International Standards for Biologicals: Future challenges

Chris Burns

IRSS Conference
Strasbourg, March 2019

Medicines and Healthcare Products Regulatory Agency

Statutory Responsibilities for Biological Medicines

Biological Standards Act (1975): Health & Social Care Act (2011)

- *Establishment of NIBSC as a specialist centre of excellence to provide independent assurance of the quality of biological medicines within the UK:* ***Standardisation – control testing – underpinning research***

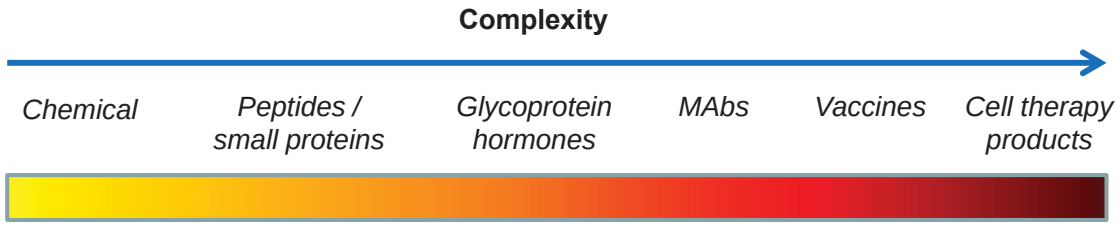
Standardisation

Medicines Control

Underpinning Research

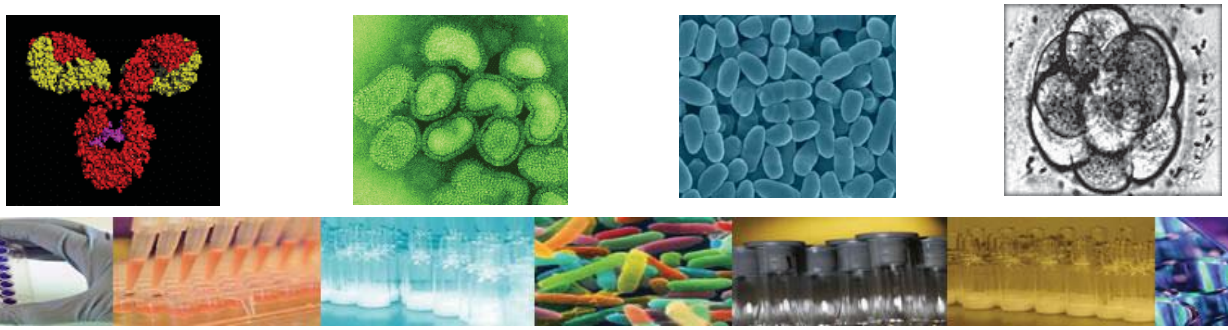
Providing Advice
Responding to Incidents

Characterisation of biological materials



Physicochemical tests play an important role in product characterisation, but more complex biologicals cannot be completely characterised by physicochemical means alone…

Despite advances in analytical technology, bioassays remain an essential requirement for characterisation of more complex biologicals – particularly for measurement of potency



International/Regional Standards

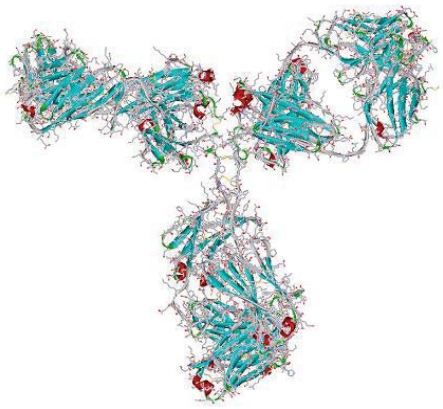
- WHO International Standards
  - Principal role is to define the Unit, a measure of biological activity
  - Formulated for long term (>10 yrs) stability
  - Protein-containing excipient
  - Limited quantity (<1microgram)

- Compendial Standards (biological and physicochemical)
  - Secondary (working) standard, unit in terms of the IS.
  - Usually non-protein containing excipients (often >100micrograms)
  - Supports comparative Biological and Physicochemical tests in the Ph. Eur., USP etc

The Biologics Revolution

- Huge explosion in importance: 8/10 top selling pharmaceuticals are now biologics

No.	Trade name	Type
1	Humira®	Biological
2	Harvoni®	Small molecule
3	Enbrel®	Biological
4	MabThera®	Biological
5	Remicade®	Biological
6	Revlimid®	Small molecule
7	Avastin®	Biological
8	Herceptin®	Biological
9	Lantus®	Biological
10	Prevenar-13	Biological (vaccine)

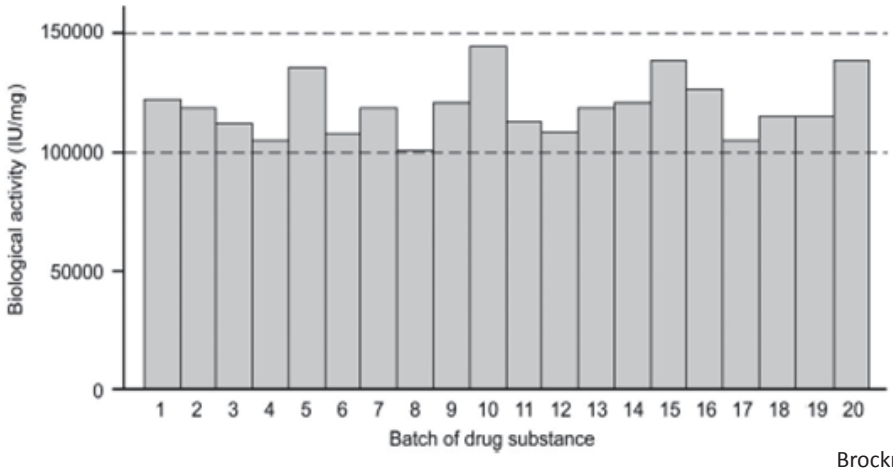


Source: https://www.genengnews.com/the-lists/the-top-15-best-selling-drugs-of-2016/77900868

- But measurement problems have not changed
  - Biologicals are inherently variable
  - How can you measure what you don't fully understand?
  - Sophisticated analytical technologies do not always provide solutions

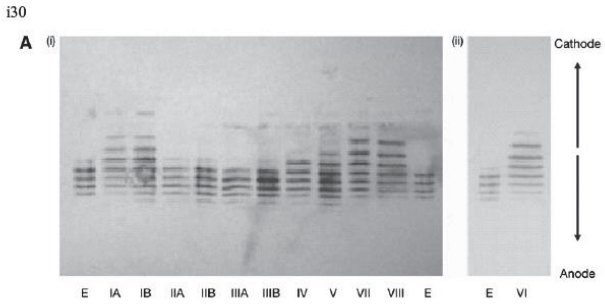
Variability of Biologicals

- "Non- identical" is an accepted principle in biotechnology
- No batch of any biological is "identical" to another



Brockmeyer and Seidel, EJHP 2009

Variability of biologicals



Schellekens H, NDT Plus (2009)

Medicines & Healthcare products Regulatory Agency

NIBSC

WHO International Standard
3rd WHO International Standard for Erythropoietin, recombinant, for bioassay
NIBSC code: 11/170
Instructions for use
(Version 1.0, Dated 30/10/2012)

1. INTENDED USE
The second International Standard (IS) for Erythropoetin (EPO) in ampoules coded 88/574 has been widely used for the calibration of preparations of recombinant DNA-derived EPO by bioassay. Stocks of the 2nd IS are exhausted and the World Health Organization (WHO) Expert Committee on Biological Standardization (ECBS) has recognized (2010) the need for a replacement International Standard for EPO for the assignment of potency to therapeutic preparations of recombinant human EPO used in the treatment of anaemia.

A new preparation of recombinant EPO has been filled into ampoules (NIBSC Code 11/170) and has been characterized by in vivo bioassay in an international collaborative study with expert laboratories and was established as the 3rd International Standard at the 63rd meeting of the ECBS. This material replaces the 2nd IS.

2. CAUTION
This preparation is not for administration to humans or animals in the human food chain

The preparation contains material of human origin, and either the final product or the source materials, from which it is derived, have been tested and found negative for HBsAg, anti-HIV and HCV RNA. As with all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures should include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

3. UNITAGE
Each ampoule contains 1650 IU of EPO

4. CONTENTS
Country of origin of biological material: USA.
Each ampoule contains the residue after freeze-drying of 0.5 ml of a solution that contained:

Recombinant human EPO	approximately 11µg
Human serum albumin	0.2 % (w/v)
Trehalose	1.0 % (w/v)
NaCl	0.12 % (w/v)

5. STORAGE
Unopened ampoules should be stored at -20°C.
Please note: because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.

6. DIRECTIONS FOR OPENING
DIN ampoules have an 'easy-open' coloured stress point, where the narrow ampoule stem joins the wider ampoule body.
Tap the ampoule gently to collect the material at the bottom (labeled) end. Ensure that the disposable ampoule safety breaker provided is pushed down on the stem of the ampoule and against the shoulder of the ampoule body. Hold the body of the ampoule in one hand and the disposable ampoule breaker covering the ampoule stem between the thumb and first finger of the other hand. Apply a bending force to open the ampoule at the coloured stress point, primarily using the hand holding the plastic collar.

Care should be taken to avoid cuts and projectile glass fragments that might enter the eyes, for example, by the use of suitable gloves and an eye shield. Take care that no material is lost from the ampoule and no glass falls into the ampoule. Within the ampoule is dry nitrogen gas at slightly less than atmospheric pressure. A new disposable ampoule breaker is provided with each DIN ampoule.

7. USE OF MATERIAL
No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution.
For practical purposes, each ampoule contains the same quantity of recombinant human EPO. The entire content of each ampoule should be completely dissolved in an accurately measured amount of buffer solution. The use of water to reconstitute ampoule contents is not recommended. The material has not been sterilized and the ampoules contain no bacteriostat.

COLLABORATIVE STUDY
The preparation was evaluated in a collaborative study in which fifteen laboratories in seven countries took part, organized with the following aims:

1) To calibrate the candidate preparation, 11/170 relative to the 2nd IS (88/574) for EPO by in vivo bioassays.

2) To determine the stability of the candidate preparation 11/170 by comparison with ampoules stored at elevated temperatures as part of an accelerated degradation stability study.

The geometric mean potency for the candidate standard was 1648 IU per ampoule (n=15; 95% confidence limits 1562 - 1738; GCV 10.1%)

The candidate preparation 11/170 is sufficiently stable to serve as an International Standard. Analysis of the thermally accelerated degradation samples in this study demonstrated no detectable loss of potency at these elevated temperatures. This suggests that 11/170 is likely to be highly stable under long term storage at -20°C.

8. STABILITY
Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label.
It is the policy of WHO not to assign an expiry date to their international reference materials. They remain valid with the assigned potency and status until withdrawn or amended.
In addition, once reconstituted, diluted or aliquoted, users should determine the stability of the material according to their own method of preparation, storage and use. Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.

NIBSC follows the policy of WHO with respect to its reference materials.

9. REFERENCES
Further information can be obtained from the report of the collaborative study which is available from the WHO website: http://www.who.int/biologicals/en/

10. ACKNOWLEDGEMENTS
We gratefully acknowledge the important contributions of all the participants and the manufacturer of the therapeutic EPO for the kind donation of material.

11. FURTHER INFORMATION
Further information can be obtained as follows:
This material: enquiries@nibsc.org
WHO Biological Standards:
http://www.who.int/biologicals/en/
JCTLM Higher order reference materials:

National Institute for Biological Standards and Control,
Potters Bar, Hertfordshire, EN6 3QG. T +44 (0)1707 641000, nibsc.org
WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory

World Health Organization

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What are the challenges?

- Biological medicines are now produced and marketed globally.
- Biosimilar route to licensure.
- Huge proliferation of next generation biologicals e.g. (monoclonal) antibodies, pegylated versions of existing products etc
- Vaccine standards for priority pathogens
- Complex Cell and Gene therapies

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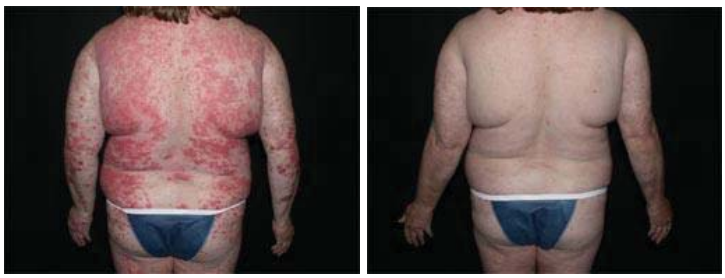
Biological medicines are global medicines

- Innovative biotherapeutics have revolutionised modern medicine and are now manufactured and marketed around the world



Before treatment

After treatment

Biological medicines are global medicines

What is important for the patient?

- Safety: where a medicine is labelled with a specific dose then the patient should expect the same actual dose each time.
- Quality: same minimum quality in all manufacturers products across countries.
- Access and Affordability: Availability and price

Global Pharmacopoeias

Collaborative working

- A more formal/efficient process making it possible to collaborate between pharmacopoeias on written and physical standards
  - Someone to take the lead in projects (Manufacturer, Expert Group, Expert committee)

- Adoption of certain metrological principles to facilitate the establishment of multiple regional (physical) standards with harmonised content.

What are the challenges?

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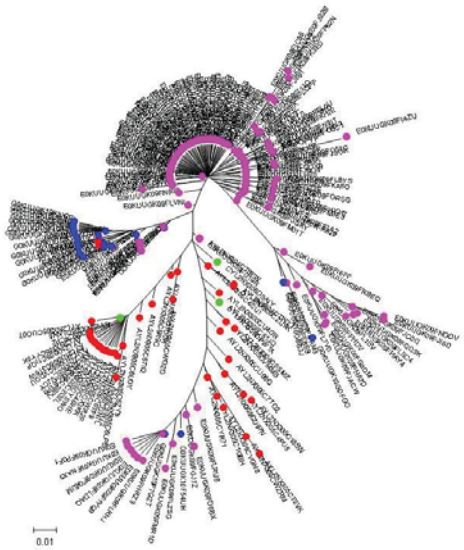
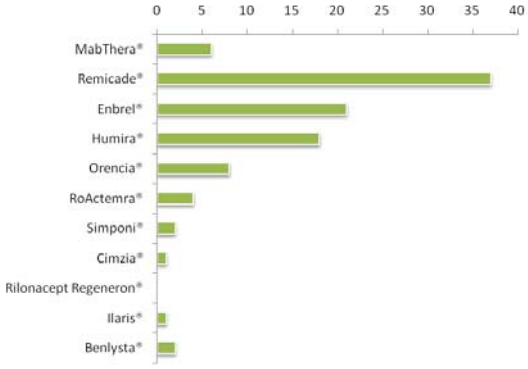
Biosimilar route to licensure

- Biosimilar route to licensure where acceptable quality is defined by the reference medicinal product
- What is the impact of authorization of multiple biosimilars?

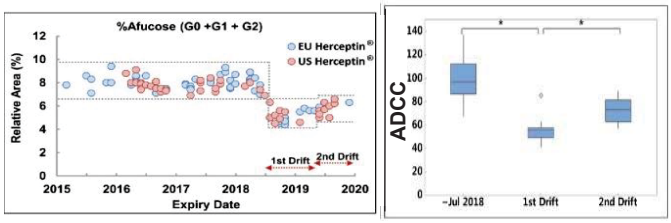


- Originator and biosimilars have their own lifecycle upon approval
- Very likely to be changes to their manufacturing processes
- Regulators have to find a way of identifying if this is a problem, and if so, how to solve it.

Changes in the manufacturing process of originator biologicals



Schneider CK. Ann Rheum Dis. 2013 Mar;72(3):315-8.
(Data source: EPARs on EMA website)



Herceptin® approved changes

Kim S., et al., MABS, 2017: 9(4):704-714

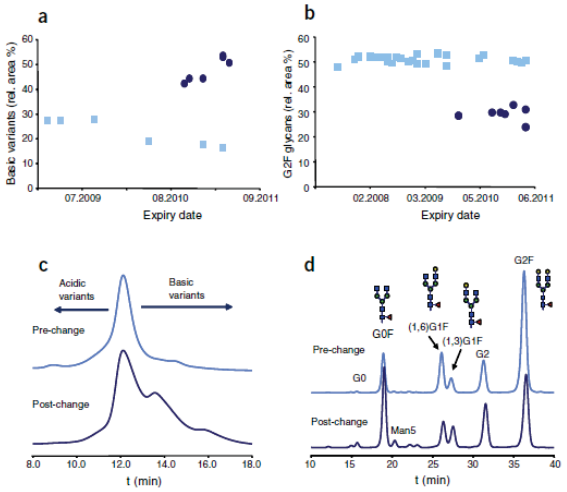
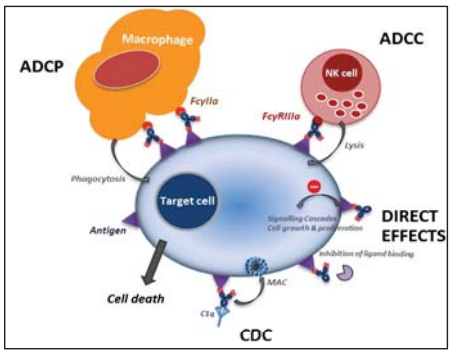


Figure 3 Comparison of the different pre- and post-change batches of Enbrel. (**a**) Relative amounts of basic variants of the pre-change ($n = 6$) and the post-change ($n = 6$) batches as measured by CEX. (**b**) Relative amount of the G2F glycan of the pre-change ($n = 25$) and the post-change ($n = 9$) batches. (**c**) Exemplary CEX chromatograms. (**d**) Exemplary glycan mapping chromatograms.

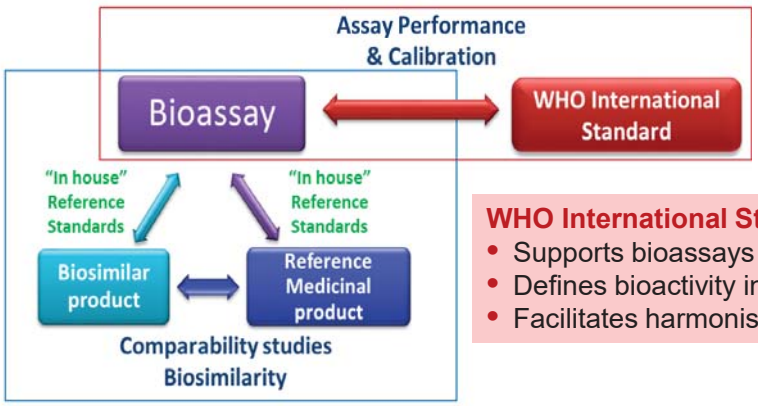
Schiestl M et al. Nat Biotechnol. 2011 Apr;29(4):310-2.

Supporting product consistency: WHO International Standards for mAbs

- Biotherapeutic mAbs are complex & heterogeneous products: small changes in PTM can affect product consistency, safety & efficacy
- Development of Biosimilars: potential cumulative drifts in bioactivity over time may be a concern



Typical MoA related to the clinical effects of mAbs



WHO International Standard
- Supports bioassays & local standards
- Defines bioactivity in IU: traceability
- Facilitates harmonisation of reported data

Established WHO IS for mAbs: Rituximab (NIBSC 14/210) & Infliximab (NIBSC 16/170)
Standardisation programs in the pipeline: Trastuzumab, Adalimumab, Bevacizumab, Cetuximab

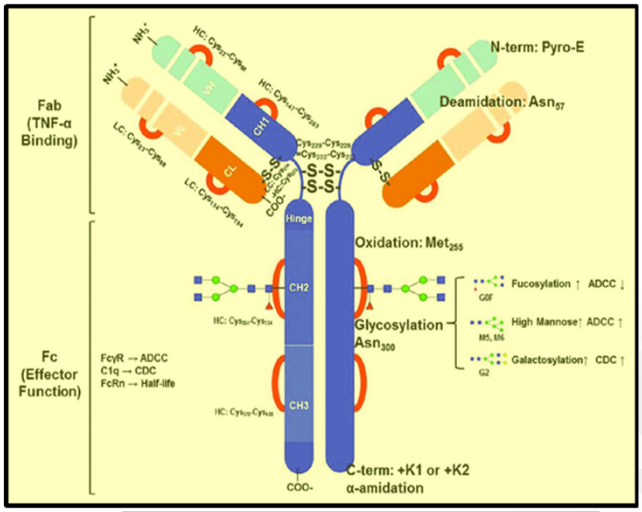
Reference standards supporting the new Ph. Eur. monograph on infliximab concentrated solution

13th International Symposium on Pharmaceutical Reference Standards

13-14 March 2019, Strasbourg, France

Dr Frank JUNG
Study Director
Laboratory Department / Biology Section
EDQM – Council of Europe

Dr Marie-Emmanuelle Behr-Gross
Scientific Officer
Department of Biological Standardisation and OMCL
EDQM – Council of Europe



$C_{6462}H_{9960}N_{1728}O_{2036}S_{44}$
M_r approx. 145 kDa (dimer without glycosylation)

Infliximab

2 glycosylation sites (one per HC);

exact composition of glycans can vary

EU marketing authorisations of medicinal products containing infliximab

Name	Marketing-authorisation holder	Date of authorisation
Remicade ®	Janssen	13/09/1999
Inflectra ®	Pfizer	09/09/2013
Remsima ®	Celltrion	09/09/2013
Flixabi ®	Samsung	26/05/2016
Zessly ®	Sandoz	18/05/2018

https://www.ema.europa.eu (EPAR)

Ph. Eur. monograph 01/2019:2928

01/2019:2928

INFLIXIMAB CONCENTRATED SOLUTION

Infliximabum solutio concentrata

- first compendial monograph for a specific therapeutic monoclonal antibody
- published in Ph. Eur. 9.6 in July 2018
- implementation date 01/01/2019

Monograph tests and compendial reference standards

PRODUCTION SECTION	**TESTS SECTION**
• Glycan analysis ***Infliximab CRS (SST)***	• Related proteins by CE-SDS ***Infliximab CRS (SST)***
• Charged variants by IEF ***Infliximab CRS (SST)***	• Impurities with molecular masses differing from that of infliximab (SEC) ***Infliximab CRS (SST)***
• Charged variants by ion exchange chromatography ***Infliximab CRS (SST)***	
IDENTIFICATION SECTION	**ASSAY SECTION**
• Peptide mapping ***Infliximab CRS (SST and identification)***	• Protein content (UV 280 nm, **specific absorbance given in the monograph**, ***no RS required***)
	• Potency by cell based bioassay ***Infliximab BRP***

Infliximab CRS batch 1 - established by the EDQM laboratory

INFORMATION LEAFLET Ph. Eur. Reference Standard

Infliximab CRS batch 1

1. **Identification**

Catalogue code: Y0002047 Unit Quantity: ca 100 mg

2. **Scientific Information**

2.1 Intended use

Reference Standard for laboratory tests as prescribed in the European Pharmacopoeia only.
Established for use with the monograph(s): 2928.

2.2 Analytical information related to intended use

Chromatogram(s)/spectrum : See annexes

One vial of inflixmab CRS 1 contains approximately 89 mg of protein. When reconstituted with 2.5 mL of water R, a concentrated solution with a protein concentration of approximately 35.6 mg/mL is obtained.

Infliximab CRS - use in glycan analysis

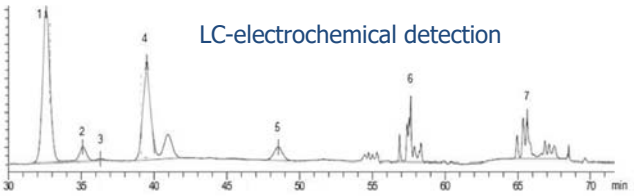
The glycan profile depends on the manufacturing process and in particular on the cell line used for the manufacturing. This is why **glycan analysis is described in the *production section* of the monograph.**

Flexibility is given as far as the method's choice, however a validated LC method is described in the monograph, where **peak ID relies on comparison with the chromatogram of the monograph (*documentary standard*).**
***Infliximab CRS* (*material standard)* is used to compare the LC profile to assess system suitability**.

The LC profile of the batch under test is then compared with that of a *in-house reference standard*, representative of the manufacturing process, whereas quantification is made by area normalization (no RS needed). No acceptance criteria given in the monograph ("as authorised by the competent authority").

Infliximab CRS - glycan analysis - SST

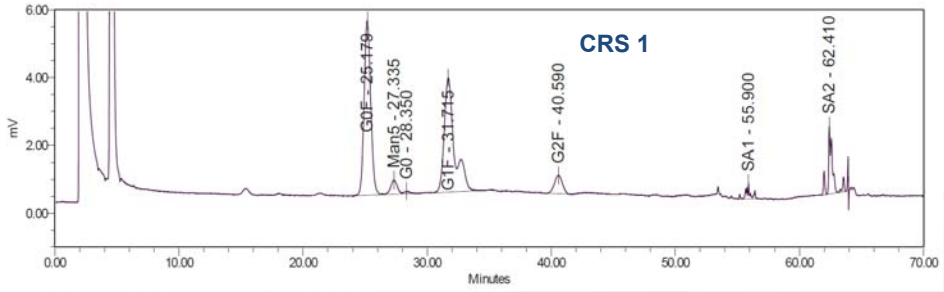
LC-electrochemical detection



Peak	Charged	Glycoform	Peak	Charged	Glycoform
1.	No	Fucosylated G0F (absence of terminal galactose)	5.	No	Fucosylated G2F (2 terminal galactoses)
2.	No	Afucosylated Man5 (addition of 2 mannoses instead of terminal *N*-acetylglucosamine)	6.	Yes	Sialylated SA1 (addition of 1 sialic acid)
3.	No	Afucosylated G0 (absence of fucose)	7.	Yes	Sialylated SA2 (addition of 2 sialic acids)
4.	No	Fucosylated G1F (1 terminal galactose)			

- peaks are identified using the monograph chromatogram (documentary standard)
- SST: the chromatogram obtained with infliximab CRS has to be qualitatively similar to the chromatogram given in the leaflet provided with infliximab CRS; peaks 1 to 7 must be clearly visible

Infliximab CRS 1 - establishment



Peaks were assigned by

a) comparison with monograph chromatogram (documentary standard)

b) **comparison with a chromatogram of manufacturer's in-house working standard run in the same chromatographic series**

Peak assignment is traceable to EU regulatory filing

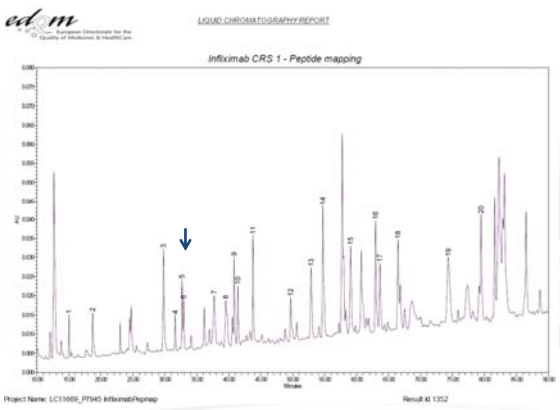
Infliximab CRS - use in peptide mapping

IDENTIFICATION IS NOT STUCTURE ELUCIDATION !
The elucidation of structure, which involves extensive characterisation of the substance using for example mass spectrometry of tryptic peptides is part of the regulatory filing, not part of testing for a monograph.

Ph. Eur. general notices

The tests given in the Identification section are not designed to give full confirmation of chemical structure / composition of the substance but are intended to confirm, with an acceptable degree of assurance, that the article conforms to the description on the label.

Infliximab CRS - use in peptide mapping (SST and identification)



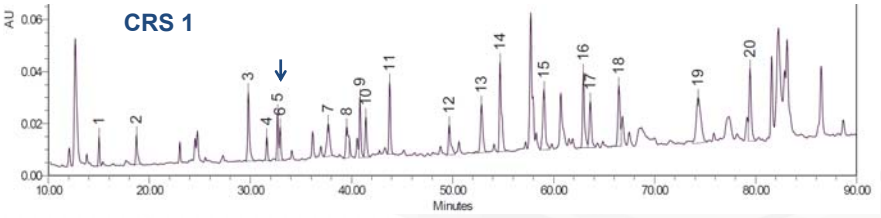
SST:

- the chromatogram obtained with infliximab CRS has to be qualitatively similar to the chromatogram supplied with infliximab CRS
- **Peaks 5 and 6** are separated as shown in the chromatogram supplied with infliximab CRS

Results:

- the profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with the reference solution
- no additional peaks with an area >0.5% of the sum of the areas of peaks 1 to 20

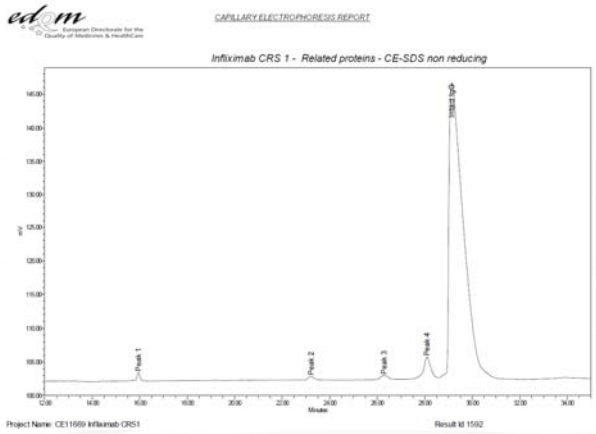
Infliximab CRS 1 - establishment



Peaks 1 to 20 were assigned by **comparison with a chromatogram of manufacturer's in-house working standard run in the same chromatographic series**

Peak assignment is traceable to EU regulatory filing

Infliximab CRS 1 - use in CE test for related proteins - SST



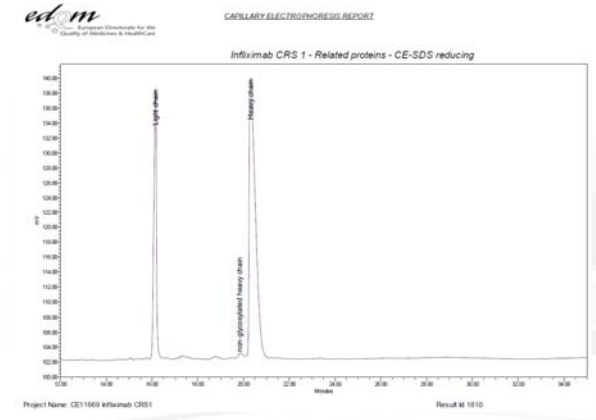
SST:

- the electropherogram obtained with infliximab CRS has to be qualitatively similar to the electropherogram supplied with infliximab CRS

Results:

- the profile of the electropherogram obtained with the test solution corresponds to that of the electropherogram obtained with the reference solution, except for minor peaks, that may be absent

Infliximab CRS 1 - use in CE test for related proteins - SST



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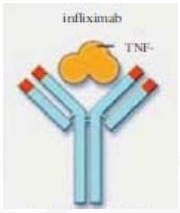
Infliximab Biological Reference Preparation (BRP) batch 1 – use in Infliximab potency bioassay in 2928

Principles

Suitable cell-based assay allowing to determine the **inhibitory action of infliximab on the biological activity of TNF-α**

Suitable read out for assessing this effect

Standard Infliximab BRP

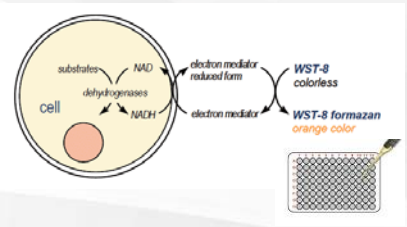


Adapted source: Nestle F. et al. 2009

Example procedure

Cytotoxicity assay, measuring the cytotoxic effect of TNF-α on **WEHI-164 cells.** Cell growth assessed through a **colorimetric assay**

Ability of Infliximab to block inhibition of proliferation of WEHI-164 assessed against BRP1



Infliximab BRP batch 1 – Establishment

Frame

Biological Standardisation Programme **(BSP**) a programme for the establishment of **Biological Reference Preparations (BRP)** carried out with funding by the European Union and by the Council of Europe

Infliximab BRP1 establishment study (BSP146)

Performed jointly with 1st WHO International Standard (IS) establishment study through a collaborative study involving 26 laboratories using different in vitro cell-based bioassays (TNF-α neutralisation, ADCC and complement dependent cytotoxicity) and binding assays.

WHO/BS/2017.2323, https://doi.org/10.1080/19420862.2018.1532766

Infliximab BRP batch 1 – Establishment some data

Materials tested

Candidate freeze dried reference preparation (16/170) prepared from a single batch of bulk recombinant Infliximab samples A and C

Comparator freeze dried product (16/160) prepared from a commercial pharmaceutical preparation sample B

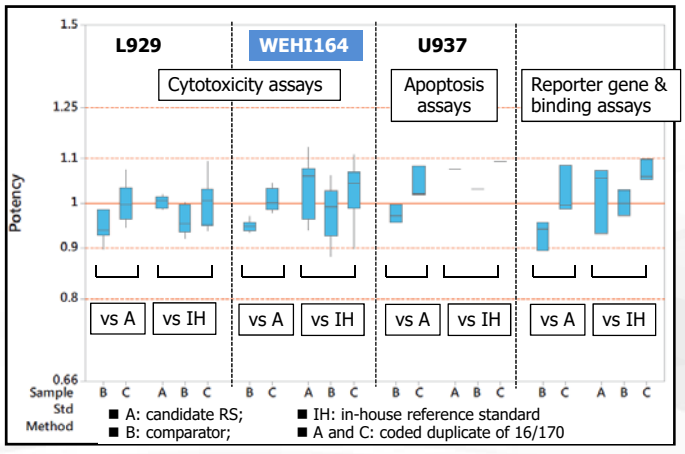
Where available in-house standards IH

Final outcome

Candidate freeze dried reference preparation 16/170 was adopted as the 1st WHO IS and as Ph. Eur. BRP with an assigned potency of 500 IU/ampoule

*

Ph. Eur. BRP for Infliximab – WHO/EDQM Study Results –



Adapted from C. Metcalfe et al, *The first World Health Organization International Standard for infliximab products: A step towards maintaining harmonized biological activity*, MABS, 2018

Ph. Eur. BRP for Infliximab
– WHO/EDQM Study Results –

Method	Sample	Potencies relative to Candidate A					Potencies relative to IH reference				
		GM	LCL	UCL	GCV	n	GM	LCL	UCL	GCV	n
WEHI-164 cytotoxicity assay	A						1.04	0.97	1.10	7.3%	7
	B	0.95	0.94	0.96	1.3%	9	0.98	0.92	1.04	6.7%	7
	C	1.01	0.99	1.03	2.6%	9	1.03	0.96	1.10	7.5%	7
Overall cell-based neutralisation assays*	A						1.02	0.99	1.06	5.9%	16
	B	0.95	0.94	0.96	2.7%	22	0.98	0.96	1.01	5.1%	16
	C	1.01	1.00	1.03	3.9%	21	1.03	1.00	1.07	6.5%	17

* Cytotoxicity using L929, WEHI-13 cell-lines; U937 apoptosis assay and reporter gene assays used in the WHO study.

WEHI-164 cytotoxicity assay – individual laboratory results:

- potency estimates obtained by the different labs are very similar for preparations relative to A with GCVs < 11.6%.

(EDQM article under preparation for publication in Pharmeuropa Bio & Scientific Notes)

Conclusions

- The Ph. Eur. reference standards **infliximab CRS and infliximab BRP are qualified for the intended use(s)** described in Ph. Eur. monograph 01/2019:2928 for infliximab concentrated solution
- Their intended use is for the determination of pharmaceutical quality in line with compendial standards and as such they are **not to be used for other purposes and notably in vivo assays, clinical comparability studies nor for defining biosimilarity**, and should not be inferred as serving this purpose.

Thank you very much for your attention.

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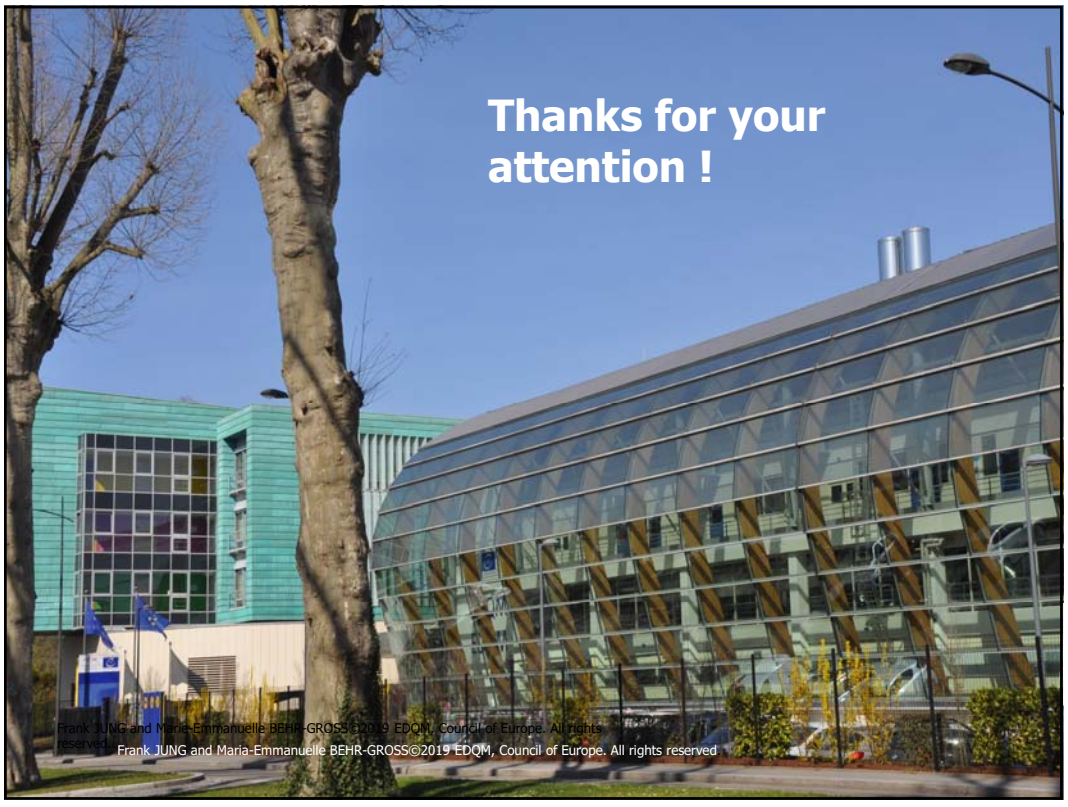
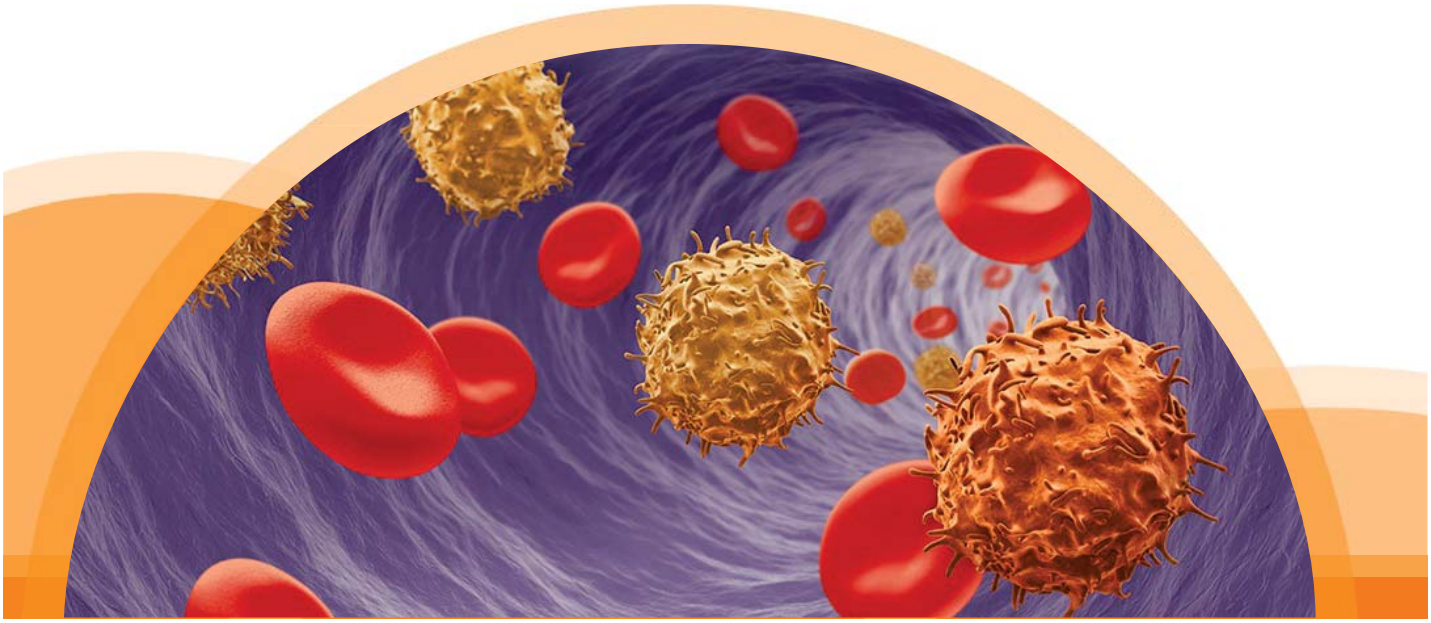


Automated and decentralized CAR-T cell manufacture

Boro Dropulić, PhD, MBA
General Manager & Chief Science Officer
Lentigen, a Miltenyi Biotec company



Miltenyi Biotec: Enabling Cell & Gene Therapy

- Miltenyi Biotec is a 2500-person company with global operations
- Headquarters near Cologne, Germany
- Over 500 scientists and engineers working in R&D
- 14,000 products: magnetic cell isolation reagents, buffers, devices, media, antibodies, stimulation reagents, plasmids and vectors
- Lentigen was acquired in August 2014 by Miltenyi Biotec GmbH
- Integration of LV manufacturing competency with MB automated work flows for the manufacture of gene-modified cell therapy products

Lentigen, a Miltenyi Biotec company

- Located in suburbs of Washington D.C.
- Specialize in Lentiviral vector (LV) design, construction, pre-clinical & GMP manufacture
- 34,000 ft^2 facility meets all US and EU requirements for commercial manufacture of Lentiviral vectors
- BMF filed with FDA on our Lentiviral vector MFG methods; similar for EU, Canada, etc.
- 2 x 200L CD-SFS LV manufacturing process – $5x10^8$ to 10^{10} TU/ML = Vector copy number (VCN) in transduced cells
- Hundreds to thousands of doses per lot
- Dramatic reduction of cost per dose = VCN
- **Reference standards to determine vector copy number in transduced cells becoming important for field**

How we assist investigators: vectors are the first step to generate the final product – gene modified cells

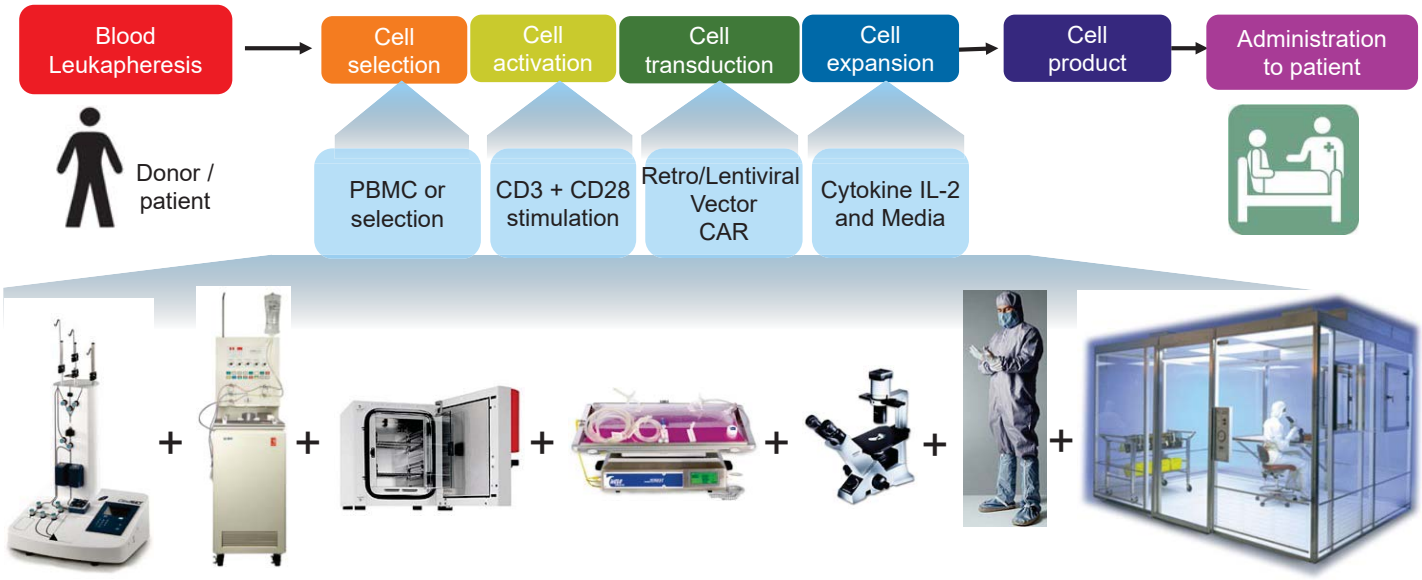
Clinical Centers

- Provide our LV-CARs as investigative products to qualified sites under an Investigator Initiated clinical Trial (IIT) agreement
- Contract and collaborative models for development of investigator IP
- Design & manufacture of LVs – research, pre-clinical, GMP grades
- Enable automated T cell manufacturing at the clinical center
- Regulatory support – assist CMC, clinical protocol, cross reference BMF

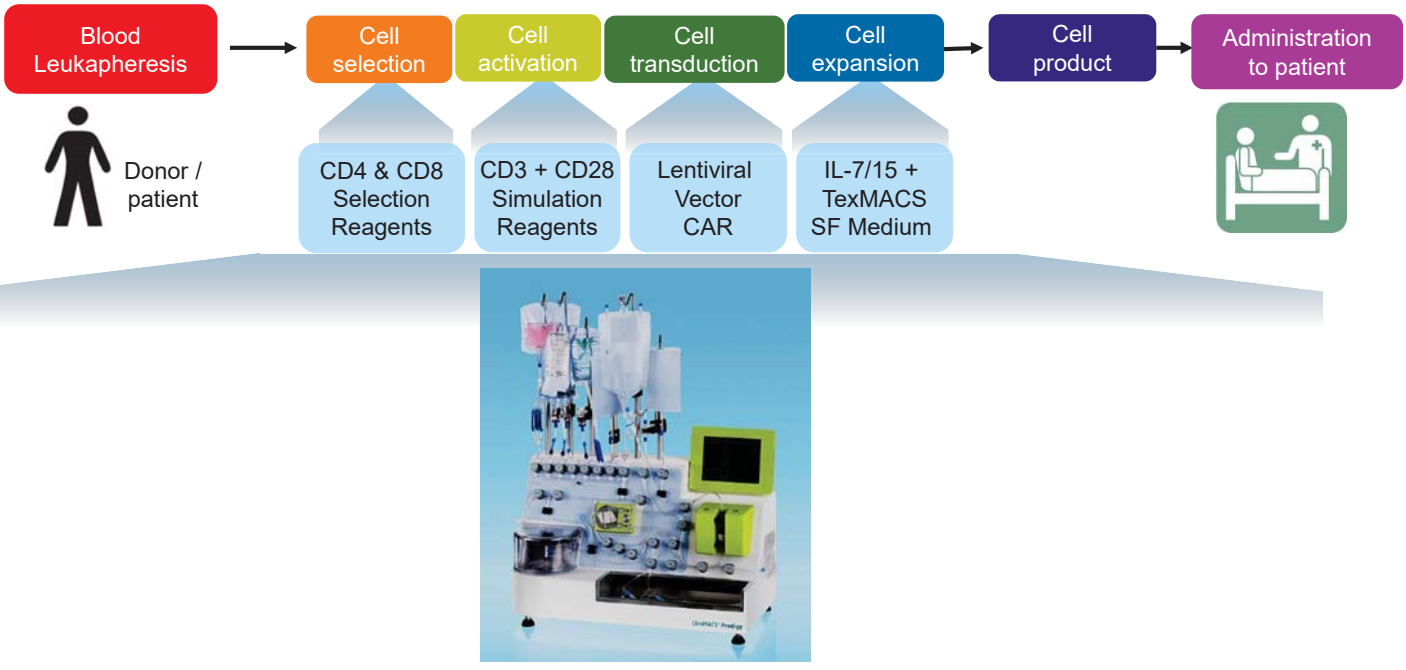
Companies

- Supply of LV with company payloads – R&D, Clinical, Commercial
- Contract fee-for-service only relationship
- Design & manufacture of LVs – research, pre-clinical, GMP grades
- Enable automated T cell manufacturing at desired site
- Regulatory support – assist CMC, cross reference BMF

Generation of CAR-T cell products using manual processes are complex and difficult to integrate



Integration of unit operations into a single device: Automation of cell processing



The CliniMACS Prodigy®

Robust T cell expansion from donor and patient cells

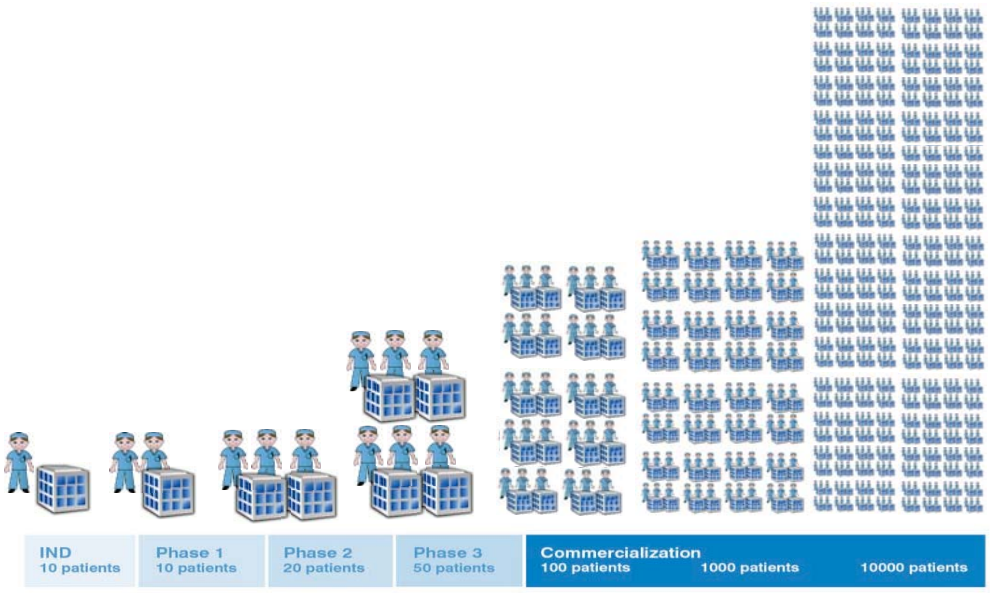
Healthy donor	T cell count for culture	final T cell count	T cell expansion	Final CAR+ T cell count
non-transduced (n=5)	0.87E8 (±0.2)	2.80E9 (±1.3)	36 fold (±23)	-
Transduced (n=7)	1.00E8 (±0.0)	3.06E9 (±0.9)	32 fold (±12)	**9.07E8 (±5.4)**

Patient material	T cell count for culture	final T cell count	T cell expansion	Final CAR+ T cell count
Melanoma (M35-WB)	0.21E8	2.35E9	112 fold	**1.38E9**
Lymphoma (L42-LP)	1.00E8	3.68E9	37 fold	**1.54E9**
Lymphoma (L43-LP)	1.00E8	3.31E9	33 fold	**Not CAR**
Lymphoma (L55-LP)*	0.55E8	4.90E9	89 fold	**1.09E9**
Lymphoma (L56-LP)*	0.55E8	3.20E9	58 fold	**0.72E9**
SUM	**0.66E8 (±0.33)**	**2.89E9 (±0.7)**	**66 fold (±34)**	**11.8E8 (±3.6)**

* frozen starting material

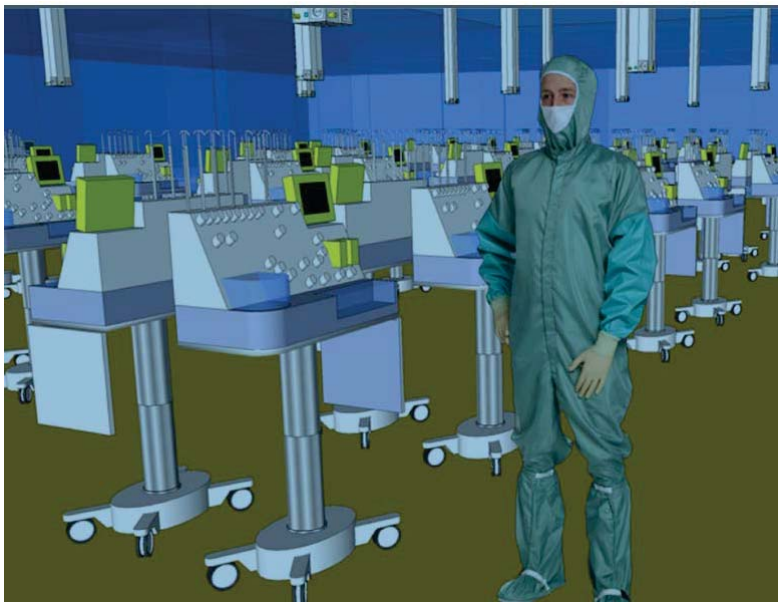
VCN or FACS

Current state of the art: Manual manufacturing of gene-modified cell products



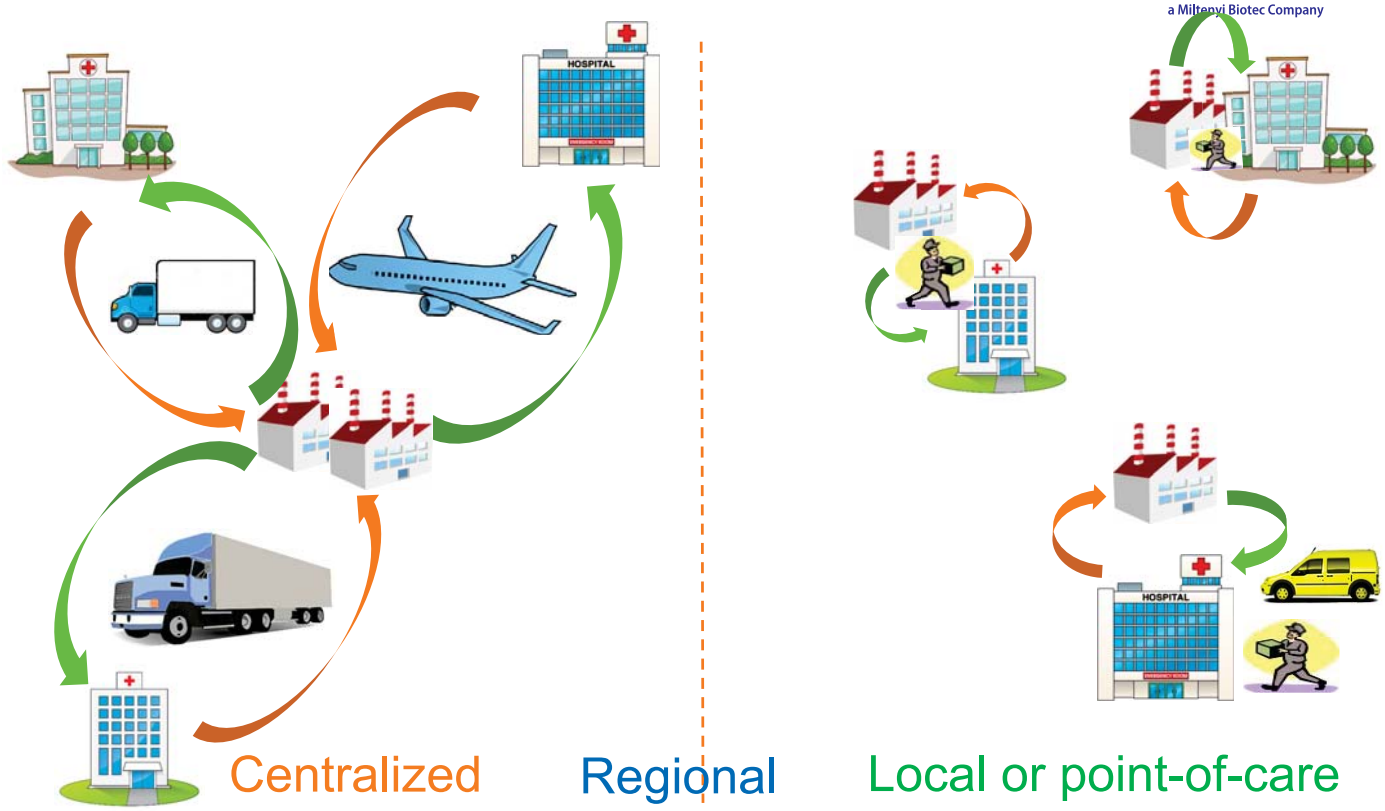
- Manual methods require many highly experienced technical staff
- Manual methods are difficult to scale for a large number of patients
- Manual methods are difficult to scale beyond single site – comparability issue

Automation will improve the economics for production of CAR-T cells and other patient-specific products



- Automation results in significant labor cost savings → decrease cost
- Automation reduces the overall product failure rate → decrease cost
- Automation provides options for manufacture of cell products beyond one site

Automated cell processing provides options for the manufacture of patient specific cell therapies



Demonstrating consistency of local CAR-T cell manufacture: A phase I/II clinical trial in Germany

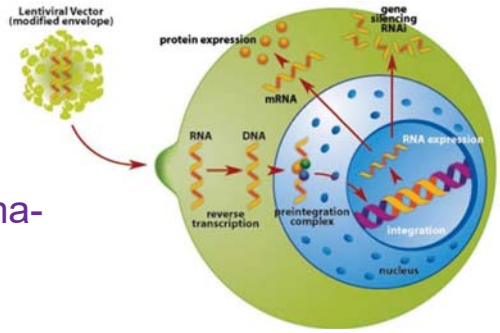
- GMP LV19CAR modified CAR-T cells are to be manufactured at 7 sites
- A Phase I/II clinical trial is about to start at 12 clinical sites in Germany (Ped + Adult)
- Main goal is to investigate product comparability and accrue outcome data
- Dosing will be fresh with option for frozen
- Global IIT network - most IIT sites will start with LV19CAR-T to show consistency
- Data from IIT sites will be collected to demonstrate CAR-T cell product manufacturing consistency globally
- Dose is important for consistency

vector copy number = dose

Lentiviral vectors: most efficient vector system for robust genetic modification of many types of primary human cells

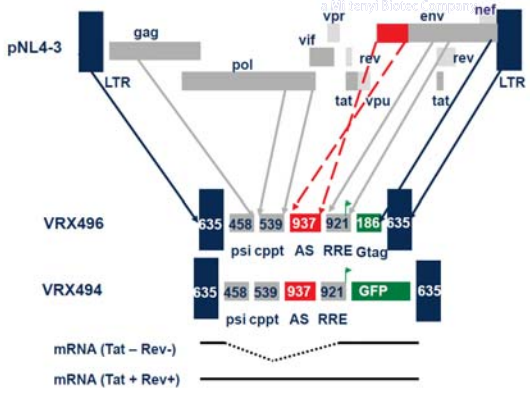
Clinically proven vector workhorse:

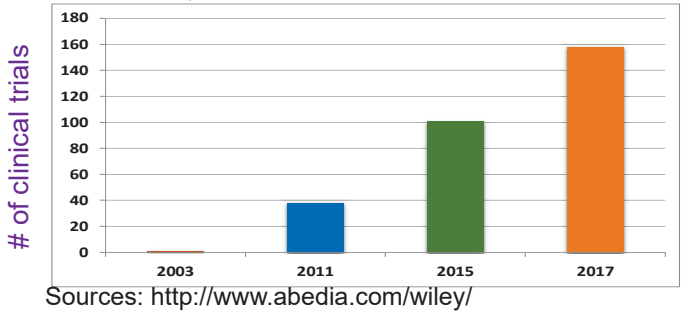
- Highly efficient stable transduction of dividing and non-dividing cells – up to 100%, without toxicity
- High gene payload capacity (2 to 6kb is the norm)
- Efficient transduction occurs *in absence of significant stimulation* – in contrast to gamma-retroviral vectors
- Very stealth – no genotoxicity observed in numerous clinical studies; in contrast to gamma-retroviral vectors
- Lack of genotoxicity observed in HIV natural infection: Over 30 million people infected with virus, billions of integration events with no evidence of T cell leukemia, even in the era of HAART



Our team's experience goes back to the very first Lentiviral vector clinical trial in humans

- HIV vector for the treatment of HIV infection
  - AIDS ideal disease to first test HIV vectors
- Vector expressed an 937nt anti-HIV antisense
  - Developed first LV MFG & QC methods
  - RAC, BRMAC FDA meetings for Ph. I trial
- Safety was established in this Ph. I clinical trial
  - 5 patients, no LV related adverse events
  - 15 years later, thousands of patients infused with LVs, no SAE related to LV



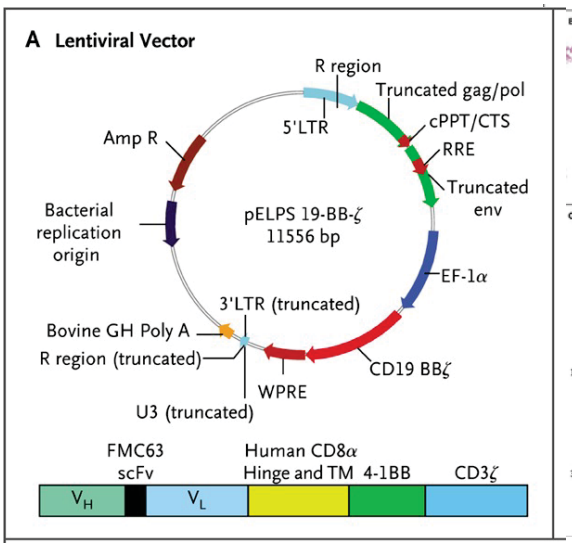


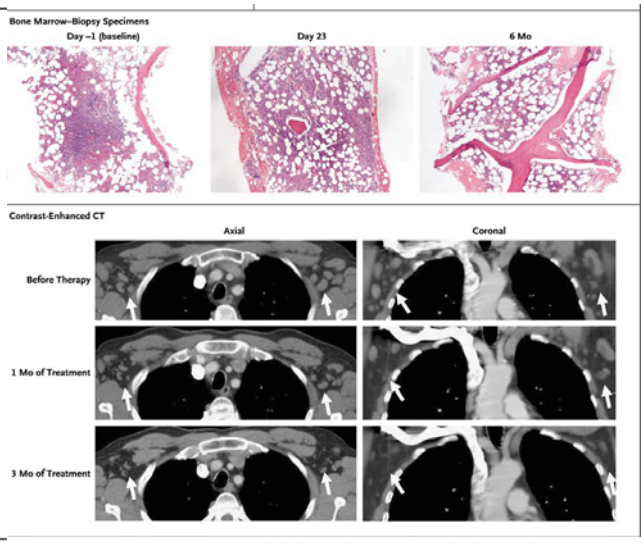
Sources: http://www.abedia.com/wiley/

Dropulic, RAC/OBA meeting June 8, 2004
Levine, Dropulic, June et al PNAS et al 2006

Moment of first-ever infusion of Lentiviral vector transduced T cells at the University of Pennsylvania GCRC on July 21, 2003

Lentigen designed and manufactured the Lentiviral vector for the UPenn anti-CD19CAR CLL clinical trial



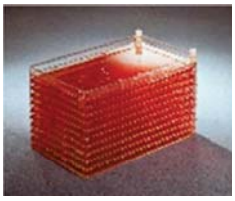


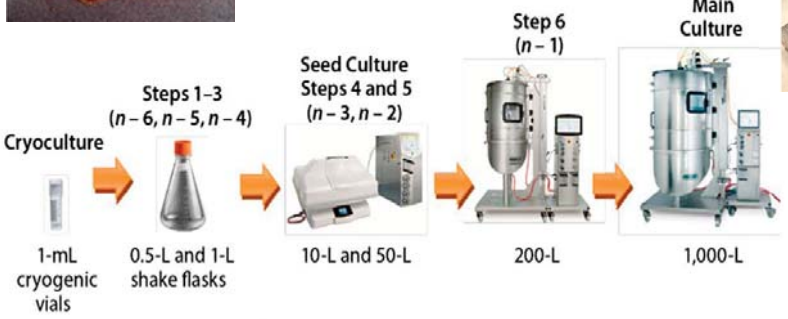
New Eng. Journal Med 2011; 365:725-733

Implementation of Chemically-Defined, Serum-Free Suspension (CD-SFS) GMP LV Manufacturing

Lentigen
a Miltenyi Biotec Company

Adherent serum method





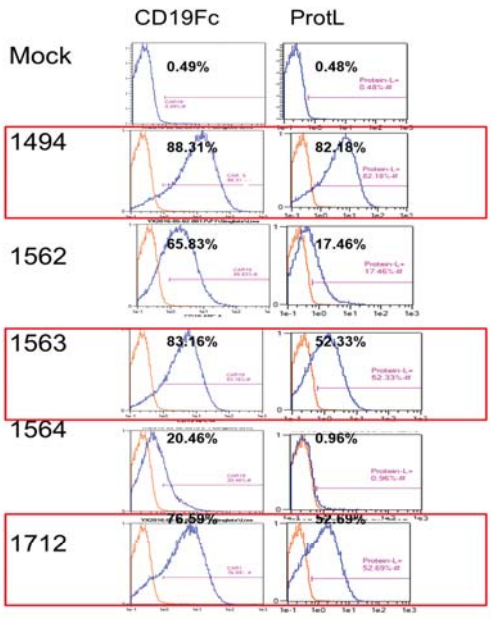
CD-SFS method

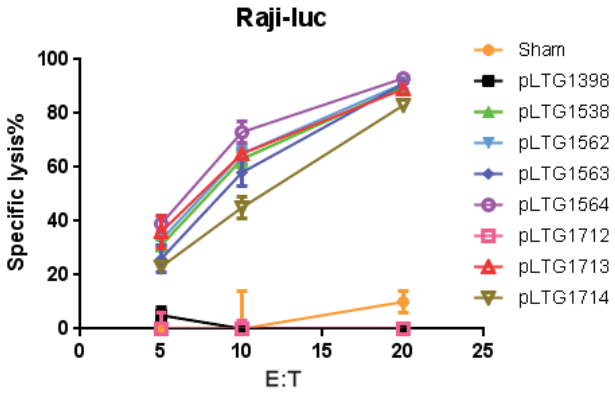
LV titers 5 x 10^8 - 10^{10}TU/ml by VCN
200/400L Commercial Process
1000s doses per lot
dramatic reduction in cost per vector dose

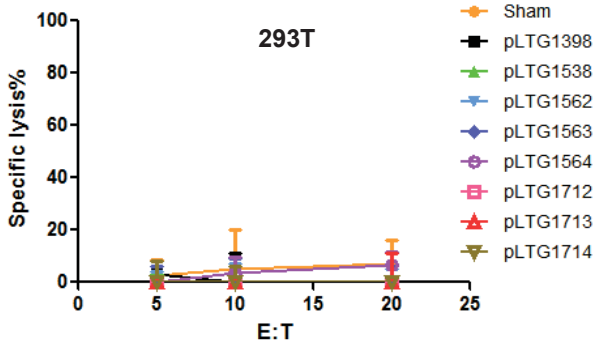
Novel LV19CAR constructs demonstrate surface CAR expression and tumor specific cell killing

Lentigen
a Miltenyi Biotec Company

Novel LV19CARs



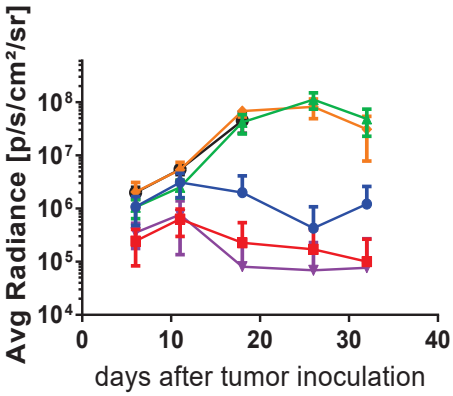


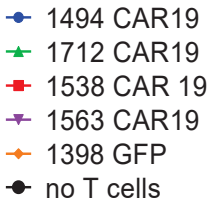


Novel LV19CAR vectors eliminate Raji CD19+ tumors in vivo

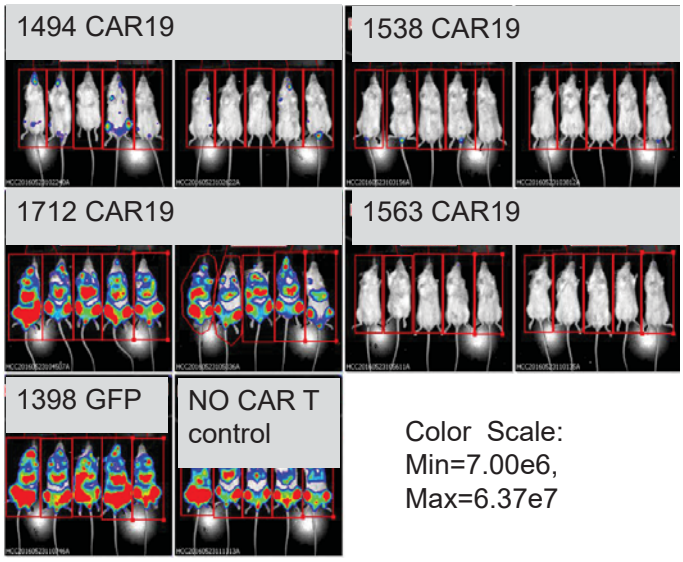
Lentigen
a Miltenyi Biotec Company

Novel LV19CARs





Day 18

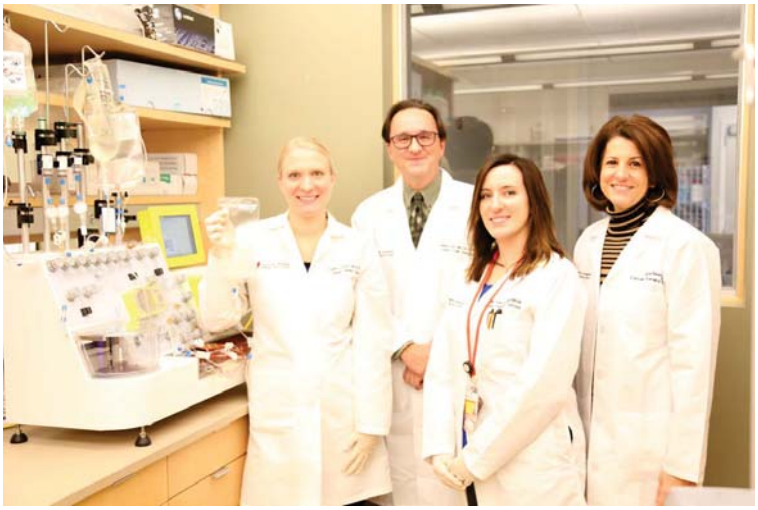


Color Scale:
Min=7.00e6,
Max=6.37e7

First-in-man clinical trial using transmembrane modified LV19CAR-T cells (LTG1563)

Lentigen
a Miltenyi Biotec Company

1. **Clinical trial at Case Western Reserve University (CWRU-UH)**
   – 4 out of 5 patients CR
   – 1 PD
   – < Grade 2 tox (CRS + NT)
2. **Clinical trial at The Dmitry Rogachev National Research Center of Pediatric Hematology, Oncology and Immunology in Moscow, Russian Federation**
   – 19 out of 21 patients CR
   – 2 PD
   – < Grade 2 tox (CRS + NT)
3. **LVCAR19 is available for free to academic clinical institutions under an IIT clinical trial agreement** – when used in combination with the Prodigy, MB materials and reagents



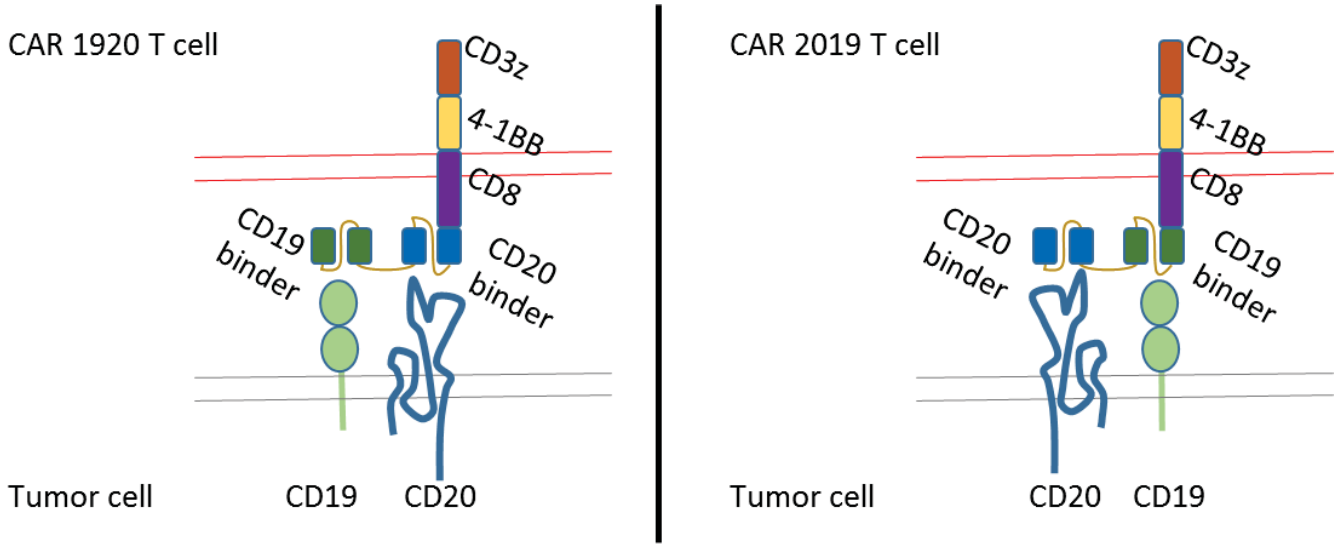
The cell processing laboratory at CWRU is a ISO7 controlled laboratory. Prodigy is not housed in the clean room since the device is a closed system.

Innovation with LV19CAR is encouraged
e.g. LV19CAR + immunoncology agent

Development of Tandem bispecific CD19-CD20 targeting CAR T cells for Adult Lymphoma

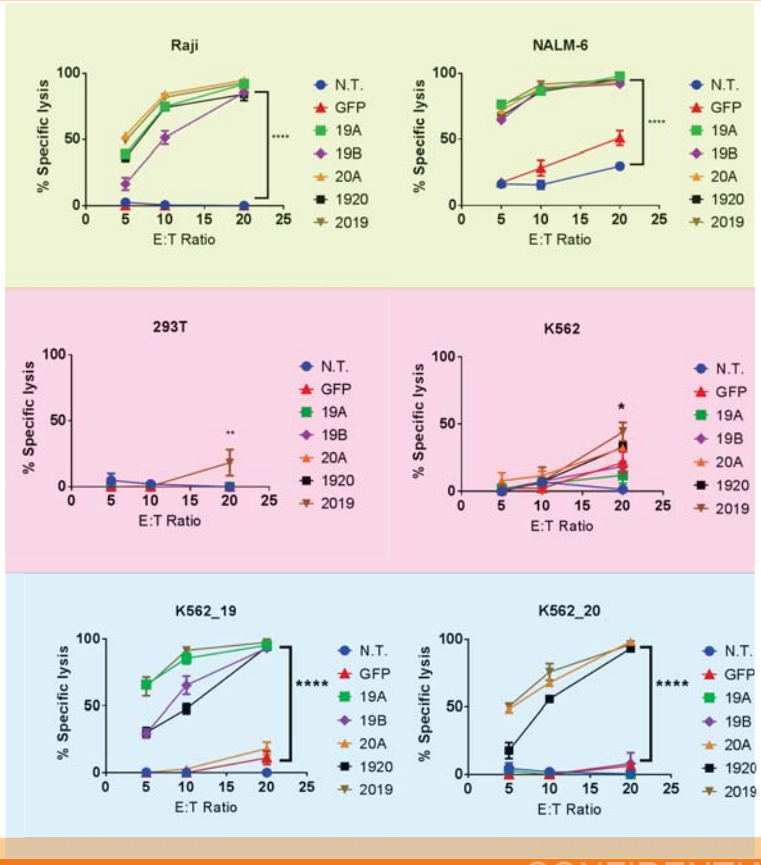
Lentigen
a Miltenyi Biotec Company

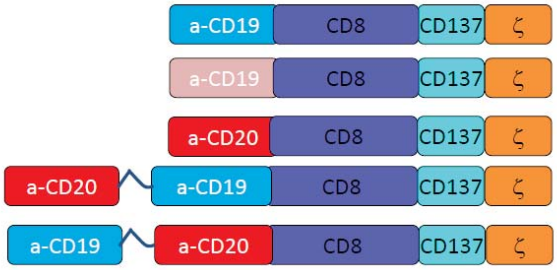
CAR T cells targeting two B cell tumor antigens at once are postulated to be more efficient, and to prevent tumor antigen escape



Monospecific and bispecific CAR-T cells demonstrate target-specific lysis of tumor cells in vitro

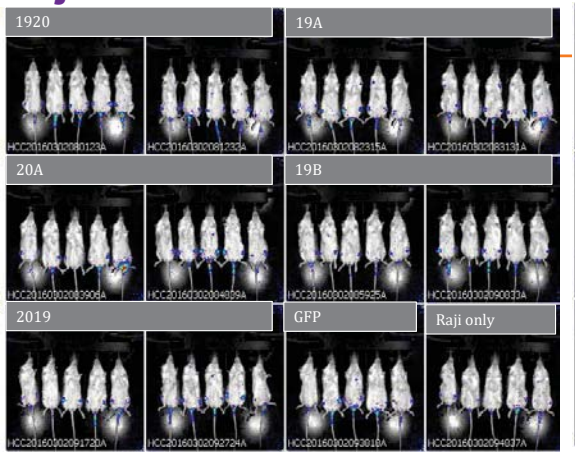
Lentigen
a Miltenyi Biotec Company



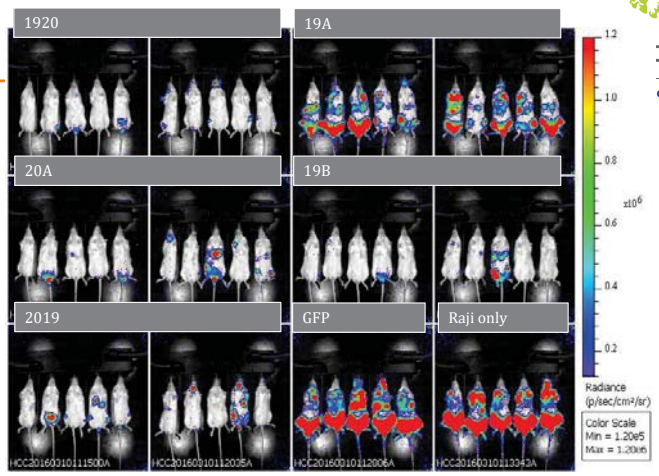


*p<0.05, ** p<0.01. **** p<0.0001
N.T. – non-transduced T cell control

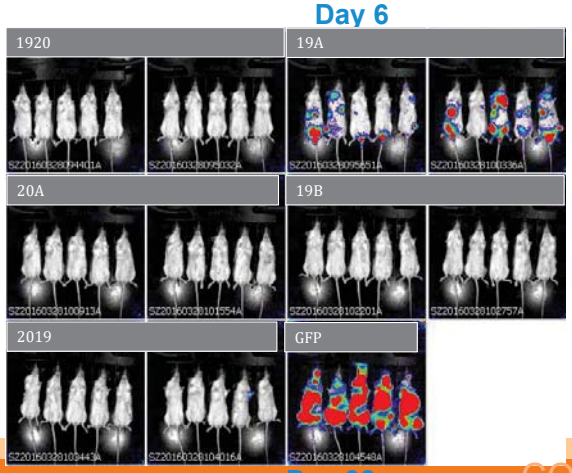
Tandem and single CD19, CD20 CAR T cells eliminate Raji tumors in vivo



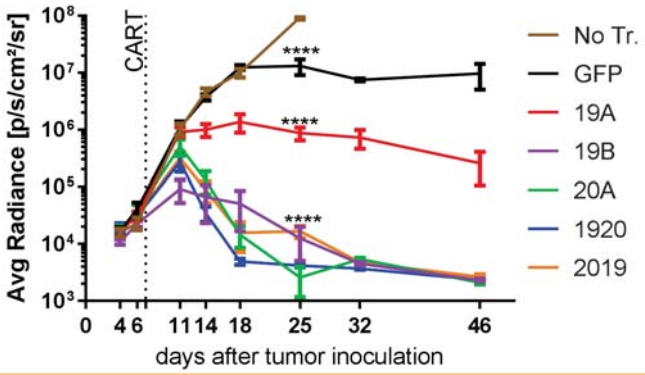
Day 6



Day 14

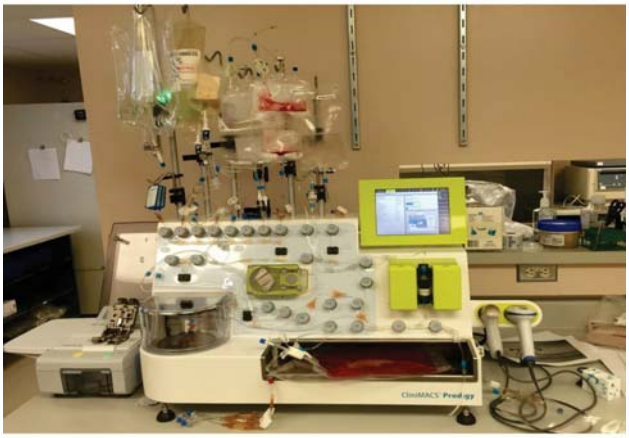


Day 32



First-in-man clinical trial using a bispecific tandem LV20.19CAR-T cells: 100%OOR after reaching target dose

1. **Day 28 Overall Response Rate: 81% (9/11 patients)**
   - –CR 6/11
   - –PR 3/11
   - –2 patients with PD at Day 28
2. **Response by Dose Level**
   - – 2.5 x 10^5 cells/kg: 1 CR, 1 PR, 1 PD
   - – 7.5 x 10^5 cells/kg: 1 CR, 1 PR, 1 PD
   - – 2.5 x 10^6 cells/kg: 4 CR, 1 PR (100% ORR)
3. **Expansion of clinical trial**
   - – Phase II clinical CIT trial
   - – Stanford and MCW sites

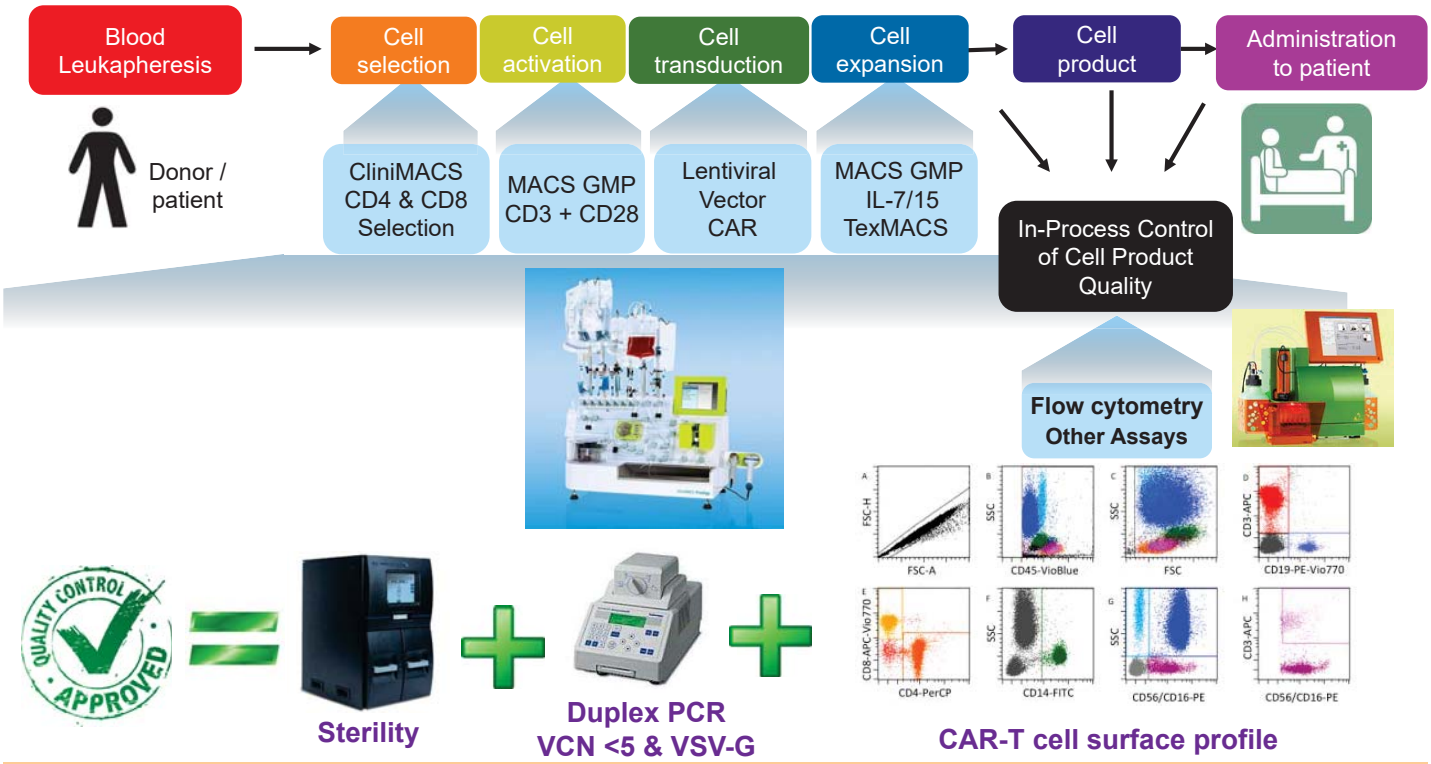


The cell processing laboratory at MCW where CAR-T cells were manufactured for first-in-man LV19.20CAR-T clinical trial

Class 10,000 (ISO7) controlled laboratory at MCW

Point-of-care cell processing facilities integrate cell manufacturing with real time analytics

Lentigen
a Miltenyi Biotec Company



CONFIDENTIAL

Development of a Cellular DNA Reference Standard for Qualification of LV Vector Copy Number (VCN)

Lentigen
a Miltenyi Biotec Company

Reference Standards Goals

- Vector copy number (VCN) important parameter for dosing gene modified cell products
- Fully characterized cell lines with copy numbers of 1, 2, 3, 4 as reference standard
- Cell line chosen that closely approximates chromosomal number in primary human cells: Jurkat cells were selected, a T cell derived cell line
- Show uniform performance among platforms, laboratories, operators and assays
- Use orthogonal methods for testing to confirm copy number in each cell line
- Determine stability of copy number in each cell line

Conclusions

- Lentigen developed and characterized a set of cell line VCN reference standards for use as controls to establish assays to quantitate integrated lentiviral vector copy numbers in clinical and non-clinical lentiviral gene therapy products

- Superior than current methods to determine copy number which spikes plasmid DNA into cellular chromosomal DNA: more precise, more similar to actual products

- Source material engineered from Jurkat cells provides a stable, renewable source of vector copy number reference standards for lentiviral gene therapy applications

- Jurkat cell line based VCN standards provide uniform VCN results across orthogonal platforms

- Starting to work with EDQM and USP to validate cell line VCN reference standards

USP Reference Standards and Methods Performance

Fouad Atouf, Ph.D.
Head, Global Biologics

March 13, 2019

Outline

- USP Reference Standards development approaches
  - opportunities for collaboration
- Reference standards to support method performance: cases studies
  - Peptides, method resolution
  - Proteins, measurement of variants
  - Cell enumeration
- USP Biologics strategic direction

1 USP Reference Standards development approaches

USP Reference Standard development approaches: opportunities for collaboration

- USP
- Industry
- Pharmacopeias
- Academia
- Gov't. Labs
- Contract Labs

Collaborative studies and types of reference standards

Types of Reference Standard

Qualitative application	Quantitative application
Identification, Peak identification, Resolution solution	Potency / Assay / Limit test
▸ Confirm identity of candidate material ▸ Evaluate chemical identity by compendial and non-compendial techniques ▸ Demonstrate the suitability for use in the compendial applications ▸ No value assigned to the RS candidate	▸ Confirm identity of candidate material ▸ Evaluate chemical identity by compendial and non-compendial techniques ▸ Value assignment (mass-balance, bioassay, etc) of the RS candidate ▸ Potency RS calibrated against the current International Standard

Reference standard development approaches

Bulk Candidate

Conventional

Collaborative study

Definitive Fill

Small Molecules

Non-routine

▸ Quantity limited, hygroscopic, stability
▸ Proposed RS presentation different from sponsor (liquid vs. solid)

Pre-characterization of bulk

Formulation/Lyophilization

Pilot Fill

Large Molecules

Definitive Fill

▸ Content of fill
▸ Homogeneity
▸ Stability studies

Collaborative study

2 Reference standards to support method performance: case studies

Case study 1: Octreotide Acetate reference standards

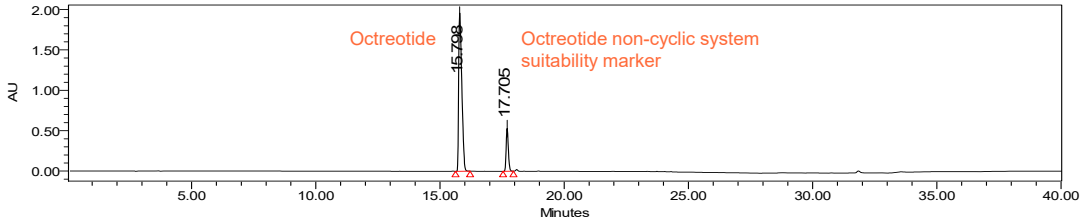
Supports Octreotide Acetate monograph

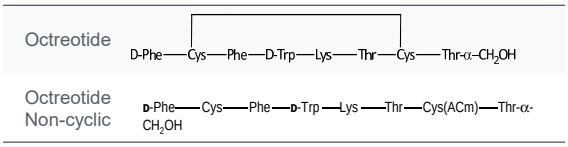
1. ***Octreotide Acetate RS***, Quantitative and qualitative applications
   - ID for IR (qualitative)
   - Assay (quantitative)
   - Related Compounds for resolution (qualitative)

   Value assignment for Octreotide on free basis by the assay against the bulk material characterized by mass balance calculation

2. ***Mixture of Octreotide Acetate RS and Octreotide Non-Cyclic System Suitability Marker RS*** , is used to demonstrate method resolution

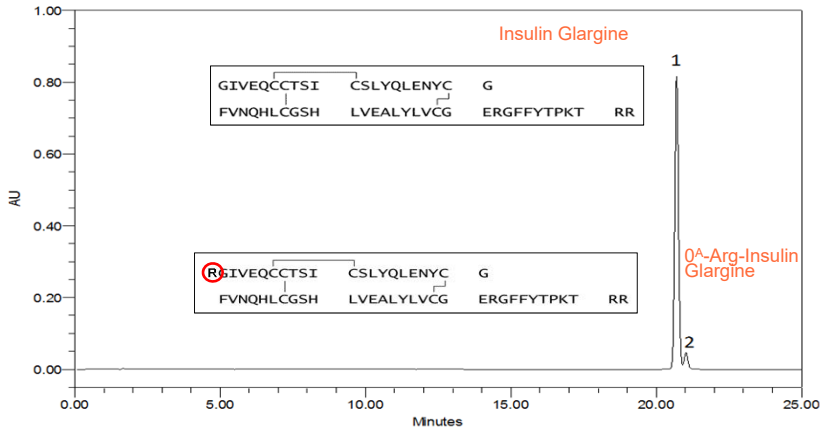
Mixture of octreotide acetate RS and octreotide non-cyclic system suitability marker RS





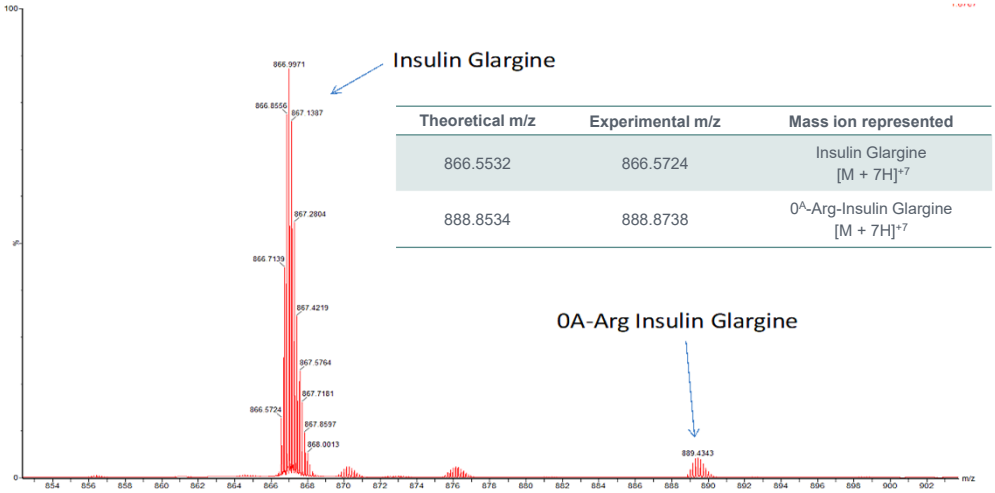
Mixture is prepared and used in assay/limit of octreotide related compounds (RP-HPLC), allows to detect difference in structure between cyclic and non cyclic

Case study 2: Insulin Glargine for peak identification RS from the tests for assay/related compounds (RP-HPLC)



This RS is a mixture of 3 mg of Insulin Glargine and 0.2 mg of 0^A-Arg-Insulin Glargine, and used as a system suitability solution (peak-to-valley ratio is NLT 2)

MS analysis for Insulin Glargine and 0^{A}-Arg-Insulin Glargine components in Insulin Glargine for peak identification RS



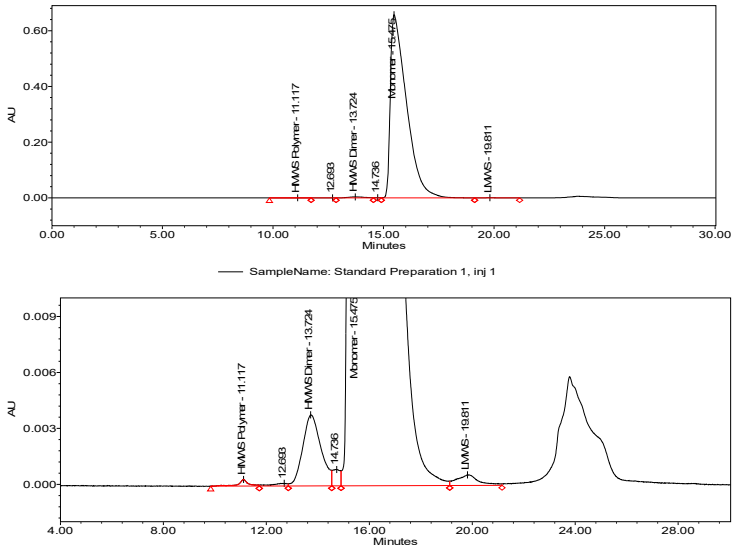
Theoretical m/z	Experimental m/z	Mass ion represented
866.5532	866.5724	Insulin Glargine $[M + 7H]^{+7}$
888.8534	888.8738	0^{A}-Arg-Insulin Glargine $[M + 7H]^{+7}$

Case study 3: Monoclonal IgG system suitability RS - General Chapter <129>

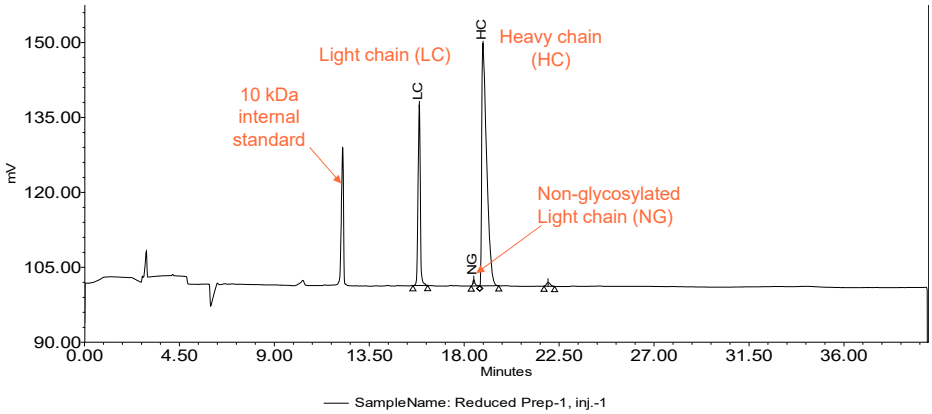
Chapter	Compendium	Test*	Test*
<129> Analytical Procedures For Recombinant Therapeutic Monoclonal Antibodies	Official in USP39 May 1, 2016	Size Exclusion Chromatography	Capillary SDS Electrophoresis (Reduced and Nonreduced)

*System Suitability Solution

SEC-HPLC chromatogram for monoclonal IgG system suitability RS (Qualitative and Quantitative)

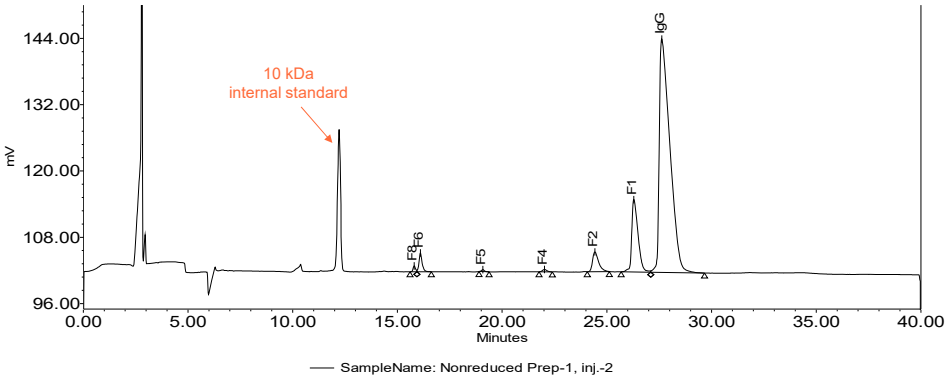


Electropherogram for monoclonal IgG system suitability RS Capillary Electrophoresis SDS under *Reduced* condition



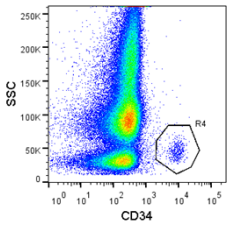
Sensitive method for the quantitation of non glycosylated vs. other forms (half antibodies, and other fragments), analysis of LC, HC

Electropherogram for monoclonal IgG system suitability RS Capillary electrophoresis SDS under *Non-Reduced* condition

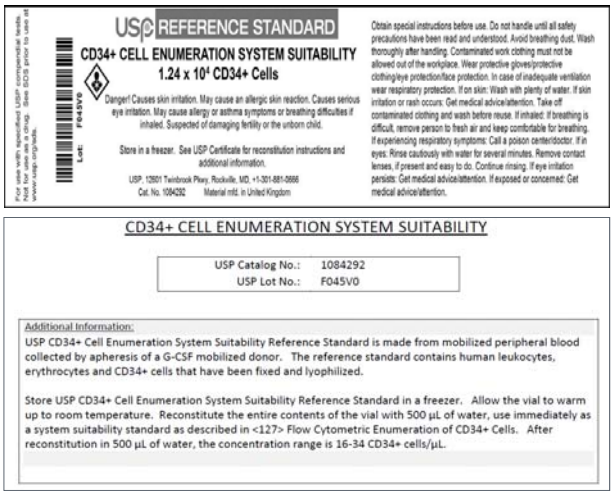


After denaturation with SDS, the method allows the analysis of the complete antibody under nonreducing conditions

Case study 4: CD34+ cell enumeration system suitability CD34+ stem cells - Flow Cytometry



USP CD34+ Cell Enumeration System Suitability Reference Standard is used to calibrate instruments, assess reagents and ensure correct gating for data acquisition and analysis



USP REFERENCE STANDARD

CD34+ CELL ENUMERATION SYSTEM SUITABILITY

1.24 x 10^4 CD34+ Cells

Danger! Causes skin irritation. May cause an allergic skin reaction. Causes serious eye irritation. May cause allergy or asthma symptoms or breathing difficulties if inhaled. Suspected of damaging fertility or the unborn child.

Store in a freezer. See USP Certificate for reconstitution instructions and additional information.

USP, 12601 Twinbrook Pkwy, Rockville, MD, +1-301-881-0666

Cat. No. 1084292 Material mfd. in United Kingdom

Lot: F045V0

Obtain special instructions before use. Do not handle until all safety precautions have been read and understood. Avoid breathing dust. Wash thoroughly after handling. Contaminated work clothing must not be allowed out of the workplace. Wear protective gloves/protective clothing/eye protection/face protection. In case of inadequate ventilation wear respiratory protection. If on skin: Wash with plenty of water. If skin irritation or rash occurs: Get medical advice/attention. Take off contaminated clothing and wash before reuse. If inhaled: If breathing is difficult, remove person to fresh air and keep comfortable for breathing. If experiencing respiratory symptoms: Call a poison center/doctor. If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: Get medical advice/attention. If exposed or concerned: Get medical advice/attention.

CD34+ CELL ENUMERATION SYSTEM SUITABILITY

USP Catalog No.: 1084292
USP Lot No.: F045V0

Additional Information:

USP CD34+ Cell Enumeration System Suitability Reference Standard is made from mobilized peripheral blood collected by apheresis of a G-CSF mobilized donor. The reference standard contains human leukocytes, erythrocytes and CD34+ cells that have been fixed and lyophilized.

Store USP CD34+ Cell Enumeration System Suitability Reference Standard in a freezer. Allow the vial to warm up to room temperature. Reconstitute the entire contents of the vial with 500 µL of water, use immediately as a system suitability standard as described in <127> Flow Cytometric Enumeration of CD34+ Cells. After reconstitution in 500 µL of water, the concentration range is 16-34 CD34+ cells/µL.

3

USP Biologics strategic direction

USP Biologics strategic direction: focus on assays and technologies

- Expand focus of reference standards beyond specific product classes
- Evaluate standards for technologies and assays with broad application and impact
- Examples:

Technology	LC, HPLC
	Electrophoresis
	MS
	NMR
	Flow cytometry
	Immunoassays
	PCR
	Genomics

Assays	Protein characterization
	Potency (Bioassays)
	Residual HCP, HC DNA
	Contaminants viral, microbial
	Particulates, metals
	Sequencing: deletion/ insertion
	Algorithms, software

call for candidates

2020–2025

Join us on the Journey

Collaborate with highly dedicated leaders from science, medicine, healthcare practitioners, industry and academia to help us establish standards that make it possible for 2 billion people around the world to have access to quality medicines, dietary supplements and foods.

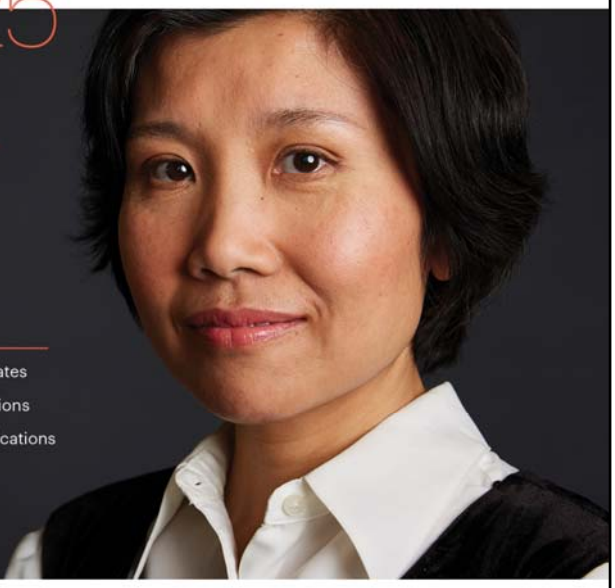
Important dates:

Jul 2018: USP launched the 2020-2025 Call for Candidates

Jan 2020: Deadline for Expert Committee chair applications

May 2020: Deadline for Expert Committee member applications

Jul 2020: 2020–2025 Council of Experts and Expert Committees begin their work



For additional information visit **callforcandidates.usp.org** or contact **USPVolunteers@usp.org.**

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Empowering a healthy tomorrow

Use of *non-compendial* Reference Standards for multi-valent vaccines

International Symposium Pharmaceutical Reference Standards

Strasbourg, 14th March 2019

Maria Silvana Bellini, EDQM - Strasbourg FR
Lorenzo Tesolin, Sciensano - Brussels BE

Outline

- Why "*non-compendial*" reference standards?
- Relevant points from Guideline of the Official Medicines Control Laboratories https://www.edqm.eu/en/discover-how-official-medicines-control-laboratories-contribute-protect-your-health-europe-and-0
- Biological non-compendial reference standards

Why "*non-compendial*" reference standards?

- Survey launched in July 2015 to the General European OMCL Network (GEON)
- **97 % of the OMCLs routinely use non-compendial reference standards**
- Focus of the **OMCL guideline** on *non-compendial* RS, since less clearly described than the rules for use of pharmacopoeial standards

What is an OMCL Guideline?

- Provides clarifications and facilitates the application of ISO 17025 requirements
- Takes into consideration the specificities of OMCL work
- Developed by the OMCL Network (ad-hoc Network experts)
- Adopted by all members of the Network

Ref: PA/PH/OMCL (10) 15 4R, Preface and Notes for Use of OMCL QM Documents

"Handling and use non-compendial RS in OMCL Network"

Guidance on the analytical/documental work to **check suitability** of non-compendial RS (received from MAH or commercially purchased), demonstrated by verification of the appropriate physico-chemical or biological **critical quality attributes** for the intended purpose/s.

Suitability checks

Intended use	Example of methods/tests in which the standard is used	Examples of work to perform
Qualitative:	IR, Raman, TLC, LC, MS, LC/DAD, LC/MS, SDS-PAGE, GC/MS, NMR	1. Plausibility check by scrutinizing the documentation accompanying the RS i.e. Certificate of analysis. 2. IR (KBr disc/ATR) or Raman: comparison with spectrum in literature or previous reference standard 3. LC/DAD: overlay of spectrum and comparison of retention times 4. LC MS/MS (high resolution) 5. Electrophoresis: comparison of gels obtained with old and new RS. 6. Immuno-diffusion: comparison of old and new RS on the same gel or between gels.
Quantitative:	LC, GC, UV, CE, NMR	1. Plausibility check by scrutinizing the documentation accompanying the RS: Certificate of analysis. If content, shelf life and traceability to International System (SI) units are proven, no additional tests required. 2. IR (KBr disc/ATR) or Raman: comparison with spectrum in literature or previous reference standard. 3. Raman 4. LC/DAD: spectrum overlay and comparison of retention time of the licensed product under test and secondary standard. 5. For screening tests a Certificate of analysis including the declared content and the shelf-life is sufficient
Quantitative (Biologicals supplied by the MAH): reference material, controls	ELISA, HPLC-PAD, HPLC, GC, ICP-OES, UV, in vivo potency assays, nephelometry	1. Reference material: OMCL to generate data and calculate bridging factors where applicable. 2. Controls supplied by manufacturer: manufacturer limits may be used or a new control chart can be set up in case of significant differences

Scope of the Guideline

- If RS used for a test **outside the established intended use** or for **different method/technique** than the one given in the MAH dossier or monographs, it is the **responsibility** of the user to carry out **supplementary tests**
- **Extent** of supplementary tests depends on intended use of the RS, based on scientific judgement

Storage of non-compendial reference standards

- According to the MAH recommendations or, in absence, based on scientific data
- Sub-division, identification, **traceability of use and disposal**
- **List of RS** (intended for multiple uses only)
- Controlled access & personnel responsibility

Monitoring of non-compendial reference standards

- To guarantee continued "**fitness for use**"
- What should be done when a RS expires?
  - Extension based on MAH's CoA or
  - Retest or
  - Change intended use of RS (chemical/bio)
- **Pre-defined acceptance criteria**

Re-testing of non-compendial reference standards

- **Critical quality attributes** should be tested, the extent depends on:
  - intended use (qualitative *versus* quantitative)
  - scientific data available
- **Re-testing intervals** based on:
  - stability data/literature information
  - experience/data generated

Apply risk-based approach !

Biological Reference Standards for multi-valent vaccines

Biological Reference Materials

Potency testing:

- **biological reference vaccines** *(in-vivo* and *in-vitro)*
- **biological reference standards** *(in-vitro*)

Validity of assay:

- **biological controls** *(in-vitro)*

Biological Reference Standards for multi-valent vaccines

Some compendial reference standards are available:

- Reference vaccines: BRP3 (Tetanus), BRP4 (Diphtheria) for challenge tests
- Reference standard: Bordetella Pertussis mouse antiserum BRP batch 2 for serology tests on mice

Biological Reference Standards for multi-valent vaccines

Manufacturers prefer in-house reference standards for the following reasons:

- homologous reference
- representative of own production
- easier to manage (supply, qualification, bridging schedule)

But mandatory to qualify in-house reference standard *versus* the International reference standards and to monitor consistency of results overtime

Biological Reference Standards for multi-valent vaccines

From the OMCL point of view:

Compendial reference standards are:

- easier to manage (single bridging study)
- products from different manufacturers can be analysed in the same run, with reduced use of animals

Biological Reference Standards for multi-valent vaccines

From the OMCL point of view: (cont')

Non-compendial reference standards:

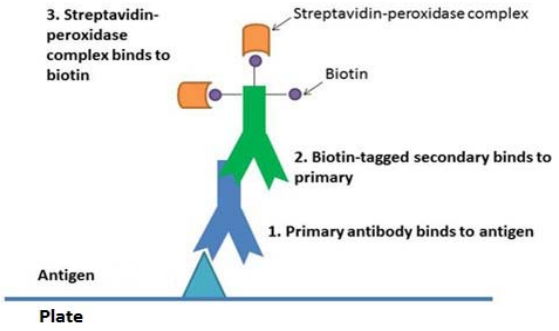
- one reference vaccine for one product (e.g. aP)
- increased use of animals for routine tests and bridging studies

Biological Reference Standards and Bridging Studies

- A **switch** from one reference standard to another may lead to a **shift** in the results obtained, therefore bridging study is required
- In any bridging study, **influences** due to other factors (e.g. assay reagents or materials) should be evaluated
- Changes of reference material should be **anticipated** in order to facilitate qualification and **continuity** of results

Biological Reference Standards

ELISA Pertussis Immunogenicity Testing



Biological Reference Standards

Biological standards are often used to **ensure traceability** to the **first clinical lot**

Potential strategies:

- Bridging study *versus* primary
- Successive bridging studies to align test results over time (with or without determination of **correction factors**)

Biological Reference Standards

- For the **bridging of controls,** the data obtained (e.g. mean, coefficient of variation) are evaluated (control chart) to keep previous **limits** of acceptability or **define new limits**
- Apply **manufacturer's control limits** in the OMCL control charts e.g. if the **same method** is used and no indication of **systematic** differences at the OMCL

Biological Reference Standards

It is **strongly** recommended to communicate in an appropriate and timely manner with the manufacturer to **avoid shortage** of reagents and materials and facilitate smooth performance of bridging studies

New Manufacturer Reference (shelf-life 3-5 years)

Bridging and lifetime use within OMCL

Biological Reference Standards

New Manufacturer Reference (shelf-life 3-5 years)

Bridging and lifetime use within OMCL

Risks:

- due to gaps (i.e. time and stability trends), the results between OMCL and manufacturer may be significantly different
- increased workload, due to lack of time/material to qualify new reference

Biological Reference Standards: Future challenges for an OMCL

- Move from *in vivo test* to *in vitro test* (3R's regulation, Vac2Vac IMI project): need to select and qualify new international or in-house standards
- Serology assays with multiplex technology (Luminex®, meso-scale®): implies increase use of in-house standards and related workload

Biological Reference Standards: Future challenges for an OMCL

- More complex vaccines (2 to 5 components for pertussis, 15 to 23-valent pneumococcal vaccines need a reference standard and biological control for total polysaccharide content, free polysaccharide content !)
- Different testing procedures and specifications between OMCL and manufacturers

Acknowledgements

- Karl-Heinz Buchheit, Michael Wierer and Richard Wanko, Department of Biological Standardisation, OMCL Network and Healthcare, EDQM - Strasbourg
- Geneviève Waeterloos, Head of section quality of vaccines and blood products, Sciensano – Brussels
- In vivo & immunology team, Sciensano - Brussels
- Wim Van Molle, Sciensano - Brussels
- Working Group - OMCL Guideline "*Handling and Use of Non-Compendial Reference Standards in the OMCL Network*"

Thank you very much for your attention.

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Use of *non-compendial* Reference Standards for multi-valent vaccines

International Reference Standard Symposium

Strasbourg, 14th March 2019

Maria Silvana Bellini, EDQM - Strasbourg FR
Lorenzo Tesolin, Sciensano - Brussels BE

Back-up

Official Medicines Control Laboratories (OMCL)

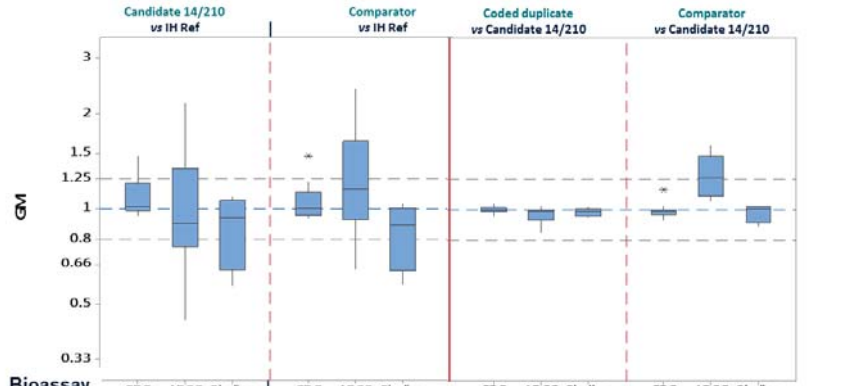
- **Public Institutions** working in close collaboration with their National Competent Authorities **to control the quality of medicines in Europe**
- Co-operate actively at European level, through the **General European OMCL Network (GEON)**, coordinated by the EDQM. EU and non-European (associated) members.
- **Quality control testing on medicines** (human and veterinary) throughout their entire life cycle (pre-authorisation, pre and post-marketing) and in cases of public health emergencies
- **Independently of manufacturers**

Official Medicines Control Laboratories (OMCL)

Added value of the GEON by:

- **Promoting work-sharing** amongst the more than 70 OMCLs from over 40 countries, favouring cost-efficient testing activities and market surveillance studies.
- Ensuring **the mutual recognition of test results** carried out by all European OMCLs to avoid duplication of work.
- Harmonising testing activities through the use of **common standards** based on legal requirements.
- Promoting **exchange of knowledge and expertise** to ensure state-of-the-art analytical procedures for all OMCLs.
- Providing **discussion platforms** where OMCLs can share scientific information, approaches and strategies.

1st WHO International Standards for Rituximab (NIBSC 14/10)



	CDC	ADCC	Binding	CDC	ADCC	Binding	CDC	ADCC	Binding	CDC	ADCC	Binding
GM	1.10	0.98	0.86	1.06	1.20	0.83	1.00	0.96	0.98	0.99	1.28	0.97
%GCV	16	62	33	14	51	29	3	6	3	5	16	7
N	13	8	4	12	8	4	16	11	5	16	11	5

When using the rituximab IS, the potency estimates for ADCC, CDC and binding activities between laboratories were **in good agreement** illustrating the benefit of using these preparations

Prior et al., Mabs 2018 10(1) 129-142

- **Rituxan®/Mabthera®** (Innovator 1997/98)
- Total of **6 approved biosimilar** products in EU & >40 biosimilars/copy products# in development

World Health Organization

1st WHO IS for RTX was established Oct 2017

- 1000 IU of CDC activity per ampoule
- 1000 IU of ADCC activity per ampoule
- 1000 IU of cell binding activity per ampoule
- 1000 IU of apoptotic activity per ampoule

(WHO/BS/2017.2309)

What next? Biosimilar mAbs Approved or in development

Molecule	Target	IS (in place) Y/N	Critical reagent	IS for critical reagent (in place)	Patent Expiry EU	Patent Expiry US	Biosim approval	Priority for Mab std
Infliximab	TNF-alpha	Y	TNF-alpha	Y	2015	2018	EU (2013); US (2016)	Done
Rituximab	CD20	Y	-	-	2013	2018	EU (2017)	Done
Adalimumab	TNF-alpha	N	TNF-alpha	Y	2018	2016	EU,US (2017)	1
Bevacizumab	VEGF	N	VEGF	Y	2022	2019	US (2017)	1
Trastuzumab	Her-2	N	-	-	2014	2019	EU (2017)	1
Cetuximab	EGF R	N	-	-	2014	2016	N	1/2

What next? Biosimilar mAbs Approved or in development

Molecule	Target	IS (in place) Y/N	Critical reagent	IS for critical reagent (in place)	Patent Expiry EU	Patent Expiry US	Biosim approval	Priority for Mab std
Ranibizumab	VEGF	N	VEGF	Y	2022	2020	N	2/3
Tocilizumab	IL-6 R	N	-	-	2017	2022	N	2/3
Denosumab	RANK L	N	RANK L	N	2018	2017	N	2/3
Ustekinumab	IL-12/IL-23 (p40)	N	IL-12/IL-23	IL-12 Y/IL-23 N	2024	2023	N	4
Pertuzumab	Her-2	N	-	-	2023	2024	N	3/4
Eculizumab	Complement prot C5	N	-	-	2020	2021	N	2/3
Omalizumab	IgE	N	-	-	2017	2017	N	2/3
Panitumumab	EGF R	N	-	-	2022	2020	N	2/3
Natalizumab	alpha-4 integrin	N	-	-	2015	2015	N	2/3

What are the challenges?

- Biological medicines are now produced and marketed globally.
- Biosimilar route to licensure.
- Huge proliferation of next generation biologicals e.g. (monoclonal) antibodies, pegylated versions of existing products etc
- Vaccine standards for priority pathogens
- Complex Cell and Gene therapies

What can we do?

Are there alternative/additional approaches to the standardization of these products?

- Written and physical standards to control the performance of the methods
  - Impurity determination for biologicals (eg dimers, oxidised forms, cleaved forms) are not always sufficiently well supported with suitable reference standards.
  - Poster describing such an approach for the EDQM Size exclusion System suitability standard for Erythropoietin

What can we do?

Are there alternative/additional approaches to the standardization of these products?

- "Class-based" standards focused, for instance, on the analytical target e.g. TNF-alpha

•TNF-alpha RS
  - Prepare a recombinant soluble TNF-alpha RS
  - Prepare a membrane-associated TNF-alpha RS

•TNF-alpha receptor?
  - Provide recombinant soluble TNF-alpha receptor
  - Cell line expressing the receptor

What next? Biosimilar mAbs Approved or in development

Molecule	Target	IS (in place) Y/N	Critical reagent	IS for critical reagent (in place)	Patent Expiry EU	Patent Expiry US	Biosim approval	Priority for Mab std
Infliximab	TNF-alpha	Y	TNF-alpha	Y	2015	2018	EU (2013); US (2016)	Done
Rituximab	CD20	Y	-	-	2013	2018	EU (2017)	Done
Adalimumab	TNF-alpha	N	TNF-alpha	Y	2018	2016	EU,US (2017)	1
Bevacizumab	VEGF	N	VEGF	Y	2022	2019	US (2017)	1
Trastuzumab	Her-2	N	-	-	2014	2019	EU (2017)	1
Cetuximab	EGF R	N	-	-	2014	2016	N	1/2

What are the challenges?

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- Vaccine standards for priority pathogens
- Complex Cell and Gene therapies

Global epidemics in the news

Vaccines for three deadly viruses fast-tracked

By Tulip Mazumdar
Global health correspondent

18 January 2017 | Health

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Scientists have named three relatively little-known diseases they think could cause the next global health emergency.

$460m pledged for vaccine initiative aimed at preventing global epidemics

Lassa, Mers and Nipah will be first diseases targeted by programme announced at Davos by coalition of governments, philanthropists and business

A health worker at an Ebola treatment centre in Guinea. Photograph: Kenzo Tribouillard/AFP/Getty Images

Need for expedited provision of reference materials in an infectious disease outbreak

- Antibody standards required for clinical trials for candidate vaccines and immune therapies
- WHO priority pathogen list includes viruses requiring high laboratory containment; hampers development work to establish standards
- Assuring safety of inactivated pathogens can not be guaranteed (also requires extensive validation)
- Sourcing antibodies and pathogens is confounded by shipping regulations from source countries, ethical issues, biosafety of reagents, governmental and institutional approvals etc.

Alternative sources

- Antibody standards produced in TC cows genetically designed to produce human antibodies
  - obviates need to source blood from infected humans (safe approach); rapid production of antibody reagents.
  - Large volume, high titre antibody suitable for producing >1000 ampoules of standard
  - Human IgG so commutable with patients samples
  - Immunogen production-6 weeks, Immunisation-8 weeks, Antibody characterisation-1 week

What are the challenges?

- Biological medicines are now produced and marketed globally.
- Biosimilar route to licensure.
- Huge proliferation of next generation biologicals e.g. (monoclonal) antibodies, pegylated versions of existing products etc
- Vaccine standards for priority pathogens
- Complex Cell and Gene therapies

Challenges and concerns

- Cell and gene therapy products are so diverse and the technology is so rapidly developing that industry-wide reference standards could restrict innovation.

- For cell and gene therapy products, perhaps the key attribute is understanding what constitutes a safe and effective dose – potency assays are not always available.

- For Cell Therapy Products, International Standards representative of a product/product type will be more difficult to develop due to heterogeneity of source material and/or limitations in quantity.

International Standards can support Innovation

- Ex…on co…

RESEARCH ARTICLES

Development of the First World Health Organization Lentiviral Vector Standard: Toward the Production Control and Standardization of Lentivirus-Based Gene Therapy Products

Yuan Zhao,[1,*] Hannah Stepto,[1] and Christian K Schneider[1,2]

[1]Division of Advanced Therapies, National Institute for Biological Standards and Control (NIBSC), Medicines and Health Products Regulatory Agency (MHRA), South Mimms, United Kingdom; and [2]Twincore Centre for Experimental and Clinical Infection Research, Hannover, Germany.

Gene therapy is a rapidly evolving field. So far, there have been >2,400 gene therapy products in clinical trials and four products on the market. A prerequisite for producing gene therapy products is ensuring their quality and safety. This requires appropriately controlled and standardized production and testing procedures that result in consistent safety and efficacy. Assuring the quality and safety of lentivirus-based gene therapy products in particular presents a great challenge because they are cell-based multigene products that include viral and therapeutic proteins as well as modified cells. In addition to the continuous refinement of a product, changes in production sites and manufacturing processes have become more and more common, posing challenges to developers regarding reproducibility and comparability of results. This paper discusses the concept of developing a first World Health Organization International Standard, suitable for the standardization of assays and enabling comparison of cross-trial and cross-manufacturing results for this important vector platform. The standard will be expected to optimize the development of gene therapy medicinal products, which is especially important, given the usually orphan nature of the diseases to be treated, naturally hampering reproducibility and comparability of results.

Keywords: LV production, WHO standard, integration analysis, genomic DNA, qPCR quantitation

HUMAN GENE THERAPY METHODS, VOLUME 28 NUMBER 4
2017 by Mary Ann Liebert, Inc.

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