Federal Institute for Vaccines and Biomedicines

Paul-Ehrlich-Institut

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Vector characterization of genetically-modified cell therapies

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GM cell therapies: MAAs and CTAs

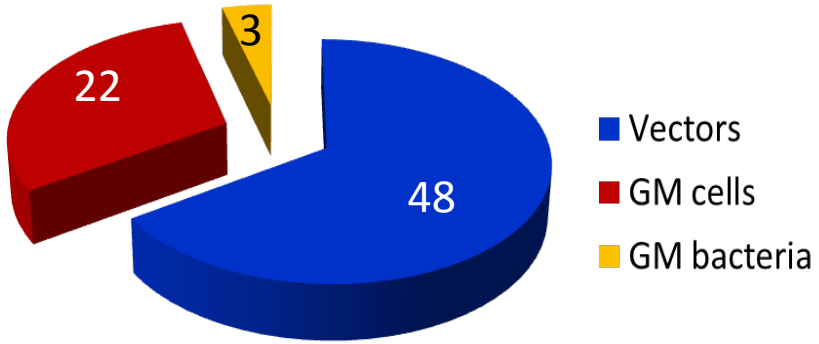
MAA:

EU: CHMP/CAT: Day 180 list of outstanding issues:

- Autologous CD34+ cells transduced with retroviral vector containing the adenosine deaminase gene (orphan)
- Allogeneic T cells genetically modified to express suicide gene (orphan)

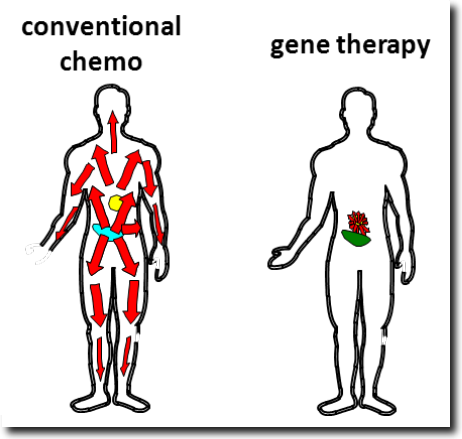
CTA:

Germany: Gene therapy CTAs 2005-2015

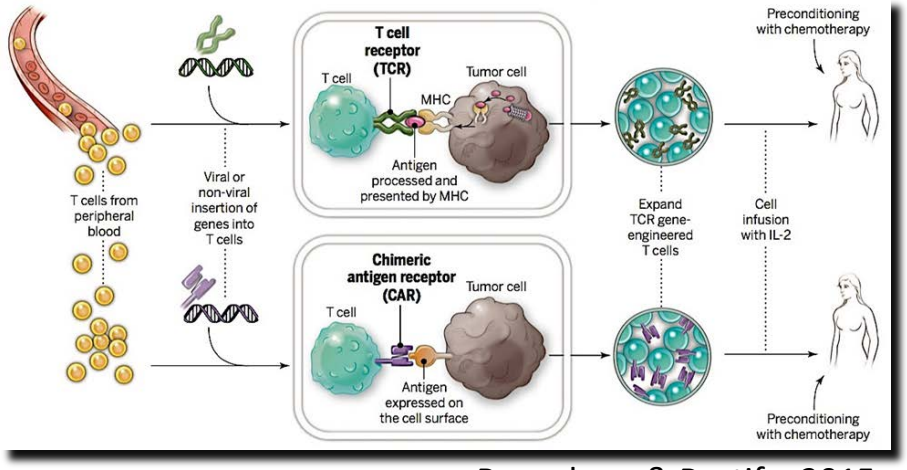


Genetically modified cells

- for suicide gene therapy, e.g. MSCs expressing suicide gene to enhance produg conversion at tumor site.
- in allogeneic use, e.g. encapsulated cells secreting insulin or recombinant proteins for neuronal cell prevention.

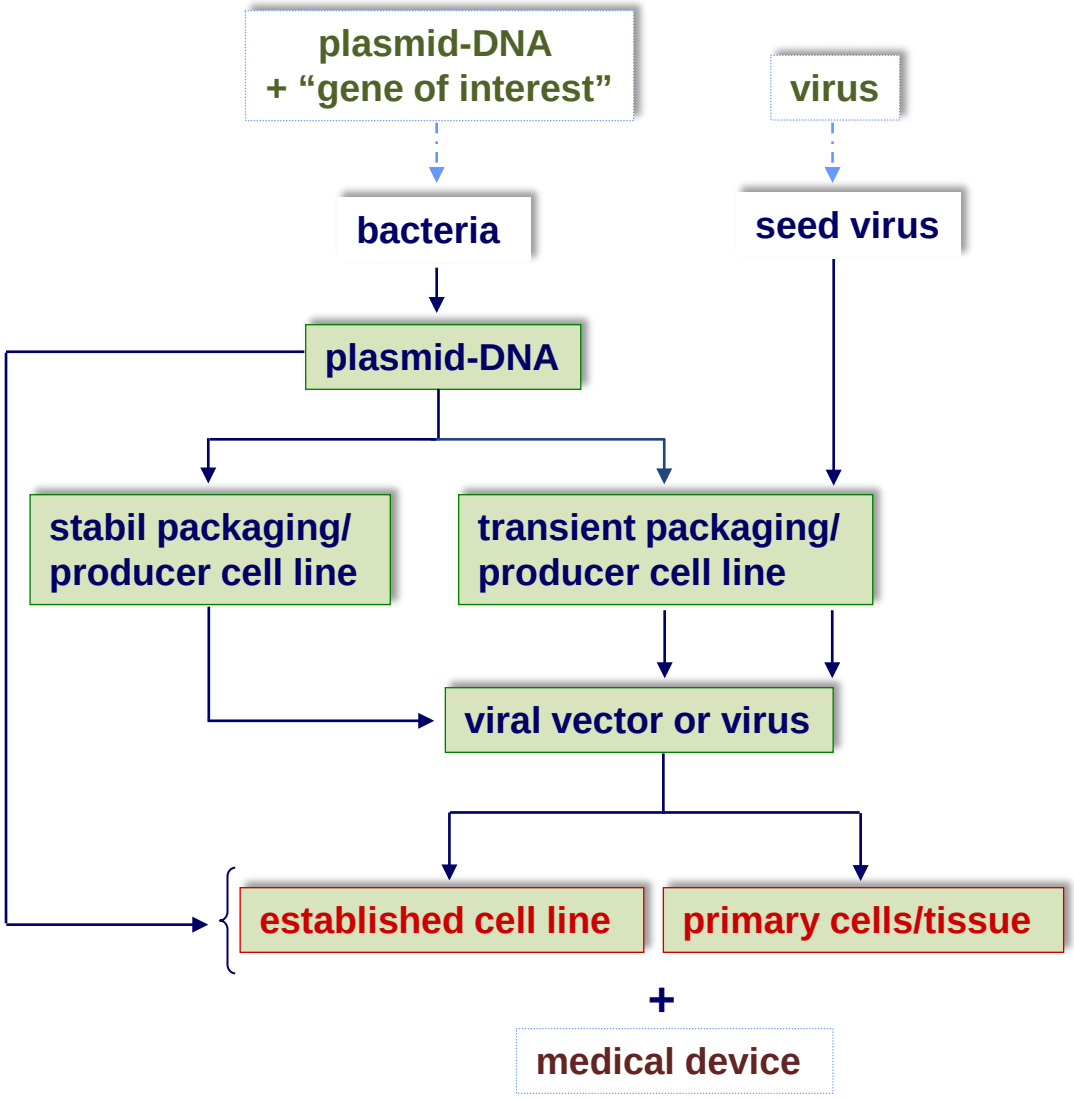


- to treat monogenic disorders, e.g. X-SCID, CGD, adrenoleukodystrophy, ADA-SCID, WAS, EB, ß-thalassemia.
- use as tumor vaccine (allogeneic or autologous), e.g. DC expressing tumor-specific antigens, TCR- or CAR-modified T-, NK-cells.
- to generate iPS cells.



Rosenberg & Restifo, 2015

GM-Cell Therapies - Generation



Starting materials for GTMPs

COMMISSION DIRECTIVE 2009/120/EC

of 14 September 2009

amending Directive 2001/83/EC of the European Parliament and of the Council on the Community code relating to medicinal products for human use as regards advanced therapy medicinal products

3.2.1.5. In the case of genetically modified cells, the starting materials shall be the components used to obtain the genetically modified cells, i.e. the starting materials to produce the vector, the vector and the human or animal cells. The principles of good manufacturing practice shall apply from the bank system used to produce the vector onwards.

EUROPEAN COMMISSION
HEALTH AND CONSUMERS DIRECTORATE-GENERAL

Health Systems and Products
Medicinal Products - Quality, safety and efficacy

Brussels,
SANCO/AM/sl/ddg1.d.6(2012)860362

EudraLex
The Rules Governing Medicinal Products in the European Union

Volume 4
EU guidelines for
Good Manufacturing Practice for
Medicinal Products for Human and Veterinary Use

Annex 2
Manufacture of Biological active substances and Medicinal Products for Human Use

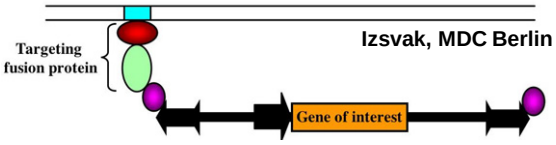
Type and source of material	Example product	Application of this guide to manufacturing steps shown in grey			
2. Virus or bacteria / fermentation / cell culture	Viral or bacterial vaccines; enzymes, proteins	Establishment & maintenance of MCB[10], WCB, MVS, WVS	Cell culture and/or fermentation	Inactivation when applicable, isolation and purification	Formulation, filling
7. Human and / or animal sources	Gene therapy: genetically modified cells	Donation, procurement and testing of starting tissue / cells[14]	Manufacture vector[13] and cell purification and processing,	Ex-vivo genetic modification of cells, Establish MCB, WCB or cell stock	Formulation, filling

[13] Where these are viral vectors, the main controls are as for virus manufacture (row 2)
[14] Human tissues and cells must comply with Directive 2004/23/EC and implementing Directives at these stages.

Vectors / tools applied for genetic modification

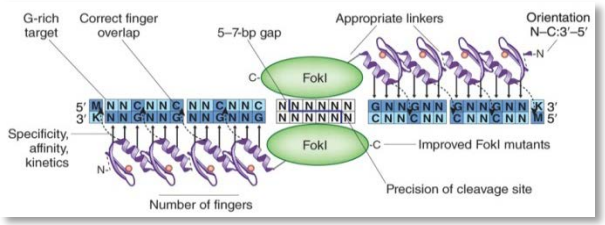
- Plasmids, RNA

- Integrating viral vectors, e.g. retro-, lentivirus
- Transposons



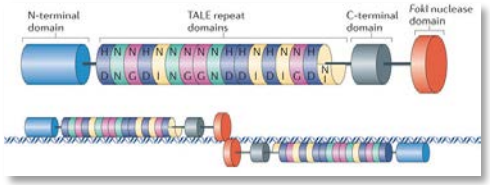
Izsvak, MDC Berlin

- Zink-finger nucleases



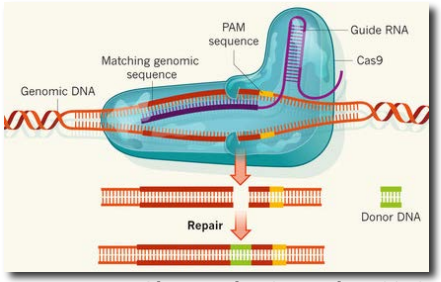
Isalan, 2012

- TALENs



Joung & Sander, 2013

- CRISPR/Cas



Charpentier & Doudna, 2013

- Transposases

Testing of cell substrates – EP 5.2.3

Test	Cell seed	Master cell bank (MCB)	Working cell bank (WCB)	Cells at or beyond the maximum population doubling level used for production
1. IDENTITY AND PURITY				
Morphology	+	+	+	+
Identification: nucleic acid fingerprinting and a relevant selection of the following tests: biochemical (e.g. isoenzymes), immunological (e.g. histocompatibility), cytogenetic markers	+	+	+	+
Karyotype (diploid cell lines)	+	+	+(1)	+(1)
Life span (diploid cell lines)	–	+	+	–
2. EXTRANEOUS AGENTS				
Bacterial and fungal contamination	–	+	+	–
Mycoplasmas	–	+	+	–
Spiroplasmas (insect cell lines)	–	+	+	–
Electron microscopy (insect cell lines)	–	+(3)	–	+(3)
Tests for extraneous agents in cell cultures	–	–	+	–
Co-cultivation	–	–	+(2)	+(2)
Tests in animals and eggs	–	–	+(2)	+(2)
Specific tests for possible contaminants depending on the origin of the cells	–	–	+(2)	+(2)
Retroviruses	–	+(3)	–	+(3)
3. TUMORIGENICITY				
Tumorigenicity	+(5)	–	–	+(4)

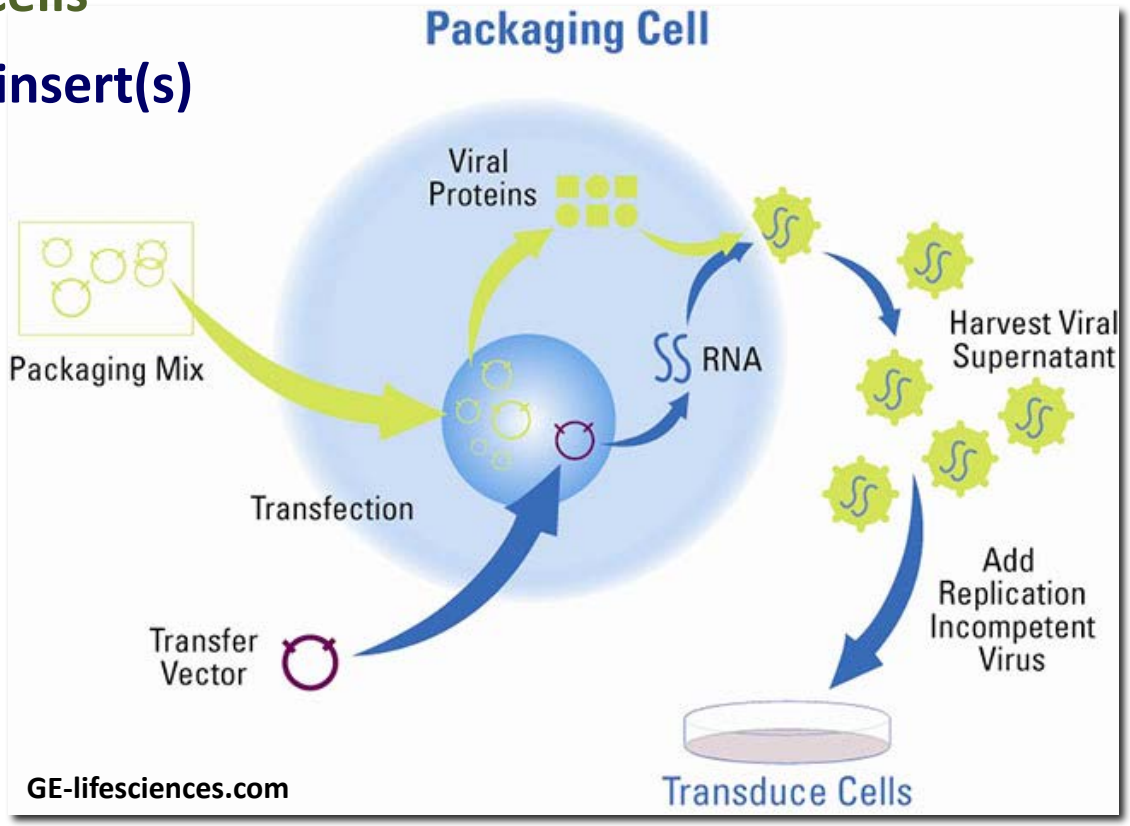
(1) The diploid character is established for each working cell bank but using cells at or beyond the maximum population doubling level used for production.

(2) Testing is carried out for each working cell bank, but using cells at or beyond the maximum population doubling level used for production.

(3) Testing is carried out for the master cell bank, but using cells at or beyond the maximum population doubling level used for production.

Characterisation of vector producer cells

- ✓ **EP 5.2.3**
- ✓ **Cell viability**
- ✓ **Morphology / growth characteristics during vector production**
- ✓ **Genetic stability of the cells**
- ✓ **Genetic integrity of the insert(s)**
- ✓ **Transgene expression**



Characterisation of vector producer cells

Preferentially

- use of a single producer clone over cell population
- use of stably genetically modified producer cells over transient system
- helper plasmid over helper virus

Characterisation and release of vector

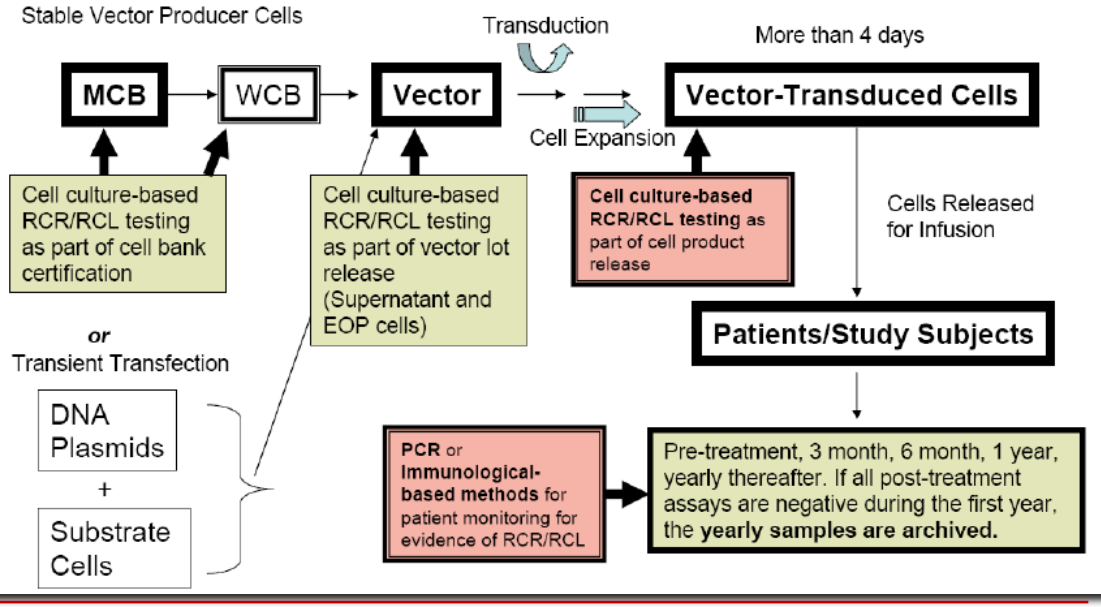
identity/genomic integrity	transgene expression, vector proteins transgene and vector sequence	immunochemically NAT, restriction digest, sequencing
quantity	vector particle count, nucleic acid concentration infectious units	UV, (RT)-qPCR, PERT infection assay
purity / impurities	HC protein HC + “production system” DNA process-related impurities (BSA, antibiotics, benzonase) RCV (replication competent virus)	BCA qPCR, picogreen assay ELISA qPCR, cell based assay

RCR/RCL testing

- **Ideally most sensitive assay employed**
- **Cell-based assay:**
  - Amplification in supernatant-inoculated cells for at least 21 days (USFDA)
  - End-point virus detection in naïve cells (marker, p24, PERT, VSV-G protein)
  - Lengthy procedure

- **Direct PCR-based assay** (less sensitive, quicker).

Briefing document – testing for replication competent retrovirus (RCR)/ lentivirus (RCL) in retroviral and lentiviral vector based gene therapy products – revisiting current FDA recommendations (2010)



Characterisation and release of vector

bioactivity / [potency]	vector particle count infectivity (titer) particles to infectious titer ratio transgene expression [bioactivity/function] DNA form	(RT)-qPCR, PERT, EM, infectious assay, FACS IF, FACS, WB AGE
safety	sterility mycoplasma bacterial endotoxin adventitious agents	acc. EP
physico-chemical characteristics	of modifying proteins aggregates appearance osmolality pH	

Genetically modified cells – Characterisation and QC

Specifications related to the genetic modification:

- Number of transduced (therapeutically active) cells
- Transgene expression levels
- Average vector copy number/cell (integrating vectors)
- Absence of RCV or modifying enzymes (TALENs, transposase, ZFNs)
- Biological activity (potency) of the therapeutic protein
- Absence of insertional oncogenesis (product-type dependent)

Issues under discussion

- Vector copy number:
  - Contributes to strength of the IMP
  - Contributes to risk level for insertional oncogenesis

RESEARCH ARTICLE | ADOPTIVE T CELL TRANSFER

Decade-Long Safety and Function of Retroviral-Modified Chimeric Antigen Receptor T Cells

John Scholler[1,*], Troy L. Brady[2,*], Gwendolyn Binder-Scholl[1], Wei-Ting Hwang[3], Gabriela Plesa[1], Kristen M. Hege[4], Ashley N. Vogel[1], Michael Kalos[1], James L. Riley[2], Steven G. Deeks[5], Ronald T. Mitsuyasu[6], Wendy B. Bernstein[7], Naomi E. Aronson[7,8], Bruce L. Levine[1], Frederic D. Bushman[2,†] and Carl H. June[1,†]

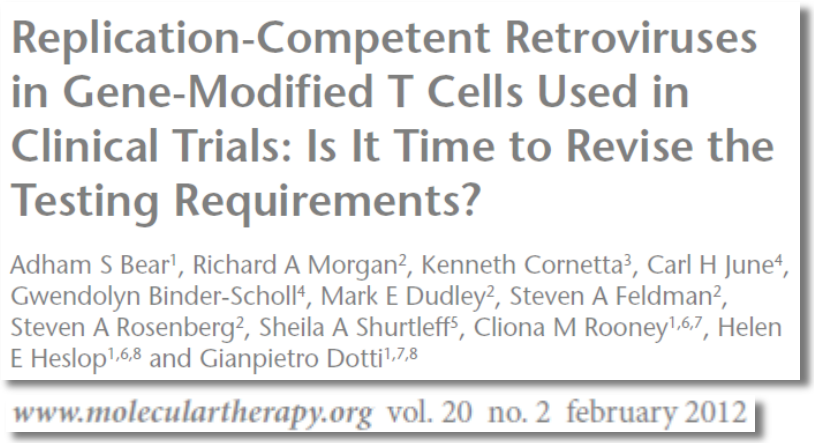
† To whom correspondence should be addressed. E-mail: bushman@mail.med.upenn.edu (F.D.B.); cjune@exchange.upenn.edu (C.H.J.)

* These authors contributed equally to this work.

Science Translational Medicine 02 May 2012:
Vol. 4, Issue 132, pp. 132ra53
DOI: 10.1126/scitranslmed.3003761

differences based on
- ✓ vector
- ✓ cell type to be transduced: CD34+ HSCs
  T-cells (rather rare)
  MSCs, NKs, DCs (??)

- Replication-competent viral vector
  - Test in vector starting material
  - DP retention samples
  - Patient retention samples



Replication-Competent Retroviruses in Gene-Modified T Cells Used in Clinical Trials: Is It Time to Revise the Testing Requirements?

Adham S Bear[1], Richard A Morgan[2], Kenneth Cornetta[3], Carl H June[4], Gwendolyn Binder-Scholl[4], Mark E Dudley[2], Steven A Feldman[2], Steven A Rosenberg[2], Sheila A Shurtleff[5], Cliona M Rooney[1,6,7], Helen E Heslop[1,6,8] and Gianpietro Dotti[1,7,8]

www.moleculartherapy.org vol. 20 no. 2 february 2012

Issues in manufacturing

In case …..

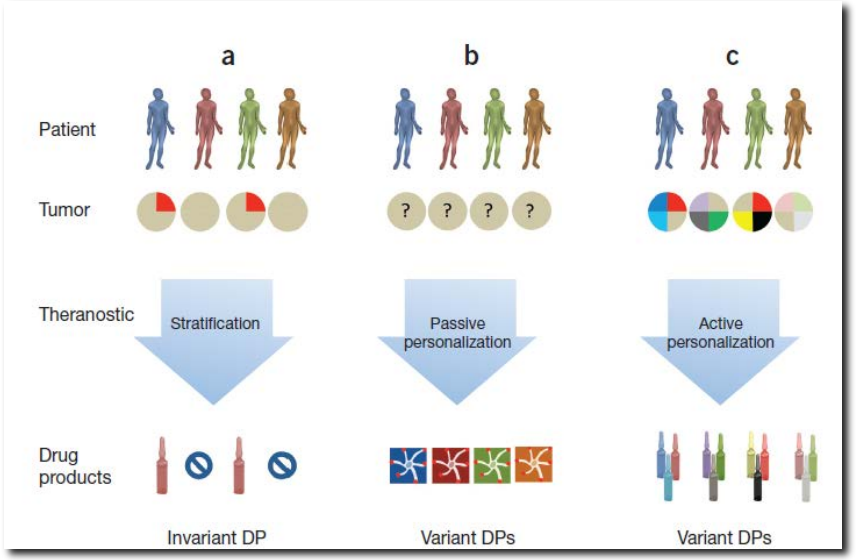
- …. final product difficult to cryopreserve without loss of activity.
- …. fresh cells do have short shelf-live (hours) before administration.
- …. patients need myeloablation before treatment.

Post administration release might be necessary:

Specification	Pre-administration	Post-administration
Quantity	Cell number	
Viability	Cell viability	
Identity/Purity	$CD34^+$ cell count	
Potency		Transduction efficiency
		Expression
		Functional assay
		Vector copy no.
Purity/Safety		Sterility
		Endotoxin
		Mycoplasma
Impurities		Process-related: beads, cytokines, vector, RCV
	Product related: $CD34^-$ cell count	

How to deal with a very personalised setting ?

e.g. individualized CAR T-cells based on patient-specific tumor antigen



Britten et al. 2013, Nat. Biotechnology

- Standardised manufacturing of vector and genetically modified cells
  Generic process validation, characterisation, stability acceptable?

- Vector development on patient base:
  - expensive
  - time-limited re. development, manufacturing, batch release (e.g. RCR testing)

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Vector characterization of genetically-modified cell therapies

Thank you !

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PEI entrance – morning view