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Lessons learnt, and still to learn, in first in human stem cell trials

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SUMMARY

Developing cellular therapies is not straightforward. This *Perspective* summarizes the experience of a group of academic stem cell investigators working in different clinical areas and aims to share insight into what we wished we knew before starting. These include (1) choosing the stem cell line and assessing the genome of both the starting and final product, (2) familiarity with GMP manufacturing, reagent validation, and supply chain management, (3) product delivery issues and the additional regulatory challenges, (4) the relationship between clinical trial design and preclinical studies, and (5) the market approval requirements, pathways, and partnerships needed.

INTRODUCTION

We are now entering an era in which increasing numbers of stem cell-based therapies are being trialed in first-in-human (FIH) studies. In this *Perspective*, we discuss some of the key issues that should be considered in such translational work, ideally beginning with the initial therapeutic concept and the scientific rationale for the therapy. This prospective approach from the outset of the work also greatly facilitates the chance of a FIH trial being acceptable to regulatory authorities without the need to go back to the drawing board. In addition, such a forward-thinking approach brings with it improved opportunities for funding support and a return on investments without compromising the scientific integrity of the work.

The areas we have chosen to concentrate on in this article are not exhaustive, but have been chosen based on our experiences in translating therapies to clinic and include many things we wished we had known when we started.

CHOOSING THE STEM CELL LINE

The manufacture of a human pluripotent stem cell (hPSC)-derived product includes (1) derivation and/or selection of the starting line, (2) genome modification (if required), (3) expansion and banking of the starting material to master and working cell banks, (4) differentiation of the stem cells to the therapeutic population (drug substance), and (5) formulation of the differentiated cells for delivery into the patient (drug product). Each of these stages has its own unique challenges (Figure 1).

We strongly recommend that the starting material for any clinical program using a stem cell product has been explicitly derived with this aim in sight. Of course, it may be possible to qualify old lines derived in research laboratories, which do not conform with standard informed consent or Good Manufacturing Practice (GMP) conditions. However, this practice constitutes a potential safety risk and may even result in the need to switch lines later in the product life cycle, with all the regulatory hurdle-related delays that this brings with it (see below).

An important aspect of cell line selection is compliance with regulatory guidelines, including Good Tissue Practices, Good Distribution Practice, and GMP, which vary in different countries; understanding this is vital to avoid selecting lines that lack the proper compliance.

In the context of generating allogeneic human induced pluripotent stem cells (iPSCs), compliance with the donor eligibility requirements (DE) (Jha et al., 2021) includes screening and testing the tissue donor at the time of the initial tissue harvest. This process is relatively straightforward when generating new iPSC lines for therapeutic use, but in the context of allogeneic human embryonic stem cell (hESC)-derived therapies, compliance with the DE is more complex and can be country dependent.

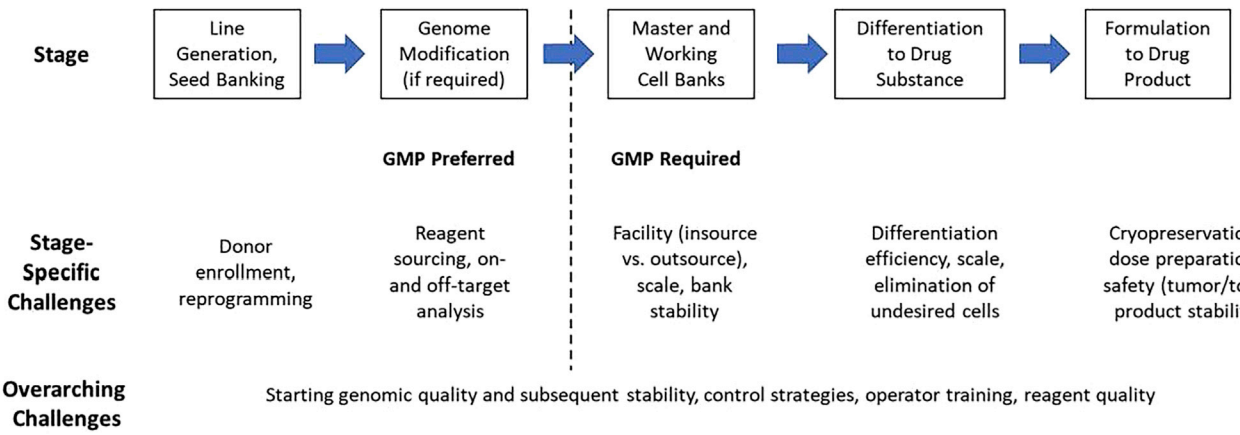

Figure 1. Manufacturing steps to create a stem cell product for clinical trials

A number of groups have entered clinical trials with hESC-derived products in which the hESCs were generated as research material, such as the H1 and H9 lines. Specifically, this means that the starting material was generated in a research lab rather than in a GMP environment. Furthermore, these early cell lines were derived by co-culturing the hESCs on mouse embryonic fibroblasts. In the United States, there is a cutoff for DE screening requirements, wherein ESCs derived after May 2005 require full DE screening, whereas those derived beforehand do not (see 21CFR1271 for details). In circumstances when compliance with DE guidelines is not feasible, manufacturers have tested the hPSC cell banks in place of donor screening and testing. The purpose of the DE screening is to prevent infectious disease transmission; therefore, directly testing the starting material for the presence of pathogens should provide sufficient information to ensure the safety of the starting material. This is a reasonable assumption for pathogens for which there are validated tests available. However, in some cases, such as transmissible spongiform encephalopathies (TSE), validated tests are unavailable, although some are starting to emerge that look promising (Concha-Marambio et al., 2020). This situation has been problematic for groups developing hPSC products from PSC lines derived from UK or EU donors that would like to initiate clinical trials in the United States. Specifically, the US Food and Drug Administration (FDA) DE requirements do not currently allow the use of materials from UK or EU donors because of concerns about TSE transmission. This may change and will likely be determined using a risk-based case-by-case assessment; e.g., age of patients in the trial, as well as the traceability of materials (and thus the risk) used in the therapy. Regarding infectious hazards, advanced therapies may require attention to microbiological agents that are not covered in traditional donor screening. Numerous viral, bacterial, and other pathogenic organisms that can grow in cell culture may need to be analyzed and fully documented in a prospective fashion with appropriate record keeping.

Fundamental biological attributes of the cell line are essential to know, including the sex of the line, ABO and Rh blood group, human leukocyte antigen typing, and the presence of mutations or variants in genes that may be of interest for its ultimate therapeutic use (detailed below). Blood antigens can be expressed on non-blood cell types (e.g., ABO on vascular endothelium), and the Y chromosome encodes minor histocompatibility antigens that may be relevant immunologically. Consideration of the history of the cell line is also important. A well characterized line will have documentation of all materials the cells have been exposed to, and this informs strategies for testing. For instance, if the cell line was exposed to bovine reagents, then the cell line should be tested for bovine viruses and so on. If the reagents were from regions where TSE is a concern, this may render the line ineligible. Experience teaches that absence of documentation decreases the probability of success with regulators; there is no presumed innocence!

Central to all of this is the critical issue for donor selection of informed consent. It is essential to ensure that ethical informed consent for the use of the harvested material has been obtained. Consent should include use of the resulting cell line in clinical applications and commercialization, if appropriate, and linked to this should also be traceability from donor to trial participant.

Determining the intellectual property and licensing requirements for use of the cell lines can also be challenging. Developing hPSC-derived products requires the use of many different technologies. For instance, for an iPSC therapy one needs to consider the technologies and reagents used for reprogramming, expansion,

cryopreservation, differentiation, enrichment and genetic modification (if used). It is incumbent on investigators with aspirations of moving their work into commercialization to determine if their technologies have freedom to operate.

The final consideration in choosing a cell line relates to the question of whether one uses an autologous versus allogeneic approach. While autologous therapies are routine in many hematological disorders and cancer immunology, to our knowledge for PSCs they have been tried in n = 1 patients; one who received an iPSC-derived dopamine neurons for their Parkinson disease (PD) (Schweitzer et al., 2020) and another who received an iPSC-derived retinal cells for age-related macular degeneration (Mandai et al., 2017). The autologous iPSC approach with iPSCs should avoid rejection of that tissue when differentiated and implanted. However, there are currently three major issues that limit the attractiveness of this strategy. The first is the cost of making personalized therapies, as the testing of any product may require the level needed for an allogeneic stem cell graft, which would thus make any final product extremely expensive. Beyond cost, the amount of work required to evaluate the genetic stability and safety of cells generated from every patient (see below) is daunting. Such genomic safety issues ultimately led to the autologous iPSC-derived retinal pigment epithelium cell trial for AMD in Japan being halted (Maeda et al., 2022). Second, there is a substantial time lag between enrolling a patient and the availability of iPSC-derived therapeutic cells. This precludes the use of autologous iPSCs for acute illness, e.g., recent myocardial infarction. Finally, in diseases with a genetic component, using the patient's own cells may promote pathology or disease recurrence in the cells so generated unless genetic editing can be done, with all the regulatory requirements and time lags that come with this. Even then, it may still be better to use unrelated allogenic cells as, e.g., in PD, human allogeneic fetal dopaminergic transplants have acquired the pathogenic alpha synuclein inclusions of PD after 10 years after implantation (Li et al., 2010). Autologous cells from a patient with PD, may acquire this pathology more readily and extensively given such cells are often used to model disease *in vitro*.

ASSESSING THE GENOME

Pluripotent and tissue-specific stem cells are thought to retain greater fidelity with regard to preserving the integrity of their genomes compared to isogenic somatic cells (Jiang et al., 2021). That said, since the goal of many therapeutic programs is to have lifelong cell replacement, genomic integrity is of the utmost importance (Rohani et al., 2018).

There are two critical components to genomic assessment for stem cell therapeutics;

(1) assessing the genome of the donor or starting cell line, and
(2) assessing the stability of the genome during the process of genetic modification (if present), banking, expansion, and differentiation.

We have learned from sequencing large populations that all humans carry germline mutations and variants of unknown significance (e.g., the 1000 Genomes Project Consortium et al., 2015). Moreover, human bodies are mosaics of cells harboring somatic mutations that are acquired during development and homeostasis. This means that every stem cell line has potentially deleterious variants, and that a perfect genome is likely non-existent (Vijg and Dong, 2020). Consequently, judgment calls will be required in line selection and process development. When evaluating stem cell genomics, it is important to consider both the qualitative nature of the mutation (ranging, in coding regions, from synonymous to gain- or loss-of-function) and the quantitative frequency of the mutant allele in the population (and whether it is increasing or decreasing with passage). Furthermore, the rigor of this approach and levels of sensitivity for genetic screening will also depend on the number of cells being grafted, as there is intrinsically less risk to engrafting a few hundred thousand cells vs. engrafting billions. Thus, when engrafting large cell numbers it may be more critical to use some sort of suicide switch.

The regulatory environment is evolving as investigators and regulators learn more about assessing genomic quality in hPSC populations. Analysis of chromosomal architecture through G-band karyotyping has long been used to assess chromosomal copy number, as well as translocations, inversions, and large-scale insertions and deletions (indels; 5 Mb or greater limit of detection) (Martin and Warburton, 2015). Array-based comparative genomic hybridization offers the ability to detect indels of approximately 10 kb or greater and, with the exception of balanced translocations and inversions, captures all of the information of karyotyping (D'Antonio et al., 2017). Next-generation sequencing affords single nucleotide resolution in the detection of sequence variants and indels (Popp et al., 2018). Exome sequencing, by definition, is restricted to variations contained within the RNA coding sequences of the genome (approximately 1% of the entire genome). Because of under-sampling, exome sequencing is not typically used to assess larger scale copy number variations (CNVs). In contrast, whole-genome sequencing is being increasingly used to assess CNVs and allelic polymorphisms at single-nucleotide resolution (Smolander et al., 2021).

Cancer risk affects virtually all pluripotent stem cell therapies, and there are no clear regulatory requirements to assess such cancer risks at present. That said, common sense indicates that genetic testing for oncogenic risk should be a part of the line selection and process development. One approach is querying public cancer genomics databases (e.g., Catalog of Somatic Mutations in Cancer [https://cancer.sanger.ac.uk/cosmic], or The Cancer Genome Atlas [https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga]) to obtain lists of candidate proto-oncogenes and tumor suppressor genes, and cross-referencing these with variants detected by DNA sequencing. A more focused approach is to use clinical cancer genomic tests using next-generation sequencing (NGS) platforms, which query fewer genes but provide more curated information to aid investigators in their interpretation (Donoghue et al., 2020) Beyond cancer, it is useful to screen cells for variants that could cause disease in the therapeutic cell type, e.g., cardiomyopathy or arrhythmia mutations for cardiomyocytes; genes linked to neurodegeneration or dysmyelination for neurons and glia, and so on. To find relevant genes in these categories, we have found it is easiest to test with gene sets used in routine clinical testing in patients. Genomic testing inevitably will yield variants of unknown significance, and consultation with medical geneticists versed in clinical testing is often very helpful in assigning risk to individual variants.

As discussed above, stem cell lines are derived with genetic variants, the significance of which are often unknown. Mutations are more common in skin fibroblast-derived iPSC lines than in blood-derived lines (particularly UV light induced) (Koh et al., 2021), while mutations in the tumor suppressor gene BCOR occurs commonly in blood-derived lines (Rouhani et al., 2022). Regardless of their derivation, stem cell lines need to be grown and maintained in the lab, and prolonged culture can lead to recurrent genomic alterations. Since every cell division induces one or more errors into the genome (Werner et al., 2020), it is perhaps not surprising that some of these alterations confer selective growth advantage and allow dominant clones to overtake the culture. Such recurrent alterations include karyotypic changes that include CNVs as well as mutations, that include gains in *BCL2L1* (Avery et al., .2013), inactivating mutations of *TP53* (Merkle et al., 2017), and, most recently, inactivating mutations in BCOR (Rouhani et al., 2022). Such aberrations vary in the magnitude of their karyotypic effects and frequency within the population. Factors that seem to contribute to the acquisition of genomic alteration include single cell cloning, feeder-free culture, and increasing passage number (Draper et al., 2004). In pluripotent stem cell culture, long-term maintenance in so-called naive conditions with dual inhibition of WNT and MAPK signaling pathways ("2i") induces global hypomethylation, chromosomal instability, and altered differentiation (Choi et al., 2017). Of late, single-cell RNA sequencing has revealed a significant level of transcriptional heterogeneity within isogenic hPSC lines (Nguyen et al., 2018).

The role of epigenetics is only now being looked at and may prove to be important going forward with such therapies. In mouse pluripotent stem cells, alterations in crucial epigenetic genes, such as *Wdr5*, have been shown to lead to global and locus-specific reduction of methylated histone lysine 4 (H3K4me)—a histone modification associated with transcription. In the case of retinal therapies, p53-dependent misspecification can lead to the development of mesoderm lineages under conditions that normally induce a retinal fate, although surprisingly *p53* loss restores global losses of H3K4me and induces *Wdr5* mutant cells back to a retinal fate (Li et al., 2020). *Wdr5* deletion in *p53*-null mESCs leads to impaired self-renewal, defective retinal neuroectoderm differentiation, and de-repression of germ cell- or meiosis-specific genes (Li et al., 2021). Indeed, the p53 family of proteins (p53/p63/p73) has been shown to regulate mesendodermal differentiation in mouse and human ESCs via WNT and Nodal signaling (Wang et al., 2017).

In human cancer cell lines, gain-of-function *TP53* mutations lead to mutant p53 proteins that target and upregulate chromatin regulatory genes that are distinct from those bound by wild-type p53 (Zhu et al., 2015). In addition, the presence of gain-of-function p53 mutants leads to increases in global histone methylation and acetylation, which alters chromatin structure, in patterns that are divergent from those related to wild-type p53 protein (Zhu et al., 2015). However, similar p53-focused studies have not yet been performed on pluripotent stem cells, despite the presence of such *TP53* mutants in a number of these cell lines, some of which are already being used in clinical trials.

Currently, karyotyping, the most common approach, does not capture the genomic and epigenomic integrity of pluripotent stem cell lines. We predict that genetic and epigenetic NGS-based approaches will provide new insights into the integrity of hPSC lines for clinical use. We encourage investigators to use NGS approaches to characterize their starting cells, their final product, and key points along the manufacturing pipeline. While some of the data so collected initially will be hard to interpret, it will be useful for product development and as a reference should adverse clinical events occur.

DEVELOPING A GMP PROTOCOL

Any clinical-grade cell product will need to be produced under GMP (Rehakova et al., 2020; Bedford et al., 2018), which is a system designed to ensure consistency and high quality in manufacturing. In the case of a cell therapy, GMP spans reagents, instrumentation, facilities,

and record keeping from first thaw of a vial of cells until the final fill-and-finish of a drug product (Rivera et al., 2020). GMP requirements vary regionally, so investigators need to be cognizant of the regulations pertinent to their own country as well as jurisdictions, where they hope to trial and market their products. While it may not be required to have a fully GMP-compliant process in FIH trials, in the long run, it is best to develop such protocols as early as is feasible to avoid manufacturing changes that may well have unexpected impacts on product performance. Furthermore, preclinical studies do not need to be done with GMP-prepared cells, but it is recommended that early on in the development of a product moves to GMP are done, as changes in the behavior of the final product can change with such modifications (e.g., Temple and Studer, 2017).

In planning a change from research grade to GMP, consideration needs to be given to the number of cells that are needed per patient (dosage), the number of patients to treat, and the amount of cells needed for analytical testing and retains. This will drive the scale of manufacturing needed that, in turn, determines the format for cell production. Investigators accustomed to working in basic research environments may be surprised when they see how few of their usual tools are available at GMP grade, and when they are, how expensive they are compared to research-use-only reagents. While some institutions have cell handling rooms of a certified quality to allow GMP work to be done within them, other researchers may have to outsource their manufacturing to contract development and manufacturing organizations (CDMOs). Some CDMOs allow investigators to put their own people into the GMP facility (the so-called person-in-plant model), and, for others, investigators must train the CDMO staff in their manufacturing practices, which may be labor intensive if the CDMO has no experience with the protocol being used.

Another important aspect to consider is supply chain management. Before embarking on a clinical trial, it is critical to ensure that all the raw materials are manufactured to GMP or the highest quality possible and will be available for the duration of the trial's manufacturing phase, at a minimum. Linked to this is the need to make sure that the reagents so made do not pass their expiry date during the manufacturing process, especially if the manufacturing process is a lengthy one. Finally, because reproducibility is a key element of GMP, it is critical to have well qualified assays that can be used to test the cellular product, both in process and at release.

Another consideration is how the dose will be prepared for administration to the patient. An ideal scenario would involve a cell or tissue product that could go directly from thawing the cryopreserved material (or the fresh cellular material, if no cryopreservation is used) into the patient. However, in many cases the product will need to be washed, centrifuged, and loaded into an appropriate delivery agent. In either case, significant attention needs to be given to training staff to reliably perform each activity. Depending on local regulations, this may require a GMP-compliant clean room for on-site preparation.

In addition, consideration needs to be given to a potency assay for the final cell product. Potency is a critical quality attribute that is related to mechanism of action and is predictive of therapeutic outcome. This can be difficult to develop, given that many therapies will consist of cells that differentiate post grafting into the desired phenotype. For example, in patients receiving hPSC-derived dopamine cell transplants for PD, the grafted cells are not dopaminergic neurons, but rather dopaminergic neuroblasts that are unable at this stage of development to produce dopamine or express markers of this neuronal type. As such, there has been a need to develop predictive markers of dopaminergic differentiation using preclinical models that can then be used to test the final product being used in a trial (Kirkeby et al., 2017).

Finally, some consideration is needed on the amount and type of preclinical animal data that supports the therapy being taken to a clinical trial. Ideally, the GMP-derived product should be tested in the most appropriate preclinical model to show efficacy (outside of any requirements from the regulatory agencies on safety and biodistribution, etc.). This presents several challenges, including;

(1) the absence of animal models that faithfully recapitulate human disease;
(2) the dose of cells being given in such models will be less than those needed in patients;
(3) the size of structure being treated in the animal compared to that which will be needed in humans;
(4) the problems of looking at delivery devices for implanting such cells (see below); and
(5) the fact that any such experiments will be xenogeneic and the issues this creates around differentiating between a failure of cell survival for non-immune reasons vs. rejection.

As such, most groups have to make do with preclinical data that often poorly corresponds with the clinical scenario. Nevertheless, the judicious use of rodents and in some cases large animal models of disease can help answer many of these questions, especially if the mechanism of action of the therapy is well defined. For example, dopamine cell therapies in PD can be tested in dopamine lesioned rodents and non-human primates (Kikuchi et al., 2017; Kriks et al., 2011), which although not modeling idiopathic PD, does nevertheless model this aspect of the disease being treated.

THE USE OF DEVICES FOR DELIVERING THERAPIES

An important consideration in cell-based interventions is determining whether a specialized device is required for the delivery of the stem cells or their differentiated product to the patient. This is not a trivial issue, as delivering the cells with an ill-suited device may significantly compromise the therapeutic value of the product.

In many cases, FDA-approved and/or EU CE-marked devices are commercially available and already used routinely in clinical practice, albeit for other approved therapies. In this respect, it should be noted that if a device is approved to deliver one type of product, one cannot automatically assume that it can be used to deliver a different product, even if the investigator thinks they are similar. By way of illustration, most clinical trials involving the trans-vitreal delivery of a suspension of stem cell-derived cells to the subretinal space in neurodegenerative diseases of the retina, such as age-related macular degeneration, have been conducted with commercially available retinal surgery instruments (Nittala et al., 2021). In contrast, cell therapy trials for PD have often used bespoke non-CE-marked instruments that have limited capacity to be used outside the institution where the device was manufactured (Barker and TRANSEURO consortium 2019).

In situations where non-FDA-approved or non-CE-marked specialized catheters, cannulae, or other devices are required to deliver a cell therapy product, additional steps are needed before their use in a clinical intervention. Most medical devices are classified by the FDA as class 2 (e.g., powered wheelchairs, etc.) and class 3 (devices that sustain or support life, are implanted or present potential unreasonable risk of illness or injury such as implantable pacemakers) (https://www.fda.gov/medical-devices/consumers-medical-devices/learn-if-medical-device-has-been-cleared-fda-marketing). If an entity can provide evidence that its device is substantially equivalent (SE) to another device already approved for marketing by the FDA, a 510(k) clearance process can be pursued for class 1 and 2 devices. In this case, clinical investigations and laboratory studies are not required before the use of the device in patients. Premarket approval (PMA), in contrast, is for class 3 devices, cases where SE cannot be proven and is a rigorous and more time-intensive process that typically requires human trials and laboratory studies.

The FDA route designates not only that the device poses no risk to the patient, but also that the device performs in a way that is consistent with its marketed use. In contrast, European CE marking affirms only that the device meets "high safety, health, and environmental protection requirements" (https://ec.europa.eu/growth/single-market/ce-marking_en). Thus, the speed with which devices are accepted by different regulatory agencies is not the same. In the case where the cell therapy product (biologic) requires a non-cleared 510(k), a non-PMA device for delivery or it is integrated into the product, the FDA designates the cell therapy and delivery device be viewed as a combination product. In this case, an Investigational New Drug application is normally required before approval, since the primary mode of action is based on the effects of cell therapy (https://www.fda.gov/combination-products/about-combination-products/frequently-asked-questions-about-combination-products#CP). In contrast in the EU, it is only considered to be a combination if truly a combined therapy and device. Finally, entities that manufacture, import, or distribute medical devices are subject to claims and lawsuits that arise from malfunction. For this reason, obtaining product liability insurance for medical devices remains critically important.

In some situations the regulators, as well as the investigators, need reassurance that the device being used accurately delivers the cells at the site and dose needed as well as in a viable way. Such work cannot be done in rodent species given the size any such device would be for use in people. As such, large animal experiments may be required, with the attendant costs that this brings with it.

WHAT WILL MY FIH TRIAL LOOK LIKE?

The design of the FIH clinical trial is important to consider early in the translational pipeline as it may take some time to identify and recruit the most appropriate patients for any such trial, and it may also influence the design of preclinical studies required for approval. Although the design of any trial is dependent to a large extent on the disease being treated and the therapy being given, the following key issues should be considered.

Which patient cohort or subtype of disease group should be targeted in FIH trials, why, and what is ultimately the optimal market population?

It is critical to know what disease one is hoping to treat and select those patients most likely to benefit from the therapy while being cognizant of the ethical aspects linked to FIH trials. This includes identifying the subtype(s) of patients who are most likely to respond to the intervention. For example, it may be that the optimal group to benefit includes patients with early stage PD who have responded well to oral L-dopa therapies (Barker et al., 2017, 2021), but have yet to develop complications from such therapies. However, treating such early stage patients, who are relatively well, with a new therapy injected directly into their brain would raise concerns with ethical committees, and thus FIH trials may only be allowed in more advanced patients where invasive therapies

(such as deep brain stimulation) are being considered anyway. However, after the initial feasibility, tolerability, and safety FIH trials, it is possible to argue for a slightly different patient cohort, although the reason for doing so needs to be clearly explained for the agencies to accept it. After approval, applying for changes in the therapeutic indication of a cellular product should be possible as for any drug therapy.

What period of run-in is ideally needed before grafting?

In many instances, having a group of patients in an observational study ahead of any intervention is useful as this will:

(1) allow for a cohort to be ready when the product with or without a device is approved for the FIH trial;
(2) enable one to have a stable baseline of measures in patients and minimizes any practice effects with assessments;
(3) allow one to compare individual patients disease progression before and after an intervention; and finally
(4) give an indication of patient commitment to the trial.

However, the use of a run-in period is not possible in all cases, such as in patients needing treatment for acute disorders (e.g., myocardial infarction or spinal cord trauma) or in those conditions where the disease progression is rapid. Indeed, in this latter case, there is a risk that having a run in period creates a bias in the sample being treated as those with slower disease progression are ultimately only recruited, creating a survival bias.

What assessments will need to be done to look at tolerability and safety in the FIH trials?

This is very dependent on the product, the disease being treated and mechanism of action but at the very least one needs to have robust measures of safety—both subjective and objective ones—as well as plans for long-term follow-up given that, in most cases, the implantation of the cells is irreversible. At its most basic level, tolerability/safety involves having as the primary endpoint the number of adverse events (AEs) and serious AEs and crucially involves looking for any abnormal proliferation of transplanted cells either at the site of delivery or elsewhere if delivered systemically.

What exploratory measures will be used to look for any signal of efficacy or target engagement?

While any FIH trial cannot hope to show definitive efficacy, it is critical to collect (as secondary or exploratory trial endpoints) some data that support some signal of efficacy and/or target engagement. Thus, being able to accurately quantify cell survival and differentiation *in vivo* using non-invasive imaging would be useful. Showing some clinical effect on a relevant endpoint is also critical for planning both phase II and III trials that are aimed at establishing dose-dependent efficacy and improved responses compared with standard of care. It is also advisable to keep FIH trials relatively simple by avoiding a large number of primary and secondary endpoints, surrogate measures, and correlative studies and to concentrate on those that are meaningful to the patient and the therapeutic candidate.

How big will the trial be in terms of patient numbers? How many sites and do they have any experience with advanced therapy medicinal products?

Trying to limit the variables in any FIH trial is critical, and thus minimizing the number of sites where it will be done is one way of trying to achieve this while also trying to ensure that enough sites are involved so that any inter-site variability can also be assessed. In addition, for FIH trials the numbers of patients treated is normally very small given the exploratory nature of what one is doing and thus can normally best be done at two centers only- ideally with teams that have some familiarity with advanced therapies of the type being tested.

Who will sponsor, oversee, and fund the trial? If using a device, who will be the legal manufacturer, and will they take on this responsibility for all proposed sites?

A whole series of contractual and procedural issues need to be resolved, and it is useful to consider these early in the planning of a trial as these can add substantial delays. This in part relates to the fact that many institutions have not trialed such therapies in the past and so are naturally nervous about the risks they are signing up for and the burden such a trial will place on the hospital. This aspect of the work necessitates working with a clinical trials unit who will then be involved in overseeing the trial and helping set up the necessary regulatory elements for it. Linked to this is the need to have secure funding for the duration of the trial and patient follow up as well as insurance coverage. In addition, establishing an independent and committed data monitoring committee (DMC) and trial steering committee (TSC) will be needed.

How long will patients be followed?

Finally, the duration of follow up may be dictated by national agencies, but it is strongly encouraged that all patients entered into FIH trials are followed long term, ideally to death or organ transplantation, with efforts made to collect declaration of intent for organ donation so histology can be done of the treated organ system. Such postmortem information can be invaluable in understanding how

the tissue has survived and integrated as well as the host reaction to it (although in some cases such information could be obtained through small biopsies in treated patients). Thus, liaising with pathological services capable of collecting such tissue and undertaking the necessary analysis is strongly advised, however untimely it might seem at such an early stage of trial development.

PRODUCT DEVELOPMENT: GETTING TO MARKET WITH THE THERAPY

Developers of advanced therapy medicinal products (ATMPs) meet several challenges during the development phases, including technical, scientific, financial, regulatory, and clinical hurdles as well as uncertain reimbursement perspectives.

A substantial number of challenges are driven by the novelty of the field, new or orphan indications, and technical and scientific uncertainties. Indeed, worldwide, there are fewer than 200 approved ATMPs or regenerative medicine products (called ATMP in the EU, Cell and Gene Therapy in the United States, or RMP in Japan) (Ramezankhani et al., 2020; Kurauchi et al., 2020; Iglesias-Lopez et al., 2019). To develop such products, it is essential to familiarize oneself with previously approved products and lessons learnt with their development (Kurauchi et al., 2020).

What is the medical need for your product?

The identification of the medical need lies at the heart of finding partners to get the product to market and for a positive health economic evaluation. Certainly, the development of complex and expensive therapies is justified only when standard therapies have definite limitations, are unavailable, or are associated with AEs, low success rates, or high recurrence rates. These conditions justify a certain amount of risk or uncertainty because the necessary investments are offered only when a real prospect of success-related reimbursement exists, assuming the therapy is approved.

In addition, for products targeting serious conditions with an unmet medical need, developers can look into expedited developmental plans for approval by regulatory authorities. In the case of orphan drugs, which represent more than 80% of these products (Kurauchi et al., 2020), a lower fee is available and conditional approval can be envisaged. Developers can use the PRIME scheme in the EU, the SAKIGAKE scheme in Japan, or the Breakthrough Therapy/Fast track in the United States, for expedited development programs, possibly demonstrating the potential to address unmet medical needs or substantial improvement over available therapies. In addition, in the United States there is a third designation for an accelerated path toward market approval that is particularly relevant for pluripotent stem cell-based products called the Regenerative Medicine Advanced Therapies (RMAT) designation. RMAT approval allows products with a successful phase II clinical trial to begin being sold or reimbursed, although it still requires a phase III trial for full market approval. Additional accelerated market access can also be obtained by conditional approval, which can be granted before the end of clinical trials. In the EU, it must be renewed annually, whereas in Japan, it can be effective for 5–7 years.

How is your product classified and what does this mean for its development?

Product classification is another step that affects the developmental plan. The classification of a biological product is not always easy; in some cases, it may be challenging to define. It is worth noting that the correct classification early on will determine the regulatory framework governing the product developmental plan. Different regulatory authorities offer scientific advice to developers for ensuring correct classification before furthering process establishment and thus early consultations with agencies are recommended. Scientific advice further enables the exchange of knowledge for a bilateral understanding of the process or product and propaedeutic to developmental planning for marketing authorization.

How will you get to a market authorization if your early trials succeed?

No investor or funder of an ATMP is interested in FIH study results unless there is a clear roadmap to market if it succeeds in clinical trials. For this, a number of issues need to be considered.

Clinical development

With regard to clinical translation, knowledge of the mode of action strongly supports product development. Knowing the mechanism of action allows one to establish critical quality attributes that must be preserved during manufacturing, regardless of scale, as well as potency assays that are essential for regulatory approval. The misinterpretation of the mechanism of action of certain ATMPs has resulted in the unusual situation of a decrease in the effectiveness of the therapy as the therapeutic progresses through clinical trial phases.

Clinical development is also affected by the different approaches of the various regulatory authorities. For example, the US regulatory agency oversees the clinical trials, whereas the EMA oversees the presented data. This in turn can create variable concerns regarding trial design, such as the different approaches to dose definition, which is mainly preclinical in Japan and derives from clinical data in Europe. There are also different approaches to control groups and sham surgery.

For the development of ATMPs in Europe, clinical trial applications are submitted to each national competent authority where the trial will take place. Some challenges can be related to country-specific requirements; therefore, a comparative evaluation of rules from different regulatory bodies can support cell product developers. The various national competent authorities often produce diverse scientific advice; to address this variance, the EMA may apply for a voluntary harmonization procedure. Finally, in clinical trials, surgeons are often considered to be investigators and clients from a commercial point of view and suppliers of the starting material for the whole process. Hence, some regulatory authorities may request official quality training on clinical procedures—from biopsy to follow-up. Such training can decrease the variability among surgeons, physicians, and nurses engaged in clinical trials and the variability in the widespread application of the therapy, thus decreasing the negative impact on time and costs.

Cost

The cost of ATMPs is a critical issue in therapy development. This is important for partners in getting the products to market and its survival thereafter.

In addition to the parameters mentioned, such as product development costs, it seems that several products (such as those in the gene therapy space) can be charged according to their overall benefit on the lifetime of the patients (i.e., saving of the costs incurred without such a curative therapy being available). This leads to costs often quite disconnected from the actual cost of goods or cost of development and could well be a model applied similarly to stem cell-based regenerative products that may require only one or few interventions. However, other factors will need to be considered in the reimbursement and access to the product (see below).

As previously described, product development has a significant influence on the cost of therapy. The high price of these therapies results from the complexity and time needed in their manufacture, quality control, intellectual property development and maintenance, preclinical and clinical development, and all related regulatory issues.

The most common picture is that early development of new ATMPs occurs in academia and hospitals, mainly with public funding. Small medium enterprises are included in the early stage of development but ultimately large companies need to be involved as they can provide the financial security and expertise in the data needed to acquire reimbursement.

Reimbursement

The reimbursement of ATMPs is considered another major obstacle because of their expense. However, positioning cell-based treatments in daily clinical practice means defining appropriate patient target groups, identifying responders through biomarkers or bioassays, and collecting all patient data and follow-up, and lays the foundation for a successful reimbursement discussion in the future.

New payment models also include the pay for success scheme. This entails that the costs be repaid by the traditional payer if the final evaluation shows that the therapy achieved agreed-upon outcomes. Pay for success centers on certain principles, such as clearly defined outcomes, data-driven decision-making, and strong accountability. This model has already been applied to some cell-based products available in the market.

Cost and market size (including accessibility to patients with rare diseases) are the two main issues that can guarantee product survival in the market and attract investors.

CONCLUSIONS

In this short *Perspective*, we have tried to draw together experiences from the authors, all of whom are involved in taking cell-based therapies to FIH trials in patients with a range of conditions. We have highlighted the key issues that we have encountered on this journey; the lessons we have learnt to date while doing this and what is still to be learnt. Obviously, such an article cannot cover all issues and eventualities given many of these are cell type, disease, and country specific, but we hope that we have covered issues that those working in this space will find useful.

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CONFLICT OF INTERESTS

C.J. is a co-founder of Aspera Biomedicines and Impact Biomedicines and receives royalties from Forty Seven Inc. C.M. is a senior vice president and equity holder in Sana Biotechnology. G.P. is a consultant for J-TEC, Gamagori, Japan, a member of the board of directors, and R&D director of Holostem Terapie Avanzate, Modena, Italy. J.S. is the founder and CEO of iPS Bio, Inc. The remaining authors report no conflicts.

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