ATMPs and Virus Safety

History has shown that ensuring the viral safety of biological medicinal products in regenerative medicine is essential. One should avoid contamination and maintain the quality of the products. Well-characterised cell banks are just one method of assisting this task

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Advanced therapy medicinal products (ATMPs) are for human use, often in the field of regenerative medicine. They can be stratified into one of the following three categories of product: gene therapy; somatic cell therapy; and tissue engineering and regenerative medicine.

Figures 1 and 2 provide an overview of the distribution of ATMPs currently in development or on the market according to clinical application (1). The sponsors of ATMPs differ widely from the classical, heavily regulated, pharmaceutical market (see Figure 3). Currently, roughly 75% of ATMPs are being developed within the non-commercial sector (universities, hospitals, institutes and so forth) – an aspect that adds unique and significant regulatory challenges when guaranteeing that such products comply with the strict requirements for biological medicinal products.

The regulatory arena is an area often new to such not-for-profit organisations and can lead to problems ensuring compliance.

In the EU, ATMPs are regulated as pharmaceutical products under a consolidated framework for advanced therapies, governed by Regulation 1394/2007 (2). ATMPs containing cells, tissues or materials of biological origin need to be manufactured in accordance with the Good Manufacturing Practice (GMP) guidelines for medicinal products for human use (eg Directive 2003/94/EC), ensuring controls over consistency, reproducibility and uniformity. Furthermore, Annex 2 of the EU Guidelines for GMP concerning medicinal products for human and veterinary use (Eudralex Vol 4) has been updated to include GMP specific to ATMPs and recognises the inherent variability and increased risks for microbial contamination and transfer of pathogens with such ATMPs. These ATMP guidelines have raised the possibility of using potentially lower levels of GMP compliance within the hospital setting, for example.

Some examples of already licensed ATMPs are shown in Table 1. Products for regenerative medicine require special handling –

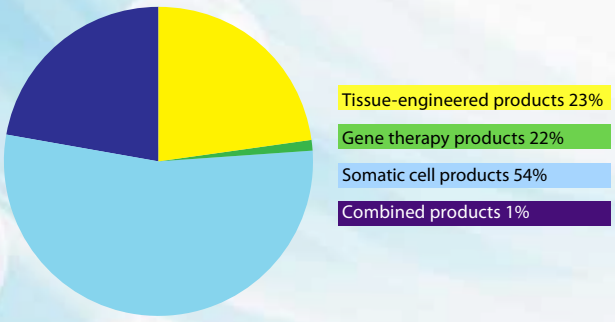

Figure 1: Types of ATMPs and clinical targets

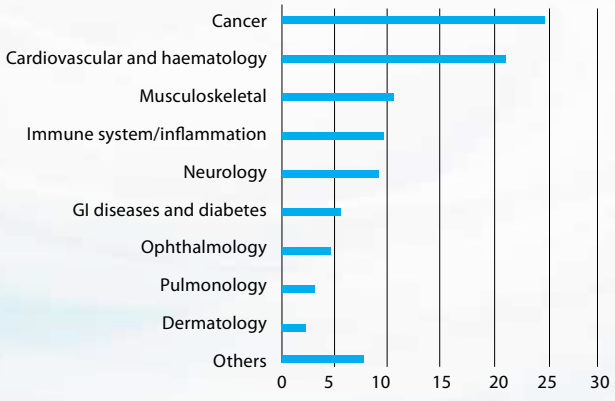

Figure 2: Major disease areas targeted

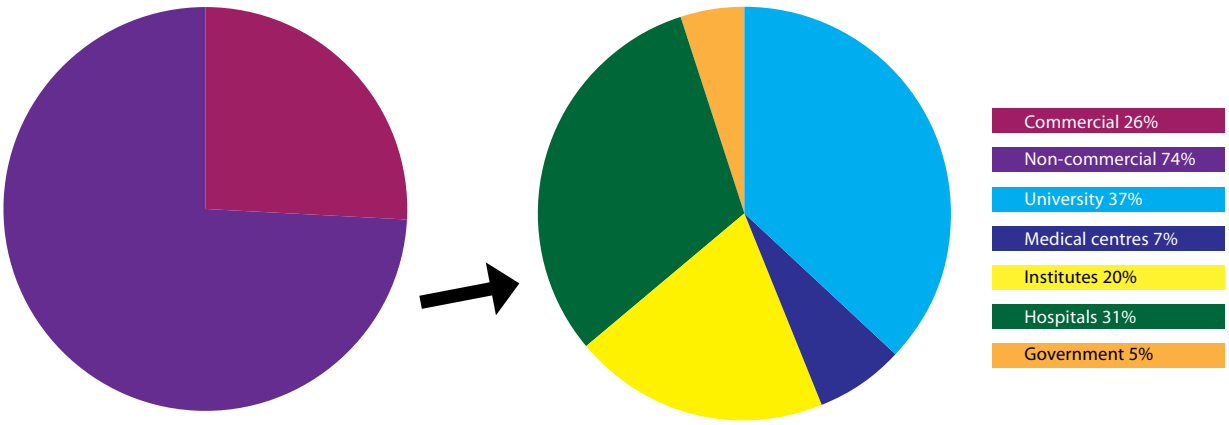

Figure 3: Sponsors of ATMPs clinical trials

often on-site at the clinic – and, under *Regulation EC 1394/2007* (and also Japanese pharma law), such ATMPs are exempt from centralised regulation. Stem cells in this regard have received specific focus.

Although all stem cells share the same principal characteristics of potential for self-renewal and differentiation, they represent a spectrum of different cell types, often limited scientific knowledge and clinical experience available. Mesenchymal stem cells or haematopoietic stem cells have been more extensively used in clinical applications, whereas human embryonic stem cells (hESCs) or induced pluripotent stem cells are less well-studied.

The level of risk associated with specific types of stem cells is therefore often difficult to quantify, but the risks will include:

- Purity of the cell preparation and application of a single cell type
- Tumorigenicity (eg hESCs when teratomas or benign tumours can form in permissive hosts)
- Virus and transmissible spongiform encephalopathies safety, including a thorough understanding of the source history, the virus risk from the cell line and the materials used during manufacture

These aspects have raised serious concerns about the safety and efficacy of stem cell treatments that use poorly defined stem cell preparations, which are discussed further in this article.

Controlling Virus Safety Risks

Given the potentially complex nature of ATMPs, ensuring the safety and quality of such products requires a rigorous, science-based approach. The virus safety of biological products is normally assured through a three-tiered approach, controlling the following aspects of the overall manufacturing process:

- Characterisation of the starting material or additives in the manufacturing process, such as the use of characterised master cell banks or careful selection of stem cell donors

Product	Manufacturer	Type of ATMP	Date licensed	Indication
Chondrocelect®	Sobi/TiGenix	Tissue-engineered	2009	Cartilage defects of the femoral condyle of the knee
Glybera®	uniQure	Gene therapy	2012	Lipoprotein lipase deficiency
MACI®	Vericel Corporation	Tissue-engineered	2013	Cartilage defects of the knee
Provenge®	Dendreon	Somatic cell therapy	2013	Castrate-resistant prostate cancer
Holoclar®	Holostem	Tissue-engineered	2015	Severe limbal stem-cell deficiency
Kymriah™	Novartis	Gene therapy	2017	B-cell acute lymphoblastic leukemia

Table 1: Examples of licensed ATMPs

Virus	Cell	Year	Company	Animal component suspected?
Epizootic hemorrhagic disease virus (EHDV)	Chinese hamster ovary (CHO)	1988	Bioferon GmbH	Yes
Mouse Minute virus (MMV)	CHO	1993	Genentech	Unknown
MMV	CHO	1994	Genentech	Unknown
Reovirus	Human with one kidney	1999	Abbott Labs	Yes
Cache Valley virus	CHO	2000	(not publicly available)	Yes
Human Adenovirus	HEK 293	2002	Eli Lilly	Unknown
Cache Valley virus	CHO	2003	(not publicly available)	Yes
MMV	CHO	2008	Amgen	Unknown
MMV	CHO	2009	Merrimack	Unknown
MMV	Baby hamster kidney fibroblasts (BHK)	2010	Foot and Mouth Disease (Institute of Turkey)	Unknown
Vesivirus 2117	CHO	2003	Boehringer Ingelheim	Unknown/Yes
	CHO	2008	Genzyme, Belgium	Unknown/Yes
	CHO	2008	Genzyme, US	Unknown/Yes
	CHO	2009	Genzyme, US	Unknown/Yes
PCV-1	Vero	2010	GlaxoSmithKline	Yes
PCV-1/PCV-2	Vero	2010	Merck	Yes

Table 2: Virus contamination events in GMP recombinant cell culture systems

- Selection of an appropriate range of infectivity or polymerase chain reaction (PCR) tests based on a defined risk management programme. The testing programme applies as much to the stem cell preparation as it does to any media additive or material used in the manufacturing process
- Validation of the manufacturing process for the removal of potential contamination. For cell-based therapies this option for reducing risk is not available, but for purified gene therapy vectors, steps for clearing potential contaminants can be considered

Virus contamination events have been reported in cell culture systems used for the manufacture of biopharmaceutical products (see Table 2). This underlines the need for stringent controls around virus safety.

Well-Characterised Cell Banks

The use of qualified cell banks provides the opportunity to detect and identify (and thereby exclude) possible contaminating viruses prior to their use in manufacture. However, virus contamination events (see Table 2) underscore the need for effective control and adherence to regulatory guidelines such as ICH Q5A (3). Cells could be contaminated either endogenously or exogenously (via animal-derived components or other routes, such as mouse minute virus [MMV]); understanding how such contamination occurred or was missed provides significant insight for controlling the virus risks with ATMPs.

The largest single root cause of virus contamination in well-characterised systems has been the use of animal-derived components. Media formulations may contain animal-derived

> **Prevention is always better than cure, so it is important to understand from where the risks might originate and implement the most appropriate steps to reduce that risk**

growth factors (often essential for stem cell growth) that can present a virus risk. Important lessons can be learned through a root cause analysis focusing on why contamination events with viruses from animal components were missed, which include:

- The virus was not permissive for the cell lines used for testing (eg porcine cirovirus [PCV], bovine polyomavirus [BPyV])
- Detection of the virus was masked by the presence of neutralising antibodies (eg bovine viral diarrhoea virus [BVDV]). Current guidelines require the inclusion of controls for neutralising antibodies to BVDV (4)
- The virus was below the limit of detection. The limit of sensitivity for current cell culture and PCR-based tests can never assure viral sterility
- No steps were present for the effective removal of the virus (not applicable though for cell-based therapies)
- Introduction of virus contaminants from other sources – eg facility rodents, as has been demonstrated for contamination with MMV and V2117 (5)

ATMPs and Virus Safety Risks

Some of the biggest virus safety challenges are encountered within cell-based therapies when it is impossible to implement robust virus clearance steps. For such products, the donor cells require full documentation history to properly understand and evaluate the risk. Such documentation requires the correct implementation of GMP procedures from the outset – an aspect that is often challenging for small university or hospital institutes that are the main drivers of ATMP therapies.

Most somatic or tissue-engineered cells require cell culture either to expand the number of available cells for therapy or to differentiate the cells. Since their culture can often be challenging, it may require the use of bovine serum or bovine-derived supplements; recombinant growth factors are not yet sufficiently developed or understood. Such supplements – even when recombinant-derived – must fulfil the GMP requirements for virus safety, including appropriate testing and a virus clearance capacity that has been appropriately validated. A frequent mistake encountered with ATMPs is the use of R&D-grade supplements for growth or differentiation – reagents that, unfortunately, often have little supporting virus safety information.

One of the questions often asked around ATMPs is whether a manipulation of engineered cells can be considered as substantial or non-substantial. According to the regulatory guidance, the following are examples of the non-substantial sort: cutting, grinding, shaping, centrifugation, soaking in antibiotic or antimicrobial solutions, sterilisation, irradiation, cell separation, concentration or purification, filtering, lyophilisation, freezing and cryopreservation (2).

All other manipulations, including cell culture, are considered to be substantial manipulations and need to be appropriately controlled and validated following GMP requirements.

Contamination with Unclear Aetiology

An aspect that has received increasing scrutiny has been the control of suppliers of components (eg cell culture medium, medium additives, active pharmaceutical ingredients [APIs]). Incidents of virus contamination with no clear aetiology (eg MMV and V2117) and the conclusion that such contaminants might have been introduced through, for example, a component of the cell culture medium raises the question of supplier control. The case study of MMV highlights the difficulty in controlling such risks.

Contamination events with MMV started to be noted in the 1990s in carbohydrate fermenters and resulted in multiple contamination events (5). In all instances, the root cause of the contamination could not be traced, but it was assumed to have originated from facility rodents – either at the facility or at the suppliers for excipients or media components.

MMV is an endemic virus in mice with sero-prevalence of up to 70% (6). The virus is present in high titres in multiple tissues (titres up to 10^7/mL), and is excreted in the urine of infected animals, which is the most likely source of contamination where exposure to mice is not controlled. Furthermore, the virus is highly resistant to inactivation procedures. Parvoviruses are among the most resistant strains used in virus validation studies, and so will also survive for extended periods in the environment (7).

Potential controls for controlling MMV risk could include the following:

Pest Control

Controlling exposure to mice for areas of risk that should include effective pest control procedures both at the GMP manufacturing plant, but also at the suppliers of media components or APIs. How far back in the supply chain needs to be evaluated should be carefully defined through systematic risk evaluation procedures.

MMV

Incoming materials could also be tested for MMV, but, as a risk control measure, this is unlikely to prevent entry into GMP-manufactured product as such testing would often miss low levels of contamination.

Inactivation
The risk from incoming materials could be reduced through the implementation of effective virus inactivation or removal steps. The success of gamma-irradiation in reducing the residual risks from bovine serum (ie the risk remaining following appropriate sourcing and testing) or the use of microwave treatment (ultra short/high heat) of growth media prior to fermentation for controlling MMV contamination are good practices currently used in licensed biopharmaceuticals (8).

Given the negative impact that virus contamination events cause, both in terms of company image as well as the resources required to investigate and clean contaminated facilities (including cleaning validation data), consideration of such control measures becomes more attractive.

V2117 was first identified as a contaminant of CHO bioreactors at the end of the 1990s where 40nm calicivirus-like virus particles in CHO cultures demonstrating cytopathic changes were observed (9). In all incidents of contamination with vesivirus, statements have been made that the virus is most likely of bovine origin, but direct data in support of this are lacking. Sequence comparisons with nucleotide databases continue to show the highest levels of homology with canine calicivirus, and, in fact, homology with bovine caliciviruses does not score high in such searches.

Therefore, the question of the origin of V2117 appears to be still open, and the assertation that this virus is of bovine origin has not yet been proven. The MMV precedent has shown us that manufacturers should be prepared to expect unusual sources of potential contamination.

Other Potential Contamination Sources

As always, the ATMPs of manufacturers should be proactive in identifying where potential risks from viruses could arise from. Potential sources of risk could include:

Arboviral Insect Vectors
A large number of viruses are arboviruses (transmitted by insects) and dead insects present in powdered media, for instance, could theoretically be a source of contamination. Most arboviruses are enveloped viruses, so might not survive for long periods in the environment, but there are exceptions to this rule.

Infected Workers
Many viruses are asymptomatic, so would not necessarily be apparent in workers in a GMP facility. Furthermore, viruses could be carried by fomites from infected pets.

Controlling the Supply Chain
A major issue is how far back in the supply chain to go. Some viruses have high resistance to inactivation and could potentially survive for extended periods in the environment. The extent of supplier auditing that is required should be given careful consideration. Prevention is always better than cure, so it is important to understand from where the risks might originate and implement the most appropriate steps to reduce that risk. This should be a combination of sourcing, quality control testing and dedicated robust virus inactivation/removal steps.

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About the author

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