogs/understanding-process. [Accessed: 21-Sep-2016].

[202] B. C. McDaniel, "Six-Sigma Conversion Table," MoreSteam.com, 2011. [Online]. Available: https://www.moresteam.com/toolbox/six-sigma-conversion-table.cfm. [Accessed: 21-Sep-2016].

[203] O. Sezer, K. Possinger, B. Metzner, H. Illiger, M. Wattad, W. Heit, H. Fuss, and W. Schultze, "Optimal CD34(+) cell dose in autologous peripheral-blood stem-cell transplantation." Comment on 'Therapeutic relevance of CD34 cell dose in blood cell transplantation for cancer therapy,'" Journal of Clinical Oncology, vol. 18, no. 18, pp. 3319–3320, 2000.

[204] D. E. Pegg, "Principles of cryopreservation.," Methods in Molecular Biology, vol. 368, pp. 39–57, 2007.

[205] "GSK's Strimvelis approved in Europe to treat very rare disease, ADA-SCID," Thepharmaletter, 2016. [Online]. Available: http://www.thepharmaletter.com/article/gsk-s-strimvelis-approved-in-europe-to-treat-very-rare-disease-ada-scid. [Accessed: 21-Sep-2016].

[206] W. Kruskal and A. Wallis, "Use of Ranks in One-Criterion Variance Analysis," Journal of the American Statistical Association, vol. 47, pp. 583–621, 1952.

[207] J. Parnaby, "A systems approach to the implementation of JIT methodologies in Lucas Industries," International Journal Production Research, vol. 26, no. 3, pp. 483–492, 1988.

[208] A. Ludwig, N. Jochmann, A. Kertesz, C. Kuhn, S. Mueller, C. Gericke, G. Baumann, K. Stangl, and V. Stangl, “Smoking decreases the level of circulating CD34+ progenitor cells in young healthy women--a pilot study.,” BMC Womens. Health, vol. 10, p. 20, Jan. 2010.

[209] B. T. Tran, A. Halperin, and J. W. Chien, “Cigarette Smoking and Outcomes after Allogeneic Hematopoietic Stem Cell Transplantation,” Biology of Blood and Marrow Transplantation, vol. 17, no. 7, pp. 1004–1011, 2010.

[210] J. I. Garaycoechea, G. P. Crossan, F. Langevin, M. Daly, M. J. Arends, and K. J. Patel, “Genotoxic consequences of endogenous aldehydes on mouse haematopoietic stem cell function,” Nature, vol. 489, no. 7417, pp. 571–575, 2012.

[211] A. Rolls, W. Pang, I. Ibarra, D. Colas, P. Bonnavion, B. Korin, C. Heller, I. Weissman, and L. de Lecea, “Sleep disruption impairs haematopoietic stem cell transplantation in mice,” Nature Communications, vol. 6, 2015.

[212] A. Nelson, C. Coe, M. Juckett, M. Rumble, P. Rathouz, P. Hematti, and E. Costanzo, “Sleep Quality Following Hematopoietic Stem Cell Transplantation: Longitudinal Trajectories and Biobehavioral Correlates,” Bone Marrow Transplantation, vol. 49, no. 11, pp. 1405–1411, 2014.

[213] E. Hacker, M. Kapella, C. Park, C. Ferrans, and J. Larson, “Sleep Patterns During Hospitalization Following Hematopoietic Stem Cell Transplantation.,” Oncolology Nursing Forum, vol. 42, no. 4, pp. 371–379, 2015.

[214] B. Goldacre, “The NHS plan to share our medical data can save lives – but must be done right,” The Guardian, 2014. [Online]. Available: https://www.theguardian.com/society/2014/feb/21/nhs-plan-share-medical-data-save-lives. [Accessed: 21-Sep-2016].

[215] B. Goldacre, “Care.data is in chaos. It breaks my heart,” The Guardian, 2014. [Online]. Available: https://www.theguardian.com/commentisfree/2014/feb/28/care-data-is-in-chaos. [Accessed: 21-Sep-2016].

[216] “Revealed: Google AI has access to huge haul of NHS patient data,” New Scientist, 2016. [Online]. Available: https://www.newscientist.com/article/2086454-revealed-google-ai-has-access-to-huge-haul-of-nhs-patient-data/. [Accessed: 21-Sep-2016].

[217] M. W. Naing, D. A. Gibson, P. Hourd, S. G. Gomez, R. B. V Horton, J. Segal, and D. J. Williams, “Improving umbilical cord blood processing to increase total nucleated cell count yield and reduce cord input wastage by managing the consequences of input variation,” Cytotherapy, vol. 17, no. 1, pp. 58–67, 2015.

8.0 Appendix

This section contains list of the references used to create the literature meta-analysis results in **Chapter 3.**

A.C. Gac, S. Chantepie, E. Marin, Q. Cabrera, S. Cheze, H. Johnson, K. Benabed, V Leferve, M. Macro, and O. Reman, "Semi-ambulatory autologous peripheral blood stem-cell transplantation in 79 patients with multiple myeloma," Haematologica, vol. 97, no. 7, pp. 715–716, 2012.

M. Aglietta, L. Barkholt, F. C. Schianca, D. Caravelli, B. Omazic, C. Minotto, F. Leone, P. Hentschke, G. Bertoldero, A. Capaldi, G. Ciccone, D. Niederwieser, O. Ringdén, and T. Demirer, "Reduced-intensity allogeneic hematopoietic stem cell transplantation in metastatic colorectal cancer as a novel adoptive cell therapy approach. The European group for blood and marrow transplantation experience.," Biology of Blood and Marrow Transplantation, vol. 15, no. 3, pp. 326–35, 2009.

E. T. Alexander, J. A. Towery, A. N. Miller, C. Kramer, K. R. Hogan, J. E. Squires, R. K. Stuart, and L. J. Costa, "Beyond CD34+ cell dose: Impact of method of peripheral blood hematopoietic stem cell mobilization (granulocyte-colony-stimulating factor [G-CSF], G-CSF plus plerixafor, or cyclophosphamide G-CSF/granulocyte-macrophage [GM]-CSF) on number of colony-forming," Transfusion, vol. 51, no. 9, pp. 1995–2000, 2011.

D. S. Allan, M. Keeney, J. Popma, K. Weir, M. Bhatia, and I. H. Chin-yee, "Peripheral blood stem cells Number of viable CD34 + cells reinfused predicts engraftment in autologous hematopoietic stem cell transplantation Summary :," Bone Marrow Transplantation, pp. 967–972, 2002.

a Anagnostopoulos, a Aleman, Y. Yang, M. Donato, D. Weber, R. Champlin, T. Smith, R. Alexanian, and S. Giralt, "Outcomes of autologous stem cell transplantation in patients with multiple myeloma who received dexamethasone-based nonmyelosuppressive induction therapy.," Bone Marrow Transplantation, vol. 33, no. 6, pp. 623–8, 2004.

D. Anderson, T. DeFor, L. Burns, P. McGlave, J. Miller, J. Wagner, and D. Weisdorf, "A comparison of related donor peripheral blood and bone marrow transplants: importance of late-onset chronic graft-versus-host disease and infections.," Biology of Blood and Marrow Transplantation: the Journal of the American Society for Blood and Marrow Transplantation, vol. 9, no. 1, pp. 52–59, Jan. 2003.

J. R. Andolina, M. Kletzel, W. T. Tse, D. A. Jacobsohn, R. E. Duerst, J. Schneiderman, I. Helenowski, A. Rademaker, and S. Chaudhury, "Allogeneic hematopoetic stem cell transplantation in pediatric myelodysplastic syndromes: Improved outcomes for de novo disease," Pediatric Transplantation, vol. 15, no. 3, pp. 334–343, 2011.

G. Andreola, A. Babic, C. Rabascio, M. Negri, G. Martinelli, and D. Laszlo, "Plerixafor and Filgrastim as a first line peripheral blood stem cell mobilisation strategy in patients with multiple myeloma and lymphoma candidated to autologous bone marrow transplantation," European Journal of Haematology, vol. 88, no. 2, pp. 154–158, 2012.

A. T. Anguita-Compagnon, M. T. Dibarrart, J. Palma, L. Paredes, C. Mosso, R. Montalva, L. Salas, D. Araos, I. Delgado, and A. Majlis, "Mobilization and Collection of Peripheral Blood Stem Cells: Guidelines for Blood Volume to Process, Based on CD34-Positive Blood Cell Count in Adults and Children," Transplantation Proceedings, vol. 42, no. 1, pp. 339–344, 2010.

S. Ataergin, F. Arpaci, M. Turan, L. Solchaga, T. Cetin, M. Ozturk, A. Ozet, S. Komurcu, and B. Ozturk, “Reduced dose of lenograstim is as efficacious as standard dose of filgrastim for peripheral blood stem cell mobilization and transplantation: A randomized study in patients undergoing autologous peripheral stem cell transplantation,” American Journal of Hematology, vol. 83, no. 8, pp. 644–648, 2008.

A. Atalla, A. E. Hallack Neto, D. B. Siqueira, and G. C. Toledo, “Allogeneic hematopoietic stem cell transplantation in mycosis fungoides.,” An. Bras. Dermatol., vol. 88, no. 6 Suppl 1, pp. 216–9, 2013.
W. Y. Au, A. K. W. Lie, L. L. P. Siu, E. C. Chan, G. C. Ooi, A. Y. H. Leung, R. Liang, and Y. L. Kwong, “Treatment of lymphoma relapses after allogeneic hematopoietic stem cell transplantation with intensive chemotherapy followed by infusion of hematopoietic stem cell from the original donor,” Annals of Hematology, vol. 82, no. 9, pp. 548–551, 2003.

A. D. Auerbach, C. S. Landefeld, and K. G. Shojania, “The tension between needing to improve care and knowing how to do it.,” The New England Journal of Medicine, vol. 357, no. 6, pp. 608–13, 2007.

A Bacigalupo, T. Lamparelli, F. Gualandi, D. Occhini, S. Bregante, a M. Raiola, a Ibatici, C. di Grazia, a Dominietto, G. Piaggio, M. Podesta, B. Bruno, a Lombardi, F. Frassoni, C. Viscoli, N. Sacchi, and M. T. Van Lint, “Allogeneic hemopoietic stem cell transplants for patients with relapsed acute leukemia: long-term outcome.,” Bone Marrow Transplantation, vol. 39, no. 6, pp. 341–346, 2007.

M. Bakr, W. Rasheed, S. Y. Mohamed, F. Al-Mohareb, N. Chaudhri, F. Al-Sharif, H. Al-Zahrani, G. Al-Dawsari, A. J. Saleh, A. Nassar, S. Ahmed, A. Elghazaly, S. O. Ahmed, K. Ibrahim, W. Chebbo, G. M. El Gohary, M. H. Al Mahayni, F. Hussain, Z. Nurgat, T. A. Elhassan, C. U. Walter, and M. Aljurf, “Allogeneic Hematopoietic Stem Cell Transplantation in Adolescent and Adult Patients with High-Risk T Cell Acute Lymphoblastic Leukemia,” Biology of Blood and Marrow Transplantation, vol. 18, no. 12, pp. 1897–1904, 2012.

A. Ballestrero, D. Boy, R. Gonella, M. Miglino, M. Clavio, V. Barbero, A. Nencioni, M. Gobbi, and F. Patrone, “Pegfilgrastim compared with filgrastim after autologous peripheral blood stem cell transplantation in patients with solid tumours and lymphomas,” Annals of Hematology, vol. 87, no. 1, pp. 49–55, 2008.

G. W. Basak, O. Jaksic, Z. Koristek, G. Mikala, S. Basic-Kinda, J. Mayer, T. Masszi, S. Giebel, B. Labar, and W. Wiktor-Jedrzejczak, “Haematopoietic stem cell mobilization with plerixafor and G-CSF in patients with multiple myeloma transplanted with autologous stem cells,” European Journal of Haematology, vol. 86, no. 6, pp. 488–495, 2011.

Q. Bashir, H. Khan, P. F. Thall, P. Liu, N. Shah, P. Kebriaei, S. Parmar, B. Oran, S. Ciurea, Y. Nieto, R. Jones, C. M. Hosing, U. R. Popat, Y. T. Dinh, G. Rondon, R. Z. Orlowski, J. J. Shah, M. De Lima, E. Shpall, R. Champlin, S. Giralt, and M. H. Qazilbash, “A randomized phase II trial of fludarabine/melphalan 100 versus fludarabine/melphalan 140 followed by allogeneic hematopoietic stem cell transplantation for patients with multiple myeloma.,” Biology of Blood and Marrow Transplantation,vol. 19, no. 10, pp. 1453–8, 2013.

Y. Beguin, E. Baudoux, B. Sautois, V. Fraipont, N. Schaaflufontaine, M. Pereira, and D. Sondag, “Hematopoietic recovery in cancer patients after transplantation of autologous peripheral blood,” Transfusion, vol. 38, no. February, pp. 199–208, 1998.

H. Bertz, A. Spyridonidis, G. Ihorst, M. Engelhardt, C. Grüllich, R. Wäsch, R. Marks, and J. Finke, “Marrow versus blood-derived stem cell grafts for allogeneic transplantation from unrelated

donors in patients with active myeloid leukemia or myelodysplasia.," Biology of Blood and Marrow Transplantation,vol. 18, no. 6, pp. 894–902, Jun. 2012.

D. Bhutani, J. Zonder, J. Valent, N. Tageja, L. Ayash, A. Deol, Z. Al-Kadhimi, J. Abrams, L. Lum, V. Ratanatharathorn, J. Uberti, and M. H. Abidi, "Evaluating the effects of lenalidomide induction therapy on peripheral stem cells collection in patients undergoing autologous stem cell transplant for multiple myeloma," Supportive Care in Cancer, vol. 21, no. 9, pp. 2437–2442, 2013.

H. Bittencourt, V. Rocha, S. Chevret, G. Socié, H. Espérou, A. Devergie, L. Dal Cortivo, J. P. Marolleau, F. Garnier, P. Ribaud, and E. Gluckman, "Association of CD34 cell dose with hematopoietic recovery, infections, and other outcomes after HLA-identical sibling bone marrow transplantation," Blood, vol. 99, no. 8, pp. 2726–2733, 2002.

J. Blay and D. Bouhour, "High-dose chemotherapy with autologous hematopoietic stem-cell transplantation for advanced soft tissue sarcoma in adults," Annals of Oncology, vol. 18, no. 21, pp. 3643–3650, 2000.

Auner HW, Pavlu J, Szydlo R, Giles C, Kanfer E, Macdonald D, Marin D, Milojkovic D, Rezvani K, Goldman JM, Apperley JF, Landgren O, Rahemtulla A. "Autologous haematopoietic stem cell transplantation in multiple myeloma patients from ethnic minority groups in an equal access healthcare system," British Journal of Haematology, vol. 12, pp. 0–2, 2010.

K. Bogunia-Kubik, A. Gieryng, K. Gebura, and A. Lange, "Genetic variant of the G-CSF receptor gene is associated with lower mobilization potential and slower recovery of granulocytes after transplantation of autologous peripheral blood progenitor cells," Cytokine, vol. 60, no. 2, pp. 463–467, 2012.

M. Bornhäuser, B. Storer, J. T. Slattery, F. R. Appelbaum, H. J. Deeg, J. Hansen, P. J. Martin, G. B. McDonald, W. G. Nichols, J. Radich, A. Woolfrey, A. Jenke, E. Schleyer, C. Thiede, G. Ehninger, and C. Anasetti, "Conditioning with fludarabine and targeted busulfan for transplantation of allogeneic hematopoietic stem cells," Blood, vol. 102, no. 3, pp. 820–826, 2003.

S. Brauninger, H. Bialleck, K. Thorausch, T. Felt, E. Seifried, and H. Bonig, "Allogeneic donor peripheral blood 'stem cell' apheresis: Prospective comparison of two apheresis systems," Transfusion, vol. 52, no. 5, pp. 1137–1145, 2012.

N. D. Bueno, F. L. Dulley, R. Saboya, J. U. Amigo Filho, F. L. Coracin, and D. D. A. F. Chamone, "Busulfan and melphalan as conditioning regimen for allogeneic hematopoietic stem cell transplantation in acute myeloid leukemia in first complete remission.," Revista Brasileira de Hemtatologia e Hemoterapia, vol. 33, no. 3, pp. 179–84, 2011.

D. Bunjes, C. Duncker, M. Wiesneth, M. Stefanic, D. Dohr, S. Harsdorf, T. Karakas, S. N. Reske, and L. Bergmann, "A randomised study of allogeneic transplantation with stem cells from blood or bone marrow.," Bone Marrow Transplantation, vol. 25, no. 11, pp. S9–S11, 2000.

L. Calvet, A. Cabrespine, N. Boiret-Dupré, E. Merlin, C. Paillard, M. Berger, J. O. Bay, O. Tournilhac, and P. Halle, "Hematologic, immunologic reconstitution, and outcome of 342 autologous peripheral blood stem cell transplantations after cryopreservation in a -80°C mechanical freezer and preserved less than 6 months," Transfusion, vol. 53, no. 3, pp. 570–578, 2013.

R. de la Cámara and A. Alonso, “Allogeneic hematopoietic stem cell transplantation in patients 50 years of age and older,” Haemat, vol. 87, no. september, pp. 965–972, 2002.
L. Castagna, S. Bramanti, a Levis, M. G. Michieli, a Anastasia, R. Mazza, L. Giordano, B. Sarina, E. Todisco, a I. Gregorini, and a Santoro, “Pegfilgrastim versus filgrastim after high-dose chemotherapy and autologous peripheral blood stem cell support.,” Annals of Oncology, vol. 21, no. 7, pp. 1482–1485, 2010.

S. Cesaro, A. G. Zanazzo, S. Frenos, R. Luksch, A. Pegoraro, G. Tridello, and S. Dallorso, “A Phase II study on the safety and efficacy of a single dose of pegfilgrastim for mobilization and transplantation of autologous hematopoietic stem cells in pediatric oncohematology patients,” Transfusion, vol. 51, no. 11, pp. 2480–2487, 2011.

S. P. Chantepie, M. Mohty, R. Tabrizi, M. Robin, E. Deconinck, a Buzyn, N. Contentin, N. Raus, V. Lhéritier, and O. Reman, “Treatment of adult ALL with central nervous system involvement at diagnosis using autologous and allogeneic transplantation: a study from the Société Française de Greffe de Moelle et de Thérapie Cellulaire.,” Bone Marrow Transplantation, vol. 48, no. 5, pp. 684–90, 2013.

B. T. Chen, A. O. Ortiz, A. Dagis, C. Torricelli, P. Parker, and H. Openshaw, “Brain imaging findings in symptomatic patients after allogeneic haematopoietic stem cell transplantation: Correlation with clinical outcome,” European Radiology, vol. 22, no. 10, pp. 2273–2281, 2012.

Y. Chen, Y. Xu, G. Fu, Y. Liu, J. Peng, B. Fu, X. Yuan, H. Xin, Y. Zhu, Q. He, D. Wu, Y. Shu, X. Li, X. Zhao, and F. Chen, “Allogeneic hematopoietic stem cell transplantation for patients with acute leukemia.,” Chinese Journal of Cancer Research, vol. 25, no. 4, pp. 389–96, 2013.

Y. Chen, L. Xu, D. Liu, H. Chen, X. Zhang, W. Han, F. Wang, J. Wang, Y. Wang, X. Huang, and K. Liu, “Comparative outcomes between cord blood transplantation and bone marrow or peripheral blood stem cell transplantation from unrelated donors in patients with hematologic malignancies: a single-institute analysis.,” Chinese Medical Journal (English)., vol. 126, no. 13, pp. 2499–503, 2013.

Y. C. Cheng, G. Rond??n, L. F. Sanchez, J. D. McMannis, D. R. Couriel, M. J. De Lima, C. Hosing, I. F. Khouri, S. A. Giralt, R. E. Champlin, and N. T. Ueno, “Interleukin-2 and granulocyte-macrophage-colony-stimulating factor immunomodulation with high-dose chemotherapy and autologous hematopoietic stem cell transplantation for patients with metastatic breast cancer,” International Journal of Hematology, vol. 90, no. 5, pp. 627–634, 2009.

Chepovetsky J. Choo Y.S. Blouin A. Tindle S. Bertinelli A. Nash E.M. Wu D.W., “Clinical use of peripheral blood CD34+ cell count and midpoint collection CD34+ cell yield for peripheral blood stem cell collections from autologous patients mobilized by G-CSF and plerixafor.,” Vox Sanguinus, vol. 6, no. 2, pp. 293–294, 2013.

P. Chevallier, R. M. Szydlo, D. Blaise, R. Tabrizi, M. Michallet, M. Uzunov, N. Fegueux, F. Guilhot, S. Lapusan, N. Gratecos, J.-Y. Cahn, G. Socié, I. Yakoub-Agha, A. Huynh, S. Francois, J.-O. Bay, S. Maury, A. Buzyn, N. Contentin, and M. Mohty, “Reduced-intensity conditioning before allogeneic hematopoietic stem cell transplantation in patients over 60 years: a report from the SFGM-TC.,” Biology of Blood and Marrow Transplantation,vol. 18, no. 2, pp. 289–94, 2012.

B.-S. Cho, C.-K. Min, K.-S. Eom, Y.-J. Kim, H.-J. Kim, S. Lee, S.-G. Cho, Y. Kim, D.-W. Kim, J.-W. Lee, W.-S. Min, and C.-C. Kim, “Clinical impact of thrombotic microangiopathy on the outcome of patients

with acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation.," Bone Marrow Transplantation, vol. 41, pp. 813–820, 2008.

B. S. Cho, J. H. Yoon, S. H. Shin, S. A. Yahng, S. E. Lee, K. S. Eom, Y. J. Kim, S. Lee, C. K. Min, S. G. Cho, D. W. Kim, J. W. Lee, W. S. Min, C. W. Park, and H. J. Kim, "Comparison of Allogeneic Stem Cell Transplantation from Familial-Mismatched/Haploidentical Donors and from Unrelated Donors in Adults with High-Risk Acute Myelogenous Leukemia," Biology of Blood and Marrow Transplantation,vol. 18, no. 10, pp. 1552–1563, 2012.

R. Chu, R. Brazauskas, F. Kan, A. Bashey, C. Bredeson, B. Camitta, K. Y. Chiang, H. Frangoul, R. P. Gale, A. Gee, B. George, F. D. Goldman, T. G. Gross, V. Gupta, G. A. Hale, L. Isola, A. U. Ispizua, H. Lazarus, J. Marsh, J. Russell, M. Sabloff, E. K. Waller, and M. Eapen, "Comparison of outcomes after transplantation of g-csf-stimulated bone marrow grafts versus bone marrow or peripheral blood grafts from hla-matched sibling donors for patients with severe aplastic anemia," Biology of Blood and Marrow Transplantation,vol. 17, no. 7, pp. 1018–1024, 2011.

N. H. Collins, A. P. Gee, A. G. Durett, F. Kan, J. Zhang, R. E. Champlin, D. Confer, M. Eapen, A. Howard, R. King, M. J. Laughlin, R. J. Plante, S. Spellman, C. Keever-taylor, J. E. Wagner, and D. J. Weisdorf, "NIH Public Access," vol. 16, no. 2, pp. 612–625, 2011.

R. Conrad, M. Remberger, K. Cederlund, O. Ringdén, and L. Barkholt, "A comparison between low intensity and reduced intensity conditioning in allogeneic hematopoietic stem cell transplantation for solid tumors," Haematologica, vol. 93, no. 2, pp. 265–272, 2008.

G. Cook, G. M. Smith, K. Kirkland, J. Lee, R. Pearce, K. Thomson, E. Morris, K. Orchard, S. Rule, N. Russell, C. Craddock, and D. I. Marks, "Outcome following reduced-intensity allogeneic stem cell transplantation (RIC AlloSCT) for relapsed and refractory mantle cell lymphoma (MCL): A study of the British society for blood and marrow transplantation," Biology of Blood and Marrow Transplantation,vol. 16, no. 10, pp. 1419–1427, 2010.

J. J. Cornelissen, B. Van Der Holt, E. J. Petersen, L. Vindelov, C. a Russel, M. Höglund, J. Maertens, H. C. Schouten, E. Braakman, M. M. C. Steijaert, M. J. M. Zijlmans, I. Slaper-Cortenbach, M. a Boogaerts, B. Löwenberg, and L. F. Verdonck, "A randomized multicenter comparison of bone marrow and peripheral blood in recipients of matched sibling allogeneic transplants for myeloid malignancies.," Blood, vol. 31, no. 10, pp. 1525–1531, Sep. 2002.

R. F. Cornell, P. Hari, M.-J. Zhang, X. Zhong, J. Thompson, T. S. Fenske, M. M. Horowitz, R. Komorowski, J. Palmer, M. C. Pasquini, J. D. Rizzo, W. Saber, M. Thomas, and W. R. Drobyski, "Divergent effects of novel immunomodulatory agents and cyclophosphamide on the risk of engraftment syndrome after autologous peripheral blood stem cell transplantation for multiple myeloma.," Biology of Blood and Marrow Transplantation,vol. 19, no. 9, pp. 1368–73, 2013.

A. Czajczynska, A. G??nther, R. Repp, A. Humpe, N. Schub, T. Raff, M. Nickelsen, A. Schrauder, M. Schrappe, M. Kneba, and M. Gramatzki, "Allogeneic stem cell transplantation with BEAM and alemtuzumab conditioning immediately after remission induction has curative potential in advanced T-cell non-hodgkin's lymphoma," Biology of Blood and Marrow Transplantation,vol. 19, no. 11, pp. 1632–1637, 2013.

E. Derenzini, C. Pellegrini, R. Maglie, V. Stefoni, A. Broccoli, L. Gandolfi, B. Casadei, F. Quirini, L. Argnani, S. Fanti, M. R. Motta, M. Baccarani, and P. L. Zinzani, "Collection of hematopoietic stem cells after previous exposure to ittrium-90 ibritumumab tiuxetan (zevalin) is feasible and does not

impair autologous stem cell transplantation outcome in follicular lymphoma," Blood, vol. 120, no. 21, pp. 1695–1701, 2012.

B. R. Dey, J. Shaffer, a J. Yee, S. McAfee, M. Caron, K. Power, D. T. Ting, C. Colby, F. Preffer, K. Ballen, E. Attar, S. Saidman, N. Tarbell, D. Sachs, M. Sykes, and T. R. Spitzer, "Comparison of outcomes after transplantation of peripheral blood stem cells versus bone marrow following an identical nonmyeloablative conditioning regimen.," Bone Marrow Transplantation, vol. 40, no. 1, pp. 19–27, Jul. 2007.

M. Ditschkowski, a H. Elmaagacli, R. Trenschel, N. K. Steckel, M. Koldehoff, and D. W. Beelen, "Myeloablative allogeneic hematopoietic stem cell transplantation in elderly patients.," Clinical Transplantation, vol. 20, no. 1, pp. 127–31, 2005.

E. A. Eksioglu, J. Kielbasa, S. Eisen, and V. Reddy, "Granulocyte-macrophage colony-stimulating factor increases the proportion of circulating dendritic cells after autologous but not after allogeneic hematopoietic stem cell transplantation.," Cytotherapy, vol. 13, no. 7, pp. 888–896, 2011.

J. El-Cheikh, R. Crocchiolo, J.-M. Boher, S. Furst, A.-M. Stoppa, P. Ladaique, C. Faucher, B. Calmels, L. Castagna, C. Lemarie, J.-M. S. De Colella, D. Coso, R. Bouabdallah, C. Chabannon, and D. Blaise, "Comparable outcomes between unrelated and related donors after reduced-intensity conditioning allogeneic hematopoietic stem cell transplantation in patients with high-risk multiple myeloma.," European Journal of Haematology, vol. 88, no. 6, pp. 497–503, 2012.

A. H. Elmaagacli, R. Peceny, N. Steckel, R. Trenschel, H. Ottinger, H. Grosse-Wilde, U. W. Schaefer, and D. W. Beelen, "Outcome of transplantation of highly purified peripheral blood CD34+ cells with T-cell add-back compared with unmanipulated bone marrow or peripheral blood stem cells from HLA-identical sibling donors in patients with first chronic phase chronic myeloid l," Blood, vol. 101, no. 2, pp. 446–53, Jan. 2003.

K.-S. Eom, S.-H. Shin, J.-H. Yoon, S.-A. Yahng, S.-E. Lee, B.-S. Cho, Y.-J. Kim, H.-J. Kim, C.-K. Min, D.-W. Kim, J.-W. Lee, W.-S. Min, C.-W. Park, and S. Lee, "Comparable long-term outcomes after reduced-intensity conditioning versus myeloablative conditioning allogeneic stem cell transplantation for adult high-risk acute lymphoblastic leukemia in complete remission.," American Journal of Hematology, vol. 88, no. 8, pp. 634–41, 2013.

T. Eto, K. Takase, T. Miyamoto, Y. Ohno, T. Kamimura, K. Nagafuji, Y. Takamatsu, T. Teshima, H. Gondo, S. Taniguchi, K. Akashi, and M. Harada, "Autologous peripheral blood stem cell transplantation with granulocyte colony-stimulating factor combined conditioning regimen as a postremission therapy for acute myelogenous leukemia in first complete remission," International Journal of Hematology, vol. 98, no. 2, pp. 186–196, 2013.

C. Fabre, S. Koscielny, M. Mohty, N. Fegueux, D. Blaise, N. Maillard, R. Tabrizi, M. Michallet, G. Socié, I. Yakoub-Agha, F. Garban, M. Uzunov, S. François, N. Contentin, S. Lapusan, and J.-H. Bourhis, "Younger donor's age and upfront tandem are two independent prognostic factors for survival in multiple myeloma patients treated by tandem autologous-allogeneic stem cell transplantation: a retrospective study from the Société Française de Greffe de Moelle," Haematologica, vol. 97, no. 4, pp. 482–90, Apr. 2012.

B. Federmann, M. Bornhauser, C. Meisner, L. Kordelas, D. W. Beelen, G. Stuhler, M. Stelljes, R. Schwerdtfeger, M. Christopeit, G. Behre, C. Faul, W. Vogel, M. Schumm, R. Handgretinger, L. Kanz, and W. A. Bethge, "Haploidentical allogeneic hematopoietic cell transplantation in adults using

CD3/CD19 depletion and reduced intensity conditioning: a phase II study.," Haematologica, vol. 97, no. 10, pp. 1523–31, 2012.

S. Flommersfeld, T. Bakchoul, G. Bein, A. Wachtel, C. Loechelt, and U. J. Sachs, "A single center comparison between three different apheresis systems for autologous and allogeneic stem cell collections," Transfususion and Apheresis Science, vol. 49, no. 3, pp. 428–433, 2013.

U. Forslöw, O. Blennow, K. Leblanc, O. Ringdén, B. Gustafsson, J. Mattsson, and M. Remberger, "Treatment with mesenchymal stromal cells is a risk factor for pneumonia-related death after allogeneic hematopoietic stem cell transplantation," European Journal of Haematology, vol. 89, no. 3, pp. 220–227, 2012.

H. Frangoul, A. Woolfrey, S. Khan, M. Pulsipher, J. Levine, D. Baker, M. Walters, M. Ayas, Y. Ravindranath, S. Grupp, D. Billheimer, and E. Nemecek, "A prospective study of G-CSF primed bone marrow from pediatric donors as a stem cell source for allogeneic bone marrow transplant: A pediatric blood and marrow transplant consortium (PBMTC) study," Blood, vol. 106, no. 13, p. 556a–556a, 2005.

F. Frassoni, M. Labopin, R. Powles, J.-Y. Mary, W. Arcese, A. Bacigalupo, D. Bunjes, E. Gluckman, T. Ruutu, U. W. Schaefer, J. Sierra, J. P. Vernant, R. Willemze, T. de Witte, and N. C. G. Transplantation, "Effect of centre on outcome of bone-marrow transplantation for acute myeloid leukaemia," The Lancet, vol. 355, no. 9213, pp. 1393–1398, Apr. 2000.

A. Gerds, M. Fox-Geiman, K. Dawravoo, T. Rodriguez, A. Toor, S. Smith, K. Kiley, D. Fletcher-Gonzalez, C. Hicks, and P. Stiff, "Randomized Phase III Trial of Pegfilgrastim versus Filgrastim after Autologus Peripheral Blood Stem Cell Transplantation," Biology of Blood and Marrow Transplantation,vol. 16, no. 5, pp. 678–685, 2010.

A. Ghavamzadeh, M. Iravani, A. Ashouri, S. A. Mousavi, N. Mahdavi, A. Shamshiri, M. Hadjibabaie, R. Namdar, L. Nedaeifard, H. Ghaffari, and K. Alimoghaddam, "Peripheral blood versus bone marrow as a source of hematopoietic stem cells for allogeneic transplantation in children with class I and II beta thalassemia major.," Biology of Blood and Marrow Transplantation,vol. 14, no. 3, pp. 301–8, 2008.

E. Z. Giannopoulou, R. Puff, A. Beyerlein, I. Von Luettichau, H. Boerschmann, D. Schatz, M. Atkinson, M. J. Haller, D. Egger, S. Burdach, and A. G. Ziegler, "Effect of a single autologous cord blood infusion on beta-cell and immune function in children with new onset type 1 diabetes: A non-randomized, controlled trial," Pediatric Diabetes, vol. 15, no. 2, pp. 100–109, 2014.

E. Gluckman and V. Rocha, "Cord blood transplantation: state of the art," Haematologica, vol. 94, no. 4, pp. 451–454, 2009.

D. Gómez-Almaguer, "Higher doses of CD34+ progenitors are associated with improved overall survival without increasing GVHD in reduced intensity conditioning allogeneic transplant recipients with clinically advanced disease," Journal of Clinical Apheresis, vol. 28, no. April 2013, pp. 349–355, 2013.

N. C. Gorin, M. Labopin, J. M. Boiron, N. Theorin, T. Littlewood, S. Slavin, H. Greinix, J. Y. Cahn, E. P. Alessandrino, A. Rambaldi, A. Nagler, E. Polge, and V. Rocha, "Results of genoidentical hemopoietic stem cell transplantation with reduced intensity conditioning for acute myelocytic leukemia: Higher doses of stem cells infused benefit patients receiving transplants in second remission or beyond - The Acute Leukemia ," Journal of Clinical Oncology, vol. 24, no. 24, pp. 3959–3966, 2006.

Grubovic R.M. Georgievski B. Cevreska L. Velkova E. Genadieva-Stavric S. Useini S. Pivkova A. Veljanovski N. Stojanoski Z. Cadievski L. Grubovic M., “Safety and efficacy of mobilized hematopoietic stem cell collection from peripheral blood in hematological patients and healthy donors-14 years of experience.,” Bone Marrow Transplantation, vol. 114, no. 18, pp. S348–S349, 2015.

M. Guo, K. X. Hu, C. L. Yu, Q. Y. Sun, J. H. Qiao, D. H. Wang, G. X. Liu, W. J. Sun, L. Wei, X. D. Sun, Y. J. Huang, J. X. Qiao, Z. Dong, and H. S. Ai, “Infusion of HLA-mismatched peripheral blood stem cells improves the outcome of chemotherapy for acute myeloid leukemia in elderly patients,” Blood, vol. 117, no. 3, pp. 936–941, 2011.

M. Hadjibabaie, H. Tabeefar, K. Alimoghaddam, M. Iravani, K. Eslami, H. Honarmand, M. R. Javadi, F. Khatami, A. Ashouri, and A. Ghavamzadeh, “The relationship between body mass index and outcomes in leukemic patients undergoing allogeneic hematopoietic stem cell transplantation,” Clinical Transplantation, vol. 26, no. 1, pp. 149–155, 2012.

73. [77] D. K. Han, H. J. Baek, S. Y. Kim, T. J. Hwang, and H. Kook, “Implication of early lymphocyte recovery after allogeneic hematopoietic stem cell transplantation in children with leukemia,” Yonsei Medical Journal, vol. 54, no. 1, pp. 62–70, 2013.

D. Hauzenberger, M. Schaffer, O. Ringdén, Z. Hassan, B. Omazic, J. Mattsson, A. C. Wikström, and M. Remberger, “Outcome of haematopoietic stem cell transplantation in patients transplanted with matched unrelated donors vs allele-mismatched donors: A single centre study,” Tissue Antigens, vol. 72, no. 6, pp. 549–558, 2008.

C. Hicks, A. Isaacs, R. Wong, and B. H. Chong, “CXCR4 expression on transplanted peripheral blood CD34+ cells: Relationship to engraftment after autologous transplantation in a cohort of multiple myeloma patients,” Annals of Hematology, vol. 90, no. 5, pp. 547–555, 2011.

J. Holowiecki, S. Giebel, J. Wojnar, M. Krawczyk-Kulis, B. Stella-Holowiecka, L. Kachel, M. Wojciechowska, M. Markiewicz, and D. Kata, “Autologous hematopoietic stem cell transplantation for high-risk Hodgkin’s disease: a single-center experience with the first 100 patients,” Transplantation Proceedings, vol. 34, no. 8, pp. 3378–3383, 2002.

J. Holowiecki, M. Wojciechowska, S. Giebel, M. Krawczyk-Kuliś, J. Wojnar, Kachel, D. Kata, and M. Markiewicz, “Ifosfamide, etoposide, epirubicine, and G-CSF: An effective mobilization regimen for PBSCT in heavily pretreated patients,” Transplantation Proceedings, vol. 32, no. 6, pp. 1412–1415, 2000.

J. Hong, S. M. Moon, H. K. Ahn, S. J. Sym, Y. S. Park, J. Park, Y. K. Cho, E. K. Cho, D. B. Shin, and J. H. Lee, “Comparison of characteristics of bacterial bloodstream infection between adult patients with allogeneic and autologous hematopoietic stem cell transplantation,” Biology of Blood and Marrow Transplantation,vol. 19, no. 6, pp. 994–999, 2013.

F. Hoodin, L. Zhao, J. Carey, J. E. Levine, and C. Kitko, “Impact of psychological screening on routine outpatient care of hematopoietic cell transplantation survivors,” Biology of Blood and Marrow Transplantation,vol. 19, no. 10, pp. 1493–1497, 2013.

R. K. Hopman and J. F. DiPersio, “Advances in stem cell mobilization,” Blood Reviews, vol. 28, no. 1. pp. 31–40, 2014.

T. Horie, R. Onodera, M. Akamastu, Y. Ichikawa, J. Hoshino, E. Kaneko, C. Iwashita, A. Ishida, T. Tsukamoto, S. Teramukai, M. Fukushima, and A. Kawamura, “Long-term clinical outcomes for patients with lower limb ischemia implanted with G-CSF-mobilized autologous peripheral blood mononuclear cells,” Atherosclerosis, vol. 208, no. 2, pp. 461–466, 2010.

P. Hourd, A. Chandra, N. Medcalf, and D. J. Williams, “Regulatory challenges for the manufacture and scale-out of autologous cell therapies,” in StemBook, pp. 1–13.

M. M. Hsieh, C. D. Fitzhugh, R. P. Weitzel, M. E. Link, W. A. Coles, X. Zhao, G. P. Rodgers, J. D. Powell, and J. F. Tisdale, “Nonmyeloablative HLA-matched sibling allogeneic hematopoietic stem cell transplantation for severe sickle cell phenotype.,” Journal of the American Medical Association, vol. 312, no. 1, pp. 48–56, 2014.

C. Huang, W. Li, W. Wu, Q. Chen, Y. Guo, Y. Zhang, D. Xu, and W. Cui, “Intra-day and inter-day biological variations of peripheral blood lymphocytes,” Clin. Chim. Acta, vol. 438, pp. 166–170, 2014.
F. Hutchinson, S. Heimfeld, and K. Blume, “Transplantation of bone marrow as compared with peripheral-blood cells from HLA-identical relatives in patients with hematologic cancers,” New England Journal of Medicine, vol. 344, no. 3, pp. 175–181, 2001.

S.-H. Lee, M. H. Lee, J.-H. Lee, Y. H. Min, K.-H. Lee, J.-W. Cheong, J. Lee, K. W. Park, J. H. Kang, K. Kim, W. S. Kim, C. W. Jung, S.-J. Choi, J.-H. Lee, and K. Park, “Infused CD34+ cell dose predicts long-term survival in acute myelogenous leukemia patients who received allogeneic bone marrow transplantation from matched sibling donors in first complete remission.,” Biology of Blood and Marrow Transplantation,vol. 11, no. 2, pp. 122–8, Feb. 2005.

J. C. Ianotto, A. Tempescul, P. Delepine, G. Guillerm, E. Hardy, J. R. Eveillard, and C. Berthou, “Delayed G-CSF stimulation after PBSCT does not seem to modify the biological parameters of bone marrow recovery,” American Journal of Hematology, vol. 86, no. 4, pp. 351–352, 2011.

Y. Ito, T. Miyamoto, T. Kamimura, K. Takase, H. Henzan, Y. Sugio, K. Kato, Y. Ohno, T. Eto, T. Teshima, and K. Akashi, “Clinical outcomes of allogeneic stem cell transplantation for relapsed or refractory follicular lymphoma: A retrospective analysis by the Fukuoka Blood and Marrow Transplantation Group,” International Journal of Hematology, vol. 98, no. 4, pp. 463–471, 2013.

D. a Jacobsohn, B. Hewlett, M. Ranalli, R. Seshadri, R. Duerst, and M. Kletzel, “Outcomes of unrelated cord blood transplants and allogeneic-related hematopoietic stem cell transplants in children with high-risk acute lymphocytic leukemia.,” Bone Marrow Transplantation, vol. 34, pp. 901–907, 2004.

G. Jang, O. B. Ko, S. Kim, D. H. Lee, J. Huh, and C. Suh, “Prospective randomized comparative observation of single- versus split-dose lenograstim to enhance engraftment after autologous stem cell transplantation in patients with multiple myeloma or non-Hodgkin’s lymphoma,” Transfusion, vol. 48, no. 4, pp. 640–646, 2008.

S.-Q. Ji, H.-R. Chen, H.-X. Wang, H.-M. Yan, S.-P. Pan, and C.-Q. Xun, “Comparison of outcome of allogeneic bone marrow transplantation with and without granulocyte colony-stimulating factor (lenograstim) donor-marrow priming in patients with chronic myelogenous leukemia.,” Biology of Blood and Marrow Transplantation, vol. 8, no. 5, pp. 261–267, 2002.

C. Kalpadakis, G. a Pangalis, M. N. Dimopoulou, T. P. Vassilakopoulos, M.-C. Kyrtsonis, P. Korkolopoulou, F. N. Kontopidou, M. P. Siakantaris, E. M. Dimitriadou, S. I. Kokoris, P. Tsaftaridis, E. Plata, and M. K. Angelopoulou, "Rituximab monotherapy is highly effective in splenic marginal zone lymphoma.," Hematological Oncology, vol. 25, no. 3, pp. 127–131, 2007.

J. Kato, T. Mori, K. Yokoyama, Y. Tsukada, T. Ueda, T. Shimizu, and S. Okamoto, "Safety and efficacy of high-dose ranimustine, cytarabine, etoposide and CY (MCVAC) regimen followed by autologous peripheral blood stem cell transplantation for high-risk diffuse large B-cell lymphoma.," Bone Marrow Transplantation, vol. 46, no. 7, pp. 923–8, 2011.

S. Kato, H. Yabe, M. Yasui, K. Kawa, T. Yoshida, A. Watanabe, Y. Osugi, and K. Horibe, "Allogeneic hematopoietic transplantation of CD34 selected cells from an HLA haplo-identical related donor. A long-term follow-up of 135 patients and a comparison of stem cell source between the bone marrow and the peripheral blood," Bone Marrow Transplation, vol. 26, no. 12 pp. 1281–1290, 2000.

K. Kawamura, M. Kikuchi, and K. Terasako, "Comparison of the efficacy of peripheral blood stem cell mobilization using G-CSF alone from healthy donors and patients with hematologic malignancies," Apheresis Science, vol. 49, pp. 334–340, 2013.

Y. Kawano, Y. Takaue, a Watanabe, O. Takeda, K. Arai, E. Itoh, Y. Ohno, T. Teshima, M. Harada, T. Watanabe, Y. Okamoto, T. Abe, T. Kajiume, T. Matsushita, K. Ikeda, M. Endo, Y. Kuroda, S. Asano, R. Tanosaki, K. Yamaguchi, P. Law, and J. D. McMannis, "Partially mismatched pediatric transplants with allogeneic CD34(+) blood cells from a related donor.," Blood, vol. 92, no. 9, pp. 3123–3130, 1998.

S. Kayal, A. Sharma, S. Iqbal, T. Tejomurtula, S. L. Cyriac, and V. Raina, "High-dose chemotherapy and autologous stem cell transplantation in multiple myeloma: A single institution experience at All India Institute of Medical Sciences, New Delhi, using non-cryopreserved peripheral blood stem cells," Clinical Lymphoma Myeloma and Leukemia, vol. 14, no. 2, pp. 140–147, 2014.

P. Kebriaei, R. Basset, C. Ledesma, S. Ciurea, S. Parmar, E. J. Shpall, C. Hosing, I. Khouri, M. Qazilbash, U. Popat, A. Alousi, Y. Nieto, R. B. Jones, M. de Lima, R. E. Champlin, and B. S. Andersson, "Clofarabine combined with busulfan provides excellent disease control in adult patients with acute lymphoblastic leukemia undergoing allogeneic hematopoietic stem cell transplantation ," Biology of Blood and Marrow Transplantation,vol. 18, no. 12, pp. 1819–1826, 2012.

P. Kebriaei, R. M. Saliba, C. Ma, C. Ippoliti, D. R. Couriel, M. de Lima, S. Giralt, M. H. Qazilbash, J. L. Gajewski, C. S. Ha, R. E. Champlin, and I. F. Khouri, "Allogeneic hematopoietic stem cell transplantation after rituximab-containing myeloablative preparative regimen for acute lymphoblastic leukemia.," Bone Marrow Transplantation, vol. 38, no. 3, pp. 203–209, 2006.

A. A. Kennedy-Nasser, K. S. Leung, A. Mahajan, H. L. Weiss, J. A. Arce, S. Gottschalk, G. Carrum, S. P. Khan, H. E. Heslop, M. K. Brenner, C. M. Bollard, and R. A. Krance, "Comparable Outcomes of Matched-Related and Alternative Donor Stem Cell Transplantation for Pediatric Severe Aplastic Anemia," Biology of Blood and Marrow Transplantation,vol. 12, no. 12, pp. 1277–1284, 2006.

A. Keyzner, A. D'Souza, M. Lacy, M. Gertz, S. Hayman, F. Buadi, S. Kumar, D. Dingli, A. Engebretson, C. Tong, and A. Dispenzieri, "Low levels of interleukin-1 receptor antagonist (IL-1RA) predict engraftment syndrome after autologous stem cell transplantation in POEMS syndrome and other plasma cell neoplasms," Biology of Blood and Marrow Transplantation,vol. 19, no. 9, pp. 1395–1398, 2013.

N. Khera, J. Jinneman, B. E. Storer, D. Ph, S. Heimfeld, M. M. O. Meara, T. R. Chauncey, S. J. Lee, and M. Linenberger, “NIH Public Access,” vol. 18, no. 2, pp. 220–228, 2013.

D. H. Kim, N. Jamal, R. Saragosa, D. Loach, J. Wright, V. Gupta, J. Kuruvilla, J. H. Lipton, M. Minden, and H. A. Messner, “Similar Outcomes of Cryopreserved Allogeneic Peripheral Stem Cell Transplants (PBSCT) Compared to Fresh Allografts,” Biology of Blood and Marrow Transplantation,vol. 13, no. 10, pp. 1233–1243, 2007.

H. J. Kim, C. Y. Park, Y. H. Park, Y. J. Kim, D. W. Kim, W. S. Min, and C. C. Kim, “Successful allogeneic hematopoietic stem cell transplantation using triple agent immunosuppression in severe aplastic anemia patients.,” Bone Marrow Transplantation, vol. 31, no. 2, pp. 79–86, 2003.

H. Kim, B. S. Kim, D. H. Kim, M. S. Hyun, S. H. Kim, S. H. Bae, J. H. Choi, S. K. Sohn, H. J. Shin, J. H. Won, S. S. Yoon, D. Y. Jo, Y. D. Joo, J. H. Park, and K. H. Lee, “Comparison between matched related and alternative donors of allogeneic hematopoietic stem cells transplanted into adult patients with acquired aplastic anemia: Multivariate and propensity score-matched analysis,” Biology of Blood and Marrow Transplantation,vol. 17, no. 9, pp. 1289–1298, 2011.

I. Kim, K. H. Lee, Y. Choi, B. Keam, H. K. Nam, S. S. Yoon, K. Y. Yoo, S. Park, and K. K. Byoung, “Allogeneic stem cell transplantation for patients with advanced hematological malignancies: Comparison of fludarabine-based reduced intensity conditioning versus myeloablative conditioning,” J. Korean Med. Sci., vol. 22, no. 2, pp. 227–234, 2007.

I. Kim, S. S. Yoon, K. H. Lee, B. Keam, T. M. Kim, J. S. Kim, H. G. Kim, M. D. Oh, K. S. Han, M. H. Park, S. Park, and B. K. Kim, “Comparative outcomes of reduced intensity and myeloablative allogeneic hematopoietic stem cell transplantation in patients under 50 with hematologic malignancies,” Clinical Transplantation, vol. 20, no. 4, pp. 496–503, 2006.

J. G. Kim, S. K. Sohn, D. H. Kim, J. H. Baek, K. B. Lee, W. S. Min, C. C. Kim, M. H. Lee, J.-J. Lee, I.-J. Chung, H.-J. Kim, and J. W. Lee, “Impact of ABO incompatibility on outcome after allogeneic peripheral blood stem cell transplantation.,” Bone Marrow Transplantation, vol. 35, no. 5, pp. 489–495, 2005.

J. Klaus, D. Herrmann, I. Breitkreutz, U. Hegenbart, U. Mazitschek, G. Egerer, F. W. Cremer, R. M. Lowenthal, J. Huesing, S. Fruehauf, T. Moehler, A. D. Ho, and H. Goldschmidt, “Effect of CD34+ cell dose on hematopoietic reconstitution and outcome in 508 patients with multiple myeloma undergoing autologous peripheral blood stem cell transplantation,” European Journal of Haematology, vol. 78, no. 1, pp. 21–28, 2007.

M. Kletzel, M. Olszewski, K. Danner-Koptik, K. Coyne, and P. Haut, “Red cell salvage and reinfusion in pediatric bone marrow donors.,” Bone Marrow Transplantation, vol. 24, no. 4, pp. 385–388, 1999.

T. Klingebiel, J. Cornish, M. Labopin, F. Locatelli, P. Darbyshire, R. Handgretinger, A. Balduzzi, J. Owoc-Lempach, F. Fagioli, R. Or, C. Peters, F. Aversa, E. Polge, G. Dini, and V. Rocha, “Results and factors influencing outcome after fully haploidentical hematopoietic stem cell transplantation in children with very high-risk acute lymphoblastic leukemia: impact of center size: an analysis on behalf of the Acute Leukemia and Pediatric Disea,” Blood, vol. 115, no. 17, pp. 3437–3446, 2010.

L. Knudsen and B. Nielsen, “Autologous stem cell transplantation in multiple myeloma: outcome in patients with renal failure,” European Journal of Haematology, pp. 27–33, 2005.

M. Koldehoff, D. W. Beelen, R. Trenschel, N. K. Steckel, R. Peceny, M. Ditschkowski, H. Ottinger, and a H. Elmaagacli, “Outcome of hematopoietic stem cell transplantation in patients with atypical chronic myeloid leukemia.,” Bone Marrow Transplantation, vol. 34, no. 12, pp. 1047–50, 2004.

J. Koreth, J. Aldridge, H. T. Kim, E. P. Alyea, C. Cutler, P. Armand, J. Ritz, J. H. Antin, R. J. Soiffer, and V. T. Ho, “Reduced-Intensity Conditioning Hematopoietic Stem Cell Transplantation in Patients Over 60 Years: Hematologic Malignancy Outcomes Are Not Impaired in Advanced Age,” Biology of Blood and Marrow Transplantation,vol. 16, no. 6, pp. 792–800, 2010.

P. Krishnamurthy, V. T. Potter, L. D. Barber, A. G. Kulasekararaj, Z. Y. Lim, R. M. Pearce, H. de Lavallade, M. Kenyon, R. M. Ireland, J. C. W. Marsh, S. Devereux, A. Pagliuca, and G. J. Mufti, “Outcome of donor lymphocyte infusion after T cell-depleted allogeneic hematopoietic stem cell transplantation for acute myelogenous leukemia and myelodysplastic syndromes.,” Biology of Blood and Marrow Transplantation,vol. 19, no. 4, pp. 562–8, 2013.

N. Kröger, E. Holler, G. Kobbe, M. Bornha, R. Schwerdtfeger, H. Baurmann, A. Nagler, W. Bethge, M. Stelljes, L. Uharek, H. Wandt, A. Burchert, P. Corradini, M. Kaufmann, P. Dreger, G. G. Wulf, H. Einsele, T. Zabelina, H. M. Kvasnicka, R. Brand, A. R. Zander, D. Niederwieser, and T. M. De Witte, “Allogeneic stem cell transplantation after reduced-intensity conditioning in patients with myelofibrosis : a prospective , multicenter study of the Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation,” Blood, vol. 114, no. 26, pp. 5264–5270, 2009.

L. Kumar, P. S. Malik, G. Prakash, R. Prabu, V. Radhakrishnan, S. Katyal, and R. Hariprasad, “Autologous hematopoietic stem cell transplantation-what determines the outcome: An experience from North India,” Annals of Hematology, vol. 90, no. 11, pp. 1317–1328, 2011.

B. H. Kushner, M. P. LaQuaglia, N. Wollner, P. A. Meyers, K. L. Lindsley, F. Ghavimi, T. E. Merchant, F. Boulad, N. K. Cheung, M. A. Bonilla, G. Crouch, J. F. Kelleher, P. G. Steinherz, and W. L. Gerald, “Desmoplastic small round-cell tumor: Prolonged progression-free survival with aggressive multimodality therapy,” Journal of Clinical Oncology, vol. 14, no. 5, pp. 1526–1531, 1996.

C. Kyriakou, C. Canals, D. Sibon, J. Y. Cahn, M. Kazmi, W. Arcese, K. Kolbe, N. C. Gorin, K. Thomson, N. Milpied, D. Niederwieser, K. Indrák, P. Corradini, A. Sureda, and N. Schmitz, “High-dose therapy and autologous stem-cell transplantation in Waldenstrom macroglobulinemia: the Lymphoma Working Party of the European Group for Blood and Marrow Transplantation.,” Journal of Clinical Oncology,vol. 28, no. 13, pp. 2227–2232, 2010.

P. Lang, J. Greil, P. Bader, R. Handgretinger, T. Klingebiel, M. Schumm, P.-G. Schlegel, T. Feuchtinger, M. Pfeiffer, H. Scheel-Walter, M. Führer, D. Martin, and D. Niethammer, “Long-term outcome after haploidentical stem cell transplantation in children.,” Blood Cells, Molecules and Diseasesvol. 33, no. 3, pp. 281–7, 2004.

H. Lannert, T. Able, S. Becker, M. Sommer, M. Braun, P. Stadtherr, and a D. Ho, “Optimizing BM harvesting from normal adult donors.,” Bone Marrow Transplantation, vol. 42, no. 7, pp. 443–7, Oct. 2008.

F. Lanza, D. C. Campioni, A. Hellmann, G. Milone, A. Wahlin, J. Walewski, P. Spedini, C. Fiamenghi, A. Cuneo, W. Knopi, M. Swierkowska-czeneszew, J. Petriz, S. Fruehauf, D. Farge, M. Mohty, J. Passweg, and T. Ruuto, “Individual Quality Assessment of Autografting by Probability Estimation for Clinical

Endpoints : A Prospective Validation Study from the European Group for Blood and Marrow Transplantation," vol. 19, pp. 1670–1676, 2013.

A. Le Bourgeois, C. Mohr, T. Guillaume, J. Delaunay, F. Malard, M. Loirat, P. Peterlin, N. Blin, V. Dubruille, B. Mahe, T. Gastinne, S. Le Gouill, P. Moreau, M. Mohty, L. Planche, L. Lode, M. C. Bene, and P. Chevallier, "Comparison of outcomes after two standards-of-care reduced-intensity conditioning regimens and two different graft sources for allogeneic stem cell transplantation in adults with hematologic diseases: A single-center analysis," Biology of Blood and Marrow Transplantation,vol. 19, no. 6, pp. 934–939, 2013.

K.-H. Lee, S.-J. Choi, J.-H. Lee, J.-H. Lee, D.-Y. Kim, M. Seol, Y.-S. Lee, Y.-A. Kang, M. Jeon, S.-C. Yun, Y.-D. Joo, W.-S. Lee, M.-J. Kang, H. Kim, J.-H. Park, S.-H. Bae, H.-M. Ryoo, M.-K. Kim, and M.-S. Hyun, "Clinical effect of reduced-intensity conditioning regimen containing antithymocyte globulin for hematopoietic cell transplantation from unrelated-donors.," American Journal of Hematology, vol. 86, no. 5, pp. 399–405, 2011.

S. Lee, B.-S. Cho, S.-Y. Kim, S.-M. Choi, D.-G. Lee, K.-S. Eom, Y.-J. Kim, H.-J. Kim, C.-K. Min, S.-G. Cho, D.-W. Kim, J.-W. Lee, W.-S. Min, W.-S. Shin, and C.-C. Kim, "Allogeneic stem cell transplantation in first complete remission enhances graft-versus-leukemia effect in adults with acute lymphoblastic leukemia: antileukemic activity of chronic graft-versus-host disease.," Biology of Blood and Marrow Transplantation,vol. 13, no. 9, pp. 1083–94, 2007.

Z. Y. Lim, A. Y. L. Ho, W. Ingram, M. Kenyon, L. Pearce, B. Czepulkowski, S. Devereux, R. F. Duarte, A. Pagliuca, and G. J. Mufti, "Outcomes of alemtuzumab-based reduced intensity conditioning stem cell transplantation using unrelated donors for myelodysplastic syndromes," British Journal of Haematology vol. 135, no. 2, pp. 201–209, 2006.

S.-C. Lin, P.-Y. Hsieh, P.-W. Shueng, H.-J. Tien, L.-Y. Wang, and C.-H. Hsieh, "Total marrow irradiation as part of autologous stem cell transplantation for Asian patients with multiple myeloma.," Biomed Research International, vol. 2013, p. 321762, 2013.

H. Link, L. Arseniev, O. Bähre, R. J. Berenson, K. Battmer, J. G. Kadar, R. Jacobs, J. Casper, J. Kühl, J. Schubert, H. Diedrich, and H. Poliwoda, "Combined transplantation of allogeneic bone marrow and CD34+ blood cells.," Blood, vol. 86, no. 7, pp. 2500–8, 1995.

H. Liu, X. Zhai, Z. Song, J. Sun, Y. Xiao, D. Nie, Y. Zhang, F. Huang, H. Zhou, Z. Fan, S. Tu, Y. Li, X. Guo, G. Yu, and Q. Liu, "Busulfan plus fludarabine as a myeloablative conditioning regimen compared with busulfan plus cyclophosphamide for acute myeloid leukemia in first complete remission undergoing allogeneic hematopoietic stem cell transplantation: a prospective and multicen," Journal of Hematological Oncology, vol. 6, no. 1, p. 15, 2013.

Q.-F. Liu, C. Liu, Y. Zhang, J. Sun, Z.-S. Yi, Z.-P. Fan, D. Xu, Q.-L. Jiang, and X.-J. Xu, "Peripheral blood stem cell transplantation compared with bone marrow transplantation from unrelated donors in patients with leukemia: a single institutional experience.," Blood Cells, Molecules and Diseases, vol. 45, no. 1, pp. 75–81, Jun. 2010.

F. R. Loberiza, D. S. Serna, M. M. Horowitz, and J. D. Rizzo, "Transplant center characteristics and clinical outcomes after hematopoietic stem cell transplantation: what do we know?," Bone Marrow Transplantation, vol. 31, no. 6, pp. 417–21, 2003.

F. Locatelli, N. Kabbara, A. Ruggeri, A. Ghavamzadeh, I. Roberts, C. K. Li, F. Bernaudin, C. Vermylen, J. H. Dalle, J. Stein, R. Wynn, C. Cordonnier, F. Pinto, E. Angelucci, G. Soci??, E. Gluckman, M. C. Walters, and V. Rocha, "Outcome of patients with hemoglobinopathies given either cord blood or bone marrow transplantation from an HLA-identical sibling," Blood, vol. 122, no. 6, pp. 1072–1078, 2013.

L. Lombardini, A. Bosi, S. Grosz, D. Pamphilon, D. Fehily, M. Mareri, C. Carella, M. Pia Mariani, S. Pupella, G. Grazzini, and A. N. Costa, "Haematopoietic stem cell transplantation: A comparison between the accreditation process performed by competent authorities and JACIE in Italy," Vox Sanguinus, vol. 105, no. 2, pp. 174–176, 2013.

J. Magenau and D. R. Couriel, "Hematopoietic stem cell transplantation for acute myeloid leukemia: To whom, when, and how," Curr. Oncol. Rep., vol. 15, no. 5, pp. 436–444, 2013.
F. Malard, N. Kröger, I. H. Gabriel, K. Hübel, J. F. Apperley, G. W. Basak, K. W. Douglas, C. Geraldes, O. Jaksic, Z. Koristek, F. Lanza, R. Lemoli, G. Mikala, D. Selleslag, N. Worel, M. Mohty, and R. F. Duarte, "Plerixafor for autologous peripheral blood stem cell mobilization in patients previously treated with fludarabine or lenalidomide.," Biology of Blood and Marrow Transplantation,vol. 18, no. 2, pp. 314–7, 2012.

D. Marenchino, L. Maddalena, R. Balbo, I. Avonto, G. Menardi, M. Prucca, L. Perotti, N. Mordini, and G. Peano, "Consequences of ABO incompatibility in multiple myeloma patients undergoing peripheral blood stem cell transplantation after reduced intensity conditioning.," Blood Transfusion, vol. 9, no. 1, pp. 79–85, 2011.

M. Markova, J. N. Barker, J. S. Miller, M. Arora, J. E. Wagner, L. J. Burns, M. L. MacMillan, D. Douek, T. DeFor, Y. Tan, T. Repka, B. R. Blazar, and D. J. Weisdorf, "Fludarabine vs cladribine plus busulfan and low-dose TBI as reduced intensity conditioning for allogeneic hematopoietic stem cell transplantation: a prospective randomized trial.," Bone Marrow Transplantation, vol. 39, no. 4, pp. 193–9, 2007.

C. Martins, J. F. Lacerda, F. Lourenço, J. a Carmo, and J. M. F. Lacerda, "Autologous stem cell transplantation in acute myeloid leukemia. Factors influencing outcome. A 13 year single institution experience.," Acta Medica Portuguesa, vol. 18, no. 5, pp. 329–37, 2005.

J. Matthijnssens, M. Ciarlet, M. Rahman, H. Attoui, M. K. Estes, J. R. Gentsch, M. Iturriza-gómara, C. Kirkwood, P. P. C. Mertens, O. Nakagomi, J. T. Patton, and M. Franco, "NIH Public Access," vol. 153, no. 8, pp. 1621–1629, 2009.

J. Mehta, L. I. Gordon, M. S. Tallman, J. N. Winter, a M. Evens, O. Frankfurt, S. F. Williams, D. Grinblatt, L. Kaminer, R. Meagher, and S. Singhal, "Does younger donor age affect the outcome of reduced-intensity allogeneic hematopoietic stem cell transplantation for hematologic malignancies beneficially?," Bone Marrow Transplantation, vol. 38, no. 2, pp. 95–100, 2006.

R. Meisel, H.-J. Laws, S. Balzer, B. Bernbeck, C. Kramm, S. Schönberger, K. Sinha, A. Tröger, M. Schmitz, J. Fischer, U. Göbel, J. Enczmann, and D. Dilloo, "Comparable long-term survival after bone marrow versus peripheral blood progenitor cell transplantation from matched unrelated donors in children with hematologic malignancies.," Biology of Blood and Marrow Transplantation, vol. 13, no. 11, pp. 1338–1345, Nov. 2007.

B. Metzner, T. H. M??ller, W. Gebauer, J. Casper, D. Kraemer, B. Rosien, S. Schumann-Binarsch, R. Thole, C. H. Khne, M. Dreyling, E. Hoster, and C. Pott, "Long-term clinical and molecular remissions in

patients with mantle cell lymphoma following high-dose therapy and autologous stem cell transplantation," Annals of Hematology, vol. 93, no. 5, pp. 803–810, 2014.

I. N. Micallef, P. J. Stiff, E. A. Stadtmauer, B. J. Bolwell, A. P. Nademanee, R. T. Maziarz, A. M. Partisano, S. Marulkar, and J. F. Dipersio, "Safety and efficacy of upfront plerixafor + G-CSF versus placebo + G-CSF for mobilization of CD34+ hematopoietic progenitor cells in patients ???60 and <60 years of age with non-Hodgkin's lymphoma or multiple myeloma," American Journal of Hematology, vol. 88, no. 12, pp. 1017–1023, 2013.

M. Michallet, Q. H. Le, M. Mohty, T. Prébet, F. Nicolini, J. M. Boiron, H. Esperou, M. Attal, N. Milpied, B. Lioure, P. Bordigoni, I. Yakoub-Agha, J. H. Bourhis, B. Rio, E. Deconinck, M. Renaud, Z. Chir, and D. Blaise, "Predictive factors for outcomes after reduced intensity conditioning hematopoietic stem cell transplantation for hematological malignancies: a 10-year retrospective analysis from the Société Française de Greffe de Moelle et de Thérapie Cellulaire," Experimental Hematology vol. 36, no. 5, pp. 535–544, 2008.

R. Mitchell and I. Nivison-Smith, "Outcomes of hematopoietic stem cell transplantation in primary immunodeficiency: a report from the Australian and New Zealand Children's Haematology Oncology," … Marrow Transplant., vol. 19, pp. 338–343, 2013.

I. Mitrus, A. Smagur, S. Giebel, J. Gliwinska, M. Prokop, M. Glowala-Kosinska, A. Chwieduk, M. Sadus-Wojciechowska, A. Tukiendorf, and J. Holowiecki, "A faster reconstitution of hematopoiesis after autologous transplantation of hematopoietic cells cryopreserved in 7.5% dimethyl sulfoxide if compared to 10% dimethyl sulfoxide containing medium," Cryobiology, vol. 67, no. 3, pp. 327–331, 2013.

T. Miyamoto, G. Yoshimoto, T. Kamimura, T. Muta, S. Takashima, Y. Ito, M. Shiratsuchi, I. Choi, K. Kato, K. Takenaka, H. Iwasaki, Y. Takamatsu, T. Teshima, and K. Akashi, "Combination of high-dose melphalan and bortezomib as conditioning regimen for autologous peripheral blood stem cell transplantation in multiple myeloma," International Journal of Hematology, vol. 98, no. 3, pp. 337–345, 2013.

R. Morariu-Zamfir, V. Rocha, a Devergie, G. Socié, P. Ribaud, H. Esperou, N. Parquet, P. Guardiola, L. Dal Cortivo, H. Bittencourt, F. Garnier, R. Traineau, J. P. Marolleau, S. Chevret, and E. Gluckman, "Influence of CD34(+) marrow cell dose on outcome of HLA-identical sibling allogeneic bone marrow transplants in patients with chronic myeloid leukaemia.," Bone Marrow Transplantation, vol. 27, no. 6, pp. 575–580, 2001.

F. Morschhauser, C. Recher, N. Milpied, R. Gressin, G. Salles, P. Brice, N. Vey, C. Haioun, P. Colombat, J. F. Rossi, E. Deconinck, F. Lazreg, L. Bergougnoux, G. Delsol, and M. Attal, "A 4-weekly course of rituximab is safe and improves tumor control for patients with minimal residual disease persisting 3 months after autologous hematopoietic stem-cell transplantation: results of a prospective multicenter phase II study in patients with," Ann. Oncol., vol. 23, no. 10, pp. 2687–95, 2012.
R. Nakamura, N. Auayporn, D. D. Smith, J. Palmer, J. Y. Sun, J. Schriber, V. Pullarkat, P. Parker, R. Rodriguez, A. Stein, J. Rosenthal, S. Wang, C. Karanas, K. Gaal, D. Senitzer, and S. J. Forman, "Impact of Graft Cell Dose on Transplant Outcomes following Unrelated Donor Allogeneic Peripheral Blood Stem Cell Transplantation: Higher CD34+ Cell Doses Are Associated with Decreased Relapse Rates," Biology of Blood and Marrow Transplantation,vol. 14, no. 4, pp. 449–457, 2008.

R. Nakamura, J. M. Palmer, M. R. O'Donnell, T. Stiller, S. H. Thomas, J. Chao, J. Alvarnas, P. M. Parker, V. Pullarkat, R. Maegawa, A. S. Stein, D. S. Snyder, R. Bhatia, K. Chang, S. Wang, J. L. Cai, D. Senitzer,

and S. J. Forman, “Reduced intensity allogeneic hematopoietic stem cell transplantation for MDS using tacrolimus/sirolimus-based GVHD prophylaxis,” Leukemia Research vol. 36, no. 9, pp. 1152–1156, 2012.

A. Okamura, M. Yamamori, M. Shimoyama, Y. Kawano, H. Kawano, Y. Kawamori, S. Nishikawa, K. Minagawa, K. Yakushijin, Y. Katayama, T. Sakaeda, M. Hirai, and T. Matsui, “Pharmacokinetics-based optimal dose-exploration of mycophenolate mofetil in allogeneic hematopoietic stem cell transplantation,” International Journal of Hematology, vol. 88, no. 1, pp. 104–110, 2008.

B. R. Oshrine, Y. Li, D. T. Teachey, J. Heimall, D. M. Barrett, and N. Bunin, “Immunologic recovery in children after alternative donor allogeneic transplantation for hematologic malignancies: Comparison of recipients of partially T cell-depleted peripheral blood stem cells and umbilical cord blood,” Biology of Blood and Marrow Transplantation,vol. 19, no. 11, pp. 1581–1589, 2013.

H. D. Ottinger, E. Albert, R. Arnold, D. W. Beelen, R. Blasczyk, D. Bunjes, S. Burdach, W. Ebell, G. Ehninger, H. Einsele, J. Enczmann, A. Fauser, W. Friedrich, J. Finke, U. Gobel, S. F. Goldmann, M. Gramatzki, W. Helbig, L. Kanz, T. Klingebiel, H. J. Kolb, P. Kuhnl, C. Loliger, C. R. Muller, H. Grosse-Wilde, and et al., “German consensus on immunogenetic donor search for transplantation of allogeneic bone marrow and peripheral blood stem cells,” Bone Marrow Transplantation, vol. 20, no. 2, pp. 101–105, 1997.

M. Ozcan, C. Ustun, E. Akcaglayan, H. Akan, O. Arslan, O. Ilhan, M. Beksac, G. Gurman, T. Demirer, M. Arat, H. Celebi, N. Konuk, A. Uysal, and H. Koc, “Recombinant human granulocyte colony-stimulating factor (rh-G-CSF) may accelerate hematopoietic recovery after HLA-identical sibling allogeneic peripheral blood stem cell transplantation,” Bone Marrow Transplantation, vol. 27, no. 5, pp. 499–505, 2001.

H. A. Ozkan, U. G. Ozer, C. Bal, and Z. Gulbas, “Daily vs every other day administration of G-CSF following autologous peripheral stem cell transplantation: A prospective randomized study,” Transfusion and Apheresis Science, vol. 49, no. 2, pp. 163–167, 2013.

S. Patil, a Spencer, a Schwarer, I. Lewis, M. Hertzberg, S. Avery, a Wei, T. Noutsos, E. Paul, Y. Taouk, and J. Muirhead, “Reduced-intensity conditioned allogeneic haematopoietic stem cell transplantation results in durable disease-free and overall survival in patients with poor prognosis myeloid and lymphoid malignancies: eighty-month follow-up.,” Bone Marrow Transplantation, vol. 45, no. 7, pp. 1154–60, 2010.

F. Patriarca, A. Bacigalupo, A. Sperotto, M. Isola, B. Bruno, M. T. van Lint, A. P. Iori, P. Di Bartolomeo, M. Musso, P. Pioltelli, G. Visani, P. Iacopino, R. Fanin, and A. Bosi, “Outcome of allogeneic stem cell transplantation following reduced-intensity conditioninig regimen in patients with idiopathic myelofibrosis: The G.I.T.M.O. Experience,” Mediterranean Journal of Hematology and Infectious Diseases,vol. 2, no. 2, 2010.

L. M. Pelus and S. S. Farag, “Increased mobilization and yield of stem cells using plerixafor in combination with granulocyte-colony stimulating factor for the treatment of non-Hodgkin’s lymphoma and multiple myeloma,” Stem Cells and Cloning: Advances and Applications,vol. 4, no. 1, pp. 11–22, 2011.

N. Pemmaraju, M. F. Tanaka, F. Ravandi, H. Lin, V. Baladandayuthapani, G. Rondon, S. a Giralt, J. Chen, S. Pierce, J. Cortes, H. Kantarjian, R. E. Champlin, M. De Lima, and M. H. Qazilbash, “Outcomes in patients with relapsed or refractory acute promyelocytic leukemia treated with or without

autologous or allogeneic hematopoietic stem cell transplantation.," Clin. Lymphoma. Myeloma Leuk., vol. 13, no. 4, pp. 485–92, 2013.

O. Pérez-López, J. Martín-Sánchez, R. Parody-Porras, I. Espigado-Tocino, P. Noguerol, M. Carmona-González, and J. a Pérez-Simón, "Lenograstim compared to filgrastim for the mobilization of hematopoietic stem cells in healthy donors.," Transfusion, pp. 10–12, Mar. 2013.

J. B. Perkins, S. C. Goldstein, J. L. Dawson, J. Kim, T. L. Field, J. S. Partyka, K. K. Fields, B. L. Maddox, C. E. Simonelli, A. M. Neuger, R. M. Lush, and D. M. Sullivan, "Phase I study of topotecan, ifosfamide, and etoposide (TIME) with autologous stem cell transplant in refractory cancer: Pharmacokinetic and pharmacodynamic correlates," Clinical Cancer Research,vol. 17, no. 24, pp. 7743–7753, 2011.

J. B. Perz, a Rahemtulla, C. Giles, R. M. Szydlo, J. Davis, D. Gopaul, J. Gillmore, C. J. Mathias, P. N. Hawkins, and J. F. Apperley, "Long-term outcome of high-dose melphalan and autologous stem cell transplantation for AL amyloidosis.," Bone Marrow Transplantation, vol. 37, no. 10, pp. 937–43, 2006.

D. Petropoulos, L. L. Worth, C. a Mullen, R. Madden, a Mahajan, M. Choroszy, C. S. Ha, R. C. Champlin, and K. W. Chan, "Total body irradiation, fludarabine, melphalan, and allogeneic hematopoietic stem cell transplantation for advanced pediatric hematologic malignancies.," Bone Marrow Transplantation, vol. 37, no. 5, pp. 463–7, 2006.

L. Pierelli, P. Perseghin, M. Marchetti, P. Accorsi, R. Fanin, C. Messina, A. Olivieri, M. Risso, L. Salvaneschi, and A. Bosi, "Best practice for peripheral blood progenitor cell mobilization and collection in adults and children: Results of a Societie Italiana di Emaferesi e Manipolazione Cellulare (SIDEM) and Gruppo Italiano Trapianto Midollo Osseo (GITMO) consensus process," Transfusion, vol. 52, no. 4, pp. 893–905, 2012.

R. Ram, H. Magen-Nativ, L. Vidal, C. Herscovici, A. Peck, P. Raanani, O. Shpilberg, and M. Yeshurun, "Toxicity of autologous hematopoietic cell transplantation in patients with multiple myeloma - comparison between two different induction regimens," Clinical Transplantation, vol. 26, no. 5, pp. 549–554, 2012.

A. Re, C. Cattaneo, C. Skert, P. Balsalobre, M. Michieli, M. Bower, A. J. M. Ferreri, M. Hentrich, J. M. Ribera, B. Allione, P. Schommers, S. Montoto, C. Almici, P. Ferremi, M. Mazzucato, S. Gattillo, S. Casari, M. Spina, J. L. Diez-Martin, U. Tirelli, and G. Rossi, "Stem cell mobilization in HIV seropositive patients with lymphoma," Haematologica, vol. 98, no. 11, pp. 1762–1768, 2013.

N. Reddy, O. Oluwole, J. P. Greer, S. Goodman, B. Engelhardt, M. H. Jagasia, and B. N. Savani, "Superior Long-Term Outcome of Patients With Early Transformation of Non-Hodgkin Lymphoma Undergoing Stem Cell Transplantation," Clinical Lymphoma, Myeloma and Leukemia,vol. 12, no. 6, pp. 406–11, 2012.

M. Remberger, J. Törlén, O. Ringdén, M. Engström, E. Watz, M. Uhlin, and J. Mattsson, "The effect of total nucleated and CD34+ cell dose on outcome after allogeneic hematopoietic stem cell transplantation" Biology of Blood and Marrow Transplantation,vol. 21, no. 5, pp. 889-93, 2015.

M. Remberger, O. Ringdén, I. W. Blau, H. Ottinger, B. Kremens, M. G. Kiehl, J. Aschan, D. W. Beelen, N. Basara, G. Kumlien, a a Fauser, and V. Runde, "No difference in graft-versus-host disease, relapse, and survival comparing peripheral stem cells to bone marrow using unrelated donors.," Blood, vol. 98, no. 6, pp. 1739–1745, Sep. 2001.

E. M. Richa, R. Kunnavakkam, L. a Godley, J. Kline, O. Odenike, R. a Larson, V. Nguyen, W. Stock, A. Wickrema, K. Van Besien, and A. S. Artz, “Influence of related donor age on outcomes after peripheral blood stem cell transplantation.,” Cytotherapy, vol. 14, no. 6, pp. 707–15, 2012.
P. G. Richardson, J. R. Berenson, D. Irwin, D. P. Schenkein, D. L. Esseltine, K. C. Anderson, and S. R. Cancer, “St or ti Fe rra ta Fo u Fe rra ta St or ti Fo u,” Haematologica, vol. 91, no. 7, pp. 10–12, 2006.
O. Ringdén, M. Labopin, N.-C. Gorin, L. Volin, G. F. Torelli, M. Attal, J. P. Jouet, N. Milpied, G. Socié, C. Cordonnier, M. Michallet, A. I. Atienza, O. Hermine, and M. Mohty, “Growth factor- associated graft-versus-host disease and mortality 10 years after allogeneic bone marrow transplantation.,” British Journal of Haematology vol. 157, no. 2, pp. 220–9, Apr. 2012.

O. Ringdén, M. Labopin, A. Bacigalupo, W. Arcese, U. W. Schaefer, R. Willemze, H. Koc, D. Bunjes, E. Gluckman, V. Rocha, A. Schattenberg, and F. Frassoni, “Transplantation of peripheral blood stem cells as compared with bone marrow from HLA-identical siblings in adult patients with acute myeloid leukemia and acute lymphoblastic leukemia,” Journal of Clinical Oncology,vol. 20, no. 24, pp. 4655–4664, 2002.

O. Ringdén, M. Labopin, D. W. Beelen, L. Volin, G. Ehninger, J. Finke, H. T. Greinix, S. Kyrcz-Krzemien, D. Bunjes, L. Brinch, D. Niederwieser, R. Arnold, M. Mohty, and V. Rocha, “Bone marrow or peripheral blood stem cell transplantation from unrelated donors in adult patients with acute myeloid leukaemia, an Acute Leukaemia Working Party analysis in 2262 patients.,” Journal of International Medicine, vol. 272, no. 5, pp. 472–83, Nov. 2012.

O. Ringdén, M. Labopin, N.-C. Gorin, K. Le Blanc, V. Rocha, E. Gluckman, J. Reiffers, W. Arcese, J. M. Vossen, J.-P. Jouet, C. Cordonnier, and F. Frassoni, “Treatment With Granulocyte Colony-Stimulating Factor After Allogeneic Bone Marrow Transplantation for Acute Leukemia Increases the Risk of Graft-Versus-Host Disease and Death : A Study From the Acute Leukemia Working Party of the European Group for Blood,” Journal of Clinical Oncology,vol. 22, no. 3, pp. 416–23, 2004.

M. Robin, R. Porcher, L. Adès, N. Boissel, E. Raffoux, a Xhaard, J. Larghero, C. Gardin, C. Himberlin, a Delmer, P. Fenaux, H. Dombret, G. Socié, and R. Peffault de Latour, “Matched unrelated or matched sibling donors result in comparable outcomes after non-myeloablative HSCT in patients with AML or MDS.,” Bone Marrow Transplantation, no. October 2012, pp. 1–6, 2013.

C. A. Rodrigues, V. Rocha, P. Dreger, C. Brunstein, H. Sengeloev, J. Finke, M. Mohty, B. Rio, E. Petersen, F. Guilhot, D. Niederwieser, J. J. Cornelissen, P. Jindra, A. Nagler, N. Fegueux, H. Schoemans, S. Robinson, A. Ruggeri, E. Gluckman, C. Canals, and A. Sureda, “Alternative donor hematopoietic stem cell transplantation for mature lymphoid malignancies after reduced-intensity conditioning regimen: Similar outcomes with umbilical cord blood and unrelated donor peripheral blood,” Haematologica, vol. 99, no. 2, pp. 370–377, 2014.

S. Roncon, I. L. Barbosa, F. Campilho, S. M. Lopes, A. Campos, and A. Carvalhais, “Mobilization and collection of peripheral blood stem cells in multiple myeloma patients older than 65 years,” Transplantation Proceedings, vol. 43, no. 1, pp. 244–246, 2011.

A. S. Roug, L. B. Hokland, E. Segel, K. Nielsen, M. Toft-Petersen, P. B. Van Kooten Niekerk, P. Hokland, and L. Nederby, “Unraveling stem cell and progenitor subsets in autologous grafts according to methods of mobilization: Implications for prediction of hematopoietic recovery,” Cytotherapy, vol. 16, no. 3, pp. 392–401, 2014.

B. N. Savani, S. Mielke, K. Rezvani, A. Montero, A. S. Yong, L. Wish, J. Superata, R. Kurlander, A. Singh, R. Childs, and A. J. Barrett, “Absolute Lymphocyte Count on Day 30 Is a Surrogate for Robust Hematopoietic Recovery and Strongly Predicts Outcome after T Cell-Depleted Allogeneic Stem Cell Transplantation,” Biology of Blood and Marrow Transplantation,vol. 13, no. 10, pp. 1216–1223, 2007.

H. G. Sayer, M. Kröger, J. Beyer, M. Kiehl, S. a Klein, K. Schaefer-Eckart, R. Schwerdtfeger, W. Siegert, V. Runde, C. Theuser, H. Martin, J. Schetelig, D. W. Beelen, a Fauser, J. Kienast, K. Höffken, G. Ehninger, and M. Bornhäuser, “Reduced intensity conditioning for allogeneic hematopoietic stem cell transplantation in patients with acute myeloid leukemia: disease status by marrow blasts is the strongest prognostic factor.,” Bone Marrow Transplantation, vol. 31, no. 12, pp. 1089–95, 2003.

R. Schulze, M. Schulze, a Wischnik, S. Ehnle, K. Doukas, W. Behr, W. Ehret, and G. Schlimok, “A comparative study of peripheral blood stem cell vs bone marrow transplantation from unrelated donors (MUD): a single center study.,” Bone Marrow Transplantation, vol. 19, no. 12, pp. S27–S31, 2003.

C. Sebban, A. Lefranc, L. Perrier, P. Moreau, D. Espinouse, A. Schmidt, L. Kammoun, H. Ghesquieres, C. Ferlay, J. O. Bay, S. Lissandre, D. Pérol, M. Michallet, and P. Quittet, “A randomised phase II study of the efficacy, safety and cost-effectiveness of pegfilgrastim and filgrastim after autologous stem cell transplant for lymphoma and myeloma (PALM study),” European Journal of Cancer, vol. 48, no. 5, pp. 713–720, 2012.

J. Y. Seo, H.-J. Huh, H.-K. Park, H.-K. Choung, D.-W. Kim, H. H. Koo, K. W. Sung, and E.-S. Kang, “Evaluation of overnight storage conditions for autologous peripheral blood stem cell products: comparison of three different conditions.,” Vox Sanguinus, vol. 103, no. 2, pp. 150–8, 2012.

P. Shaughnessy, J. Uberti, S. Devine, R. T. Maziarz, J. Vose, I. Micallef, E. Jacobsen, J. McCarty, P. Stiff, a Artz, E. D. Ball, R. Berryman, M. Dugan, R. Joyce, F. J. Hsu, D. Johns, and P. McSweeney, “Plerixafor and G-CSF for autologous stem cell mobilization in patients with NHL, Hodgkin’s lymphoma and multiple myeloma: results from the expanded access program.,” Bone Marrow Transplantation, vol. 48, no. 6, pp. 777–81, 2013.

B. E. Shaw, J. F. Apperley, N. H. Russell, C. Craddock, E. Liakopoulou, M. N. Potter, R. Wynn, B. Gibson, R. M. Pearce, K. Kirkland, J. Lee, J. A. Madrigal, G. Cook, and J. L. Byrne, “Unrelated donor peripheral blood stem cell transplants incorporating pre-transplant in-vivo Alemtuzumab are not associated with any increased risk of significant acute or chronic graft-versus-host disease,” British Journal of Haematology vol. 153, no. 2, pp. 244–252, 2011.

B. Simona, R. Cristina, N. Luca, S. Sara, B. Aleksandra, B. Paola, G. Federica, A. Pierluigi, O. Laura, S. Simona, Q. Jessica, N. Mara, and M. Giovanni, “A single dose of Pegfilgrastim versus daily Filgrastim to evaluate the mobilization and the engraftment of autologous peripheral hematopoietic progenitors in malignant lymphoma patients candidate for high-dose chemotherapy,” Transfusion and Apheresis Science, vol. 43, no. 3, pp. 321–326, 2010.

S. Singhal, R. Powles, J. Treleaven, S. Kulkarni, B. Sirohi, C. Horton, B. Millar, V. Shepherd, D. Tait, R. Saso, A. Rowland, S. Long, and J. Mehta, “A low CD34 cell dose results in higher mortality and poorer survival after blood or marrow stem cell transplantation from HLA-identical siblings : should 2 × 10 6 CD34 cells / kg be considered the minimum threshold?,” pp. 489–496, 2000.

I. Sirinoglu Demiriz, E. Tekgunduz, and F. Altuntas, “What Is the Most Appropriate Source for Hematopoietic Stem Cell Transplantation? Peripheral Stem Cell/Bone Marrow/Cord Blood,” Bone Marrow Research, vol. 2012, pp. 1–5, 2012.

S. Sivgin, E. Karakus, L. Kaynar, F. Kurnaz, C. Pala, M. Keklik, G. Zararsiz, M. Solmaz, B. Eser, M. Cetin, and A. Unal, “The comparison of Filgrastim (Neupogen®), biosimilar filgrastim (Leucostim®) and Lenograstim (Granocyte®) as a first line peripheral blood stem cell mobilization strategy in autologous hematopoieitic stem cell transplantation: A single center experience,” Transfusion and Apheresis Science, vol. 48, no. 3, pp. 315–20, Jun. 2013.

A. Smagur, I. Mitrus, S. Giebel, M. Sadus-Wojciechowska, J. Najda, T. Kruzel, T. Czerw, J. Gliwinska, M. Prokop, M. Glowala-Kosinska, A. Chwieduk, and J. Holowiecki, “Impact of different dimethyl sulphoxide concentrations on cell recovery, viability and clonogenic potential of cryopreserved peripheral blood hematopoietic stem and progenitor cells,” Vox Sanguinus, vol. 104, no. 3, pp. 240–247, 2013.

V. R. Smith, U. Popat, S. Ciurea, Y. Nieto, P. Anderlini, G. Rondon, A. Alousi, M. Qazilbash, P. Kebriaei, I. Khouri, M. de Lima, R. Champlin, and C. Hosing, “Just-in-time rescue plerixafor in combination with chemotherapy and granulocyte-colony stimulating factor for peripheral blood progenitor cell mobilization,” American Journal of Hematology, vol. 88, no. 9, pp. 754–757, 2013.

R. M. Sobecks, R. Dean, L. a Rybicki, J. Chan, K. S. Theil, R. Macklis, S. Andresen, M. Kalaycio, B. Pohlman, C. Ferraro, K. Cherni, J. Sweetenham, E. Copelan, and B. J. Bolwell, “400 cGy TBI with fludarabine for reduced-intensity conditioning allogeneic hematopoietic stem cell transplantation.,” Bone Marrow Transplantation, vol. 42, no. 11, pp. 715–22, 2008.

S. K. Sohn, J. G. Kim, K. W. Seo, Y. S. Chae, J. T. Jung, J. S. Suh, and K. B. Lee, “GM-CSF-based mobilization effect in normal healthy donors for allogeneic peripheral blood stem cell transplantation.,” Bone Marrow Transplantation, vol. 30, no. 2, pp. 81–6, Jul. 2002.

A. D. Souza, M. Lacy, M. Gertz, S. Kumar, F. Buadi, S. Hayman, D. Dingli, S. Zeldenrust, R. Kyle, S. Ansell, D. Inwards, P. Johnston, I. Micallef, L. Porrata, M. Litzow, D. Gastineau, W. Hogan, and A. Dispenzieri, “Long-term outcomes after autologous stem cell transplantation for patients with POEMS syndrome (osteosclerotic myeloma): a single-center experience,” Blood, vol. 120, no. 1, pp. 56–62, 2012.

S. Srivastava, D. Jones, L. L. Wood, J. E. Schwartz, R. P. Nelson, R. Abonour, A. Secrest, E. Cox, J. Baute, C. Sullivan, K. Kane, M. J. Robertson, and S. S. Farag, “A phase I trial of high-dose clofarabine, etoposide, and cyclophosphamide and autologous peripheral blood stem cell transplantation in patients with primary refractory and relapsed and refractory Non-Hodgkin lymphoma,” Biology of Blood and Marrow Transplantation,vol. 17, no. 7, pp. 987–994, 2011.

M. Stelljes, M. Bornhauser, M. Kroger, J. Beyer, M. C. Sauerland, A. Heinecke, B. Berning, C. Scheffold, G. Silling, T. Buchner, A. Neubauer, A. A. Fauser, G. Ehninger, W. E. Berdel, and J. Kienast, “Conditioning with 8-Gy total body irradiation and fludarabine for allogeneic hematopoietic stem cell transplantation in acute myeloid leukemia,” Blood, vol. 106, no. 9, pp. 3314–3321, 2005.

P. J. Stiff, I. Micallef, A. P. Nademanee, E. A. Stadtmauer, R. T. Maziarz, B. J. Bolwell, G. Bridger, S. Marulkar, F. J. Hsu, and J. F. DiPersio, “Transplanted CD34 + Cell Dose Is Associated with Long-Term Platelet Count Recovery following Autologous Peripheral Blood Stem Cell Transplant in Patients with

Non-Hodgkin Lymphoma or Multiple Myeloma," Biology of Blood and Marrow Transplantation,vol. 17, no. 8, pp. 1146–1153, 2011.

K. Sunami, K. Shinagawa, M. Sawamura, A. Sakai, Y. Saburi, Y. Imamura, I. Mizuno, S. Tamaki, T. Kamimura, H. Tsuda, H. Gondo, N. Hino, C. Shimazaki, A. Miyata, F. Tajima, Y. Takemoto, A. Miwa, T. Chou, and M. Harada, "Phase I/II study of tandem high-dose chemotherapy with autologous peripheral blood stem cell transplantation for advanced multiple myeloma," International Journal of Hematology, vol. 90, no. 5, pp. 635–642, 2009.

D. Szpecht, E. Gorczyńska, K. Kałwak, J. Owoc-Lempach, M. Choma, J. Styczyński, J. Goździk, A. Dłużniewska, M. Wysocki, J. R. Kowalczyk, A. Chybicka, A. Pieczonka, and J. Wachowiak, "Matched sibling versus matched unrelated allogeneic hematopoietic stem cell transplantation in children with severe acquired aplastic anemia: Experience of the Polish pediatric group for hematopoietic stem cell transplantation," Archivum Immunologiae et Therapia Experimentalis, vol. 60, no. 3, pp. 225–233, 2012.

M. Takahashi, T. S. Li, R. Suzuki, T. Kobayashi, H. Ito, Y. Ikeda, M. Matsuzaki, and K. Hamano, "Cytokines produced by bone marrow cells can contribute to functional improvement of the infarcted heart by protecting cardiomyocytes from ischemic injury," American journal of physiology. Heart and circulatory physiology, vol. 291, 2006.

S. Talhi, S. Osmani, M. Brahimi, N. Yafour, R. Bouhass, A. Arabi, and M. A. Bekadja, "The use of granulocyte colony stimulating factoR (G-CSF) (filgrastim) alone in the mobilization of stem cell in the autologous stem cell transplantation," Transfusion and Apheresis Science, vol. 49, no. 1, pp. 97–99, 2013.

R. Tanosaki, N. Uike, A. Utsunomiya, Y. Saburi, M. Masuda, M. Tomonaga, T. Eto, M. Hidaka, M. Harada, I. Choi, T. Yamanaka, M. Kannagi, M. Matsuoka, and J. Okamura, "Allogeneic hematopoietic stem cell transplantation using reduced-intensity conditioning for adult T cell leukemia/lymphoma: impact of antithymocyte globulin on clinical outcome.," Biology of Blood and Marrow Transplantation,vol. 14, no. 6, pp. 702–8, 2008.

A. Torres, J. Sanchez, D. Lakomsky, J. Serrano, M. A. Alvarez, C. Mart??n, C. Valls, L. Nevado, A. Rodriguez, J. Casa??o, F. Mart??nez, and P. G??mez, "Assessment of hematologic progenitor engraftment by complete reticulocyte maturation parameters after autologous and allogeneic hematopoietic stem cell transplantation," Haematologica, vol. 86, no. 1, pp. 24–29, 2001.

J. S. Ungerstedt, E. Watz, K. Uttervall, B.-M. Johansson, B. E. Wahlin, P. Näsman, P. Ljungman, a Gruber, U. Axdorph Nygell, and H. Nahi, "Autologous hematopoietic stem cell transplantation in multiple myeloma and lymphoma: an analysis of factors influencing stem cell collection and hematological recovery.," Medical Oncology, vol. 29, no. 3, pp. 2191–9, 2012.

C. Ustun, O. Arslan, M. Beksac, and H. Koc, "A retrospective comparison of allogeneic peripheral blood stem cell and bone marrow transplantation results from a single center: a focus on the incidence of graft-vs.-," Biology of Blood and Marrow Transplantation, vol. 35, pp. 28–35, 1999.

P. Van Wilder, "Advanced therapy medicinal products and exemptions to the Regulation 1394/2007: How confident can we be? An exploratory analysis," Frontiers in Pharmacology, vol. 3 FEB, no. February, pp. 1–9, 2012.

S. Vejbaesya, P. Luangtrakool, K. Luangtrakool, D. W. Vaughn, T. P. Endy, M. P. Mammen, D. H. Libraty, F. A. Ennis, A. L. Rothman, and A. F. Henry, “NIH Public Access,” vol. 199, no. 10, pp. 1442–1448, 2010.

R. J. Verburg, a a Kruize, F. H. van den Hoogen, W. E. Fibbe, E. J. Petersen, F. Preijers, J. K. Sont, R. M. Barge, J. W. Bijlsma, L. B. van de Putte, F. C. Breedveld, and J. M. van Laar, “High-dose chemotherapy and autologous hematopoietic stem cell transplantation in patients with rheumatoid arthritis: results of an open study to assess feasibility, safety, and efficacy.,” Arthritis and Rheumatology, vol. 44, no. 4, pp. 754–760, 2001.

A. C. Vigorito, W. M. Azevedo, J. F. Marques, a M. Azevedo, K. a Eid, F. J. Aranha, I. Lorand-Metze, G. B. Oliveira, M. E. Correa, a R. Reis, E. C. Miranda, and C. a De Souza, “A randomised, prospective comparison of allogeneic bone marrow and peripheral blood progenitor cell transplantation in the treatment of haematological malignancies.,” Haematologica, vol. 22, no. 6, pp. 1145–1151, 2001.

C. H. Villa, T. Shore, K. Van Besien, and M. Cushing, “Addition of Plerixafor to Mobilization Regimens in Autologous Peripheral Blood Stem Cell Transplants Does Not Affect the Correlation of Preharvest Hematopoietic Precursor Cell Enumeration with First-Harvest CD34+ Stem Cell Yield,” Biology of Blood and Marrow Transplantation,vol. 18, no. 12, pp. 1867–1875, 2012.

M. . C. Wach M.; Hus, M.; Jawniak, D.; Legiec, W.; Malek, M.; Manko, J.; Walter-Croneck, A.; Wasik-Szczepanek, E.; Dmoszynska, A., “Treatment of multiple myeloma patients with autologous stem cell transplantation - a fresh analysis,” Folia Histochem Cytobiol, vol. 49, no. 2, pp. 248–254, 2011.
Y. Wang, D. Wu, A. Sun, Z. Jin, H. Qiu, M. Miao, X. Tang, and Z. Fu, “Allogeneic hematopoietic stem cell transplantation for patients with chronic myeloid leukemia in second chronic phase attained by imatinib after onset of blast crisis,” International Journal of Hematology, vol. 87, no. 2, pp. 167–171, 2008.

J. M. Winter, P. Jacobson, B. Bullough, A. P. Christensen, M. Boyer, and J.-A. Reems, “Long-term effects of cryopreservation on clinically prepared hematopoietic progenitor cell products.,” Cytotherapy, vol. 16, no. 7, pp. 965–75, Jul. 2014.

B. Woog Kang, J. H. Moon, Y. Soo Chae, J. Gwang Kim, J. S. Jung, G. J. Cho, D. Y. Jo, Y. K. Kim, H. J. Kim, H. M. Ryoo, H. S. Eom, S. M. Lee, Y. D. Lee, J. H. Won, M. R. Park, M. K. Park, M. S. Hyun, and S. Kyun Sohn, “Comparative analysis of outcomes of allogeneic peripheral blood stem cell transplantation from related and unrelated donors,” Annals of Hematology, vol. 89, no. 8, pp. 813–820, 2010.

I. Wyleżoł, E. Snarski, M. Markiewicz, S. Kyrcz-Krzemień, W.-W. Jędrzejczak, and J. Walewski, “Comparision of benefits of early, delayed, and no administration of G-CSF after autologous peripheral blood stem cell transplantation in lymphoma patients.,” Annals of Transplantation, vol. 18, pp. 336–41, 2013.

N. Y., K. Y., W. T., A. T., O. Y., K. Y., and T. Y., “Hematopoietic and immune recovery after allogeneic peripheral blood stem cell transplantation and bone marrow transplantation in a pediatric population,” Pediatric Transplantation, vol. 6, no. 4, pp. 319–326, 2002.

N. Yafour, M. Brahimi, S. Osmani, A. Arabi, R. Bouhass, and M. A. Bekadja, “Biosimilar G-CSF (filgrastim) is effective for peripheral blood stem cell mobilization and non-cryopreserved autologous transplantation,” Transfusion Clinique et Biologique, vol. 20, no. 5–6, pp. 502–504, 2013.

S. A. Yahng, J. H. Yoon, S. H. Shin, S. E. Lee, B. S. Cho, D. G. Lee, K. S. Eom, S. Lee, C. K. Min, H. J. Kim, S. G. Cho, D. W. Kim, J. W. Lee, W. S. Min, T. G. Kim, C. W. Park, and Y. J. Kim, "Response to pretransplant hypomethylating agents influences the outcome of allogeneic hematopoietic stem cell transplantation in adults with myelodysplastic syndromes," European Journal of Haematology, vol. 90, no. 2, pp. 111–120, 2013.

D. H. Yoon, B. S. Sohn, G. Jang, E. K. Kim, B. W. Kang, C. Kim, J. E. Kim, S. Kim, D. H. Lee, J. S. Lee, S. J. Park, C. J. Park, J. Huh, and C. Suh, "Higher infused CD34+ hematopoietic stem cell dose correlates with earlier lymphocyte recovery and better clinical outcome after autologous stem cell transplantation in non-Hodgkin's lymphoma," Transfusion, vol. 49, no. 9, pp. 1890–1900, 2009.

A. Yoshimi, B. Strahm, I. Baumann, I. Furlan, S. Schwarz, A. Teigler-Schlegel, J.-U. Walther, B. Schlegelberger, G. Göhring, P. Nöllke, M. Führer, and C. M. Niemeyer, "Hematopoietic Stem Cell Transplantation in Children and Young Adults with Secondary Myelodysplastic Syndrome and Acute Myelogenous Leukemia after Aplastic Anemia.," Biology of Blood and Marrow Transplantation,vol. 20, no. 3, pp. 425–429, 2014.

W. Yu, L. Qi-Fa, Q. Ya-Zhen, L. Dai-Hong, X. Lan-Ping, J. Bin, J. Qian, D. Min, Y. Si-Jian, J. Xin-Miao, L. Yan-Rong, and H. Xiao-Jun, "Improved outcome with hematopoietic stem cell transplantation in a poor prognostic subgroup of patients with mixed-lineage-leukemia-rearranged acute leukemia: Results from a prospective, multi-center study," American Journal of Hematology, vol. 89, no. 2, pp. 130–136, 2014.

C. Zhang, X. Chen, X. Zhang, L. Gao, P. Kong, Q. Wang, X. Peng, and H. Liu, "Mobilization of peripheral blood stem cells for autologous transplantation patients with hematological malignancies: Influence of disease, mobilization method, age and sex," Transfusion and Apheresis Science, vol. 39, no. 1, pp. 21–28, 2008.

X. Su Zhao, Y. Chen, X. Yu Zhao, D. Hong Liu, L. ping Xu, Y. Wang, W. Han, Y. Hong Chen, H. Chen, X. hui Zhang, K. Yan Liu, and X. Jun Huang, "Improved outcomes using G-CSF-mobilized blood and bone marrow grafts as the source of stem cells compared with G-PB after HLA-identical sibling transplantation in patients with acute leukemia," Clinical Transplantation, vol. 27, no. 6, pp. 844–851, 2013.

C. Zheng, B. Tang, W. Yao, J. Tong, X. Zhu, K. Song, L. Geng, H. Liu, and Z. Sun, "Comparison of unrelated cord blood transplantation and HLA-matched sibling hematopoietic stem cell transplantation for patients with chronic myeloid leukemia in advanced stage.," Biology of Blood and Marrow Transplantation,vol. 19, no. 12, pp. 1708–12, 2013.

T. Ben Othman, L. Torjemane, A. Abdelkefi, A. Lakhal, S. Ladeb, L. Ben Hamed, H. Slama, and A. Ben Abdeladhim, "Allogeneic hematopoietic stem cell transplantation in Tunisia," Bone Marrow Transplantation, vol. 42, pp. S139–S141, Aug. 2008.

J. M. Zaucha, T. Gooley, W. I. Bensinger, S. Heimfeld, T. R. Chauncey, R. Zaucha, P. J. Martin, M. E. Flowers, J. Storek, G. Georges, R. Storb, and B. Torok-Storb, "CD34 cell dose in granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cell grafts affects engraftment kinetics and development of extensive chronic graft-versus-host disease after human leukocyte antigen-identical sibling transpla," Blood, vol. 98, no. 12, pp. 3221–3227, Dec. 2001.

W. I. Bensinger, P. J. Martin, B. Storer, R. Clift, S. J. Forman, R. Negrin, A. Kashyap, M. E. D. Flowers, K. Lilleby, T. R. Chauncey, R. Storb, K. Blume, S. Heimfeld, S. Rowley, and F. R. Appelbaum,

"Transplantation of Bone Marrow as Compared with Peripheral-Blood Cells from HLA-Identical Relatives in Patients with Hematologic Cancers," New England Journal of Medicine, vol. 344, no. 3, pp. 175–181, Jan. 2001.

A. Busca, C. Anasetti, G. Anderson, F. R. Appelbaum, C. D. Buckner, K. Doney, P. J. Martin, E. Petersdorf, J. E. Sanders, and J. a Hansen, "Unrelated donor or autologous marrow transplantation for treatment of acute leukemia.," Blood, vol. 83, no. 10, pp. 3077–84, 1994.

B.-S. Cho, J.-H. Yoon, S.-H. Shin, S.-A. Yahng, S.-E. Lee, K.-S. Eom, Y.-J. Kim, S. Lee, C.-K. Min, S.-G. Cho, D.-W. Kim, J.-W. Lee, W.-S. Min, C.-W. Park, and H.-J. Kim, "Comparison of Allogeneic Stem Cell Transplantation from Familial-Mismatched/Haploidentical Donors and from Unrelated Donors in Adults with High-Risk Acute Myelogenous Leukemia," Biology of Blood and Marrow Transplantation,vol. 18, no. 10, pp. 1552–1563, Oct. 2012.

S. Chow, A. Lazo-Langner, G. Ormond, K. Howson-Jan, and A. Xenocostas, "Predictors of unsuccessful mobilization with granulocyte colony-stimulating factor alone in patients undergoing autologous hematopoietic stem cell transplantation," Journal of Clinical Apheresis vol. 28, no. 4, pp. 285–292, Aug. 2013.

E. Derenzini, V. Stefoni, R. Maglie, B. Casadei, C. Pellegrini, A. Broccoli, G. Stefani, S. Fanti, M. R. Motta, R. Narducci, L. Argnani, and P. L. Zinzani, "Collection of Hematopoietic Stem Cells after Previous Radioimmunotherapy is Feasible and Does Not Impair Engraftment after Autologous Stem Cell Transplantation inFollicular Lymphoma," Biology of Blood and Marrow Transplantation,vol. 19, no. 12, pp. 1695–1701, 2013.

A. H. Elmaagacli, R. Peceny, N. Steckel, R. Trenschel, H. Ottinger, H. Grosse-Wilde, U. W. Schaefer, and D. W. Beelen, "Outcome of transplantation of highly purified peripheral blood CD34+ cells with T-cell add-back compared with unmanipulated bone marrow or peripheral blood stem cells from HLA-identical sibling donors in patients with first chronic phase chronic myeloid l," Blood, vol. 101, no. 2, pp. 446–453, 2003.

K.-S. Eom, S.-H. Shin, J.-H. Yoon, S.-A. Yahng, S.-E. Lee, B.-S. Cho, Y.-J. Kim, H.-J. Kim, C.-K. Min, D.-W. Kim, J.-W. Lee, W.-S. Min, C.-W. Park, and S. Lee, "Comparable long-term outcomes after reduced-intensity conditioning versus myeloablative conditioning allogeneic stem cell transplantation for adult high-risk acute lymphoblastic leukemia in complete remission," American Journal of Hematology, vol. 88, no. 8, pp. 634–641, Aug. 2013.

A. A. Fauser, N. Basara, I. W. Blau, and M. G. Kiehl, "A comparative study of peripheral blood stem cell vs bone marrow transplantation from unrelated donors (MUD): a single center study.," Bone Marrow Transplantation, vol. 25 Suppl 2, pp. S27-31, May 2000.

G. Favre, M. Beksaç, a Bacigalupo, T. Ruutu, a Nagler, E. Gluckman, N. Russell, J. Apperley, J. Szer, K. Bradstock, a Buzyn, J. Matcham, a Gratwohl, and N. Schmitz, "Differences between graft product and donor side effects following bone marrow or stem cell donation.," Bone Marrow Transplantation, vol. 32, no. 9, pp. 873–880, Nov. 2003.

F. Ferrara, T. Izzo, C. Criscuolo, C. Riccardi, A. Viola, R. Delia, A. Carbone, and M. Celentano, "Comparison of fixed dose pegfilgrastim and daily filgrastim after autologous stem cell transplantation in patients with multiple myeloma autografted on a outpatient basis," Hematological Oncology vol. 29, no. 3, pp. 139–143, Sep. 2011.

N.-C. Gorin, “Results of Genoidentical Hemopoietic Stem Cell Transplantation With Reduced Intensity Conditioning for Acute Myelocytic Leukemia: Higher Doses of Stem Cells Infused Benefit Patients Receiving Transplants in Second Remission or Beyond--The Acute Leukemia W,” Journal of Clinical Oncology,vol. 24, no. 24, pp. 3959–3966, Aug. 2006.

D. Heldal, G. Tjønnfjord, L. Brinch, D. Albrechtsen, T. Egeland, R. Steen, B. G. Solheim, and S. A. Evensen, “A randomised study of allogeneic transplantation with stem cells from blood or bone marrow,” Bone Marrow Transplantation, vol. 25, no. 11, pp. 1129–1136, Jun. 2000.

M. Iravani, E. Tavakoli, M. H. Babaie, A. Ashouri, F. Khatami, and A. Ghavamzadeh, “Comparison of peripheral blood stem cell transplant with bone marrow transplant in class 3 thalassemic patients.,” Exp. Clinical Transplantation, vol. 8, no. 1, pp. 66–73, Mar. 2010.

S. Ji, H. Chen, H. Wang, J. Ma, S. Pan, M. Xue, L. Zhu, J. Liu, M. Xiao, and L. Zhou, “Low incidence of severe aGVHD and accelerating hemopoietic reconstitution in allo-BMT using lenograstim stimulated BM cells.,” Chinese Medical Journal (Engl)., vol. 114, no. 2, pp. 191–5, Feb. 2001.

P. Kebriaei and M. Detry, “Long-term follow-up of allogeneic hematopoietic stem-cell transplantation with reduced-intensity conditioning for patients with chronic myeloid leukemia,” Blood, vol. 110, no. 9, pp. 3456–3462, 2007.

L. Kumar, J. Ghosh, P. Ganessan, A. Gupta, R. Hariprasad, and V. Kochupillai, “High-dose chemotherapy with autologous stem cell transplantation for multiple myeloma: what predicts the outcome? Experience from a developing country.,” Bone Marrow Transplantation, vol. 43, no. 6, pp. 481–9, 2009.

S. T. RM Lemoli, G Bandini, G Leopardi, G Rosti, A Bonini, A Fortuna, D Rondelli, S Mangianti, MR Motta, S Rizzi, C Tassi, M Cavo, C Remiddi, A Curti, R Conte, “Allogeneic Peripheral Blood Stem Cell Transplantation In Patients With Early-Phase Hematologic Malignancy: A Retrospective Comparison Of Short-Term Outcome With Bone Marrow Transplantation,” Haematologica, vol. 83, pp. 48–55, 1998.

Y.-F. Lin, D. R. Lairson, W. Chan, X. L. Du, K. S. Leung, A. A. Kennedy-Nasser, C. A. Martinez, H. E. Heslop, M. K. Brenner, and R. A. Krance, “Children with acute leukemia: A comparison of outcomes from allogeneic blood stem cell and bone marrow transplantation,” Pediatric Blood Cancer, vol. 56, no. 1, pp. 143–151, Jan. 2011.

Z. N. Ozkurt, Z. A. Yegin, I. Yenicesu, S. Z. Aki, M. Yagci, and G. T. Sucak, “Impact of ABO-Incompatible Donor on Early and Late Outcome of Hematopoietic Stem Cell Transplantation,” Transplantation Proceedings, vol. 41, no. 9, pp. 3851–3858, 2009.

J. Pavlů, H. W. Auner, S. Ellis, R. M. Szydlo, C. Giles, A. Contento, A. Rahemtulla, J. F. Apperley, K. Naresh, D. H. MacDonald, and E. J. Kanfer, “LACE-conditioned autologous stem cell transplantation for relapsed or refractory diffuse large B-cell lymphoma: treatment outcome and risk factor analysis from a single centre.,” Hematological Oncology vol. 29, no. 2, pp. 75–80, Jun. 2011.

M. Remberger, M. Ackefors, S. Berglund, O. Blennow, G. Dahllöf, A. Dlugosz, K. Garming-Legert, J. Gertow, B. Gustafsson, M. Hassan, Z. Hassan, D. Hauzenberger, H. Hägglund, H. Karlsson, L. Klingspor, G. Kumlien, K. Le Blanc, P. Ljungman, M. Machaczka, K. J. Malmberg, H. U. Marschall, J. Mattsson, R. Olsson, B. Omazic, D. Sairafi, M. Schaffer, B. M. Svahn, P. Svenberg, L. Swartling, A. Szakos, M. Uhlin, M. Uzunel, E. Watz, A. Wernerson, A. Wikman, A. C. Wikström, J. Winiarski, and O.

Ringdén, “Improved survival after allogeneic hematopoietic stem cell transplantation in recent years. A single-center study,” Biology of Blood and Marrow Transplantation,vol. 17, no. 11, pp. 1688–1697, 2011.

M. Remberger, J. Mattsson, R. Olsson, and O. Ringdén, “Second allogeneic hematopoietic stem cell transplantation: A treatment for graft failure,” Clinical Transplantation, vol. 25, no. 1, pp. 68–76, 2011.

E. M. Richa, R. Kunnavakkam, L. a Godley, J. Kline, O. Odenike, R. a Larson, V. Nguyen, W. Stock, A. Wickrema, K. Van Besien, and A. S. Artz, “Influence of related donor age on outcomes after peripheral blood stem cell transplantation.,” Cytotherapy, vol. 14, no. 6, pp. 707–15, 2012.

V. Rocha, M. Labopin, G. Sanz, W. Arcese, R. Schwerdtfeger, A. Bosi, N. Jacobsen, T. Ruutu, M. de Lima, J. Finke, F. Frassoni, and E. Gluckman, “Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia,” N Engl J Med, vol. 351, no. 22, pp. 2276–2285, 2004.

S. K. Sohn, D. H. Kim, J. G. Kim, W. J. Sung, J. H. Baek, N. Y. Lee, D. I. Won, J. S. Suh, K. S. Lee, and K. B. Lee, “Outcome of allogeneic peripheral blood stem cell transplantation using matched sibling donors in patients with high-risk hematological diseases” European Journal of Haemtology, vol. 72, no. 6, pp. 430-6, 2004.