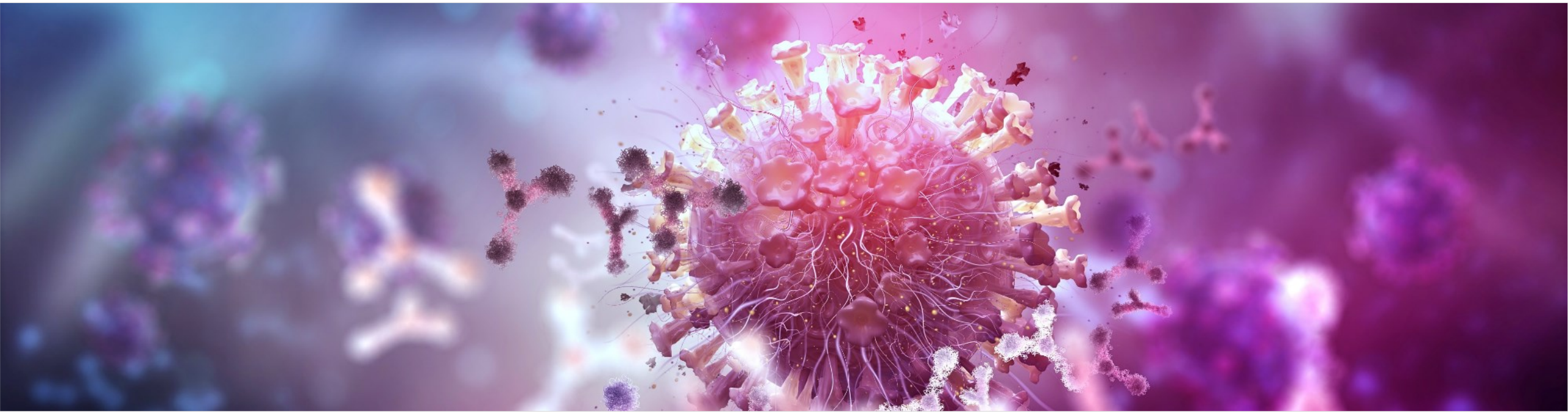

Current Challenges Ensuring the Virus Safety of Advanced Therapy Medicinal Products

FCPAE European Forum, 2022

Andy Bailey, CEO ViruSure GmbH

Presentation Overview

- Introduction to ATMPs
  - Regulatory framework
  - Concerns around virus contamination
- Upstream processes and the risk of virus contamination
- Barrier technologies for controlling virus risk
  - Why barrier technologies are an effective way to control virus risk
  - Examples & regulatory expectations

ATMPs- Basic Definitions

- ATMPs (Advanced Therapy Medicinal Products) are medical products for human use, often in regenerative medicine, and include:
  - Gene therapy products
  - Somatic cell therapy products
  - Tissue engineering and regenerative medicinal products
- In the EU, ATMPs are regulated under consolidated framework for advanced therapies (Regulation 1394/2007) as pharmaceutical products
- ATMP Regulation, Article 3
  - Where an ATMP contains human cells or tissues, the donation, procurement and testing of those tissues or cells shall be made in accordance with Directive 2004/23/EC
  - Gene therapy products are regulated as medicinal products according to 2001/83/EC

Regenerative Medicines: Exemptions from Centralised Regulation

Under EC 1394/2007:

- Allows for the use of an ATMP without a marketing authorization under certain circumstances
  - A non routine basis,
  - Specific quality standards
  - Custom made for an individual patient under the responsibility of the requesting physician
  - For use in a hospital setting within the same Member State in which they are manufactured

In the revised Japanese Pharmaceutical law:

- Regenerative medicines may receive an expedited approval once initial safety has been confirmed in order to ensure timely provision of the medicines to patients.

ATMPs- GMP Manufacture & Virus Safety

- **ATMPs need to be manufactured in accordance with the GMP guidelines for human medicinal products for human use (e.g. Directive 2003/94/EC)**
  - Control of consistency, reproducibility and uniformity are key aspects
  - Annex 2 of the EU Guidelines for GMP for medicinal products for human and veterinary use (Eudralex Vol 4) has been updated to include GMP specific to ATMPs
    - *The Annex recognizes the inherent variability and increased risks for microbial contamination and transfer of pathogens associated with biological culturing processes and materials*
- ***Given the potentially complex nature of ATMPs, ensuring the safety and quality of such products requires a science based and rigorous approach***
- ***Virus safety is one of the main areas of concerns for ATMPs and requires careful consideration:***
  - *Careful sourcing of tissues and materials*
  - *Cell sourcing*
  - *Reagents and materials used for culture*
  - *Careful testing of product intermediates and/or reagents to ensure safety*
  - *Careful design of the manufacturing process of all components to minimise the risk*

Concerns Around Stem Cells?

Ouagadougou, Burkina Faso (AO); and University of Newcastle, Newcastle, UK (SC)

1 Kok M, de Souza DK. Young Voices demand health research goals. *Lancet* 2010; 375: 1416–17.
2 Anon. Declaration of the International Students' Meeting on Public Health (ISMOPH). April 27, 2009; Istanbul,Turkey. http://www.t-hasak.org/english/word%20congres/ismoph.pdf (accessed July 27, 2010).

Use of unregulated stem-cell based medicinal products

Advanced-therapy medicinal products (ATMPs) include stem cells, gene therapy, or engineered tissues, and hold promise for a large number of currently incurable diseases. Yet no marketing authorisation has been granted for any stem-cell medicinal products in the European Union.

ATMPs are complex and their evaluation requires specific expertise. For this reason, the Committee for Advanced Therapies (CAT) was established in the European Medicines Agency. The CAT is responsible, among other tasks, for preparing a draft opinion on the quality, safety, and efficacy of ATMPs that follow the centralised marketing authorisation procedure.[1]

The CAT is concerned about a phenomenon known as stem-cell tourism in which severely ill patients travel to clinics around the world where unauthorised stem-cell-based treatments are offered in the absence of rigorous scientific and ethical requirements. Some clinics offer these unauthorised therapies to desperate patients with incurable diseases at a high cost without ethics approval from independent bodies and potentially without documentation of adequate quality standards necessary for the protection of patients' safety.

There are serious concerns about the safety and efficacy of such experimental treatments that use poorly defined stem-cell preparations from a variety of sources. These preparations are often inadequately characterised[2] and they can lack pharmacological or toxicological data from non-clinical studies to establish reasonable evidence of safety and efficacy.[2,3] Generally, there are no peer-reviewed publications to demonstrate their efficacy. The retrospective data analyses that are sometimes found on the clinics' websites lack transparency; hence, such data cannot be properly assessed. However, there are already well documented cases of where so-called stem-cell therapy has resulted in serious adverse effects, including brain tumours,[2] meningitis, or other life-threatening infections.[3]

The CAT strongly encourages high-quality research leading to the development of stem-cell-based medicinal products in approved programmes of research and development. Before clinical use, rigorous non-clinical studies should be done to establish the safety and effectiveness.[4]

To ensure the safety of patients involved in clinical research, development of stem cells should comply with the highest standards, as for any investigational medicinal product, under the supervision of statutory regulatory bodies. Those planning the development of such treatments are encouraged to engage in dialogue with the CAT at an early stage of this process.

We declare that we have no conflicts of interest.

Committee for Advanced Therapies and CAT Scientific Secretariat
advancedtherapies@ema.europa.eu
European Medicines Agency, London E14 4HB, UK

For members of the Committee for Advanced Therapies and the CAT Scientific Secretariat see webappendix

1 European Union. Regulation (EC) No 1394/2007 of the European Parliament and of the Council of 13 November 2007 on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:324:0121:0137:en:PDF (accessed July 21, 2010).
2 Regenberg AC, Hutchinson LA, Schanker B, Mathews DJ. Medicine on the fringe: stem cell-based interventions in advance of evidence. *Stem Cells* 2009; 27: 2312–19.
3 Barclay E. Stem-cell experts raise concerns about medical tourism. *Lancet* 2009; 373: 883–84.
4 Committee for Advanced Therapies. Reflection paper on stem cell-based medicinal products. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2010/03/WC500079932.pdf (accessed July 27, 2010).

- *"There are serious concerns about the safety and efficacy of such experimental treatments that use poorly defined stem-cell preparations from a variety of sources. These preparations are often inadequately characterised and they can lack pharmacological or toxicological data from non-clinical studies to establish reasonable evidence of safety and efficacy"*

- *From Lancet 2010; Vol. 376:514*

GMP Requirements for Starting Materials for ATMPs?

- *"A GMP certificate is not required for manufacturing and testing sites of starting materials for ATMPs. For certain starting materials of biological origin, (such as e.g. linear DNA used as template for ex vivo transcription into mRNA, plasmids to generate viral vectors and/or mRNA, and vectors) used to transfer genetic material for the manufacturing of ATMPs it is, however, mandatory that the principles of GMP are complied with."*

24 February 2021
EMA/246400/2021
Inspections, Human Medicines, Pharmacovigilance and Committees Division

Questions and answers on the principles of GMP for the manufacturing of starting materials of biological origin used to transfer genetic material for the manufacturing of ATMPs

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Upstream Processes for Cell Culture: A Focus for ATMP Products?

AsahiKASEI BIOPROCESS

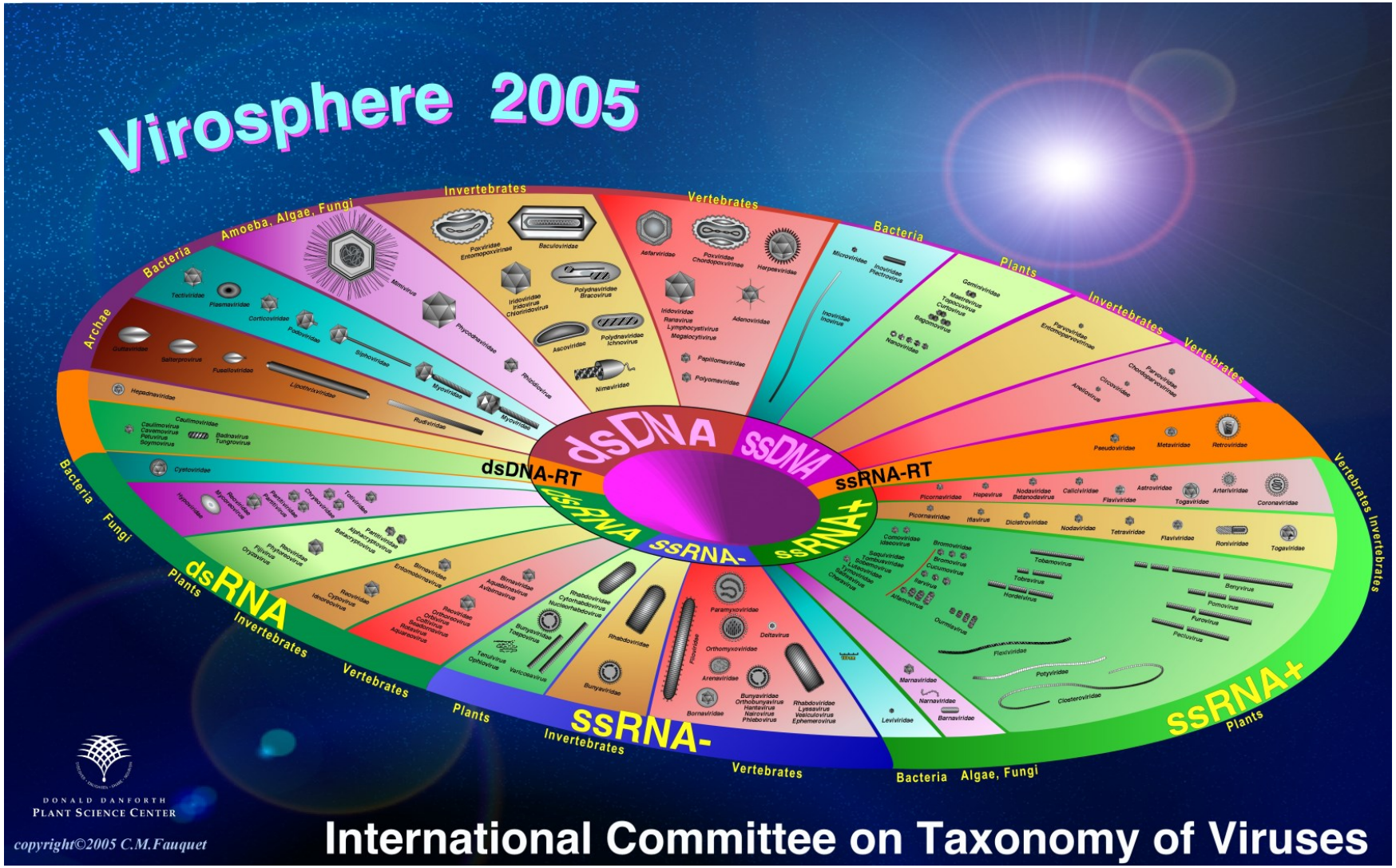
Why Focus on Upstream Processes?

- **The reasons why dedicated virus reduction steps upstream of cell culture may be considered are different for different types of products:**
  - Recombinant cell culture derived products
    - Steps are implemented downstream of cell culture to reduce virus risk and medium is often chemically defined:
      - Chemically defined though does not necessarily mean there is no virus risk (see later)
    - The impact though of a contamination even upstream can be significant:
      - Supply of product is impacted
      - Significant investigation / clean-up costs
      - Impact on company image
  - Cell based or virus based therapies
    - Steps downstream for controlling virus risk may not be feasible
    - Virus might be inherently carried in the cells (latent or inapparent infections)
    - Medium is often complex including human or animal derived components where the virus risk is higher
      - FBS or purified bovine / porcine proteins
      - Platelet derived growth factor
    - → there is a greater need to control the virus risk in such components

ATMPS- Available Risk Reduction Measures for Assuring Virus Safety?

	Gene therapy vectors (enveloped)	Gene therapy vectors (unenveloped)	Somatic cells
Upstream:			
QC testing of start materials?	☑	☑	☑
QC testing of cell culture medium & components?	☑	☑	☑
Barrier technologies for cell culture medium?	☑	☑	☑
Downstream:			
Virus filtration	X	☑	X
Detergent treatment	X	☑	X
Chromatography	☑	☑	X
UV inactivation	X	X	X
Gamma-irradiation	X	X	X
HTST	X	☑???	X

The Realm of Viruses



- Viruses are found in all spheres of life in all shapes and sizes
- Testing for every possible contaminant is a virtual impossibility
- Controlling virus safety therefore requires a holistic approach

The History of Virus Contamination Events in Mammalian Cell Culture Derived Biopharmaceuticals

Year of Contamination	Contaminations (virus / host cell)	Total
1985-1989	Bluetongue / CHO EHDV / CHO	2
1990-1994	Herpesvirus / Primary Monkey Herpesvirus / Vero MMV / CHO (x2) Parainfluenza virus / MRC-5 Reo3 / MRC-5 Simian adenovirus / Primary monkey	7
1995-1999	Cache valley virus / CHO Reovirus / Human primary kidney Vesivirus 2117 / CHO	3
2000-2004	CVV / Unknown (x2) Human adenovirus / HEK293	3
2005-2010	CVV / CHO MMV / CHO (x2) Vesivirus 2117 / CHO (x3)	6
2010-Present	MMV / CHO MMV / BHK-21 PCV-1 / Vero	3
Unknown	MMV / BHK-21 Reovirus / Unknown	2
	Total:	26

Data from Barone et. al.; Nature Biotechnology (2020); Vol 38; pp 563-572

What were the Sources of Contamination in these Historical Events?

Contaminated Cell Line	Contaminating Virus	Pathogenic to Humans?	Suspected and Confirmed Sources of Contamination					
			Serum	Recombinant Medium Component	Undetermined Medium Component	Operator	Host Cell Line	Not Found
Viruses found to contaminate CHO cell culture								
CHO	Bluetongue virus	No	1					
CHO	Cache valley virus	Yes	2					
CHO	Minute virus of mice	No		1	3			1
CHO	Vesivirus 2117	No	4					
Viruses found to contaminate human or primate cell lines								
Primary monkey, Vero	Herpesvirus	Yes				1	1	
HEK293	Human adenovirus type 1	Yes				1		
MRC5	Parainfluenza virus type 3	Yes				1		
MRC5	Reovirus type 3	Yes				1		
Primary monkey	Simian adenovirus	No					1	

Comments:

- Data from Barone *et. al.*; Nature Biotechnology (2020); Vol 38; pp 563-572

Testing Alone is not going to Provide Sufficient Assurance

	Risk Calculation 1 (no Inactivation Step)	Risk Calculation 2 (with a single 5 log inactivation step)
LOD of test cell culture test (10ml tested):	~<0.3 IU/ml (at 95% CI)	~<0.3 IU/ml (at 95% CI)
Volume of FBS at which theoretical risk exceeds >1IU in cell culture system:	3.33 ml	3.33 ml
Potential load in 100ml of FBS:	<30 IU	<30 IU
Potential load in 1,000ml of FBS:	<300 IU	<300 IU
Inactivation or removal of virus?:	0 log	>5 log
Final Residual Risk (100ml):	<30 IU	<0.0003 IU
Final Residual Risk (1,000ml):	<300 IU	<0.003 IU

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Upstream Processes- Barrier Technologies for Controlling Virus Risk

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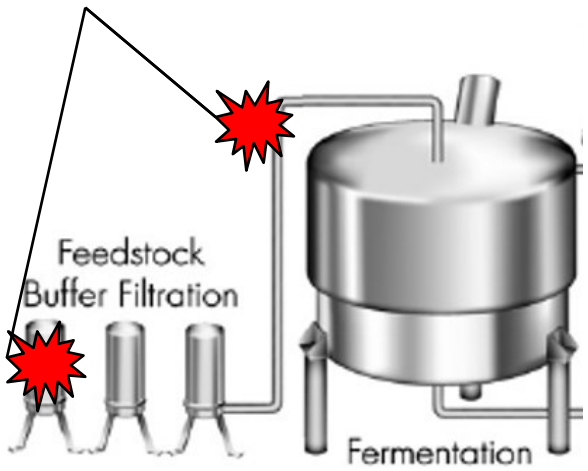
Barrier Technologies

Barrier Technology

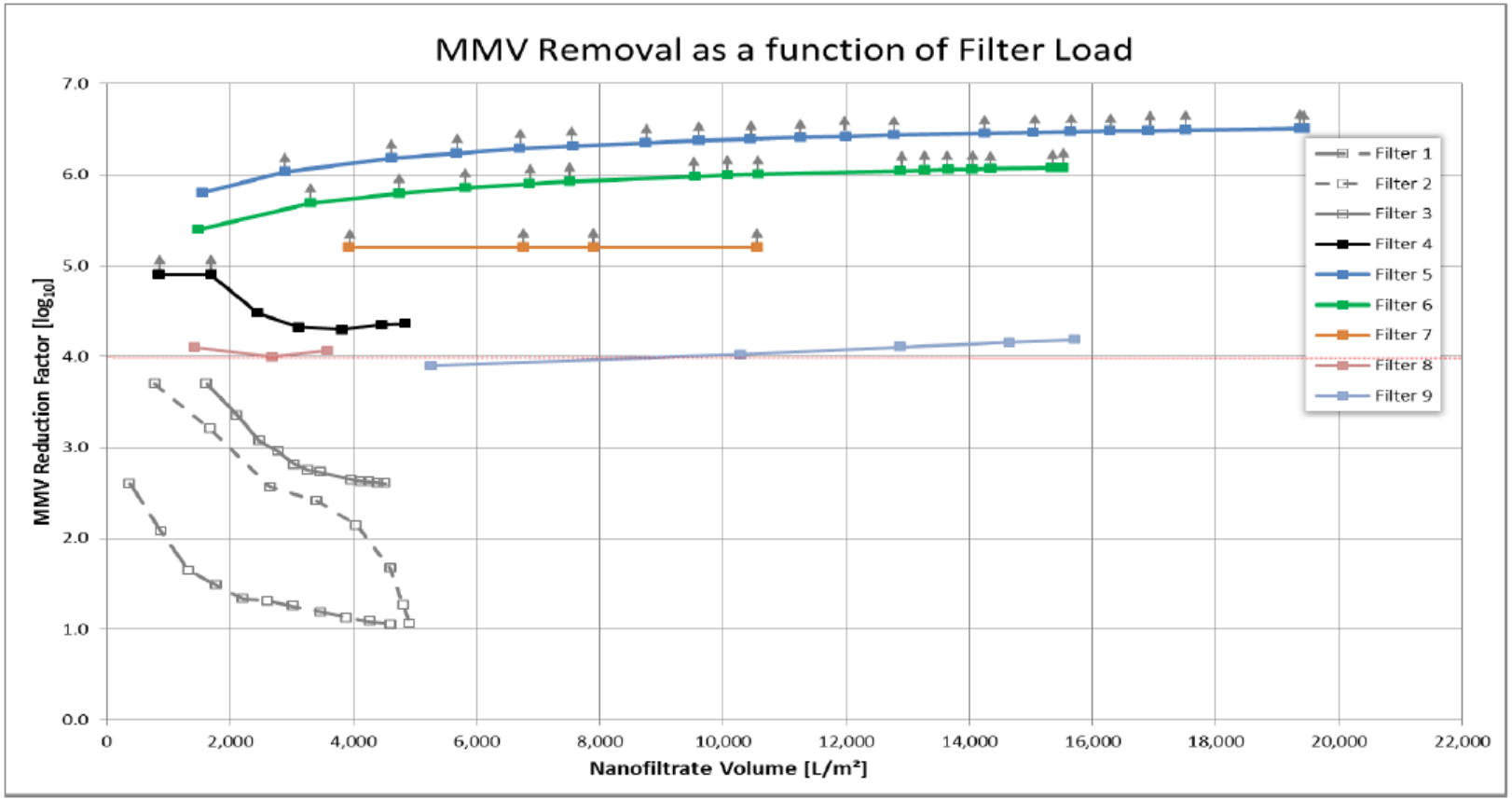


- The implementation of barrier technologies presents one of the best control measures for reducing the risk of introducing adventitious agents into the cell culture system

- Technologies include:
  - HTST
  - Small virus filtration
  - Heat
  - UV treatment
  - Gamma irradiation
  - Other membrane technologies

Filtering Large Volumes of Chemically Defined Medium (1)

Virus Filtration as Upstream Barrier



Presented by: Andreas Wieser at the Planova Workshop, Prague 2017

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Robustness of Gamma Irradiation at Higher Doses

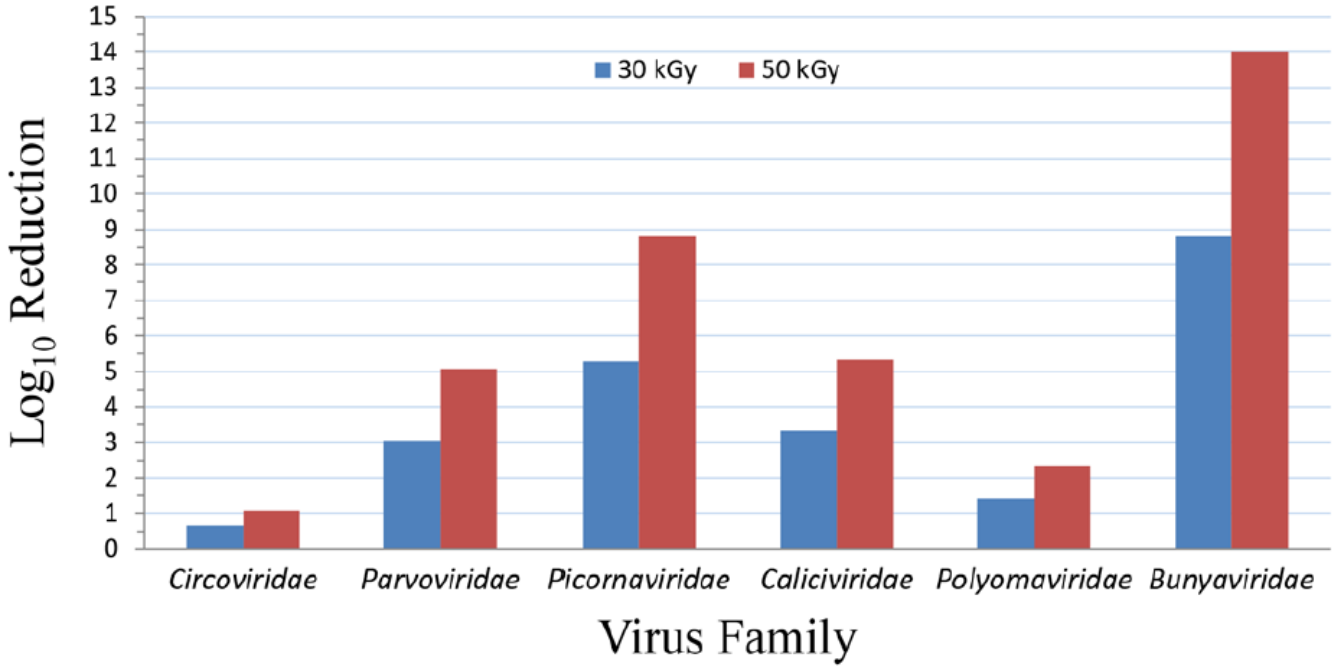
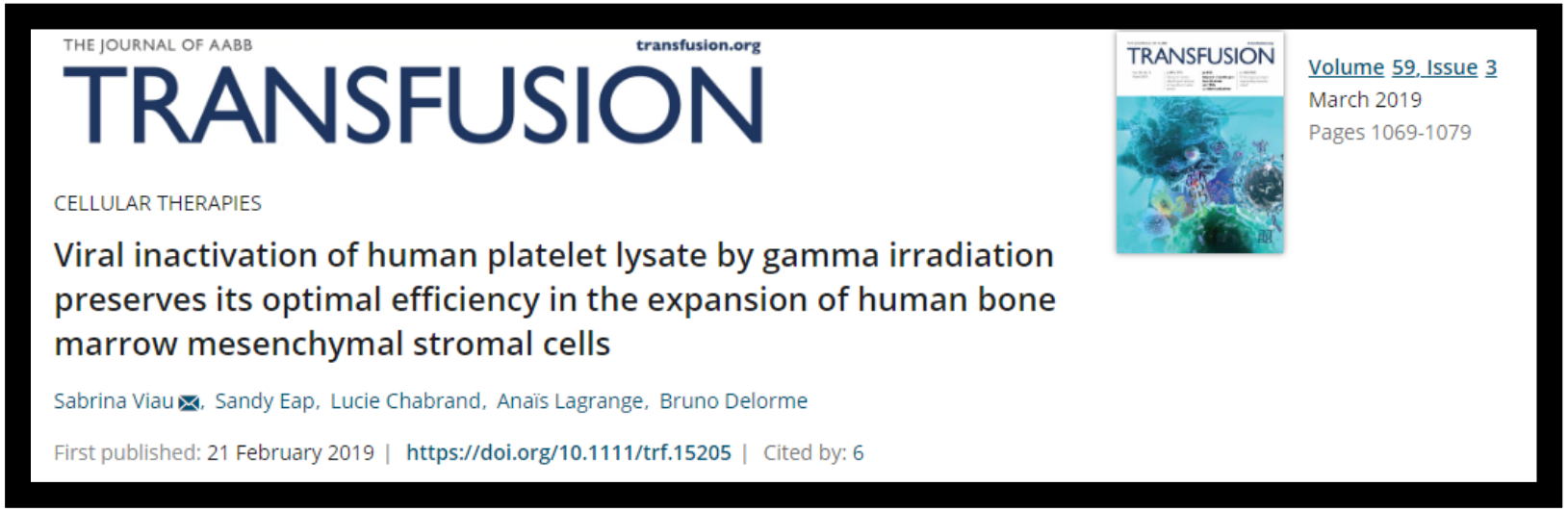


FIGURE 1. Comparison of the resistance of viruses to the direct effects of gamma irradiation in a frozen bovine serum matrix. The bars indicate the expected $\log_{10}$ reduction in titer resulting from irradiation doses of 30 and 50 kGy (which were modeled as they represent the typical fluency range used). The lower the bar, the more resistant the virus is to inactivation.

- Data from Plavsic et. al. (2016; BioProcessing Journal, Vol.15/No.2,)
- Robustness of gamma irradiation is significantly improved at 50 kGy for most viruses

High Dose Treatment of Human Platelet Lysate



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Viral inactivation of human platelet lysate by gamma irradiation preserves its optimal efficiency in the expansion of human bone marrow mesenchymal stromal cells

Sabrina Viau, Sandy Eap, Lucie Chabrand, Anaïs Lagrange, Bruno Delorme

First published: 21 February 2019 | https://doi.org/10.1111/trf.15205 | Cited by: 6

Volume 59, Issue 3
March 2019
Pages 1069-1079

- A dose of 55 kGy of human platelet lysate did not affect:
  - The proliferation or clonogenic potential of BM-hMSCs
  - The differentiation potential of adipocytes or osteoblasts
- → i.e. Authorities will likely request data confirming any claims that higher doses of gamma-irradiation are indeed detrimental to product quality

Planova 15N Filtration for Removal of Circoviruses

Sample ID	Planova 20N	Planova 15N	BioEX	Planova 35N
0.1µm Pre-filtration	0.00	0.00	0.00	0.00
MAb Solution	≥2.81*	≥2.40*	≥2.84*	1.71*
DMEM (1x Filtration)	0.00*	1.51§	-	0.00*
DMEM (2x Filtration)	-	2.79§	-	-

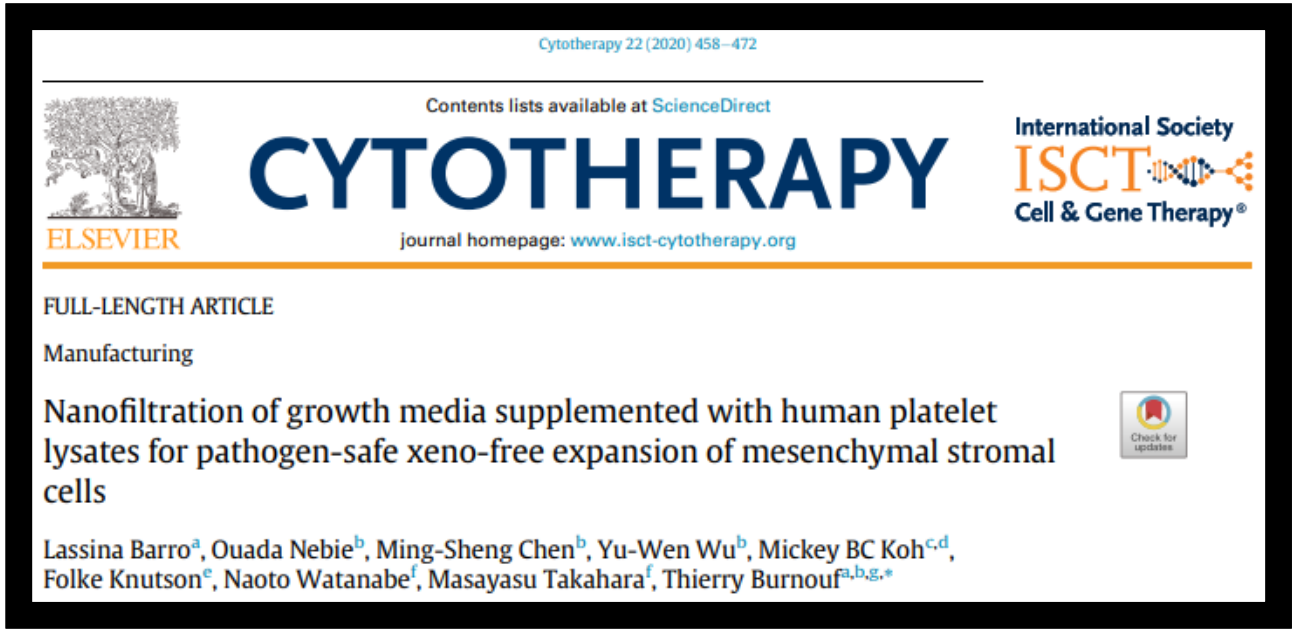
i.e. Removal of virus in simple cell culture medium may be more difficult than in protein containing solutions

Data presented by Natascha Hodosi, ViruSure GmbH, at the 17th Planova Workshop, Washington 2014

* Experiments performed with PCV2

§ Experiments performed with PCV1

Nanofiltration of Human Platelet Lysates



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ELSEVIER

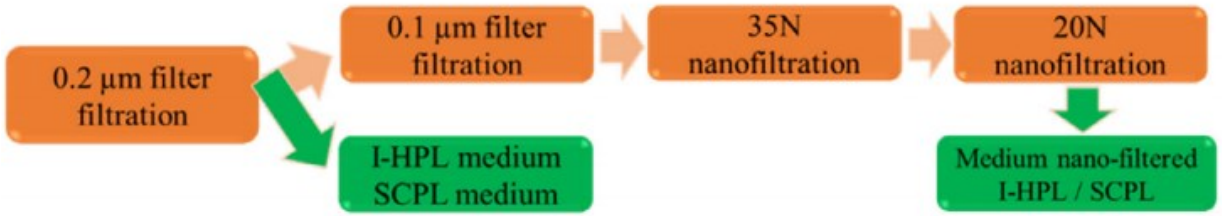
International Society ISCT Cell & Gene Therapy®

FULL-LENGTH ARTICLE

Manufacturing

Nanofiltration of growth media supplemented with human platelet lysates for pathogen-safe xeno-free expansion of mesenchymal stromal cells

Lassina Barro[a], Ouada Nebie[b], Ming-Sheng Chen[b], Yu-Wen Wu[b], Mickey BC Koh[c,d], Folke Knutson[e], Naoto Watanabe[f], Masayasu Takahara[f], Thierry Burnouf[a,b,g,*]

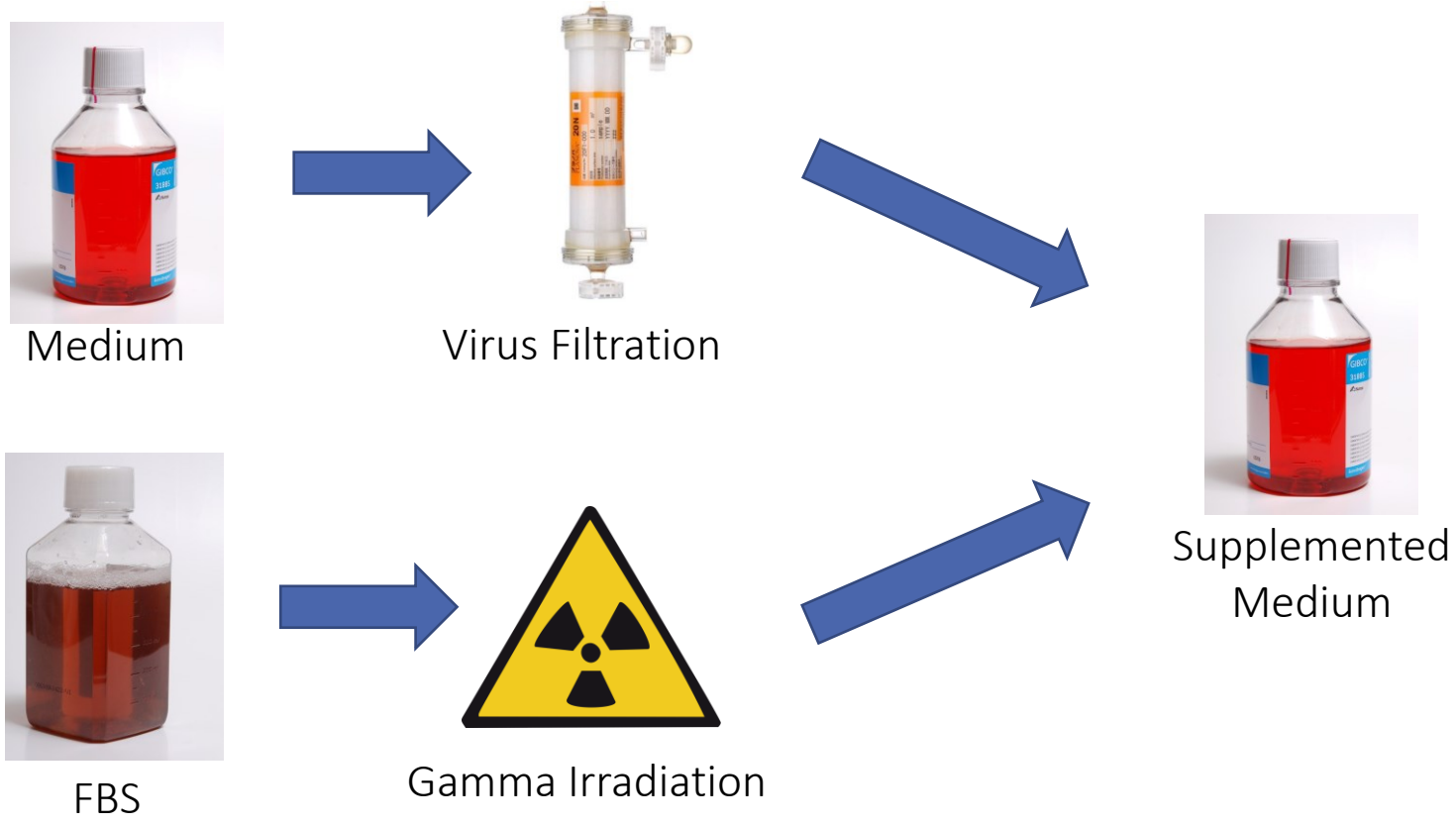


- Human platelet lysate (HPL) made from clinical-grade platelet concentrates is a highly effective xenofree supplement used for for propagating human cells

- Highly effective supplement of cell culture medium for stem cells
  - Normally very difficult to filter
  - Filtration of medium supplemented with HPL through an Asahi Kasei Planova 35N/20N filtration was feasible without significant impact on cell growth or differentiation properties of the medium
  - HPL was effectively subjected to 2 dedicated virus clearance steps:
    - Intercept™ virus inactivation
    - Planova 20N virus filtration

Combined Strategies for Multi-Component Medium

- For multi-component medium, strategies where components are treated separately can also be considered



Summary

- The virus safety risk for medium and upstream processes is very real and needs to be controlled to provide assurances for
  - The virus safety of biopharmaceutical manufacturing facilities
  - The virus safety of ATMPS

- Testing alone will only get you part of the way- it can never reduce risk alone to an acceptable level

- The feasibility of implementing upstream virus safety steps, at large volume, have now been demonstrated and provide options for manufacturers looking to control this source of risk

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감사합니다 Gracias Danke

Благодаря 谢谢 Tack धन्यवाद Dziękuję

Спасибо Thank You Obrigado

Děkuju Grazie Ευχαριστώ Merci Köszönöm

ありがとうございました Teşekkür ederim