CELL & GENE THERAPY INSIGHTS

PRECLINICAL & TRANSLATIONAL INSIGHTS

SPOTLIGHT

From laboratory to patients: dissecting obstacles in cell & gene therapy development

EDITORIAL

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Looking at the cellular therapy landscape, there are more 34,000 clinical studies reported in https://clinicaltrials.gov (searching for 'cellular therapy' closed, past, active, enrolling, non-enrolling) [1]. While this number most probably over-estimates the clinical impact of cells as therapeutics, it is beyond doubt that cells are progressively entering into the

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clinical scenario for a variety of clinical indications [2].

A recent report by The Alliance for Regenerative Medicine (ARM) describes more than 900 cell and gene therapy companies worldwide currently carrying out 1052 clinical trials: 363 phase I, 594 phase II and 95 phase III [3]. While these studies do cover the full spectrum of human diseases, the great majority deals with oncology (65%), compared with only 5% for each of neurological and skeletal diseases.

Among cell-based products (Figure 1), mesenchymal stromal/stem cells (MSC) represent a promising option for a variety of clinical indications, with approximately 1,000 studies overall, 250 recruiting investigations, and 9 MSC-based products with market authorization [2,4,5].

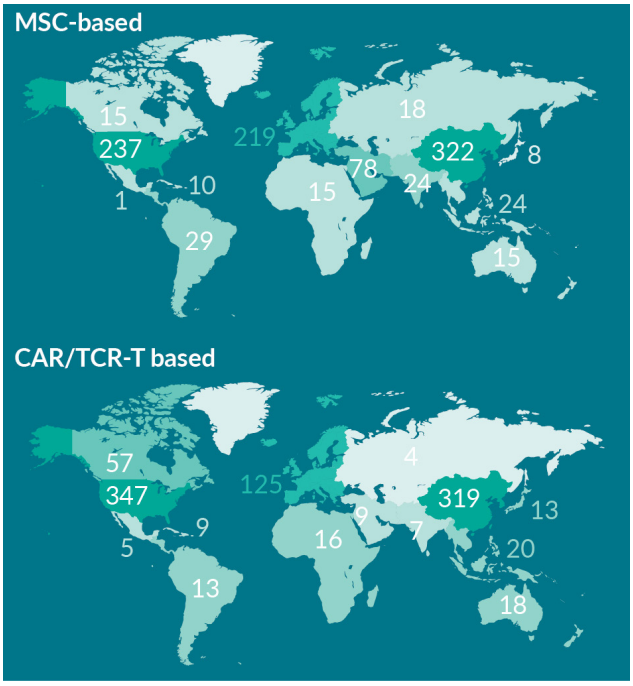
The second cell-based product area that has rapidly emerged as real a driver in cell and gene therapy is the chimeric antigen receptor (CAR)/ T-cell receptor (TCR) engineered cell therapy field; here, approximately 900 studies are reported with about 400 recruiting [6]. However, despite all these increasing numbers only 2 CAR-T cell-based products have so far obtained market authorization [2,7].

Focusing on oncology, we and others uncovered that MSC can be redirected to target tumors, becoming powerful anti-cancer molecules that deliver tools increasing the microenvironment bioavailability upon specific recognitions. Starting from this concept, we developed two main strategies based on both gene modified MSC and lymphocytes.

On the one hand, we aimed to modify MSC inducing expression of ligands capable of generating selective cancer death [8,9], and on the other, to modify lymphocytes by CAR targeting solid tumors [10]. In the first case, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) variants can be delivered to different cancer models, such as pancreatic adenocarcinoma and sarcomas. Here, adipose MSC were armed by lentiviral vectors to target a large variety of tumor lines and primary cancer cells both *in vitro* and *in vivo*. We showed that MSC can successfully deliver TRAIL variants to rapidly induce tumor death thanks also to synergizing chemotherapy agents within a novel combinatory strategy of multimodal chemo-gene therapy.

FIGURE 1

MSC and CAR-T cells reported clinical trials.



https://clinicaltrials.gov, Feb 2020.

Regarding CAR-T and TCR-T, we were able to generate lymphocytes modified to successfully target neuroblastoma, glioblastoma, hepatobiliary diseases and epithelial cancers [10–12]. Finally, in the attempt to further refine the MSC delivering approach, for the first time we included a CAR on the surface of MSC delivering TRAIL variants [13].

Moving these concepts from academic research towards a clinical translation strategy, we have been challenged by a variety of issues including the foundation of a university start-up (Rigenerand srl) that, with more than 500 sqm of classified environment in a cell factory, aims now to produce cell and gene therapy products for solid tumors. For this reason, we wanted to share the challenges that have been faced (and that we are still facing) during the development of MSC

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BOX 1

Key challenges in cell-based translation from laboratory to patients

1. Cells-related obstacles
2. Process and scale-up related obstacles
3. The "original sins" of academia

and CAR/TCR-T projects. We have classified these into 3 main obstacle areas (Box 1). While this may be somewhat reductive, we hope it may have utility for cell therapists around the word.

CELL RELATED OBSTACLES

Cell-based products are classified as drugs accordingly to regulators [14,15]. While this warrants high manufacturing standards and safe clinical translation, it does not completely encompass that cells are living entities coming from human subjects that have both intrinsic and acquired variabilities. These varibilities can impact on cell-based product translation towards a solid clinical scenario, and need to be considered when developing cell-based therapeutic approaches from early laboratory discovery onwards, as is the case for (gene modified) MSC and CAR/TCR-T therapies.

In this sense, there are several issues that can impact on products' clinical translation, as represented in Table 1. In particular, the source of cells can directly and indirectly influence clinical translation. For MSC, different tissue sources are associated with different performance *ex vivo* and *in vivo* [16]. In the case of TRAIL, for instance, the bone marrow derived MSC could not perform as well as adipose MSC while producing TRAIL (unpublished data). Also, for CAR/TCR modified cells, the cell source may make a relevant difference, as is the case when considering peripheral blood or cord blood cytotoxic CD8+ cells or NK cells. This may also impact upon the possible clinical uses (i.e allogeneic, autologous) [17–20].

Similarly, different individuals may see differing performance in terms of *ex vivo* proliferation, viral transduction and *in vivo* therapeutic potential [21]. For MSC, it is known that a fraction of the cells are incapable of generating bone, while for CAR/TCR-T cells, a fraction of patients' lymphocytes may not be efficiently manipulated and transduced [22,23]. Regarding MSC, this calls for ways (i.e., potency assays) to predict the mechanisms of action, either in the unfortunate circumstances of an autologous setting, or in the more dramatic situation of allogeneic cells obtained from a single non-performing donor. The issue of predicting cell performance may also be related to the capacity to predict

TABLE 1

Key issues impacting on MSC and CAR-T clinical translation

Issue	Impact on MSC clinical translation	Impact on CAR/TCR cells clinical translation
Different sources to different cell type	+++	++
Different donors to different performance (variability)	++	++ (some patients cannot generate CAR-T)
Defining MoA to differentiation soluble factor/s secretion, EV	+++	++ (more on side effects)
Animal models to challenge cells performance	++	+++ (more on side effects)
Delivery method: i.v., i.a., i.p. endotracheally etc.	+++	+ (hematological diseases) +++ (solid tumors)

Relevance. +: poor; ++: relevant; +++: very relevant.
MoA: Mechanism of action; EV: Extracellular vehicles.

side effects after CAR/TCR-T infusion, since the current pre-clinical *in vitro* and *in vivo* therapeutic models are not yet able to fully mimic the human clinical scenario.

Finally, the delivery method may also impact clinical translation, not so much for CAR-T in hematological malignancies, but more for solid tumors where the homing of the infused cells has yet to be properly address. Similar situation applies for MSC targeting cancer. In our case, to avoid bias due to cancer homing, we decided during pre-clinical development to move towards an intra tumoral infusion of MSC-TRAIL [8].

PROCESS AND SCALE-UP RELATED OBSTACLES

The translation of cells towards the clinic is not generally dissimilar to any other biotechnological product and involves a series of generic steps.

There is a **first step of concept** evaluation to generate basic research that can be transferred into pre-clinical investigation (generally *in vitro*). This is then followed by the creation of 'proof-of-concept' *in vivo* studies, where cell survival kinetics/ distribution and toxicology studies are carried out to define a cellular product's desired characteristics.

In this phase, rudimentary methods of cell manufacturing (i.e., for animal studies in the best-case scenario) and product testing are generally conceived, and appropriate laboratory instrumentation and reagents are identified. However, even in this early phase, obstacles in the translation of cells towards the clinic may be apparent, such as the fact that technologies used in early studies may not be optimized or transferrable for larger/clinical-scale cell production. In particular, the selected reagents (i.e., tissue culture media, dissociation and ancillary reagents) in the labs may not be suitable for human uses, and cell features may not be robustly defined. While all these aspects have been generally tolerated in the discovery phase, current advancements in the field call for special attention to be paid for solid translation from the early phases of academic research around cell therapeutics.

In a **second step** of process development, there is a **phase of scale-up and optimization**. This is associated with the development of a reproducible, large-scale manufacturing process that parallels the creation of a clinical study design. This also involves translational development into a cGMP laboratory to develop tools, to scale-up the process, and to optimize manufacturing (incorporating regulatory-grade product characterization and QC). In the case of gene therapy, this is also linked with viral vector development and manufacturing, conducted either in house or by CMO. Here, there is also the transition from laboratory experiment book to SOP, and in the best-case scenario, batch production records (BPR) are developed.

This second step is the most critical part of a cell-based product's development. It might be described as the 'teenage years' of the product: lots of excitement for things to come, but also severe uncertainties about the future. Here, research-based methods are frequently not perfectly refined, and the experimental book from "academic wild lab" may be difficult to translate into SOP, hindering a smooth transition into cGMP manufacture. Importantly for gene therapy, viral vector comparability between R&D grade and cGMP grade may become a relevant issue to be accounted for not only for safety reasons, but also for the vector backbone, for the type of promoter, and for manufacturing steps that would allow generation of an adequate quantity of vector to execute early clinical trials in the respect of related regulatory issues (e.g. gene copy number).

Theoretically, this phase has to be carefully designed from the start by cell and gene therapy developers since obstacles here may be so relevant to, and dramatically impair, product development. To underline the critical nature of this moment (generally taking place during Phase I/IIa trial preparation and execution), clinical trial design, patient selection, delivery methods and read-out must also be defined

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and presented to regulatory bodies within pre-IND and IND.

Finally, there is a **third very advanced step with methods validation**. This begins when a clinically appropriate and optimized method has been defined. Here, SOP are established and a validation plan is generated and executed. In this phase, the CMC section of the investigational new drug application and the validation results are provided to the regulatory body and to the institutional review board for approval. The major obstacle is related to the fact that the CMC of a living cell-based drug is challenging, in particular for autologous products [24].

THE 'ORIGINAL SINS' OF ACADEMIA

Since most cell-based product have emerged from academia, they may carry what we call the "original sin" of academia. In some cases, GMP laboratories in Hospitals/Universities spin out from research and development laboratories, and so may carry the "bugs" of early academic research. This is not necessarily a negative aspect, but it does require a cultural step in the direction of cell manufacturing, cell culture standardization and quality control/assurance.

Aspects related to academic cGMP facilities may negatively impact large cell-based product development in that environment: limited space, small teams and underestimation of long-term sustainability of the maintenance costs and personnel. Other, not inconsiderable aspects relate to regulatory and economic hurdles for phase I/II studies that are generally developed with little consultation with regulatory experts. This may generate obstacles when the product would be moving from a first-in-man study towards advanced Phase II/III studies.

Finally, while academia generally dreams of partnering with biopharmaceutical companies that would empower the process/product with required investment, this tends to take place only very late in the process and scale-up development. This in turn may have a negative impact on the "cGMPification" process and the subsequent industrialization. Therefore, there is the need to combine early academical research with the biopharmaceutical world from the earliest phases of cell (and gene) therapy product development, and in particular, from the beginning of the second step described above.

HOW TO FILL THE GAPS (OR BEGIN TO...)

Having recognized these obstacles during the transition from a research laboratory to a cGMP manufacturing environment, we would like to propose some strategies on how to fill the gaps:

1. to conceive and foster dedicated training programs in collaboration with stakeholders (Universities, Regulators, Scientific Societies, such as ISCT and Pharma Industry) to train scientists to think as cellular therapists from the early steps of concept evaluation;

2. to create dedicated know-how (i.e about media, reagents, instruments) around the translational processes in cell and gene therapy;

3. to continue facilitating and enforcing phase I/IIa studies performed at the academic level by accelerated regulatory pathways;

4. a better (earlier) integration between academia and pharmaceutical industry;

5. to create non-profit national/regional Authority/ies to facilitate the early links between academia and industry;

6. to allocate financial resources for infrastructure and maintenance by national/regional founding bodies favoring networking between research laboratory and cell factories;

7. to allocate financial resources supporting laboratory services for a number of cGMP facilities;

8. to identify novel *ex vivo* cell manipulation procedures/devices (i.e close systems, bioreactors, isolators) capable of delivering innovative, consistent, safer and sustainable cell manufacturing and gene modification steps.

Recognition of these challenges and the proposed strategies may represent fundamental first steps towards faster, safer development of cell and gene therapies for patients with unmet medical needs.

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AUTHORSHIP & CONFLICT OF INTEREST

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