Post-Thaw Viability Of Cryopreserved Peripheral Blood Stem Cells (PBSC) Does Not Guarantee Functional Activity: Important Implications For Quality Assurance Of Stem Cell Transplant Programmes

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Abstract

Standard quality assurance (QA) of cryopreserved peripheral blood stem cells (PBSC) uses post-thaw viable CD34+ cell counts. In 2013, concerns arose at Great Ormond Street Hospital (GOSH) about 8 patients with delayed engraftment following myeloablative chemotherapy with cryopreserved cell rescue, despite adequate post-thaw viable cell counts in all cases. Root cause analysis was undertaken; investigations suggested the freeze process itself was a contributing factor to suboptimal engraftment. Experiments were undertaken in which a single PBSC product was divided into three and cryopreserved in parallel using a control-rate freezer (CRF) or passive freezing method (-80°C freezer) at GOSH, or the same passive freezing at another laboratory. Viable CD34+ counts were equivalent and adequate in each. CFU-GM assays demonstrated colonies from the products cryopreserved using passive freezing (both laboratories), but no colonies from products cryopreserved using the CRF. The CRF was shown to be operating within manufacturer's specifications with freeze-profile within acceptable limits. This experience has important implications for quality assurance for all transplant programmes, particularly those using cryopreserved products. The failure of post-thaw viable CD34+ counts, the most widely used routine QA test available, to ensure PBSC function is of great concern and should prompt reassessment of protocols and QA procedures.

Introduction

Myeloablative chemotherapy is an important component of treatment for a number of diagnoses in adult and paediatric oncology. Bone marrow reconstitution following chemotherapy is typically achieved with the reinfusion of cryopreserved autologous peripheral blood stem cells (PBSC) previously obtained by apheresis; cryopreserved cells are less commonly used in the setting of allogeneic transplants (Watt *et al*, 2007). The procedures involved in haematopoietic progenitor cell (HPC) transplantation are governed by international standards produced by FACT (Foundation for the Accreditation of Cell Therapy) and JACIE (Joint Accreditation Committee of International Society of Cellular Therapy [ISCT] and European Society for Bone Marrow Transplantation [EBMT]). These standards require accredited cell therapy laboratories (CTLs) to have policies and procedures in place for cell cryopreservation and release for clinical use. Viable CD34 enumeration assays are a mandatory standard for the collection of HPC products (JACIE v.6 standard D8.1.3.2)[1], almost universally met using flow cytometric measurement of CD34+ cells and 7AAD (7-aminoactinomycin D) viability stain (Gratama *et al*, 2003; Barnett *et al*, 1998; Serke & Johnsen, 2001). Criteria for testing cryopreserved cells are, however, much less well defined, with most centres using post-thaw viable CD34 enumeration rather than functional testing such as colony assays (Lee *et al*, 2008; Allan *et al*, 2002). Post-thaw CD34-viability counts are reported to correlate well with colony assays and may be more predictive for engraftment outcome (Yang *et al*, 2005).

Great Ormond Street Hospital (GOSH) is one of the largest paediatric oncology centres in Europe undertaking approximately 20 autologous and 70 allogeneic transplants annually and is accredited by JACIE and UK Human Tissue Authority (HTA) regulatory bodies. Patients undergo apheresis as in-patients, with cells processed and cryopreserved on-site by the clinical CTL before storage in liquid nitrogen vapour phase (LNVP). In October 2013, a series of patients with unusually delayed bone marrow reconstitution following reinfusion of cryopreserved stem cells was identified. This prompted the declaration of a Serious Incident[2]

and a detailed analysis of transplant programme processes. The transplantation centre at University College London Hospital (UCLH) routinely performs HPC colony assays and undertook a joint investigation with GOSH that included these functional tests.

Patients and Methods

In October 2013, a series of 6 patients undergoing myeloablative chemotherapy with autologous PBSC rescue at GOSH who experienced significantly delayed count recovery was identified (see Table I). An additional patient also underwent autologous transplant during this period and recovered promptly (15 days to ANC $>0.5x10^9$/L, 28 days to platelets $>20x10^9$/L). Of note, PBSC for this patient were cryopreserved elsewhere and transferred frozen to GOSH for storage and re-infusion. Two additional patients were subsequently identified who had received cryopreserved allogeneic bone marrow-derived HPCs and also experienced delayed engraftment. In each of these 8 patients, stem cell viability assessed by CD34+ 7-AAD vital dye flow cytometry on a pilot vial sample defrosted at the time of administration of the cell product confirmed adequate post-thaw viable CD34+ cell doses of $\geq 2.5x10^6$/kg in all cases suggesting satisfactory cryopreservation.

Root-cause analysis was undertaken to review all aspects of the transplant process including mobilisation, apheresis, cryopreservation, PBSC reinfusion, and patient factors including conditioning chemotherapy and concomitant medications; together with external review of CTL processes. Information was obtained from hospital paper and computer records and staff interviews.

Despite satisfactory thaw viable CD34 doses, the involvement of two allogeneic transplant patients who had received cryopreserved products and the successful marrow reconstitution in a patient who received PBSC that had been cryopreserved elsewhere suggested a potential problem with cryopreservation by the GOSH CTL. Consequently, cryopreservation was suspended whilst investigations were undertaken. Review of laboratory processes showed no

deviations from standard quality control (QC) practices, no recent change in cell handling protocols, reagents or equipment, and no common individual responsible for cell processing in all cases.

Fresh and thaw viable CD34 assays

CD34 assays for cell counts and viability (assessed by 7AAD) were undertaken using standard flow cytometry methods (BD FACS Canto II flow cytometer and antibodies from BD, Oxford, UK). Daily internal quality control used the stem cell control kit (BD), with external QC for CD34 counts via participation in the NEQAS scheme[3]. Cryopreserved pilot vials were defrosted using a 37^0C waterbath prior to assay.

Short-Term Colony Forming Cell Assays (CFC)

Colony Forming Unit-Granulocyte-Monocyte (CFU-GM) assays were used to assess potency. These assays were set up by dilution of fresh HPC samples with Iscove's media and 2% human albumin solution (HAS), in the case of thawed samples adding warmed media dropwise, to prepare cell plating concentrations of $2.5x10^4$/ml in methylcellulose media enriched with growth factors (StemMACS HSC-CFU complete with EPO, Miltenyi Biotec) (Watts *et al*, 2002; 2008). Each sample was then dispensed in four 0.5ml aliquots in 24 well plates (Costar) and colonies counted after 14 days incubation at 37^0C in a 4% $C0_2$ atmosphere, taking the mean of the four well readings as the colony number/well.

Stored clinical harvest testing

There were no additional test vials or unused cells available for colony assays from the affected patients and in the first investigation five clinical autologous PBSC harvests that had been cryopreserved and stored at GOSH and were not required for clinical use (so would otherwise have been discarded as per hospital protocol) were thawed and tested for viable CD34+ cell counts and colony assays.

Split fresh apheresis product cryopreservation experiments

In subsequent experiments cells from fresh single apheresis cell products were divided into three and processed in parallel. To obtain the most immediately relevant clinical information these tests were performed using the same routine methods, materials and scale used for processing PBSC for clinical use. Excess fresh PBSCs are not frequently available. To ensure that experiments were completed in a reasonable timescale, two products used were CD34-poor flow-through 'waste' fractions of two clinical CD34-purification procedures (Watts *et al*, 2002) run at UCLH (known to have low progenitor numbers), and in the remaining cases were collections where there was an excess of progenitors above clinical requirement so that the surplus cells (which would otherwise have been discarded) could be used for these assays. In each experiment, the cell product was mixed with an equal volume of cryoprotectant (20% dimethyl sulfoxide (DMSO) in HAS) at 4°C (Watt *et al*, 2007), transferred to a clinical cryobag (CryoMACS, Miltenyi Biotec, Bisley, UK), inserted in its supplied overwrap bag and then placed in stainless steel heat-transfer plates for freezing. In three of the paired freeze experiments, thermocouple probes were placed inside the secondary overwrap to record the sample cooling curves (A&D50 temperature logger with T1 thermocouple, A&D Company, Japan). Thereafter, cells were frozen using either a controlled-rate freezer (Kryo560, Planer plc, Sunbury-on-Thames, UK) or were placed directly into a -80°C freezer (passive freezing) at GOSH. A third aliquot of the cells was frozen using identical passive freezing at UCLH. After 7 days, each cryopreserved aliquot was defrosted and assessed for cell viability by trypan blue exclusion assay and for total white cell count. Functional activity of PBSCs was determined using CFU-GM assays. In additional experiments, a single apheresis cell product was again cryopreserved in parallel, using either passive freezing or controlled-rate freezer with the programme altered to match profiles used at other UK laboratories or to mimic the passive freeze profile ('CRF passive freeze mimic').

Statistical methods

Differences between engraftment times for historical cohorts were evaluated by Mann-Whitney U test using Prism 5 software (Graphpad), with values of $P<0.05$ being considered significant.

Results

External review of processes failed to identify an obvious cause for delayed engraftment in these patients. The first investigation in collaboration with UCLH was to test clinical harvests cryopreserved at GOSH contemporaneously with the affected patients for colony activity (Table II). Five such harvests were available for analysis and in all good viable thaw CD34+ cell doses were confirmed but all showed unexpectedly poor CFU-GM numbers (Serke *et al*, 1996). This discrepancy was particularly surprising as GOSH and UCLH used the same cryopreservation method, reagents, plastics and disposables. However, the centres differed in the final freezing step and subsequent investigations focussed on the potential impact of this freeze stage in the preservation of clonogenic activity. The freeze process at GOSH used a controlled-rate freezer (CRF), in which regulated liquid nitrogen injection is used to reduce the temperature of a chamber within the freezer according to a pre-specified programmable freeze profile, while the UCLH laboratory used an alternative 'passive freezing' method in which cells were placed directly into a -80°C freezer. A series of experiments were subsequently conducted using available fresh cell products not required for clinical use to compare these methods, with cells frozen in parallel using either the CRF or -80°C freezer, before being defrosted and assayed. Thermocouple probes were used to record the sample cooling curves from the paired products for both freeze methods for comparison purposes. In the case of the CRF the sample cooling rates pre- and post-liquid and solid phase transition followed the chamber rates closely (Figure 1) and were significantly more rapid than passive freezing; mean 5.2^0C/min versus 1.1^0C/min ($n=3$, $p=0.0001$).

Although there were no major differences in the WBC concentration or cell viability, CFU-GM assays demonstrated a clear difference between the methods. Whilst cells cryopreserved

using passive freezing at either GOSH or UCLH were capable of growing colonies, CFU-GM assays consistently showed no colonies from the cells cryopreserved using the GOSH CRF (see Table III).

In further experiments, the CRF freeze profile was amended to reflect profiles used at other UK CTLs (see Figure 2 and Table SI). In two experiments, CFU-GM showed broadly equivalent results using the passive freeze method and the CRF with amended profiles: mean CFU-GM/well=12 using profile A versus 16 with passive freeze; mean CFU-GM/well=13 using profile B versus 11 with passive freeze. In a third experiment, the pre-existing GOSH CRF profile was compared with an alternative CRF freeze profile program to mirror passive freezing. The mean CFU-GM/well=3 using the original GOSH CRF profile was dramatically lower than the CFU-GM/well=30 obtained with the 'CRF passive mimic' profile, but using the same CRF machine. Thus, although functional activity of cells (as estimated by CFU-GM assay) cryopreserved using the GOSH CRF appeared significantly reduced compared to those cryopreserved using the passive freeze method, this difference did not appear related to the operation of the CRF device itself, but rather to the freeze profile. This profile had been used in the laboratory since the early 1990s and had not been amended during that time. No concerns had previously been raised with the functioning of the CRF and no abnormalities in the functioning of the CRF were detected during the investigation or based on its full service record history. Nevertheless, in view of the problems that occurred, an alternative cryopreservation method based on the passive freezing method in routine use at UCLH since 1995 was introduced into regular practice at GOSH from the start of 2014.

In view of the issues raised by the serious incident, we undertook a retrospective review of count recovery following all autologous procedures performed at GOSH from 2003–2015. During this 13-year period, there were 181 procedures with a median time to neutrophil engraftment (ANC $>0.5x10^9$/L) of 13 days. There was however marked variation in recovery time with 26 (14%) taking >30 days to recover (Figure 3a). Importantly, following suspension and subsequent reintroduction of cryopreservation at GOSH at the beginning of 2014, there

has been a significant reduction in time to neutrophil engraftment: 2002-2013 median time 13 days versus 2014-15 median time 11.5 days ($p<0.0001$ Mann-Whitney, Figure 3c). A similar significant reduction in the median time to platelet recovery (>$20x10^9$/L unsupported) has also been seen: 2010-2013 42 days versus 20 days for 2014-15 ($p<0.0001$ Mann-Whitney, Figure 3b/c). A similar result was obtained when limiting the analysis only to patients conditioned with busulfan/melphalan (BuMel), as the largest consistently treated group, indicating that this improvement in engraftment was not due to a change in conditioning protocols. Comparison of duration of admission (limited to patients conditioned with BuMel) also showed a significant reduction from a mean of 43 days in 2002-2013 to 30 days 2014-15 ($p<0.0001$, t-test) consistent with more rapid engraftment (Supplementary Figure 1).

Discussion

Cryopreserved granulocyte colony stimulating factor (G-CSF)-mobilised peripheral blood stem cells (PBSC) are used in the vast majority of autologous transplants and a lower proportion of allogeneic procedures (Watt *et al*, 2007). The processes governing transplant programmes are closely regulated – such as by JACIE accreditation and, in the UK, the Human Tissue Authority. Nevertheless, cell processing and cryopreservation procedures have been devised empirically by many individual centres prior to the regulatory era and are poorly standardised with the notable exception of standardised flow cytometric CD34+ cell counts (Gratama *et al*, 2003; Barnett *et al*, 1998). The details of quality control procedures for products varies significantly between countries and between laboratories in the same country (Rosskopf et al, 2011). Although historically CFU-GM assays were used, based on previously available evidence and the real-time and convenient nature of viability assays, the majority of laboratories have moved to using quantification of CD34+ cells and confirmation of post-thaw viability for routine QC. Few laboratories continue to use CFU-GM assays routinely. Although CFU-GM for QC provides an indication of the functional activity of PBSC and has

proved to be of value in individual centres (Page *et al*, 2011; Watts *et al*, 1998; Watts, 1999), attempts to introduce inter-laboratory standardisation have been fraught with difficulty (Lumley *et al*, 1999). Based on our experience, we believe, however, that this does not preclude a role for CFU-GM assays in direct comparative studies or intermittently as part of the on going QC process within CTLs.

At GOSH, where a transplant programme has been operating for more than 30 years, we identified a series of patients with unexpectedly delayed engraftment. In each case, the cells reinfused had met standard QC criteria (CD34+ number and viability). In view of the involvement of two patients who had received allogeneic cryopreserved products and the successful marrow reconstitution in a patient who received PBSC that had been cryopreserved elsewhere, a potential problem with cryopreservation at GOSH was identified. Subsequently experiments in which cells were cryopreserved in parallel using different freeze methods indicated that although PBSC frozen using the GOSH CRF had equivalent viability, their functional activity based on CFU-GM was significantly reduced. Ultimately, no problem with the CRF device itself was identified and indeed with amended freeze profiles using the same machine CFU-GM assays showed equivalent results with other freeze methods.

The investigation and discussions with other UK CTLs has confirmed that there is no single standard freeze profile in use in those laboratories using a CRF for cryopreservation (Figure 2). The freeze profile and the rate of change of temperature within the cell product is crucial for successful cryopreservation with there being a need to balance sufficiently rapid cooling to minimise the transition phase, whilst avoiding excessively rapid cooling thereafter (Baust *et al*, 2009). In particular, cooling after the transition phase greater than 5-6°C/min has been reported to be associated with CFU-GM loss and delayed re-engraftment after reinfusion into patients (Abrams *et al*, 1980; Gorin *et al*, 1983). In the current investigation the CRF freeze rate did not exceed 5^0C/min so that the major losses of CFU-GM activity seen is surprising. The CRF programme in use at GOSH since the early 1990s was established when bone marrow was the exclusive stem cell source at the time of these early reports and it is possible

that the more recent use of PBSC could be a factor in greater susceptibility to a lower range of freeze rates. In the absence of a 'gold-standard' freeze profile, it is imperative that individual CTLs have measures in place to validate their methods. Critically, assessment of post-thaw CD34+ viability is unlikely to be adequate to guarantee functional activity.

A recent study using umbilical cord blood (UCB) confirms the negative impact of excessively rapid cooling and may be relevant to the clinical incident as an example of major CFU-GM activity loss without comparable CD34+ cell death related to rapid freezing conditions. In this study the number of viable CD34+ cells was not significantly reduced under any of the conditions tested but CFU-GM were specifically and significantly lower if cryopreservation using a CRF was interrupted at different temperature points and cells transferred directly to liquid nitrogen vapour phase (LNVP, usually below -150^{0}C) if the cells had not reached a sufficiently low temperature (-40°C) before the transfer. In contrast, no significant differences were seen in CFU-GM or CD34+ cell viability in paired samples transferred to a -80°C mechanical freezer from the CRF at any temperature point (Yang *et al*, 2015).

We believe that the experiences and subsequent investigation at GOSH have significant implications for other CTLs and transplant programmes, and for the cryopreservation of UCB, as well as PBSC. It is clear that the widely accepted criteria of thaw CD34+ quantification and measurement of viability does not guarantee the functional activity of stem cells post-cryopreservation. Real-time collection and analysis of re-engraftment data from patients receiving cryopreserved cells is crucial and ultimately only successful bone marrow reconstitution confirms the effectiveness of the cryopreservation process. The previous lack of such a system for real-time monitoring of outcomes for patients treated at GOSH almost certainly contributed to delays in identifying issues with prolonged engraftment times. Although it is important for individual centres to review their results, in the paediatric setting in particular, the number of procedures undertaken by any individual centre may preclude the early appreciation of adverse trends in count recovery. For individual patients with delayed engraftment, there is often the possibility of attributing other explanations (such as

intercurrent infection) rather than investigating a potential underlying issue with cryopreservation. Consequently, in the UK there is a plan to develop a prospective national audit process for paediatric autografts; a retrospective audit of engraftment following paediatric autografts is also underway.

CRF profiles may involve several multi-stage cooling ramps and it is difficult to establish the 'optimal freeze profile' but the findings of this study support recommendations that the post-fusion rate should be between 1-2^{0}C/min rate down to at least -40^{0}C (Rowley, 1992). Passive freezing at a fixed temperature such as -80^{0}C does not allow the same degree of precision and documentation as CRF but has the advantages of low cost, simplicity and more rapid throughput. It also represents an option for validation in that a CRF programme that fails to preserve clonogenic activity at least as effectively as passive freezing should be subject to review.

The GOSH laboratory has now moved to using a passive freezing technique. Previous studies have suggested improved CFU-GM yields and more rapid engraftment with use of a CRF rather than passive freeze (Montanari *et al*, 2003; Perez-Oteyza *et al*, 1998), although this is not supported in other reports (Takaue *et al*, 1994; Katayama *et al*, 1997). Crucially, the GOSH data on count recovery post-autografts for the period since the beginning of 2014 when this new cryopreservation process was introduced appear to support the effectiveness of this technique. Ultimately, whatever method is used, measurement of post-thaw viable CD34+ numbers is not sufficient to guarantee functional activity and laboratories and transplant programmes need to be aware of the risk of impaired functional activity of cryopreserved stem cells even if they have passed standard QC criteria. The considerable variability in processing and cryopreservation between centres represents a major QC challenge so that sessional validation of the methods assuring adequate potency of the final product is essential. GOSH now stores a second pilot vial on all products. The CTL undertakes a CFU-GM on every fifth product as routine QC to monitor ongoing validity of the freeze process, as well as undertaking CFU-GM assays on any product where there is

delayed engraftment. Of note, however, a small pilot vial may not be representative of the whole clinical product (given differences in freeze rate) and therefore further work is required to establish a better surrogate sample for potency testing (e.g. by freezing pilot vials using an isopropyl alcohol freezing container).

A new potency assurance standard for cryopreserved products was introduced into the JACIE version 6 standards in 2015[1]. This standard could be met by sessional colony assays for HPC function however, as previously noted, standardisation issues remain a barrier for widespread adoption. In contrast, the standardisation of flow cytometric CD34+ cell counting between transplantation centres has been remarkably successful (Gratama *et al*, 2003; Barnett *et al*, 1998) and may present a model for a flow based test where viability relies not on simple permeability but on metabolic function such as ALDH and Syto-16 activity. These activities have been shown in a number of flow cytometric studies to be closely associated with progenitor cell clonogenic activity (de Boer *et al*, 2002; Schuurhuis *et al*, 2001; Lee *et al*, 2014; Fallon *et al*, 2003) and may provide the required validation to assure the potency of cryopreserved cells in the future.

Our experience has demonstrated that viability assessment may provide false reassurance of the effectiveness of long-established cell product preparation methods. All countries should ensure that their regulatory processes include adequate audit and monitoring so that deviations can be rapidly detected, particularly in smaller centres where trend analysis may be difficult.

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cryopreservation procedures. DAM and MW analysed the data and wrote the paper. All authors have reviewed and approved the final version of the manuscript.

Conflict of interest statement

The authors confirm that they have no conflicts of interest to declare.

Table I. Summary of patients with delayed bone marrow reconstitution. ATRT: atypical teratoid rhabdoid tumour. Conditioning: treosulfan (Treo), melphalan (Mel), busulfan (Bu), carboplatin (Carbo), fludarabine (Flu), cyclophosphamide (Cy). Cryopreservation date (Cryo date). Number of days to neutrophil recovery >0.5x10^9/L (ANC>0.5) or platelet recovery >20x10^9/L, at least 7 days after last transfusion (Plt >20). Rescue indicates administration of additional PBSC in view of failure of adequate BM reconstitution. ANED: alive with no evidence of disease.

Diagnosis	Conditioning	Type	Day 0	Cryo date	Fresh CD34 dose (x10^6/kg)	Post-thaw CD34 dose (x10^6/kg)	Post-thaw viability	D30 ANC (x10^9/L)	Days to ANC >0.5x10^9/L	Days to plt >20x10^9/L	Rescue?	Outcome
Neuroblastoma	Treo/Mel	Auto	Apr 2013	Feb 2013	6.06	7.56[a]	>95%	0.91	30	57	No	ANED
Neuroblastoma	Bu/Mel	Auto	Apr 2013	Mar 2013	3.88	2.94	>95%	0.44	37	53	Yes	Prolonged thrombocytopenia, ANED
Neuroblastoma	Bu/Mel	Auto	Apr 2013	Feb 2013	6.60	6.12	>95%	0.14	41	49	Yes	ANED
ATRT	Carbo/Thio	Auto	Jul 2013	Jun 2013	8.99	7.31	>95%	0.38	38	>50	Yes	ANED
Neuroblastoma	Bu/Mel	Auto	Aug 2013	Apr 2013	4.00	3.39	>95%	0.25	75	>100	Yes	Died from recurrent disease
Neuroblastoma	Bu/Mel	Auto	Aug 2013	Jun 2013	4.21	3.22	>95%	0.59	42	n/a	Yes	Died from sepsis
Rel AML	Bu/Cy/Mel	Allo[b]	Mar 2013	Feb 2013	8.56	7.68	88%	0.85	30	>96	No	Died from recurrent disease
Rel ALL	Flu/Treo/Thio	Allo[c]	Apr 2013	Apr 2013	2.92	2.55	87%	0.12	>78	>78	Yes	Died from recurrent disease

[a] post-thaw CD34 dose greater than fresh due to variability in CD34 count in pilot vials
[b] allogeneic bone marrow-derived HPC, CD3 cell dose 11.7x10^7/kg, 10/10 matched unrelated donor
[c] allogeneic bone marrow-derived HPC, CD3 cell dose 2.4x10^7/kg, 10/10 match unrelated donor

Table II. Thaw viable CD34+ cell and CFU-GM assays of clinical PBSC harvests cryopreserved at GOSH.

Date of Cryopreservation at GOSH	**Fresh viable CD34+ count (x10^6/kg)**	**Post-thaw viable CD34+ count (x10^6/kg)**	**Post-thaw CD34+ viability (%age 7AAD-)**	**CFU-GM (x10^5/kg)**
30/09/2010	3.4	2.8	94	0.16
28/09/2011	30.8	26.8	88	0.29
13/03/2013	4.8	3.6	99	0.58
19/04/2013	2.0	1.3	99	0.00
30/07/2013	6.5	6.1	91	0.13

Analysis of the fresh product showed viability (7AAD-) of >98% in all cases

Table III: Results of four split-harvest cryopreservation procedures comparing different methods

PBSC sample	Cryopreservation method	WBC ($x10^9$/L)	Viability	CFU-GM /well	CFU-GM $x10^6$/ml of product (thaw yield)
Sample 1	*Fresh*	*145*	*100%*	*32.8*	*0.38 (100%)*
	UCLH -80°C freezer	166	78%	17.0	0.23 (59%)
	GOSH -80°C freezer	175	79%	17.5	0.25 (64%)
	GOSH CRF	176	77%	0.0	0.00 (0%)
Sample 2*	*Fresh*	*204*	*99%*	*5.5*	*0.09 (100%)*
	UCLH -80°C freezer	239	90%	5.0	0.10 (106%)
	GOSH -80°C freezer	239	78%	5.25	0.10 (112%)
	GOSH CRF	242	79%	0.0	0.00 (0%)
Sample 3*	*Fresh*	*210*	*98%*	*3.25*	*0.06 (100%)*
	UCLH -80°C freezer	237	82%	3.5	0.07 (118%)
	GOSH -80°C freezer	234	64%	2.25	0.04 (47%)
	GOSH CRF	251	80%	0.0	0.00 (0%)
Sample 4	*Fresh*	*87*	*99%*	*47.0*	*0.33 (100%)*
	GOSH -80°C freezer	100	76%	28.5	0.23 (70%)
	GOSH CRF	97	89%	0.0	0.00 (0%)

*Sample 2 and 3 were CD34+ cell poor flow through 'waste' cells from two clinical CD34 enrichment CliniMACS procedures run on donor apheresis harvests at UCLH and hence lower colony numbers/well and reduced accuracy.

Figure 1. Representative cooling curves of clinical scale HPC samples cryopreserved by CRF (separate curves for chamber and sample temperatures) or passive freezing. * shows increase in temperature associated with release of latent heat of fusion at phase transition

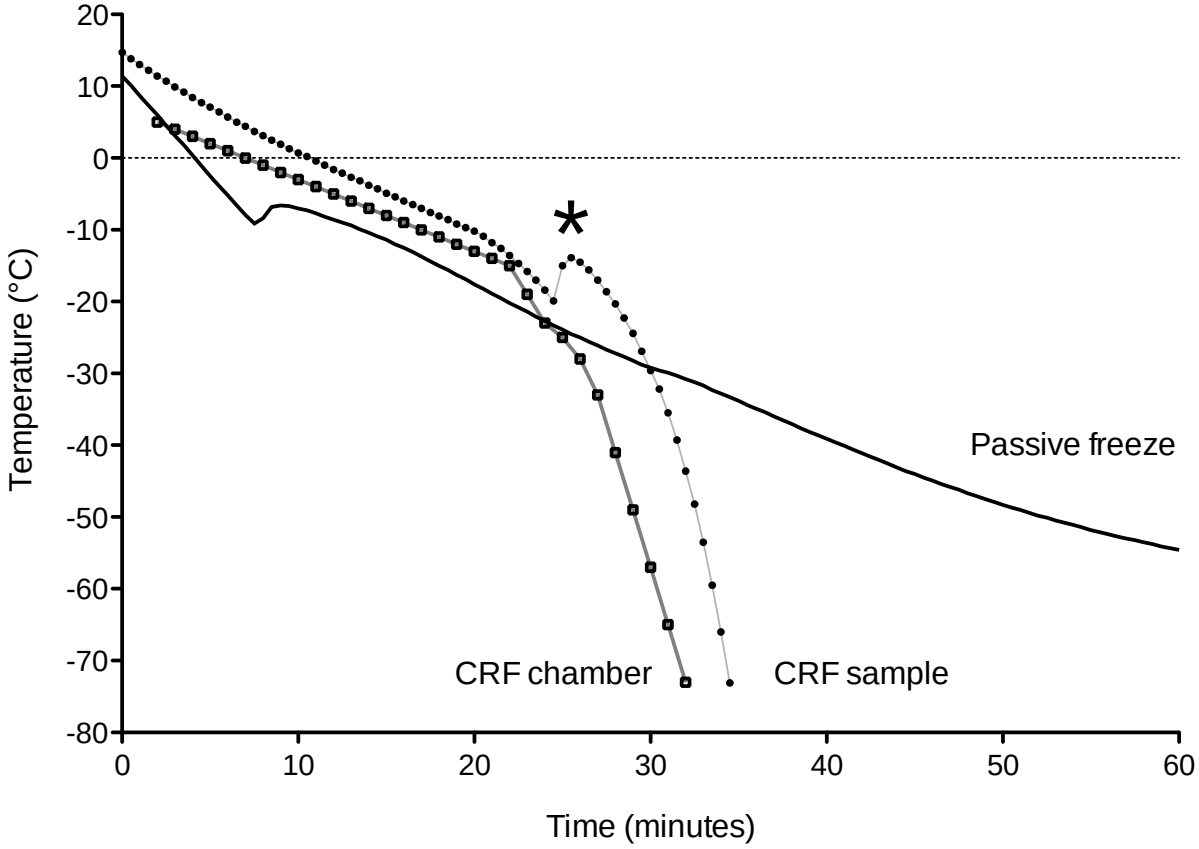
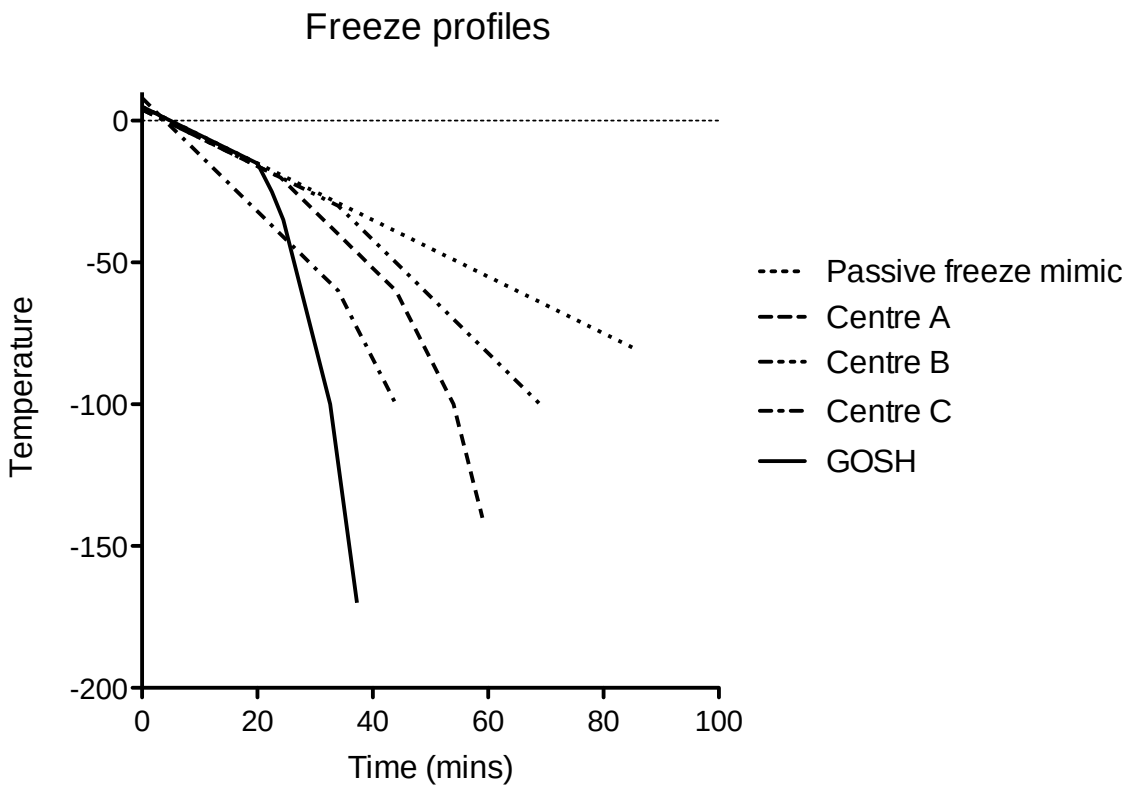


Figure 2 Different freeze profiles



Freeze rates (for temperature change between -20^{0}C (usual post phase point) and -40^{0}C):
passive freeze mimic:1.0^{0}C/min; Centre A: 1.5^{0}C /min; Centre B: 2.0^{0}C/min; Centre C: 2.0^{0}C/min; GOSH:5.0^{0}C/min

Figure 3a Time to neutrophil recovery (ANC>0.5x10^9/L) over a 13-year period at GOSH. Each point represents a sequential patient undergoing an autologous transplant procedure. Shaded area from start of 2014 highlights introduction of revised cryopreservation process.

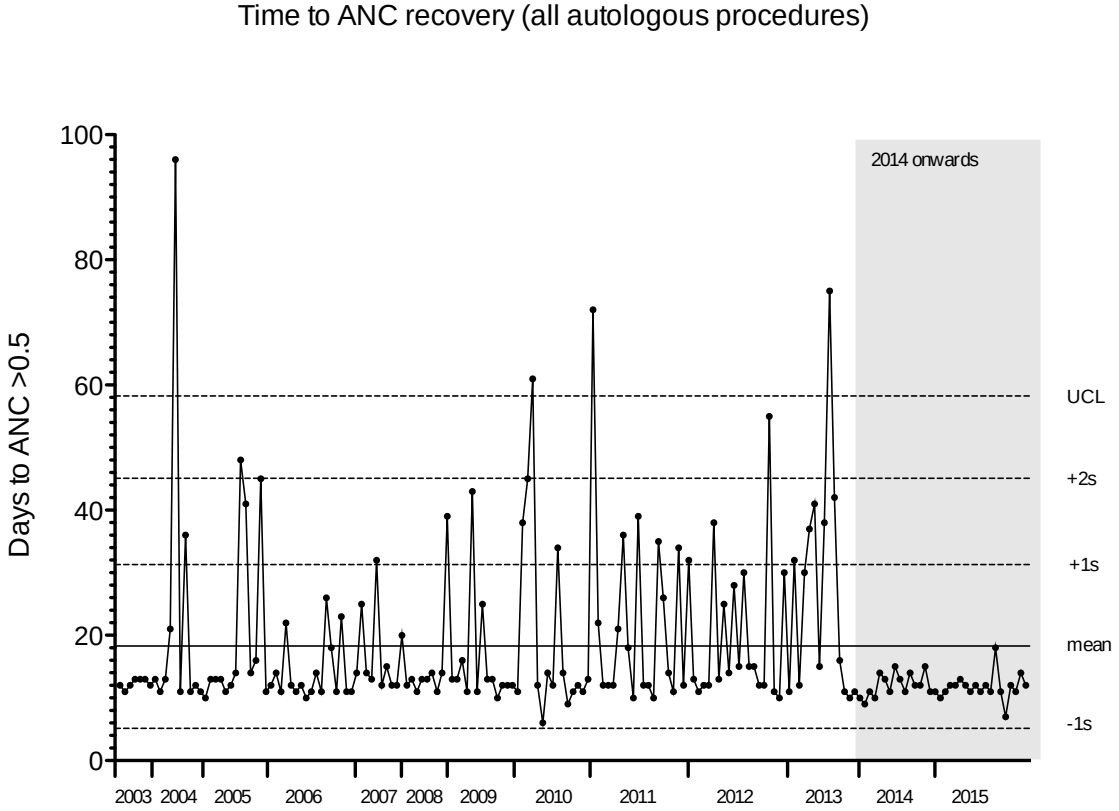


Figure 3b. Time to platelet recovery (platelet>20x10^9/L unsupported) over period since start of 2010 at GOSH. Each point represents a sequential patient undergoing an autologous transplant procedure. Shaded area from start of 2014 highlights introduction of revised cryopreservation process.

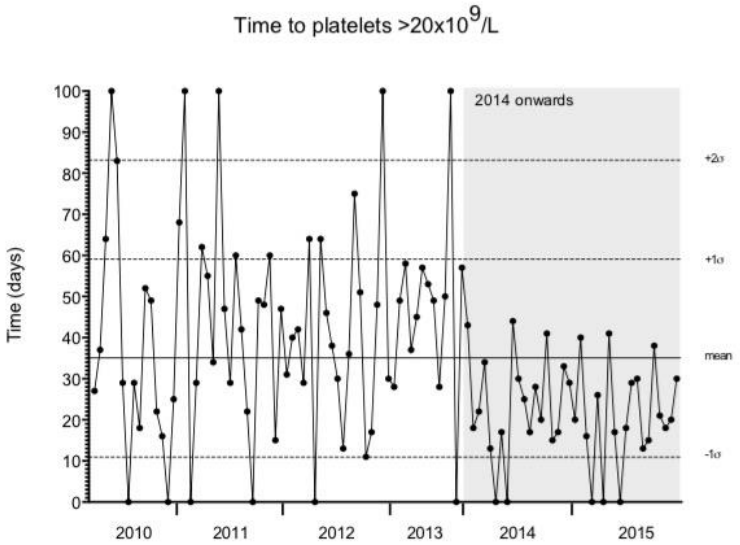
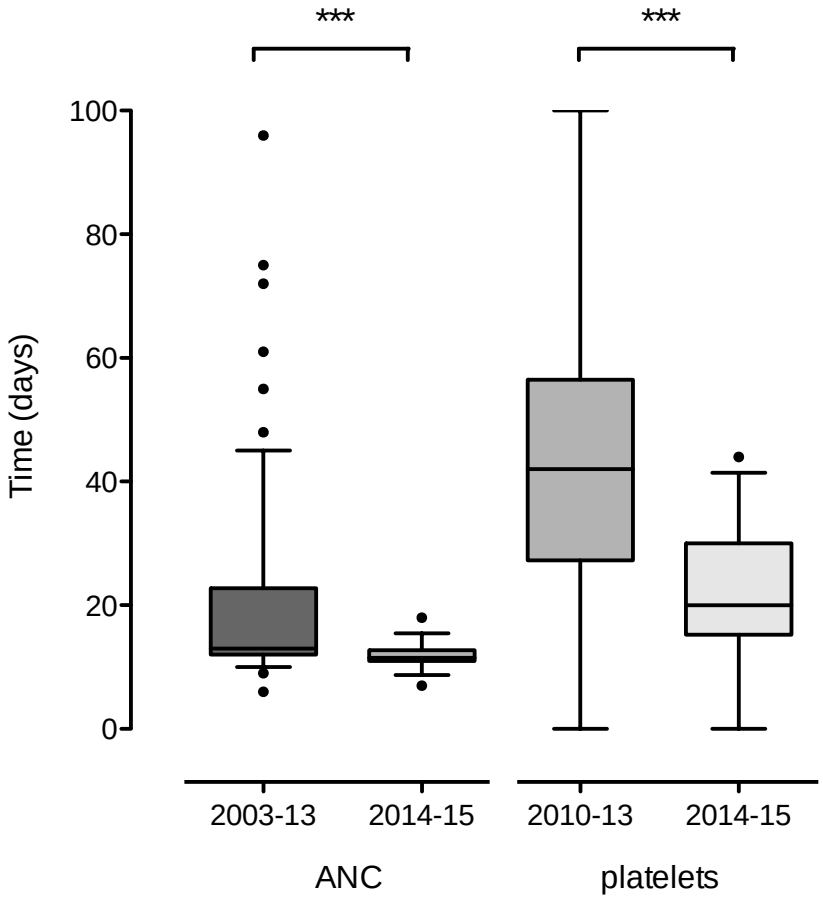


Figure 3c. Comparison of time to ANC and platelet recovery at GOSH for 2003-2013 (ANC) or 2010-13 (platelets) versus 2014-15.
*** p<0.0001 (Mann-Whitney)



Supplementary Figure 1. Duration of admission for patients receiving cryopreserved PBSC after conditioning with BuMel, 2010-2015.

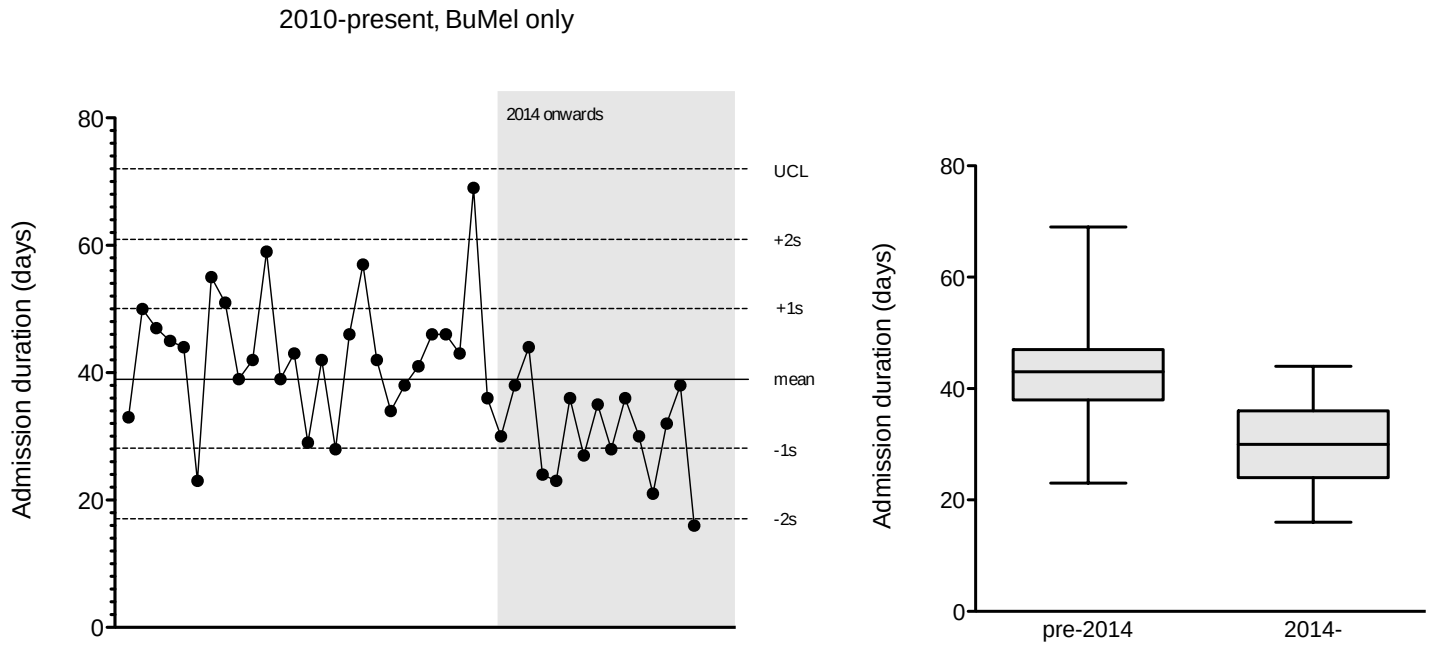


Table SI Comparison of different freeze profiles

	GOSH	Centre A	Centre B	Centre C	Passive Freeze Mimic
Start temp	+5°C	+8°C	+4°C	+4°C	+5°C
Time	35 mins	60 mins	70 mins	60 mins	80 mins
Profile	1°C/min to -15°C 4°C/min to -25°C 5°C/min to -35°C 8°C/min to -100°C 15°C/min to -170°C	2°C/min to 0°C 1°C/min to -20°C 2°C/min to -60°C 4°C/min to -100°C 8°C/min to -140°C	1°C/min to -30°C 2°C/min to -100°C	1°C/min to 0°C 2°C/min to -60°C 4°C/min to -100°C	1°C/min to -80°C

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