QUALITY CONSIDERATIONS FOR VIRAL VECTORS USED IN THE MANUFACTURE OF GM-CELL PRODUCTS

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1

Objectives Outline

- Introduction
  - Products authorised to date
- Quality considerations for vectors used to prepare GM-cell products
  - Vector status
  - *Safety aspects (not discussed as such)*
  - Production
  - Purity and impurities
  - Touch on other apsects

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EMA (EU) EXPERIENCE

Submissions and outcomes

Product (Company)	INN/description	Indication/s (Therapeutic area)	Start Date	Opinion	Finalisation Date
Cerepro (Ark Therapeutics)	*adenovirus-mediated Herpes Simplex Virus-thymidine kinase gene*	*High grade glioma*	*Oct 2005*	*(Negative)*	*Withdrawn Jul 2007*
Advexin (Gendux Molecular)	*contusugene ladenovec*	*Li-Fraumeni syndrome*	*Sep 2007*	*(Negative)*	*Withdrawn Dec 2008*
Contusugene Ladenovec (Gendux Molecular)	*contusugene ladenovec*	*squamous cell carcinoma of the head and neck*	*Jul 2008*	*(Negative)*	*Withdrawn Jun 2009*
Cerepro (Ark Therapeutics)	*adenovirus-mediated Herpes Simplex Virus-thymidine kinase gene*	*High grade glioma*	*Jan 2009*	*Negative*	*Dec 2009*

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Product (Company)	INN/description	Indication/s (Therapeutic area)	Start Date	Opinion	Finalisation Date
Glybera (uniQure biopharma)	alipogene tiparvovec (AAV1)	Hyperlipoproteinemia Type I	Dec 2009	***Positive Jul 2012***	**Oct 2012 Withdrawn (Oct 2017)**
Imlygic (Amgen)	talimogene laherparepvec	metastatic melanoma	Sep 2014	Positive Oct 2015	**Dec 2015**
Zalmoxis (MolMed SpA)	Allogeneic T cells genetically modified with a retroviral vector ΔLNGFR andHSV-TK Mut2	haploidentical haematopoietic stem cell transplantation	Apr 2014	Positive June 2016	**Aug 2016**
Strimvelis (GlaxoSmithKline)	Autologous CD34+ cells transduced with retroviral vector containing the adenosine deaminase gene	ADA-SCID	May 2015	**Positive March 2016**	**May 2016**
Luxturna (Spark Therapeutics)*	*voretigene neparvovec (AAV2-hRPE65v2)*	*Leber congenital amaurosis retinitis pigmentosa*	*Jul 2017*	*TBD*	*TBD*
Yescarta (Kite Pharma)*	*axicabtagene ciloleucel (KTE-C19; retroviral vector)*	*relapsed or refractory DLBCL, PMBCL and TFL*	*Jul 2017*	Positive (June 2018)	*TBD*
Kymriah (Novartis)*	*Tisagenlecleucel-T*	*Relapsed or refractory B-cell ALL and DLBCL*	*Nov 2017*	Positive (June 2018)	*TBD*
Axal (Advaxis, Inc)	*Axalimogene filolisbac (live attenuated Listeria monocytogenes)*	*cervical cancer*	Apr 2018	*Withdrawn July 2018*	

FDA APPROVED

FDA APPROVED

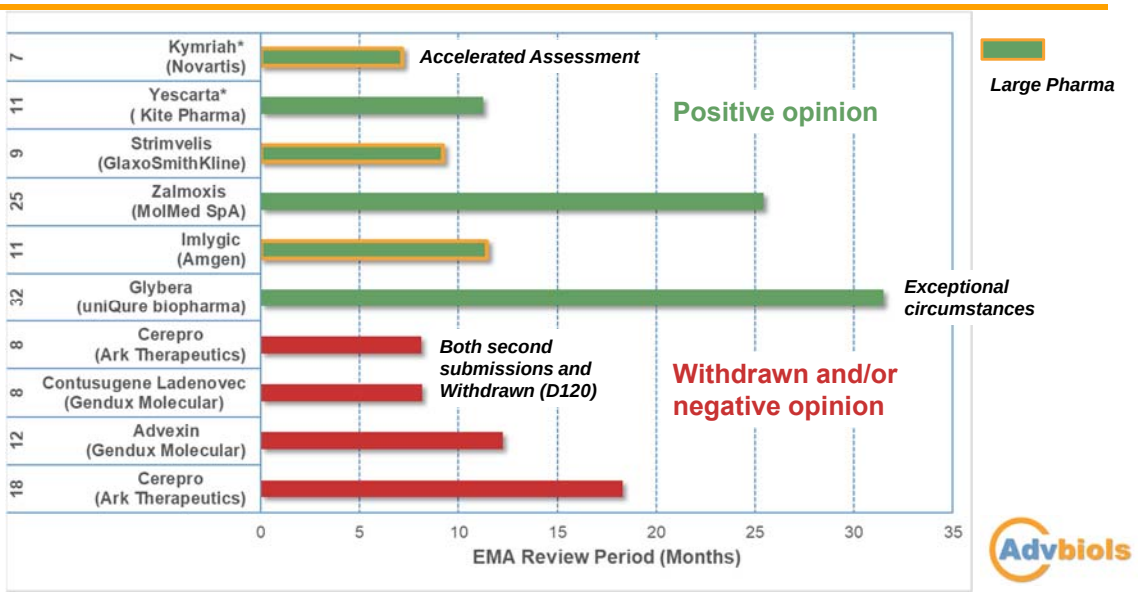
FDA APPROVED

Orphan

Not orphan

Review Times for EMA GT Submissions

(Ignores EU Commission time, typically 3m)



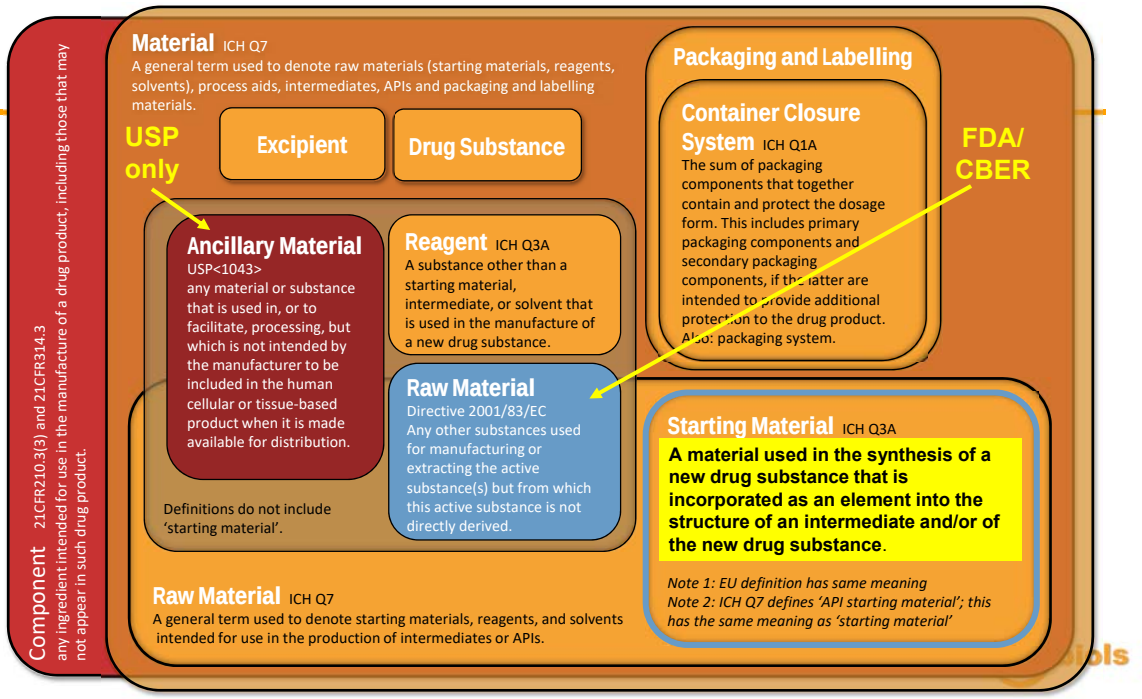
5

GENE THERAPY VECTOR MANUFACTURING

What is the status of the vector for GM-cells?

- EU EMA
  - Very clear = starting material (and all components to make the vector)
  - Annex I part IV of Directive 2001/83/EC
- US FDA
  - Less clear
  - FDA describe (if at all) as a critical raw material
- Just a matter of terminology
  - Expectations aligned
  - *Remembering individual reviewers may differ in their expectations and choice of terminology.*

6



GENE THERAPY VECTOR FOR GM-CELLS

Safety Testing (adventitious agents)

- Patient safety comes first
  - Do the same adventitious agent safety testing as for vectors that are the DP
  - *Not discussed further here, except next slide (too little time)*
  - *Focus will be purity, impurities and other testing*

8

COMMON QUESTIONS FOR IMPD'S (EU NATIONAL AGENCIES)

From voluntary harmonised procedure (c.2016)

Clinical Trial Applications –Voluntary harmonized procedure
Distribution of "Grounds for Non-Acceptance"
(initial questions) in%

Viral Safety of ATMPs and Raw Materials
Johannes Blümel, Paul-Ehrlich-Institut
PDA Europe, Berlin, 7-8June 2016

Produkt	Quality	Pre-clinics	Clinic	Viral Safety	Statistics
Allergens	67,3	47,1	83,7	4,8	43,3
Blood products	51,2	34,1	73,2	22,0	36,6
Ab-Fusion proteines	12,5	12,5	41,7	12,5	25,0
Gene-transfer MP.	85,7	78,6	78,6	50,0	21,4
Clotting factors	58,4	23,8	64,4	29,7	20,8
Immunglobuline, normal	32,3	6,5	35,5	9,7	16,1
Immunglobulin, specific	25,0	25,0	75,0	0,0	75,0
Vaccines	16,4	8,8	34,4	2,0	13,2
monoclonal Ab	24,9	16,5	50,1	25,5	14,6
Somatic Cell-therapeutics	77,9	58,1	76,7	57,0	53,5
Tumor-Vaccines/Peptides	73,0	66,2	71,6	23,0	25,7

9

VIRAL VECTOR FOR GM-CELLS

Quality of vector

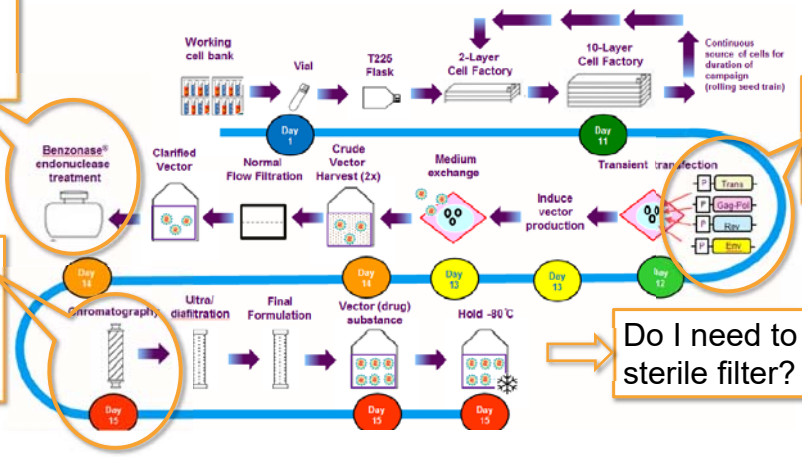
- Who uses cytokines, growth factors or other raw materials (ignore excipients) that are licensed medicines (biologics, drugs)?
- Who uses a vector for a GM-cell product that is the same quality as a vector drug product?
- Do raw materials need to be medicinal quality?

10

GENE THERAPY VECTOR MANUFACTURING

Questions when the vector is a starting material

How much residual RNA and DNA is acceptable?



Do residual plasmids matter?

How far should I clean up vector? e.g. do HCP need to be low?

Do I need to sterile filter?

Lentiviral vector production strategies: *James Miskin, Oxford BioMedica*
PDA Europe ATMPs Valencia/Spain, 27-28 June 2017

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VIRAL VECTORS

Plasmids- minimum testing

- **Identification**. Plasmids are identified by restriction enzyme analysis, sequencing or NAT (*2.6.21*).
- **Genomic integrity**. e.g. restriction enzyme analysis of the viral genes, the genetic insert and their respective regulation elements.
- **Plasmid DNA**. e.g.
  - DNA < 500 ng/mL absorbance at 260 nm.
  - DNA > 500 ng/mL by fluorescent dyes that bind DS DNA.
  - Liquid chromatography/capillary electrophoresis.

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PLASMID VECTORS*

Bacterial cell Banks

*plasmid-vector products and plasmids for preparation of viral vectors.

- Provenance of bacteria
- bacterial cell-bank system testing
  - All of this or can it be reduced?
  - Presenters view: this is minimal testing, if the plasmid was the DP you'd expected more. So I recommend doing this.
- Do the plasmids need to be prepared to GMP?
  - EMA position = principles of GMP (but far down process for a GM-cell) but not asked for IMPD.
  - FDA - ? (not asked for IND's)

Assay	Host strain	MCB	WCB	EOPCs*
Identity and purity				
Viability	+	+	+	+
Bacterial strain characterisation	+	+	–	+
Genotyping / phenotyping	+	+	–	+
Presence of the plasmid				
– Sequence of the DNA plasmid	–	+	–	+
– Copy number	–	+	+	+
– Restriction map	–	+	+	+
– Percentage of cells retaining the plasmid	–	+	+	+
Adventitious agents				
Purity by plating	+	+	+	+
Presence of bacteriophage	+	+	–	+

* EOPCs are cells with a passage number at least equivalent to that used for production. The analysis has to be done once to validate each new WCB, except for purity, which has to be tested for each fermentation.

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13

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VIRAL VECTORS

Purified harvest (drug substance)#2

- **Residual host-cell protein**
  - by a suitable immunochemical method (*2.7.1*)
  - unless suitable clearance has been validated.
- **Residual host-cell DNA**.
  - Quantitative PCR is recommended
  - other suitable techniques may also be used.
- **Residual reagents**.
  - *Process specific residual raw materials*
  - unless suitable clearance has been validated.
- **Residual plasmids** (transient production process)

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14

PROCESS-RELATED IMPURITIES
Host cell proteins (HCP)

- HCP should be tested (major process-related impurity)
- Will a generic test kit be acceptable? Considerations;
  - These tend to under-estimate HCP (protein products: specific method for approval)
  - The degree to which they do could be explored, e.g. 2D gel and Western blotting with 'kit' polyclonal antisera to demonstrate how good the 'antigen' coverage is.
  - Should you demonstrate whether HCP interfere with transduction in-process (e.g. spiking studies)?
  - Biotech products typically in the order of 100 ppm (100ng/mg) HCP (own experience - there are no guideline values, case-by-case)
  - unless HCP is detrimental to transduction step the question simply becomes how much is carried over onto the DP
    - Could test GM-Cell DP for vector HCP as part of characterisation, but need to consider if donor cells and other materials react with test (assay matrix effect)

15

VECTOR QUALITY FOR GM-CELLS
Host Cell DNA

- HCDNA – should be tested (mammalian cell line)
  - WHO recommended limit <10 ng/dose is generally accepted for a DP
  - What level is acceptable for the vector of a GM-Cell?
  - If the vector is <10 ng per amount added to the cells (assuming autologous here) in the process would this be OK?
    - Conditions of manufacture designed to favour gene transfer, so more likely than in vivo.
  - You could calculate backwards from DP 'conservatively' to estimate the highest level in the vector batch that ensures to the DP cannot be >10 ng/dose
    - Ignores increased risk of transfer to cells in process
    - Also, consider carefully the target cell for transfection, it would be prudent to be more conservative if the cell is a stem cell expected to have long-term persistence in the body, such as HSC, than if the cell was unlikely to persist for more than a few days or weeks.

16

VECTOR QUALITY FOR GM-CELLS
Other impurities

- Process-related
  - Residual raw materials
  - Same sorts of arguments as HCP and HCDNA
- Product-related impurities
  - e.g. empty vector
  - Similar arguments, e.g. empty vector may reduce transduction effciiency (competition with full vector).

17

Quality of the vector for a GM-Cell
Biological activity of Vector

- the vector is not the active substance (drug substance) for a GM-cell
  - but its biological 'activity' is a cQA of the vector as a material.
  - Some sort of vector activity should therefore be part of vector release.
- Infectivity is a biological activity of the vector particle

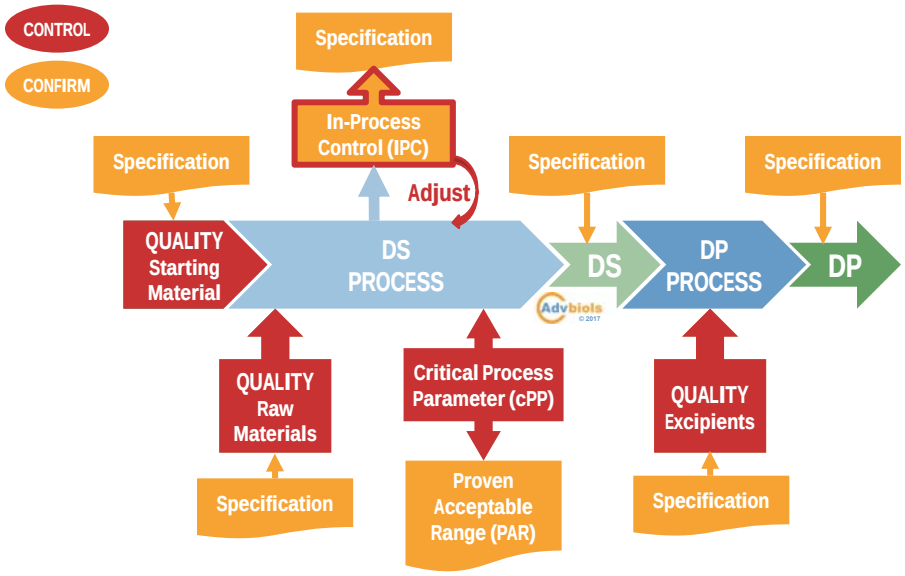
BUT……

- Infectivity doesn't mean correct gene transfer or correct expression (depending on how its measured)
  - Simply identifying the presence of the gene product e.g. by antibody staining or PCR is not measuring a biological activity, merely confirming a protein or gene sequence is present
  - not necessarily whether that protein is correctly expressed and active

18

NEED TO JUSTIFY THE CONTROL STRATEGY

Confirmation and Control



Quality of the vector for a GM-Cell

Others

- You will need to prepare a reference material for the vector
  - From FiH
- Stability
  - Accelerated, real-time, in-use

Quality of the vector for a GM-Cell
Summary

- Vector quality doesn't need to be the same as a vector product
  - You need to justify quality
  - May not be able to reduce range of tests, but some acceptance criteria can be wider then a vector DS/DP.
  - Safety aspects should be the same as GM-cell product cannot be sterilised or subject to viral reduction or elimination steps.
- Broadly the same information as a DS section
  - Recommend follow same CTD headings.
  - But belongs within S.2.3 (control of martials)

21

THANK YOU FOR LISTENING

Questions?

ADDITIONAL SLIDES AND READING

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S.2.3

Directive 2001/83/EC

GENE THERAPY MEDICINAL PRODUCT

Starting Materials

3.2.1.3. In the case of products consisting of viruses or viral vectors, the starting materials shall be the components from which the viral vector is obtained, i.e. the master virus vector seed or **the plasmids used to transfect the packaging cells** and the master cell bank of the packaging cell line.

3.2.1.4 In the case of products consisting of plasmids, non-viral vectors and genetically modified microorganism(s) other than viruses or viral vectors, the starting materials shall be the components used to generate the producing cell, i.e. the **plasmid, the host bacteria** and the master cell bank of recombinant microbial cells.

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24

POTENCY: GM cells
ICH Q6B*

- The measure of the **biological activity** using a suitably **quantitative biological assay** (also called potency assay or bioassay), based on the attribute of the product which is linked to the **relevant biological properties** [of the active substance].

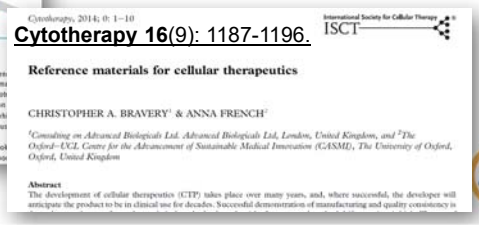
*Specifications : Test Procedures and Acceptance Criteria for Biotechnological/ Biological Products (ICH Q6B) 1999

25

What Are You Trying to Measure?

- **Measurement**: process of experimentally obtaining one or more quantity values that can reasonably be attributed to a quantity.
- **Analyte**: specific substance to be measured (e.g. IL-2, haemoglobin).
- **Measurand**: quantity to be measured

See discussion in these



26