Revamping Regulations

Safety information is required to allow clinical development of stem cell products, but clearer guidance concerning safety testing requirements is still needed

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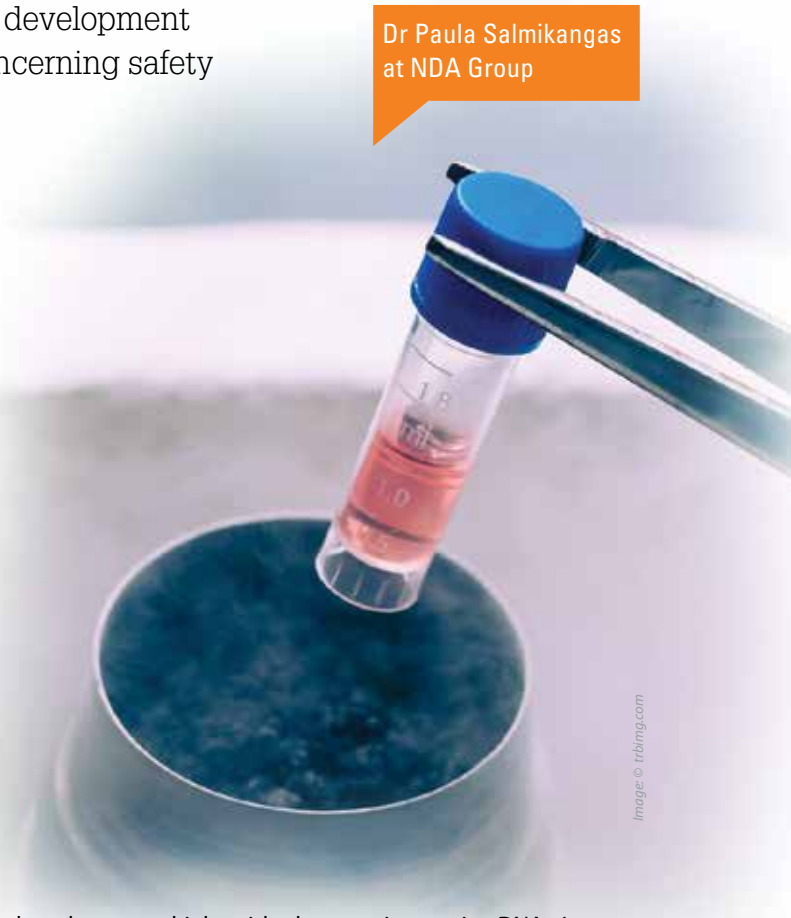

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Stem cell products hold great promise for multiple clinical indications and are actively studied in clinical trials (1). However, many related safety features raise concerns and currently generate extensive and costly testing of the final product, if intended for clinical use. The regulatory expectations for stem cell testing for clinical studies, as well as marketing authorisation applications, are not entirely clear, up-to-date or detailed. Furthermore, differences between US and EU guidance, in this respect, hamper the current development of products for EU markets.

Risk Profiling

Stem cells used for therapeutic purposes include a wide variety of cells with different type differentiation capacities, spanning from restricted multipotent up to pluripotent stem cells (2). Hematopoietic stem cells have been widely used for *ex vivo* gene therapy of monogenic inherited diseases, and mesenchymal stem cells have been utilised – eg in treatment of Graft versus Host Disease – for more than a decade due to their immunomodulatory properties (3,4). Pluripotent stem cells, ie embryonic stem cells and induced pluripotent stem cells (iPSCs), are still in early clinical development, yet some clinical data are already available (5,6).

When considering possible risks of stem cells, several issues need to be considered, such as the donor (autologous versus allogeneic), characteristics of the cells, extent of manipulation, design of vectors for genetic modification, dose and the existing knowledge of the cell type/product to be used. One of the main concerns related to stem cell products is tumourigenicity. For pluripotent stem cells, teratoma formation is part of their natural characteristics, which calls for thorough control of the cell differentiation status before clinical use.

For other stem cells, the tumourigenicity risk is mainly due to the manufacturing process – genetic modification, use of growth factors and lengthy *in vitro* culture – and may be controlled through design and validation of the production process. However, analysis of the genetic stability of the cells is recommended (7). Other possible potential hazards include immunogenicity and ectopic tissue formation. These risks are difficult to evaluate through final product testing and usually require separate non-clinical studies and close patient monitoring during clinical studies and in the post-marketing phase.

When stem cells are genetically modified, the vectors may increase the risks depending on the type used. In general, integrating RNA-viruses (retroviruses and lentiviruses) are thought to pose higher risks than non-integrating DNA-viruses (adeno and adeno-associated viruses), yet recent modifications of lentiviruses suggest their safety profiles have greatly improved (8). Nevertheless, replication competency of non-replicating vectors and possible genotoxicity of integrating vectors remain problematic.

Pluripotency

According to the guideline on human cell-based medicinal products, "cellular components should be evaluated for their tumourigenic potential by analysing e.g. proliferative capacity, dependence on the exogenous stimuli, response to apoptosis stimuli and genomic modification. Karyology and tumourigenicity testing of cells derived from a cell culture/cell banking system may be required" (9). These analyses are expected as part of characterisation studies, but if the stem cell products are autologous, some testing is inevitably expected at the drug product (DP) level. Possible analytical tools and requirements therein are not clarified, nor are the number of cells that would be required for testing. The reflection paper on stem cell-based medicinal products states that expanded stem cells are often administered in a differentiated state, yet it is acknowledged that multipotent stem cells may still be in an undifferentiated, proliferative state at the time of administration (10). In such cases, additional testing during development

is expected. This approach is unclear and confusing, as characterisation studies in such situations do not ensure full differentiation status of each cell dose to be administered, especially in the case of autologous iPSCs.

The US/FDA *Guidance on Preclinical Assessment of Investigational Cellular and Gene Therapy Products* requires that the potential for tumourigenicity, dysplasia or hyperplasia to occur is considered and addressed for each investigational cell therapy product (11). The document lists factors that may impact tumourigenicity assessment, including the differentiation status profile of cell types within the cell therapy product – ranging from undifferentiated/embryonic to terminally differentiated/specialised – and the growth kinetic profile. These factors seem relevant as analysis targets for the stem cell products, but such requirements for DP testing are not described in the applicable guidelines.

A specific meeting to discuss the genetic and epigenetic changes of human pluripotent stem cells intended for clinical use, including the significance of the findings for safety assessment, was organised in October 2016 (12). As a result, an international advisory group was proposed to assess established pluripotent stem cell lines. The work should be expanded to identify what testing is required at product level and how reliable results are achieved, taking limited sample sizes into consideration. Additionally, dialogue between such a group and regulatory experts would be necessary to identify the needs for analytical testing to ensure patient safety and avoid unnecessary regulatory hurdles early on. Furthermore, the methodologies and their capabilities or sensitivities should be explored to support clinical translation of these products.

Testing for Replication Competent Viruses (RCV)

Both US and EU guidelines on gene therapy identify the need to test viral vectors rendered non-replicating for any replication-competent viruses (retro/adenoviruses) (13,14). In this respect, the FDA guideline is quite detailed in terms of volumes/cells to be tested and expects that RCV testing is performed both at the level of the vector and the DP. From the product, the recommendation is to test 1% of the total cells or 10^8 *ex vivo* transduced cells (whichever is less). For example, considering genetically modified CD34+ cells – and also CAR-T cells, yet not stem cells – the testing requirement appears quite high and may even impact the final achievable dose for the patients.

At the same time, the donors are tested for viral contaminants, and infected patients are usually excluded from treatment. Data from past gene therapy trials are reported with no evidence of replication-competent viruses or virus reactivation when properly controlled vectors are used (15). RCV testing is labour-intensive, time-consuming and costly, and the negative impact of the testing requirements on the clinical development of genetically modified cells was raised years ago (16). Perhaps it is time to look into past experience with different vectors and update the requirements where possible.

Insertional Mutagenesis

Insertional mutagenesis is a recognised safety concern of gene therapy products. The risk is low or even theoretical for non-integrating vectors like adeno-associated virus vectors. For retroviruses and lentiviruses, the risk is higher and several cases leading to oncogenesis have been reported (17,18). In all cases where oncogenesis has been identified, the vector integration sites have been close to proto-oncogenes, leading to their activation and clinical manifestations.

According to FDA guidance on gene therapy clinical trials, hematopoietic stem cells – when transduced with an integrating vector – should be monitored for clonal outgrowths and vector integration sites when technically feasible (19). Similar expectations are presented in the corresponding EMA guidance (17). However, recent vector developments, together with known challenges related to the genotoxicity testing, question the necessity and feasibility of testing for integration sites (8,20).

Update of the Regulatory Requirements

Current regulatory guidelines (both US and EU) require analytical testing to address aforementioned risks. This may be difficult, especially in the autologous setting, due to limited amounts of cells available for analysis. On the other hand, the design of vectors and characteristics of novel stem cell-based products would require thorough evaluation of past experiences and current knowledge to define real testing needs.

Several EU guidelines for advanced therapy medicinal products (ATMPs) – cell- and gene-based products – are currently under revision, providing excellent momentum to review the current requirements against available safety data and consider the unnecessary regulatory burden that may be hampering fast patient access of innovative therapies.

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