A Blueprint for Translational Regenerative Medicine

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Abstract

The last few decades have produced a large number of proof-of-concept studies in regenerative medicine. However, the route to clinical adoption is fraught with technical and translational obstacles that frequently consign promising academic solutions to the so-called "valley of death." This review is intended to serve as a blueprint for translational regenerative medicine: we suggest principles to help guide cell and material selection, present key *in vivo* imaging modalities and argue that the host immune response should be considered throughout therapeutic development. Finally, we suggest a pathway to navigate the often complex regulatory and manufacturing landscape of translational regenerative medicine.

Key Words

Regenerative medicine; tissue engineering; cells; biomaterials; translation

Regenerative Medicine: A Key Curative Strategy for the 21st Century

The great success of vaccines, antibiotics, statins and other major therapies have reshaped the landscape of preventative and curative medicine. In particular, the aging population demographic has led to the rise of *regenerative medicine*, which typically employs combinations of cells, biomaterials and biomolecules in order to rejuvenate or replace damaged or diseased tissue (**Figure 1**). The development of clinically-viable regenerative therapies is challenging and thus, we sought to write guidelines based on our experiences as part of the UK Regenerative Medicine Platform. We outline key criteria for selecting cells and biomaterials, summarize the major clinical options for tissue assessment and provide a roadmap for navigating key immunological, regulatory, manufacturing and translational hurdles. We hope that these guidelines will enable researchers in the field to avoid the common pitfalls of translational regenerative medicine and aid in the design of smart, holistic strategies with improved prospects of clinical adoption.

The Regenerative Potential of Cells

Cells as Regenerative Agents

Natural cellular processes can be used as inspiration for regenerative medicine. During embryogenesis and post-natal growth, stem and progenitor cells generate specialized progeny that build and maintain tissues by cellular assembly, extracellular matrix (ECM) secretion and enzymatic remodeling. Normal wound healing is also regulated by coordinated cellular processes: the first responders to injury are platelets and neutrophils, which aggregate at the wound site and release growth factors and chemoattractants that trigger the recruitment of macrophages, lymphocytes, fibroblasts and endothelial cells. These cells remove damaged tissue and populate the wound site with a collagenous granulation tissue that is gradually remodeled into vascularized ECM *(1)*. The processes involved in development and wound healing highlight the regenerative potential of cells. These natural precedents offer insights into how we can repair damaged tissue and provide a rationale for therapies that seek to replace diseased or damaged cells, or modulate the host environment to promote endogenous regeneration.

The biological complexity underpinning tissue regeneration necessitates a careful selection process guided by both technical and practical considerations. A first criteria is whether cells are sourced from the patient (autologous) or a donor (allogeneic). Allogeneic therapies are attractive if cells can be readily sourced and used "off-the-shelf," however, the risks of immune rejection and iatrogenic infection mean that autologous therapies are often preferred for translation, even if these cells retain some of the defects that originally led to the disease in that individual. A second criteria is whether to use like-for-like cell replacement, such as autologous chondrocyte implantation for the repair of cartilage defects *(2)*, or to employ cells out of context, such as the injection of skeletal myoblasts for cardiac regeneration *(3)*. A third criteria is whether to use committed cell types, already specialized and functional, or stem cells that have the capacity for prolonged or unlimited self-renewal under controlled conditions and the potential to secrete a range of factors and differentiate into a variety of specialized cell types.

Mesenchymal stem cells (MSCs), hematopoietic stem cells (HSCs) and neural stem cells (NSCs) are examples of cells that exhibit *multipotency*; the capacity to differentiate into a small subset of cell lineages *(4)*. A common alternative are cells that exhibit *pluripotency*; the ability to differentiate into any cell of the three germ layers (endoderm, mesoderm, ectoderm). Embryonic stem cells (ESCs) are naturally pluripotent but are restricted by their allogenic and ethically-controversial cell source. Meanwhile, induced pluripotent stem cells (iPSCs) can be generated by reprogramming autologous somatic cells, such as fibroblasts *(5)* or other abundant cell types *(6)*. The controlled *in vitro* differentiation of iPSCs has enabled the derivation of a wide range of therapeutic cell types that would be otherwise challenging to source. However, a clinical hurdle in using iPSCs and ESCs is the inherent tumorigenicity associated with pluripotency, either through benign teratoma formation of undifferentiated cells or malignant transformation of differentiated progeny *(7)*.

Redefining the Cellular Role

Induced pluripotency radically redefined our understanding of cell state and offered a striking example of cell plasticity: the concept that cells can readily adopt different phenotypic identities. This viewpoint sparked

intensive research into reprogramming strategies that converted somatic cells without the need for an iPSC intermediate. One method appeared to show that a brief pulse of Yamanaka factors could create a transient cell state that could be biochemically differentiated to various progeny, however, it is unclear whether this method truly bypasses pluripotency *(8)*. Another approach involves somatic cell reprogramming using lineage-specific transcription factors, which has been used to generate a variety of regenerative cells, such as cardiomyocytes *(9)*, neurons *(10)*, and hepatocytes *(11)*. These "transdifferentiation" strategies are restricted by low yields and the lack of cell rejuvenation, however, they do offer a cheaper and faster route than iPSCs for deriving regenerative cell lines and, to date, appear to mitigate the inherent risk of tumorigenicity associated with pluripotent cells *(12)*.

A major consideration for the translation of pluripotent cells (ESCs and iPSCs) is the emergence of potentially dangerous genetic and epigenetic variants. *In vitro* culture creates bottlenecks, such as post-plating survival, cell-cycle checkpoints and post-mitotic survival of daughter cells, which favor cells with certain acquired mutations, such as copy-number variants overexpressing *BCL2L1 (13)*. This genetic variance can result in aberrant lineage specification, growth factor and niche independence, and increased frequency of tumor-initiating cells *(14)*. Moreover, loss of genomic imprinting and erosion of chromosome inactivation can arise during pluripotent stem cell culture *(15, 16)*. Such culture-induced perturbations represent a latent safety risk for ESC and iPSC therapies. Accordingly, there has been a focus on screening clonal cell lines to understand and catalog the functional significance and risk associated with such changes (*e.g.*, tumorigenesis), in order to aid the development of culture conditions that could slow the emergence of variant cells *(17)*.

A number of studies have indicated that screening can also be used to identify cell subpopulations with enhanced regenerative potency. For instance, Dickinson *et al.* used fluorescence-activated cell sorting to isolate a subset of highly chondrogenic human MSCs, which were used to provide quantitatively improved outcomes in the treatment of articular cartilage defects *(18)*. In another example, Kirkeby *et al.* performed a large-scale analysis into the global gene expression profile of human ESC-derived dopaminergic neuroprogenitors on the day of intrastriatal transplantation in a rat model of Parkinson's disease. Predictive

markers expressed by midbrain dopaminergic neurons close to the midbrain-hindbrain boundary region correlated with a successful graft outcome, while markers expressed in the diencephalic domain were negatively associated *(19)*. Such studies can aid in the identification of optimal culture protocols, improve clinical reliability through reduction of biological variability, help to define acceptable levels of genetic variance in cell-based products, and offer a quality control, defined release assay for cells that need to differentiate *in vivo* for efficacy.

Orchestrating Regeneration

Interactive and Dynamic Biomaterials

Stem cells reside in distinct anatomical microenvironments, known as niches, in which the surrounding ECM, soluble factors, immobilized biochemicals and local niche cells present a dynamic array of signals that instruct the resident cells to remain quiescent, undergo self-renewal, differentiate, or die *(20)*. Regenerative biomaterials are typically designed to play a similar functional role, interacting with interfaced cells and the host environment in order to elicit targeted biological responses. This can be guided by cell-material interactions that can be nonspecific (*e.g.*, electrostatic binding, lipid anchoring) or specific (e.g., ligand binding to integrins or organized proteoglycan domains) *(21, 22)*. The cellular interaction of biomaterials can also be tuned by modulating protein adsorption. For example, hydrophobic materials can be used to adsorb albumin, an abundant serum protein that blocks cell adhesion, while slightly hydrophilic substrates can be used to adsorb fibronectin, a cell-binding protein *(22)*.

Cell-material interactions are also guided by interfacial geometry. It is widely accepted that biomaterials that offer a 3D network, such as microfibrous scaffolds or nanofibrous hydrogels, can provide a more biomimetic cell culture environment than 2D substrates *(23)*. Moreover, topographical cues provide an effective means to regulate adhesion, migration, differentiation, epigenetic state or cell reprogramming *(24)*. Many of these biological responses are achieved by mechanosensing, in which the interfaced cells respond to extracellular forces through deformation, mechanosensitive ion channel activation, or integrin binding and signal transduction *(25)*. For instance, cells originating from softer tissues (*e.g.*, neural) can show improved

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biological response when cultured on low-stiffness biomaterials *(26)*. These observations have popularized the use of biomaterials with tissue-matched mechanical properties, however, this approach often overlooks the complex changes in tissue mechanics that occur during physiological development *(27)*. In this regard, biomaterials that provide spatial and temporal differences in substrate mechanics can provide a more natural biological response *(28, 29)*.

Biomaterials that can undergo dynamic phase changes are also widely used in regenerative medicine; consider the different transitions exploited in bioprinting *(30)*, micromolding *(31)* and biomaterial injection *(32)*. Meanwhile, controlled degradation can be used to enhance the maturation of engineered tissues or release cargo from biomaterial vectors. This can be achieved by using synthetic biomaterials that slowly hydrolyze into non-toxic byproducts, while additional control can be leveraged by using biomaterials susceptible to enzymatically-catalyzed degradation *(33)*. For example, cartilage engineered using chondrocyte-laden agarose can be cultured with agarase to degrade the hydrogel and provide the growing tissue with additional space for nutrient diffusion and matrix assembly *(34)*. Another example is the use of materials crosslinked with peptides subsequently cleaved by specific catabolic enzymes. This approach can be used in regenerative medicine to create drug delivery systems that are responsive to particular *in vivo* environments (*e.g.*, inflammation), or biomaterial systems with programmed degradation profiles for *in vitro* tissue engineering *(33)*.

Immunology and Regenerative Medicine

The Host Immune Response

Vertebrates have evolved an innate and an adaptive immune system that aims to eliminate invading pathogens, toxic substances and allergens without causing harm to the host. *Innate immunity* is an ancient defense mechanism found in all multicellular organisms and involves a non-specific inflammatory response after recognition of a foreign substance *(35)*. Many pathogens exhibit conserved pathogen-associated molecular patterns that can be recognized by immune cells bearing pathogen recognition receptors *(36)*. Upon detection of a foreign body, the innate immune system initiates phagocytosis, apoptosis, opsonization

and the activation of pro-inflammatory signaling pathways *(35, 36)*. *Adaptive immunity* is a highly-specific antigen response that provides long-term immunological memory *via* either cell-mediated or antibody-mediated (humoral) immunity *(37)*. Adaptive immunity involves the activation of T cells, B cells and natural killer cells, while innate immunity is regulated by polymorphonuclear cells and mononuclear phagocytes, such as dendritic cells, monocytes and macrophages.

The immune system is implicated in many diseases targeted by regenerative medicine, moreover, the recognition and elimination of foreign entities by the host immune system presents several issues for therapies that involve the administration of exogenous cells, tissues or biomaterials. For instance, non-autologous cells can activate the host adaptive immune system through either direct or indirect allorecognition, leading to cell-mediated or antibody-mediated cytotoxicity (**Figure 2A**) *(38)*. The severity of the initial allorecognition response scales with the degree of antigen mismatch between the donor and recipient cells. Major histocompatibility complex (MHC) matching between the donor and recipient is used clinically to mitigate allorecognition, however, even well-matched donors are capable of provoking unwanted immune responses *(39)*. Critically, therapies based on autologous hiPSCs can also elicit an immune response, potentially due to the use of relatively immature cells or the effect of cell reprogramming, xenogeneic supplements and/or non-physiological culture conditions *(40)*.

An immune response can also be elicited by the implantation of acellular or cellularized biomaterials *(41)*. Many regenerative therapies require the implanted biomaterial to integrate with the surrounding environment to provide long-term restoration of biological function. In reality, implanted biomaterials often provoke a host immune response, known as a foreign-body reaction, that can hinder integration and functional performance *(42)*. Initially, a blood-biomaterial interaction leads to the formation of a protein film and provisional matrix at the biomaterial surface, which is then followed by acute and chronic inflammation. At this stage, macrophages fuse to produce foreign body giant cells, granulation tissue is deposited and then a fibrotic capsule is formed around the biomaterial (**Figure 2B**). It should be noted that the foreign-body reaction is a chronic pathology and thus more of an issue for long-term, nondegradable grafts rather than transient or biodegradable materials.

Evading or Harnessing the Immune System

The nature and magnitude of the host immune response to a regenerative therapy is dependent on several factors, such as the properties of the cell or biomaterial, the implant location and the environmental conditions. For instance, while many cell types commonly provoke a host immune response, other cell types are thought to provide a level of immunosuppression *(43)*. Meanwhile, the immunogenicity of a biomaterial can be affected by a number of properties, such as porosity, surface chemistry, size and shape *(44)*. Given these complexities, it is evident that a holistic understanding can allow the design of regenerative therapies that are compatible with the host immune system. Classically, this has involved the design of *immune evasive* therapies that attempt to circumvent the immune response in order to allow the implanted therapeutic to perform its intended role with minimal interference from the host.

For example, the initial allorecognition response can be mitigated by using biomaterials with certain properties; a well-known example is the harnessing of hydrophilic polymer coatings to attenuate recognition, opsonization and clearance *(45)*. Biomaterial cell encapsulation can also be used to provide a physical barrier that aims to limit direct cell-cell interactions with the host immune system *(46)*. An elegant, biological approach is the use of cells derived from iPSCs isolated from immune-privileged sites, which appear to retain an epigenetic memory of immune evasion *(47)*. Moreover, recent work has shown that inactivation of MHC classes I and II, combined with overexpression of CD47, produces hypoimmunogenic iPSCs that can avoid rejection in allogeneic hosts (**Figure 2C**) *(48)*. Other approaches include the inhibition of T-cell activity using pharmacological or cell-based immunosuppression, the genetic modification of cells to express tolerogenic antigens or remove immunogenic epitopes, or the immunization of the host to specific cell-surface markers *(38)*.

These examples highlight the classic view that the immune response should be suppressed or evaded during therapy. However, the immune system can also be a fundamental orchestrator of tissue repair, with many regenerative processes regulated by macrophages, a heterogeneous cell population with a dynamic phenotype that resides between a pro-inflammatory (M1) state and an anti-inflammatory (M2) state. This

cellular plasticity is driven by environmental signals and is central to both the formation and degradation of scar matrix *(49)*. These features have prompted the design of immune-interactive biomaterials with the aim of polarizing macrophages towards the M2 phenotype (**Figure 2D**) *(50)*. Materials can also be used to deliver pro-regenerative factors: for instance, gold nanoparticles decorated with interleukin-4 have been used to direct M2 polarization of macrophages and improve the function of injured muscle *(51)*. These, and other findings, offer a fresh perspective on how immunomodulation can be an intrinsic component of regenerative medicine.

Measuring & Visualizing Tissue Regeneration

In Vivo Tissue Profiling

In vivo imaging has revolutionized disease diagnosis and monitoring by offering invaluable tools that can assess tissue status in a minimally-invasively, quantitative, and spatiotemporal manner. Magnetic resonance imaging (MRI), computed tomography (CT) and ultrasound (US) are the clinical state-of-the-art for visualizing structural, functional, molecular and mechanical tissue properties, and can be used in preclinical/clinical trials of regenerative therapies. For instance, multimodal MRI has been used to measure the impact of intramyocardial stem cell injections on scar size, tissue perfusion and circumferential strain in patients with heart disease *(52)*. Meanwhile, emerging modalities present new opportunities for *in vivo* interrogation of regenerative mechanisms. For example, hyperpolarized ^{13}C MRI can increase sensitivity by 40,000-fold compared to standard MRI *(53)*. This has enabled the metabolism of injected labelled metabolites to be visualized clinically *(54)*, offering the possibility for linking new metabolic diagnostic biomarkers to degeneration and repair.

Optical coherence tomography and confocal microscopy offer a clinical imaging route for assessing retinal or corneal regeneration *(55, 56)*. However, visible light is highly absorbed and scattered by most biological tissues, compromising the spatial resolution of light-based methods for deep-tissue imaging. However, by combining optical excitation with US detection, photoacoustic imaging (PAI) enables high-resolution, deep-tissue imaging with clear potential for clinical translation *(57)*. This exploits the photoacoustic effect, in

which molecular absorption of incident photons triggers a thermally-induced pressure jump that releases US waves. PAI has been used to evaluate vascular reperfusion, blood oxygenation and whole-brain neural activity in small animal disease models *(58, 59)*. An exciting development for resolving dynamic tissue processes is four-dimensional functional US imaging, which can provide volumetric recordings at high spatiotemporal resolution. This has been used to evaluate neural connectivity and map whole-brain wave propagation *(60)*, thus providing new opportunities for measuring the efficacy of neuro-regenerative therapies.

In Vivo Monitoring

Resolving the fate of an administered therapeutic, including bio-distribution, engraftment, migration, embolism and clearance, is essential for understanding the mechanism, technical constraints and safety profile of a regenerative strategy. *In vivo* imaging can be adapted to visualize implanted therapeutics, such as the CT-based monitoring of high-contrast biomaterials (*e.g.*, ceramics) *(61)* or repair processes (*e.g.*, bone ingrowth into an implanted scaffold) *(62)*. However, therapies involving smaller entities (*e.g.*, cells, particles, extracellular vesicles, biomolecules) generally require a more sensitive imaging modality. One option is nuclear imaging of therapeutics labeled with radionuclides that either release γ-rays for single-photon emission computed tomography (SPECT), or positrons that annihilate with electrons in the host tissue to produce γ-rays for positron-emission tomography (PET) *(63)*. In each case, it is important to use radiolabeling methods that provide high contrast across the imaging period, without the radionuclide dissociating, causing significant toxicity or altering the performance of the therapeutic entity.

Radiolabeling small molecules and proteins typically involves covalent or electrostatic binding *(64)*, while non-specific metabolic radiotracers can be taken up by cells, which has been used to track the fate of administered peripheral-blood CD34+ cells *(65)* and the engraftment of islet cells *(66)*. Targeted approaches can provide process-specific readouts, for instance, ^{18}F-dihydroxyphenylalanine can traverse the blood-brain barrier and be taken up by presynaptic terminals of dopaminergic projections, providing information regarding the survival and maturation of transplanted dopaminergic neurons *(67)*. Similarly, a radiolabeled

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dopamine D2 receptor antagonist, ^{11}C-raclopride, can be used to visualize synaptic dopamine release from stimulated, transplanted neurons *(68)*. Alternatively, the stable integration of reporter genes into the genome (*e.g.*, using lentiviral transfection) provides the opportunity to track cells in the body over long periods of time. For instance, the expression of an enzyme (HSV-TK1) that selectively traps ^{18}F-fluoro-3-[hydroxymethyl]butyl)guanine can be used to provide cellular contrast with PET *(69)*. The recent introduction of high-sensitivity, whole-body PET scanners have been used clinically with reduced imaging time and radionuclide dose, offering greater ease for monitoring regenerative therapeutics in patients compared to current scanner designs *(70)*.

Delivering Patient Benefit: Clinical Translation

A Blueprint for Translational Medicine

The last two years has seen the publication of results from several promising clinical trials (**Table 1**). In phase I clinical trials, Moroni *et al.* established the safety profile of peripheral infusions of autologous monocyte-derived macrophages in nine patients with compensated liver cirrhosis *(71)*. Da Cruz *et al.* reported improvements in visual acuity and no significant safety issues following the implantation of synthetic membranes, seeded with human ESC-derived retinal pigment epithelium, in two patients with age-related macular degeneration *(72)*. Curtis *et al.* reported no adverse effects, and early indications of therapeutic efficacy, after local delivery of human NSCs in four individuals with spinal cord injury *(73)*. Meanwhile, clinical investigation of iPSC-derived retinal pigment epithelium as a treatment for macular degeneration *(74)* has been followed by iPSC-based clinical trials for a range of indications, including corneal disease *(75)*, spinal cord injury *(76)*, and Parkinson's disease *(77)*.

The success of these trials, and other translational studies, will be determined by a variety of factors. Ultimately, the most important aspect of translation is the demonstration of robust patient safety and efficacy through preclinical and clinical trials. However, even safe and efficacious therapies can be thwarted by other translational pitfalls; products may be cost ineffective and not competitive against current therapies, challenging to manufacture at scale, or restricted by regulatory requirements in a particular sector or market.

Here, we provide a blueprint for translation of academic ideas into clinical regenerative medicine, focusing on key stages and milestones that researchers should address in the early stages of translation in order to build a strong basis for product development and the compilation of a technical file for regulatory submission (**Figure 3**). To provide context, we also discuss later translational stages, although by this stage, activity will typically have transferred from academic to an industrial setting (licensing or start up).

Commercial Considerations

Translating a regenerative therapy is expensive: in the United States of America (USA), it can cost US$150-250 million to take a cell-based product through phase III clinical trials *(78)*. Although there are established business models for translational regenerative medicine *(79)*, the associated costs and risks dictate that capital investment is almost always required. However, many venture capitalists are justifiably wary after the high-profile failures of the 1990s, in which several tissue engineering products were unprofitable despite being shown to be safe and efficacious *(80)*. Steps should be taken to ensure freedom to operate (FTO) in the desired area and to protect intellectual property (IP). Since these factors can greatly influence the technical components of a proposed strategy, such as cell line selection, researchers should engage technology transfer teams early in the translational process to review FTO and establish IP strategy before publishing any details of the invention.

It is also important to establish whether the translational product will meet an "unmet need." Clinicians must be consulted and competitor reviews performed to ascertain the advantages of the proposed therapy over the current standards of care and competing systems. This assessment should be carried out at the early stages of translation and must cover the treatment pathway as well as clinical outcomes and product costs. Will the proposed product be available "off-the-shelf"? Would the proposed mode of delivery reduce costs of invasive surgery and associated complications? Could the proposed therapy be used to treat a broader spectrum of patients? These practical considerations form the basis of the cost-effectiveness of a proposed solution and will greatly influence the design of the final product. This is important as the decision makers

for product adoption assess products on economic benefits in their healthcare setting, rather than on clinical promise.

Regulatory Considerations

In the USA, products are regulated as *human cells, tissues, and cellular and tissue-based products (HCT/Ps)*, *devices, biologics* or *drugs* based, in part, on their "primary intended purposes" *(78)*. The European Union (EU) has a similar classification of *advanced therapy medicinal products (ATMP)*, *medical devices* or *medicinal substances* based on the "principal intended action" *(81)*. These classifications determine the applicable regulations and are relatively easily applied to single-part therapies, such as acellular scaffolds. For multi-component systems, such as cell-seeded scaffolds, the legal manufacturer must comply with applicable regulatory requirements for all components. While the regulations are necessarily stringent, schemes such as the Breakthrough Therapy designation (USA), the Regenerative Medicine Advanced Therapy designation (USA), the Sakigake designation (Japan), and the PRIME initiative (EU) are available to support patient access to innovative medicines with high public health potential *(82)*. Regulatory strategies should ideally be devised in consultation with a regulatory expert, once the unmet need and intended use have been defined.

Preclinical and Clinical Studies

Before a product can be authorized for market release, the legal manufacturer must demonstrate safety and efficacy for its intended use, in the intended population, for the intended purpose. In the early stages, researchers should clearly define these clinical parameters (intended use, population, purpose), how the proposed product achieves a therapeutic effect, and the expected benefits over available alternatives. These claims must, ultimately, be validated by clinical data using endpoints that can quantifiably measure whether the patient undergoing treatment lives longer and/or better than if they received the gold standard therapy or no treatment at all. Real clinical endpoints include higher overall survival, reduced patient symptoms, or improved quality of life *(83)*. Surrogate endpoints, such as disease-free survival and progression-free

survival, can also provide useful indication of therapeutic efficacy, however, care should be taken as these factors may not necessarily correlate with real clinical endpoints *(83)*.

Clinical studies should be designed in collaboration with clinicians and statisticians, and aided by a systematic review of relevant clinical data. Ideally, preclinical tests are performed using well-validated, high-fidelity animal models that in some way mimic the clinical problem. However, accurate modelling can be restricted by differences in physiology, anatomical size, and disease characteristics between animal models and human patients. In addition, preclinical testing with human cells requires immunocompromised animals, which can cause problems when studying long-term effects (>6 months) *(84)*. For clinical testing (*i.e.*, in humans), proposed therapies must progress through a carefully staged process. Initial studies are performed on a small number of participants to test for tolerability and feasibility (phase I), then larger groups are used to test for any signal of therapeutic efficacy (phase II), which is then formally compared to the current standard of care (phase III). Further clinical studies are performed after approval, in order to track long-term benefits and side effects (phase IV).

Manufacturing Considerations

Successful translation requires manufacturing processes that can be performed reliably and at scale, while remaining financially viable. Unfortunately, the manual handling protocols commonly used in academia can present high technical variability, the risk of contamination, a lack of precision or power, and low throughput production. Moreover, manually-operated systems require expertise knowledge and training. Given these limitations, it is important that automated and mechanized processes are employed, where possible, during product manufacture. *Automation* uses machines to replicate manual processes, which can greatly reduce technical variance, production costs and the risk of contamination. Automated systems can also benefit from *mechanization*, in which machines outperform humans in precision, power or throughput *(85)*. Reliability can be improved through other means: by employing strict standard operating procedures, chemically-defined and batch-tested media of good manufacturing practice (GMP) standard, closed cell systems and

batch segregation to limit microbial or chemical contamination, and culture protocols known to reduce genetic or epigenetic variability *(86)*.

Harmonized standards are available to support manufacturers in establishing quality management systems (QMS) that can be implemented throughout product development and production. In particular, this framework of quality assurance practices can aid in documenting the design history, product requirements, applicable standards and state-of-the-art test methods, and in performing initial risk assessments, maintaining traceability, characterizing products and monitoring manufacturing processes. For example, it is recommended that cell and tissue culture is performed using bioreactors with integrated sensors that continuously record the media pH, glucose concentration and dissolved oxygen levels *(87)*. These online monitoring systems, and other analytical methods, should be used to generate critical-to-quality (CTQ) attributes. These quantitative metrics are used for product quality control to assess whether changes in critical process parameters (CPP) produce statistically significant differences in safety or efficacy. Equivalence testing should be performed for all process changes, including alterations in protocol, materials, equipment or manufacturing location *(88)*.

One process change that should not be underestimated during early development is scaling: therapies can require hundreds of millions of cells per dose, and manufacturers often need to navigate hurdles of large-scale cell expansion (senescence, dedifferentiation, slow proliferation, infrastructure) *(89, 90)*. Scalable cell expansion can be facilitated by automated and mechanized processes, closed cell systems and intermediate cryopreservation stages *(89, 90)*. Production rate can then be raised either by *scale-out*, which involves an increase in the number of units (*e.g.*, bioreactors) with maintained output per unit, or by *scale-up*, whereby the output per unit is increased. Scale-out is a more predictable process change with additional flexibility over production rate and reduced operating risk, however, scale-up may be more appropriate for allogeneic therapies or highly mechanized manufacture *(90, 91)*. To avoid unnecessary operational costs, manufacturing processes should be *designed to be scalable* but only scaled in line with clinical trials size or market demand *(91)*.

Risk Management

Regulations require risk assessments for the whole product, each individual part and any interactions that may occur between components. For example, an engineered graft should be considered in terms of the whole tissue, its constituent cells, materials and biomolecules, as well as any interactive processes (*e.g.*, adhesion, signaling). Technical risks include malignant transformation, component toxicity, disease transmission, immune rejection, and the increased risk of thromboembolic disease, while extrinsic risks can arise during procurement, delivery, handling, storage, surgery, or immunosuppression *(92)*. Given the variety of different factors, it is important to assemble a risk assessment team during the early stages of translation, and include technical, clinical, regulatory and manufacturing expertise to effectively identify, score, and pre-emptively mitigate potential risks by design. This can be supported by performing an early review of scientific literature, including failure reports and national registry data for similar therapies. An effective risk management strategy, implemented throughout the course of a project, can help avert setbacks that can consign an idea to the translational "valley of death."

Future Perspectives

These are truly exciting times for regenerative medicine, at the confluence of cutting-edge biological manipulation (*e.g.*, iPSCs, CRISPR/Cas9, optogenetics), powerful *in vitro* models (*e.g.*, organoids, organ-on-a-chip, 3D bioprinting, machine learning) and advanced characterisation tools (*e.g.*, spatially-resolved omics, super-resolution microscopy, machine learning) *(93)*. We must strive to use such innovative methods to improve our understanding of disease targets, better resolve the fate of implanted therapeutics and unravel the mechanisms underpinning tissue regeneration. These outcomes should provide solid scientific foundations for the major challenge of the field: to assemble efficacious, reproducible and scalable therapies that offer programmed tissue regeneration within a robust regulatory and manufacturing pipeline. We hope that the biological, material, immunological, imaging and translational aspects that we have highlighted in this review can serve as a blueprint for those seeking to turn academic ideas into clinical regenerative medicine products providing real patient benefit.

Tables

Table 1. Selection of published clinical trials of regenerative therapies since 2018

Indication	Trial Details	Regenerative Therapy	Reference
Macular degeneration	- Phase I - 2 patients	Human iPSC derived retinal pigment epithelium delivered on a PET membrane.	L. Da Cruz *et al. Nat. Biotechnol.* **2018** *(72)*
Thoracic spinal cord injury	- Phase I - 4 patients	Human NSCs (NSI-566) injected locally using a stereotactic floating cannula.	E. Curtis *et al. Cell Stem Cell* **2018** *(73)*
Ischemic stroke	- Phase I - 9 patients	Intra-carotid artery transfusion of autologous $CD34^+$ cells in the infarct area.	P.-H. Sung *et al. Am. J. Transl. Res.* **2018** *(94)*
Rheumatoid arthritis	- Phase I/II - 15 patients	Intra-articular injection of cultured, autologous mesenchymal stem cells.	S. Shadmanfar *et al. Cytotherapy* **2018** *(95)*
Compensated liver cirrhosis	- Phase I - 9 patients	Peripheral infusion of matured autologous monocyte-derived macrophages.	F. Moroni *et al. Nat. Med.* **2019** *(71)*
Knee osteoarthritis	- Phase 2 - 26 patients	Intra-articular injection of cultured, autologous mesenchymal stem cells.	L. Lu *et al. Stem Cell Res. Ther.* **2019** *(96)*
Compensated liver cirrhosis	- Phase 2 - 81 patients	Subcutaneous delivery of granulocyte colony-stimulating factor and intravenous infusion of $CD133^+$ HSCs.	P. N. Newsome *et al. Lancet Gastroenterol. Hepatol.* **2018** *(97)*

Figures

(i) Cellular remodelling or degradation of biomaterials

(ii) Biomaterial encapsulation, support or induction of cells

CELLS

BIOMATERIALS

Cell Therapies

Material Therapies

Tissue Engineering

Controlled Drug Delivery

Immunomodulation

Genetic Engineering

(i) Chemical stimulation of cell differentiation, proliferation, migration, apoptosis *etc.*

(ii) Cellular synthesis, modification, presentation, packaging or secretion of biomolecules

Molecular Therapies

(i) Biomaterial encapsulation and controlled release of biomolecules

(ii) Biomolecular assembly, remodelling or degradation of biomaterials

BIOMOLECULES

Figure 1

Figure 2

TRL 1   **TRL 3**   Project likely to have transferred to a start up / licence   **TRL 5**   **TRL 6**   **TRL 8**

EARLY RESEARCH & DEVELOPMENT | **PRE-CLINICAL PRODUCT DEVELOPMENT** | **CLINICAL STUDIES**

ACADEMIC CONCEPT

Define the clinical need

Target market/s (*e.g.*, EU/US) and product classification

Compile list of the applicable standards

Evaluate regulatory options: *e.g.*, registration as a small-medium enterprise (SME), classification requests, certification procedures

Apply quality control systems for design, manufacture and testing processes

Implement infrastructure, work environment and training controls

Concept design with user

Lab book preparation

Compile product requirements, such as customer needs, performance, regulation

Control of changes in production, suppliers and design

Selection of cells, materials, biochemical factors and manufacturing methods

Undergo iterations of product specification, manufacture and verification testing, with maintained traceability over the materials, processes, inspection methods and results

Aseptic process validation, design validation

Safety assessment for viruses, prions *etc.*

Clinical investigation and follow up using defined endpoints and controls to establish both the safety and efficacy of the product

Establish commercial strategy

Identify the optimal cell type / source. Autologous or allogeneic? From a cell bank or a commercial source?

Determine requirements for product stability, packaging, transport, delivery, administration

Prepare clinical evaluation, clinical protocols and technical files for ethics and regulatory submission, including defined clinical endpoints

Identify informative controls (*e.g.*, clinical gold standard)

Identify and collaborate with a relevant clinical partner

Initial risk assessment | Risk management

CLINICAL PRODUCT / THERAPY

Business Case & Product Type
You should have:
(**1**) established the need: how and by whom the product would be used, and the intended population,
(**2**) identified benefits of your product idea over the competition,
(**3**) reviewed your product idea, including IP/FTO considerations with regulatory and Technology Transfer experts,
(**4**) identified your target market/s and regulatory classification.

Concept Approval & Initial Risk Assessment
You should have:
(**1**) determined the basic user requirements, including the method of introduction or administration,
(**2**) started to compile a list of the applicable guidelines, regulations and standards,
(**3**) established initial product components and materials for optimum regulatory compliance,
(**4**) reviewed key safety risks and possible sterilization methods with experts.

Preliminary Design & Development
You should have:
(**1**) compiled the list of applicable regulations, guidelines and standards,
(**2**) chosen optimum materials and methods for reproducible production,
(**3**) chosen a control as a clinical comparator,
(**4**) defined initial specifications and qualified assay suitability,
(**5**) drafted target product profile / summary of product characteristics
(**6**) started the formal compilation of design input and risk analysis
(**7**) standardized documentation to maintain traceability and development history.

Design Validation & Transfer to Production
You should have:
(**1**) identified facilities for manufacture and pivotal non-clinical safety studies,
(**2**) undertaken development batch runs to provide representative material for pivotal non-clinical studies,
(**3**) finalized the product composition and frozen design,
(**4**) qualified/validated analytical assays and manufacturing processes,
(**5**) clearly defined data requirements for regulatory submission and clinical documentation, and established relationships with competent authorities/notified bodies.

*Note that some verification tests may need to be repeated on the first production series.

Regulatory Submission
You should have:
(**1**) obtained approval from the relevant competent authority,
(**2**) obtained approval from the research ethics committee,
(**3**) obtained any additional approval, *e.g.*, for the use of radiation in clinical trials, or genetically-modified cells/gene therapy.
(**4**) submitted clinical and technical files, including the clinical endpoints and any comparator product or therapy to be used during the clinical investigation.
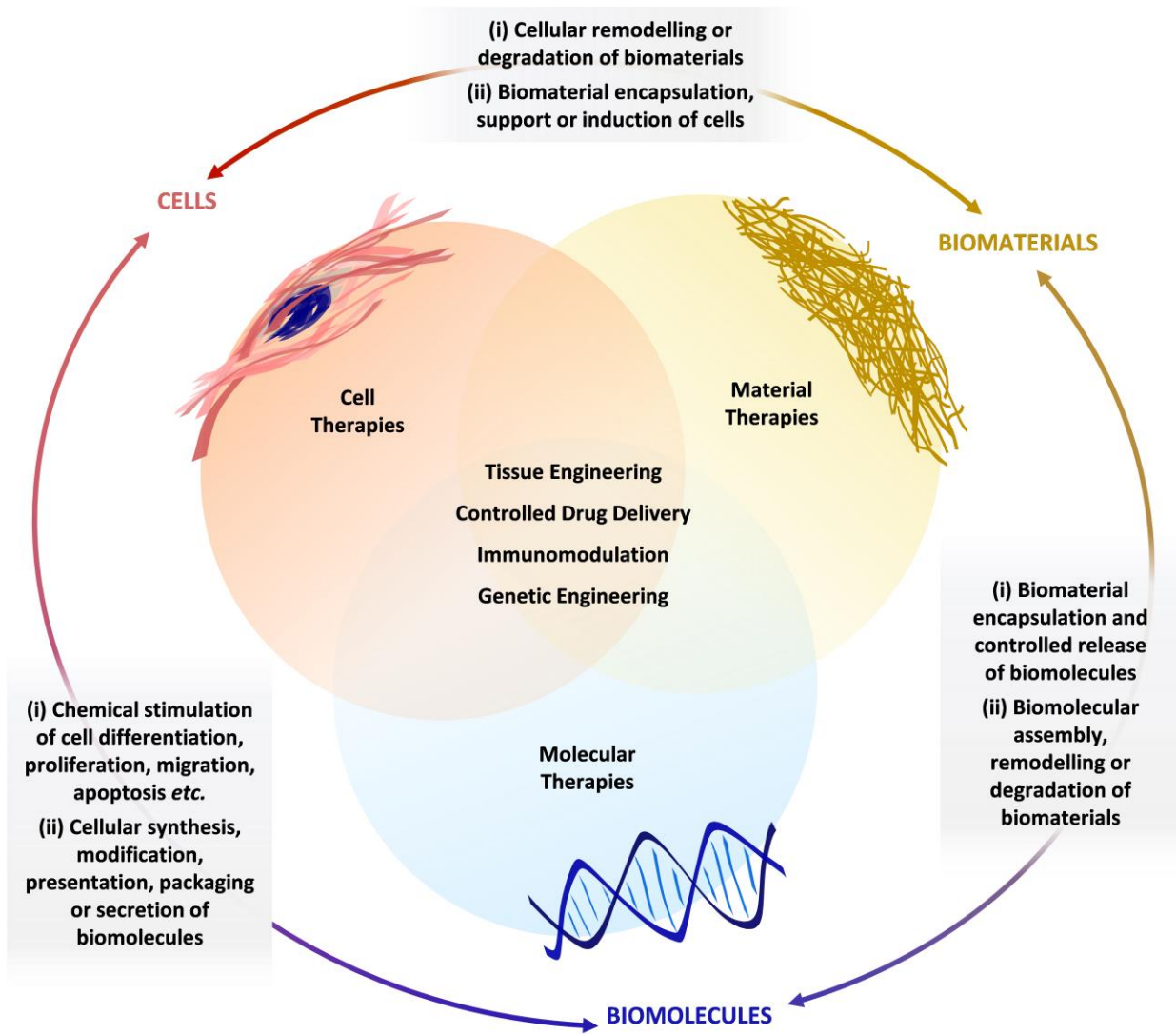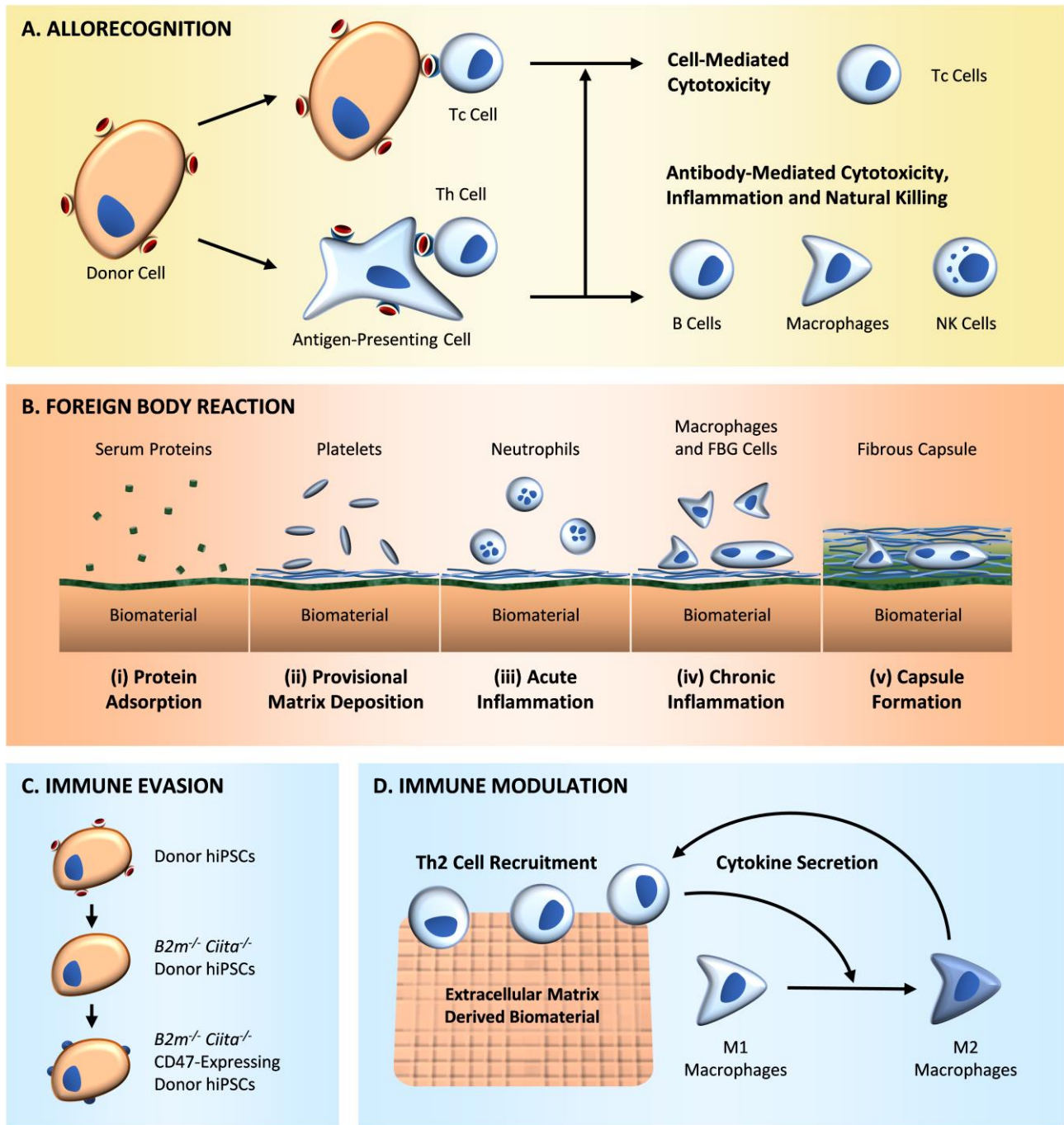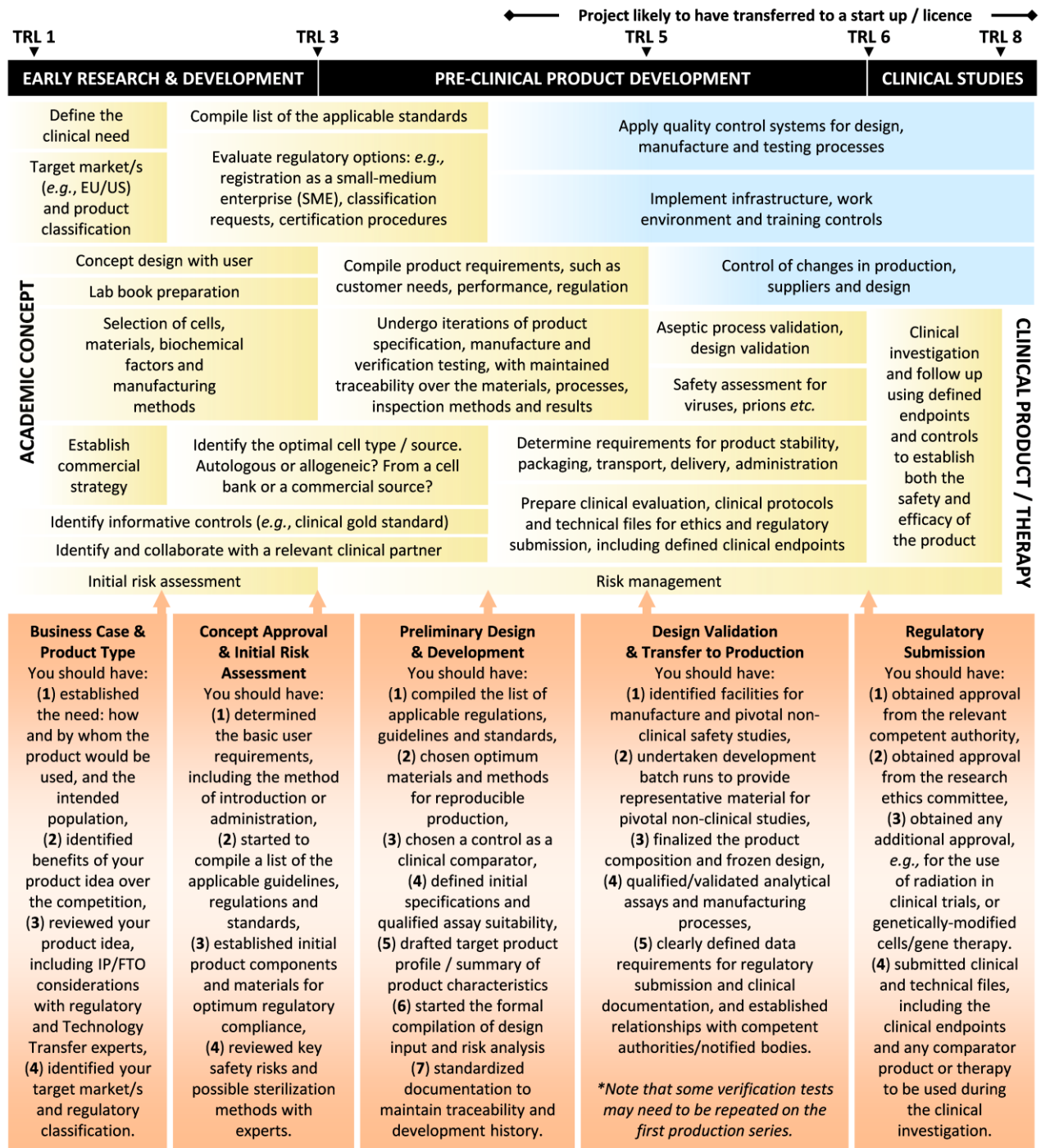
Figure 3

Figure Legends

Figure 1. Overview of some common strategies, components and interactions used in regenerative medicine. Cells, biomaterials and biomolecules exhibit a number of interdependent interactions that can be exploited for various regenerative medicine strategies, including tissue engineering, drug delivery, immunomodulation and genetic engineering.

Figure 2. The host immune response and regenerative medicine. (A) Direct allorecognition occurs by cytotoxic T cells (Tc cells) recognizing and eliminating allogeneic donor cells, while indirect allorecognition occurs by helper T cells (Th cells) recognizing donor antigens taken up and displayed by host antigen-presenting cells. (B) Implanted biomaterials can provoke a foreign body reaction resulting in the formation of a fibrous capsule containing both macrophages and foreign body giant (FBG) cells. (C) An example of immune evasion, in which transgene expression and CRISPR/Cas9 are used to generate hypoimmunogenic hiPSCs with upregulated CD47 but neither class of MHC (*B2m*$^{-/-}$ and *Ciita*$^{-/-}$). (D) An example of immune modulation, in which ECM-derived biomaterials recruit T helper 2 cells (Th2 cells), which secrete cytokines that can polarize macrophages to an M2-like phenotype. In turn, these alternatively-activated macrophages secrete cytokines that sustain Th2 cell activation.

Figure 3. Bench-to-bedside translation of regenerative medicine products. Translating an academic concept into a clinical product can be divided into three key stages: early research and development, pre-clinical product development and clinical studies. This schematic predominantly focuses on the *technical aspects* that should be considered by scientists at early technology readiness level (TRL), with higher TRL items included for context. Highlighted are the key tasks (yellow boxes), systematic quality control to fulfill all applicable requirements (blue boxes) and critical checkpoints that should be met throughout product translation (orange boxes), with each stage guided by experts in the field. The timeline of this schematic should not be considered an absolute scale, indeed, the relative time and work required at the different stages will vary considerably depending on the product or therapy that is being translated. It should also be noted

that in the EU, good manufacturing practice (GMP) is applicable for medicines and ATMPs but not for medical devices.

Acknowledgements

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