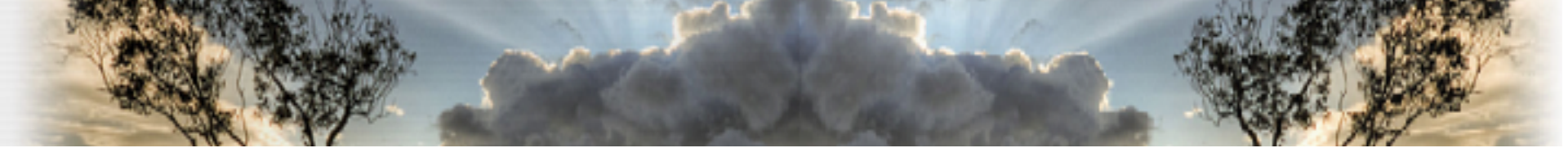


WS 4: Supporting Cell & Gene Therapies in the Bioanalytical Laboratory

12th EBF Open Symposium
Imagine! A new bioanalytical Earthrise

http://www.e-b-f..eu

Aim of session and discussion points

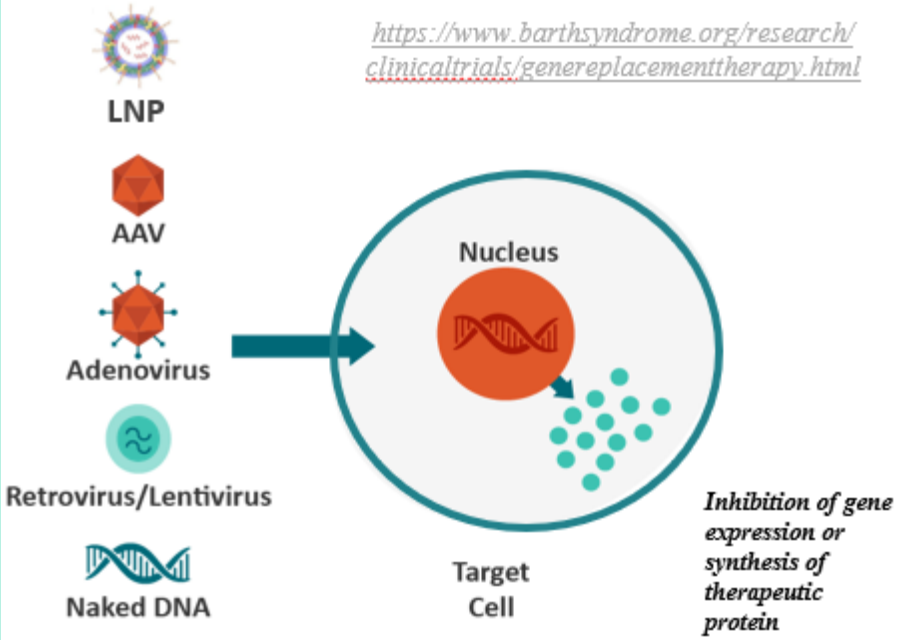
- Topic Introduction
- Survey results
- Immune response to cell and gene therapy
- Gene therapy – Exposure and integration
- Gene therapy – Transgene product assessment
- Cell Therapies – Pre-clinical development and animal models
- Cell therapies – Exposure assessment

Definitions

Gene Therapies

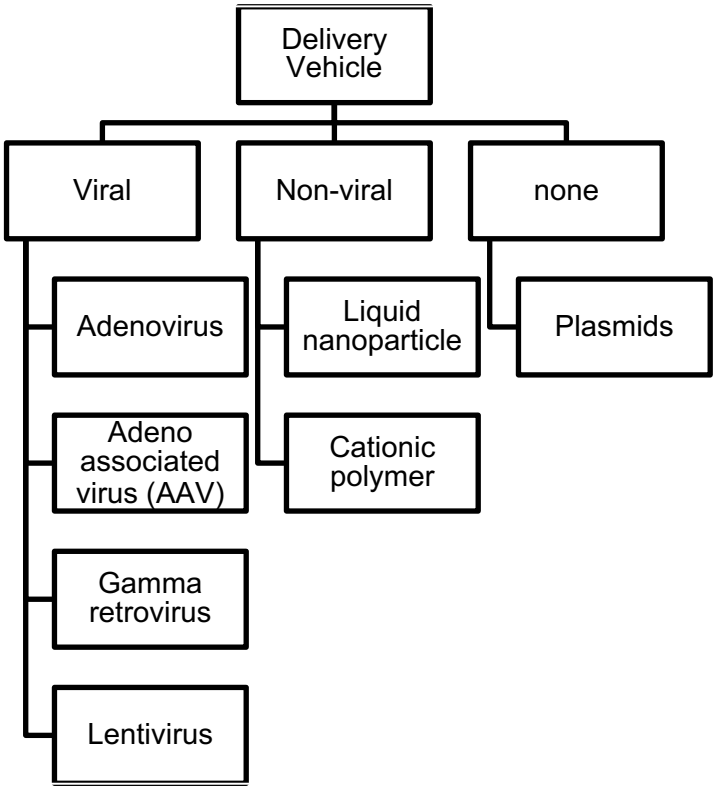
All strategies that modify an individual's protein make-up by introducing exogenous nucleic acid or nucleic acid modifiers, regardless of delivery*

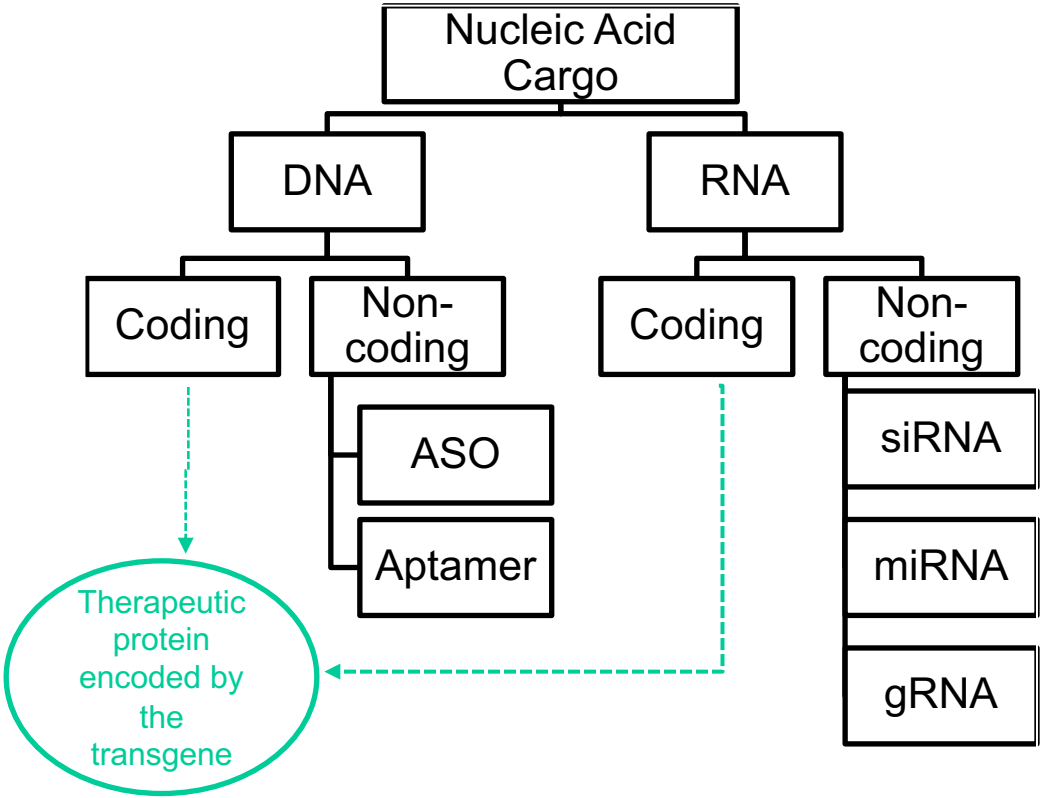
Defined by the precision of the procedure and the intention of direct therapeutic effect.



https://www.barthsyndrome.org/research/clinicaltrials/genereplacementtherapy.html

GT components define the Bioanalytical Support



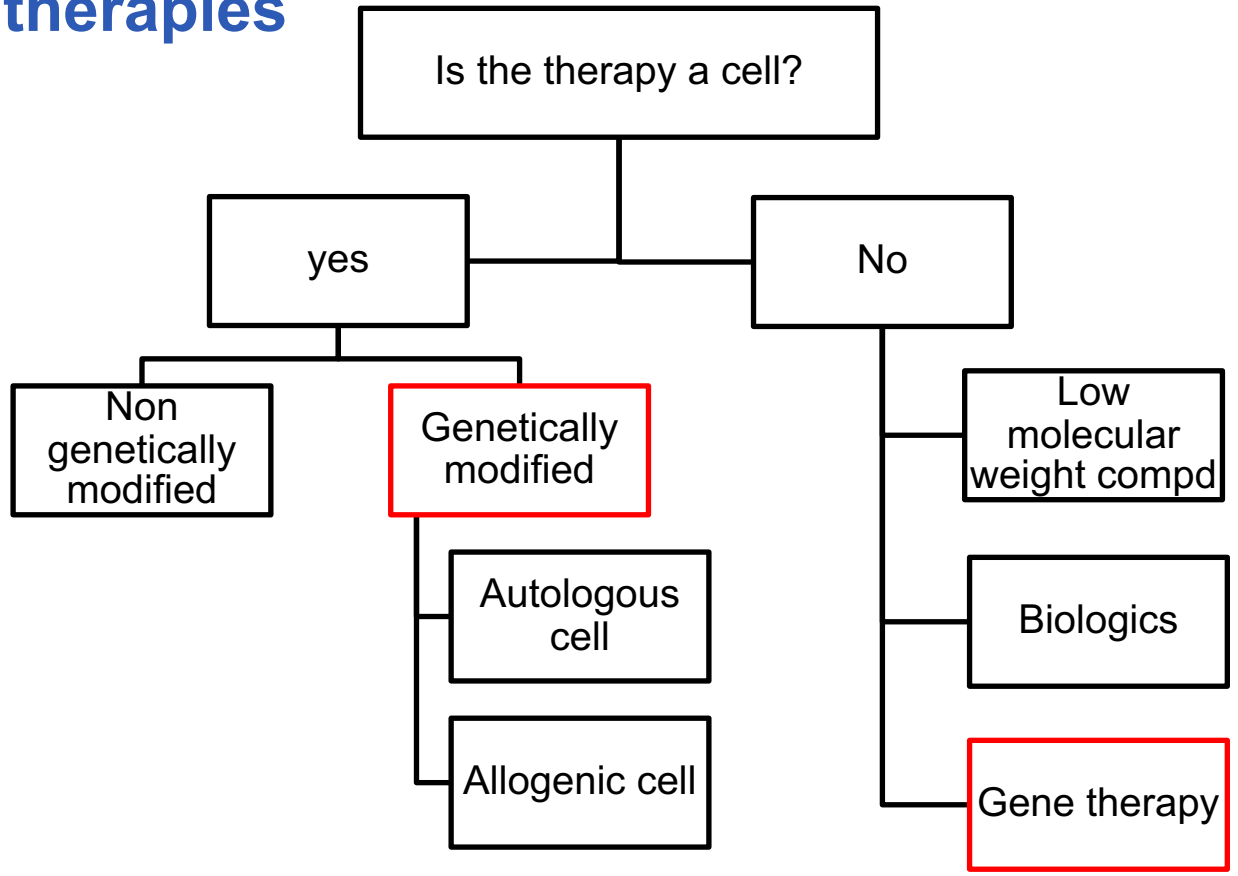


Cell *"vs"* gene therapies

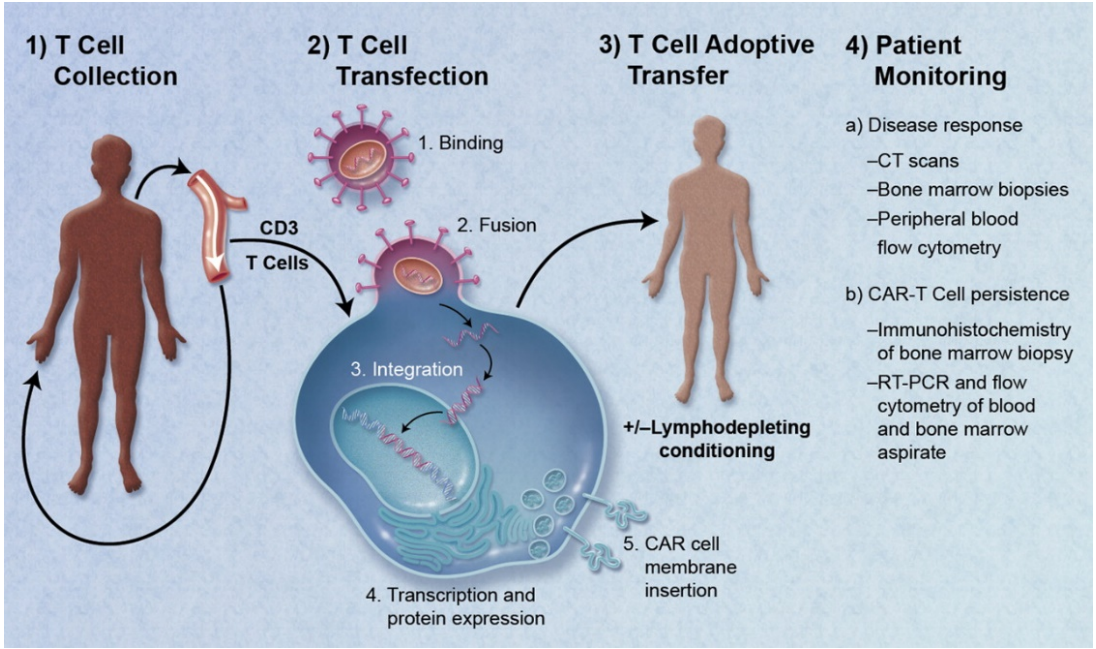
Ex-vivo gene therapies

Beyond correcting genetic deficiencies directly in the patients' cells, gene therapy can also provide a cell with capabilities not present in its natural state (e.g. CAR-T therapeutics).

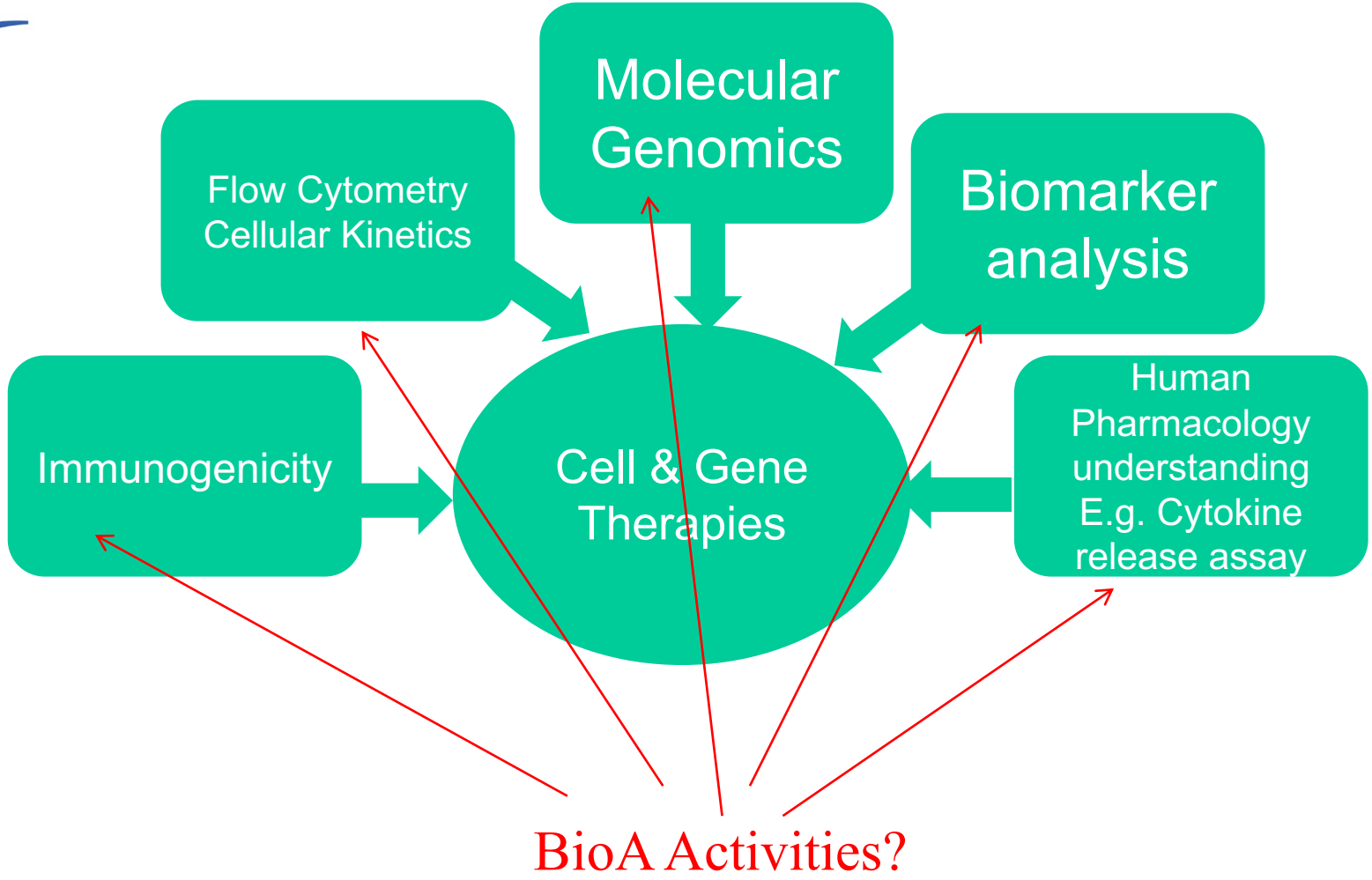
=> Not all cell therapies are gene therapies



Autologous T Cell Therapies: an example of cell therapy



- CAR-T: current paradigm of cell therapies
  - Strimvelis: autologous *ex-vivo* gene therapy: CD34+ enriched stem cells transduced with gamma retrovirus carrying ADA gene
- Allogenic ("off-the-shelf") cell therapies: cells come from donors => impact on the immunogenicity assessment


Molecular Genomics
Flow Cytometry Cellular Kinetics
Biomarker analysis
Immunogenicity
Cell & Gene Therapies
Human Pharmacology understanding E.g. Cytokine release assay
BioA Activities?

Survey results

- Companies currently working on C&GT:
  - Pharma (7) 50%
  - CRO (7) 50%

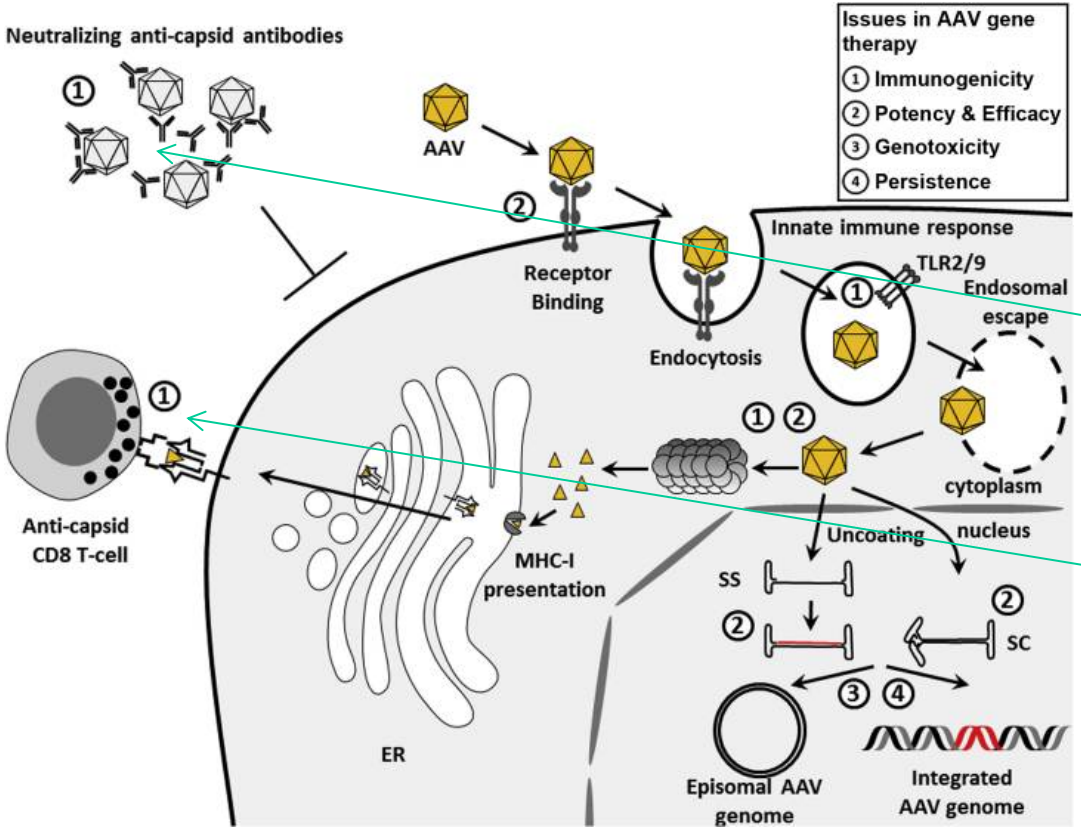
- Companies not currently in C&GT
  - 28 (of 67)

- Companies who said they will work on it in the future:
  - 3

What platforms do you use?	Replies (n)	Replies (% of companies)
PCR	11	79%
Flow cytometry	11	79%
LBA	13	93%
ELISPOT	9	64%
Cell Counters	9	64%
Mass spectrometry	10	71%
Enzymatic assays	11	79%
Clinical analysers	7	50%
other:	Multiplex cytokine analysis, and biomarkers in general Cell-based functional assay (potency and nAb) Immunhistochemistry, Imaging (PET/CT), RNASeq/Nanostring, ex vivo functional assay for viral replication (cell bioassays)	

Which endpoints do you support?		
Patient selection/stratification	6	43%
Primary or secondary endpoint	13	93%
Exploratory endpoint	13	93%
PK endpoint	11	79%
PD endpoint	13	93%
Immunogenicity endpoint	13	93%
Bio-distribution assessment	8	57%
IND submission package	10	71%
BLA/MAA submission package	9	64%

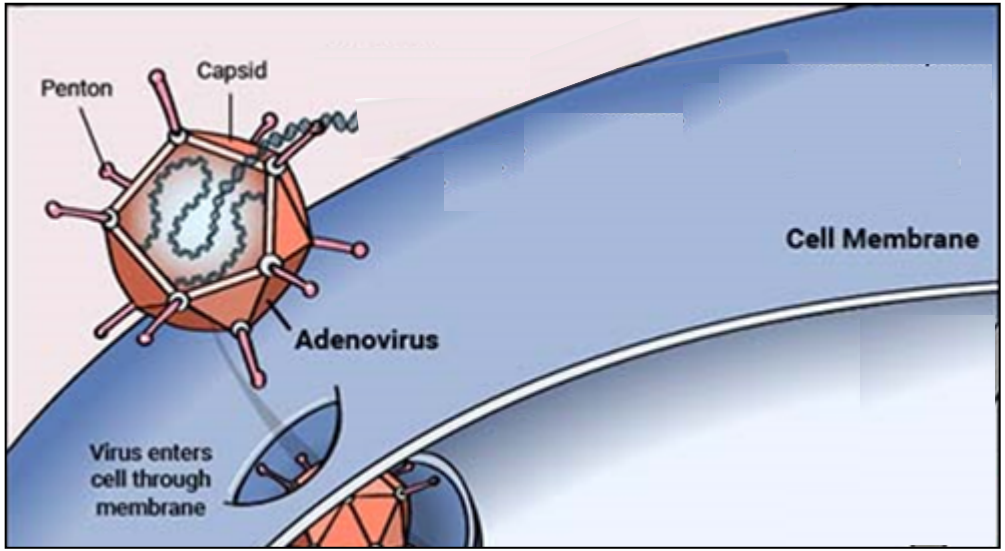
Gene Therapy – Capsid immunogenicity



Pre-existing response:
ADA & Neutralising Ab assay

Cell mediated response:
ELISPOT and/or Flow assay

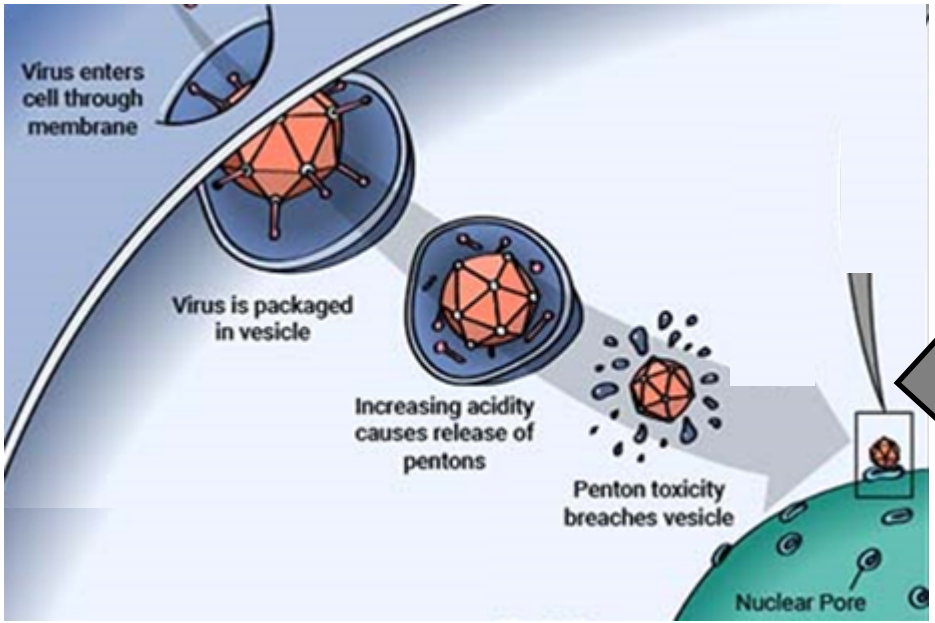
Image from: Emerging Issues in AAV-Mediated In Vivo Gene Therapy. Pasqualina Colella, Giuseppe Ronzitti, and Federico Mingozzi. Molecular Therapy: Methods & Clinical Development Vol. 8 March 2018

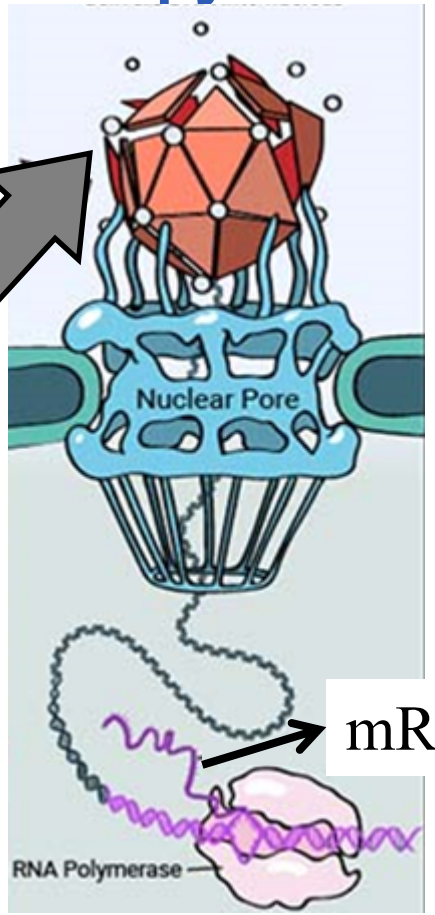


BioA Challenges:
As a community where does Bioanalysis contribute to this Class of therapeutic?

Q: Which lab should do this work and what regulation should we follow (FDA, ISO, CLIA). What are the specific considerations for BioA ?

Exposure and integration Gene therapy





Exposure and integration – Gene therapy

- qPCR / RT-PCR
- Hybridisation ELISA (ASO, siRNA)
- Branched ELISA
- Oligonucleotide by MS
- Viral Capsid detection by MS
- Lipid particle by MS

- Pre-clin biodistribution – immunohistochemistry, in-situ hybridisation
- Shedding

Q: How can we bring this under the BioA umbrella?

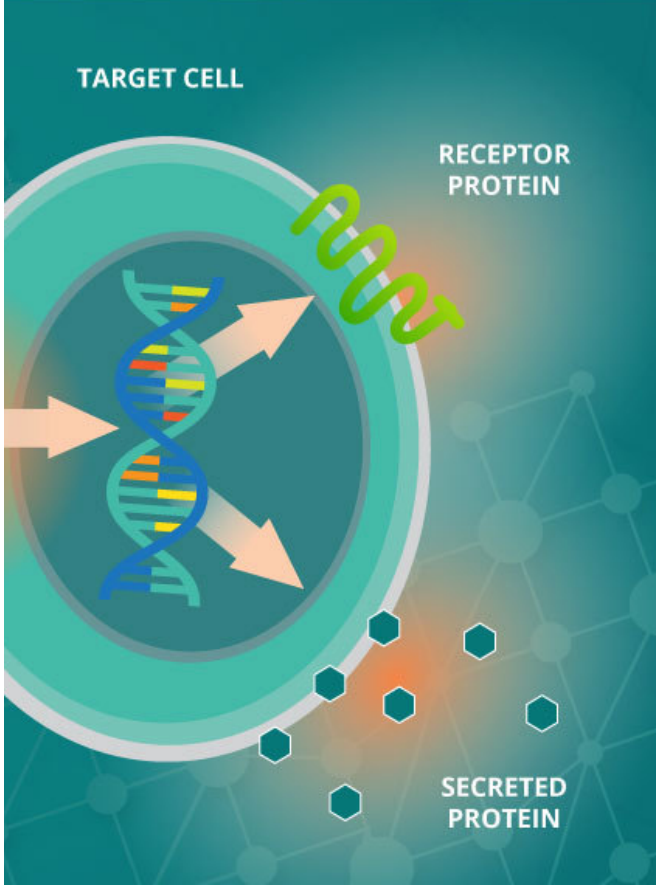
Q: Historic settings of the assays no appropriate for current needs (example hybridisation assays)

Transgene product

- Cell surface receptor
- Soluble protein or excreted protein
- Functional protein
- Functional enzyme

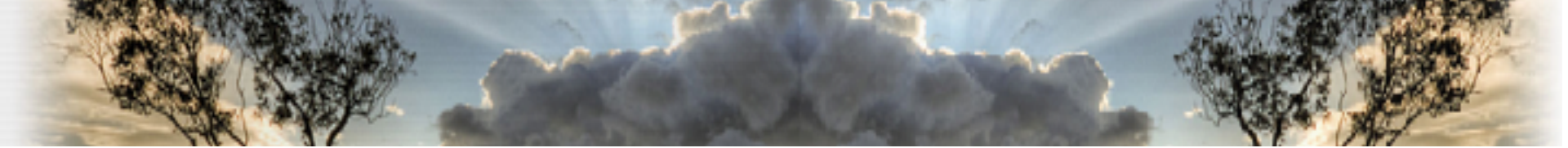
- Transgene product Immunogenicity
  - transcription factor immunogenicity in clinical

  Q: Who has seen this?
  - Native protein
  - Engineer protein



Q: Is it needed to do transgene product immunogenicity?

Q: How can we estimate the risk (safety, long lasting effects)?



Cell Therapies

CAR-T is used as case modality

Pre-clinical development and animal models

- Considerations for Immunogenicity assessment
- Building better models
- Cytokine release syndrome

Questions and discussion:

- Value of pre-clin assessments?
- Translational ability of results? (cell and gene therapy alike)

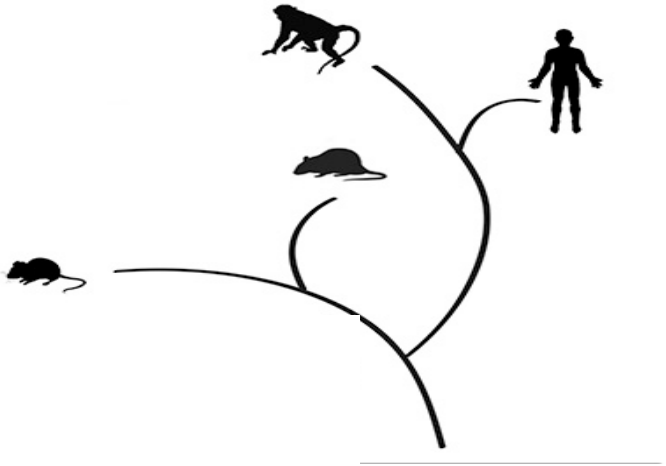
EMA/FDA – Immunogenicity Guidance

- Predictive nature is low for clinical risk
- Used to aid data interpretation
- Different classes of therapeutic compound will have different considerations for Pre-clinical immunogenicity assessment based on associated risks

The Challenge for Industry

- Nonclinical assessment of immunomodulatory therapies lags behind traditional toxicology, because of the complexity of the immune system and its interaction with disease states

Current models do not fully predict outcomes



Building better models

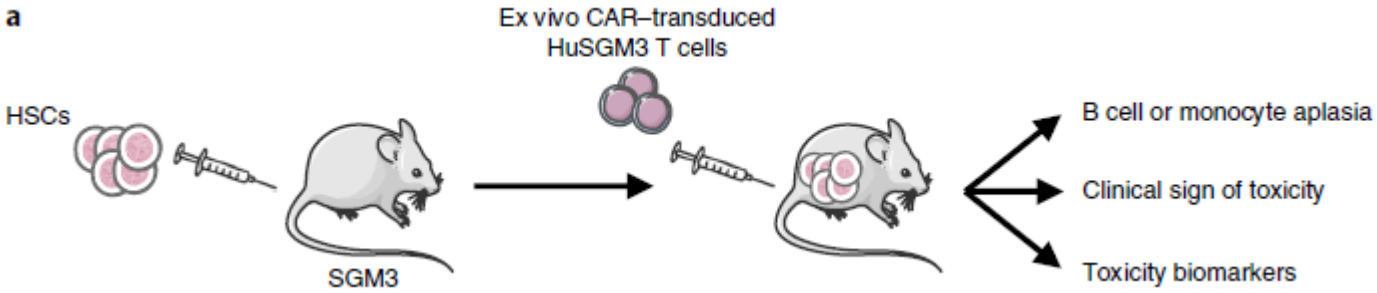


Figure 1.3: (adapted from Norelli et al. Nat Med. 2018): Generation of CRS model. SGM3 mice are i.v. infused with human hematopoietic stem cells (HSCs). Four weeks after that, they receive CAR-modified T cells and are monitored for B cell or monocyte aplasia, clinical signs of toxicity and toxicity biomarkers.

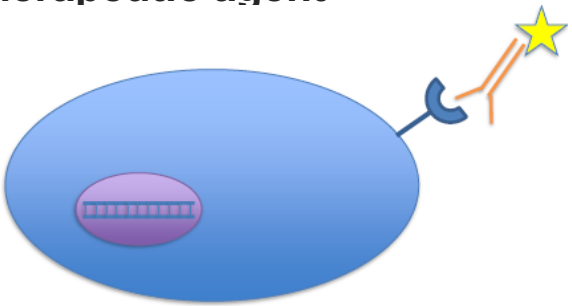
innovative medicines initiative

Q: How do we address the gaps in translating non-clinical work

Cell therapy – Exposure, persistence and immunogenicity

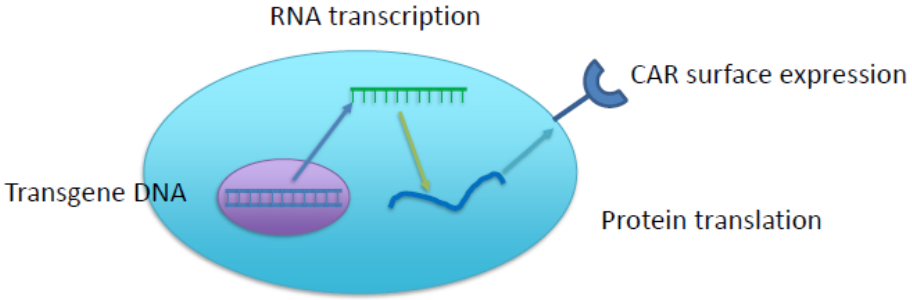
- Flow cytometry
- qPCR (real-time or endpoint, cf. WS5, Rob Nelson and Chris Cox)
- LBA, CBA

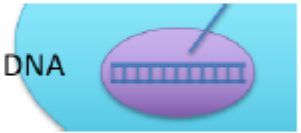
Flow cytometry:
direct measure of the CAR-T therapeutic agent



Technical and logistical limitations
Sensitivity – Stability
=> Alternative

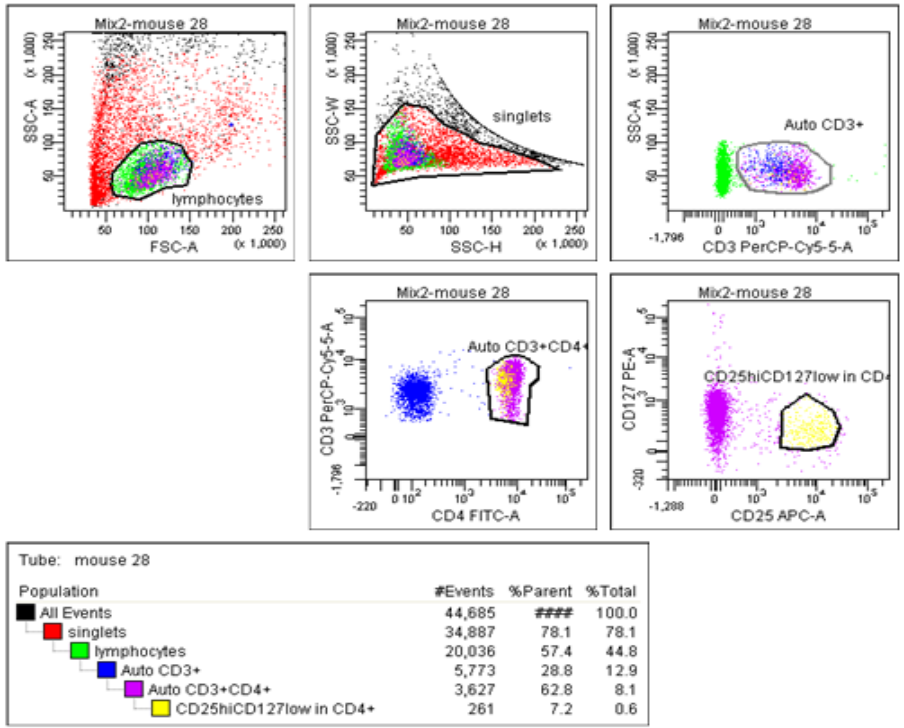
Quantitative PCR:
CAR-T concentration is inferred from the level of transgene DNA



Does  CAR surface expression = Transgene DNA  ?

Correlation between flow cytometry (cell measurement) and qPCR results (transgene measurement) for exposure to CART-T - Mueller et al (Blood, 2017): Cellular kinetics of CTL019 in relapsed/refractory B-cell acute lymphoblastic leukemia and chronic lymphocytic leukemia -> Correlation performed on the PK parameters

Flow cytometry



Tube: mouse 28

Population	#Events	%Parent	%Total
All Events	44,685	####	100.0
singlets	34,887	78.1	78.1
lymphocytes	20,036	57.4	44.8
Auto CD3+	5,773	28.8	12.9
Auto CD3+CD4+	3,627	62.8	8.1
CD25hiCD127low in CD4+	261	7.2	0.6

- Generally used to look for effects of a biotherapeutic on immune cell populations in the blood or spleen homogenate
- Antibodies are used to identify cells by detecting specific antigens expressed by these cells, which are known as phenotypic markers
- Same principles as ligand binding assay but generally performed on fresh samples

Considerations for Cell Population & Receptor Expression

- Cell population:
  - Low numbers can make it difficult to measure and may require the implementation of more cell markers

RECEPTOR EXPRESSION:

- Levels of expression can vary between healthy and disease states
- Receptor can be shed or internalised
- Expression levels can be variable across species
- Target may be on non-circulating tissues

Cell therapy – Exposure and persistence

- Flow cytometry
- qPCR

Q: What should be reported and why?
Q: How important is this data?
Q: Who should do the analysis?

BioA lab with no experience in the technology or lab with technological experience but no BioA experience?

Acknowledgment

EBF Members

- Chris Cox – Psioxus
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- Johannes Stanta – Covance
- Lydia Michaut – Bioagilytix
- Manuela Braun – Bayer
- Robert Nelson – Covance

Non-EBF members

- Paula Miranda – uniCure


Thank You !

Contact Information

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European Bioanalysis Forum vzw

www.e-b-f.eu

Additional Questions to the community

- Where do oligonucleotides fall into?
  -> DNA oligos and siRNA (ONPATTRO) approved by FDA-CDER
  ≠ other GTs are approved by FBA-CBER

- Current guidelines
  – Ambiguities in nomenclature
  – Gaps