EBF

16th Open Symposium

Science Winning the Race

The Increasing Complexity of Clinical Trials for Bioanalysis

Robert Nelson, on behalf of the EBF

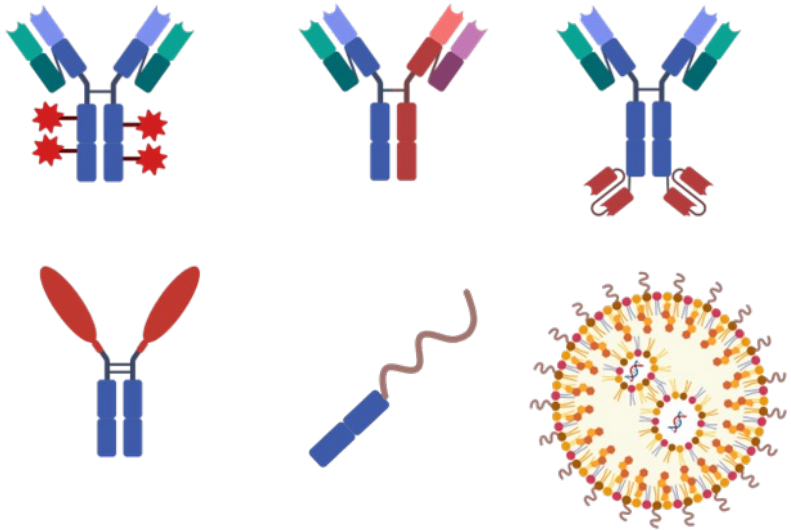
15-17 November 2023, Barcelona

Background

- The emergence of novel biotherapeutic scaffolds, gene and cell therapies, and nanoparticle-based drugs requires development of sensitive bioanalytical assays across a range of analytical platforms
- The rise of rare and orphan diseases indications necessitates innovative trial designs and endpoints
- This presentation will discuss some of the obstacles facing the bioanalytical scientist in their quest to provide their stakeholders with relevant pharmacokinetic, immunogenicity and pharmacodynamic data

The increasing complexity of therapeutic modalities

- **Many novel therapeutic scaffolds**
  - Require more complex approaches to assess PK, immunogenicity, biodistribution, …
    - May require ultrasensitive technologies to measure physiologically relevant concentrations
    - Additional characterisation tiers in ADA/Nab assessment
  - Often need multiple technologies to elucidate
    - Immunoassay, mass spectroscopy, PCR, …

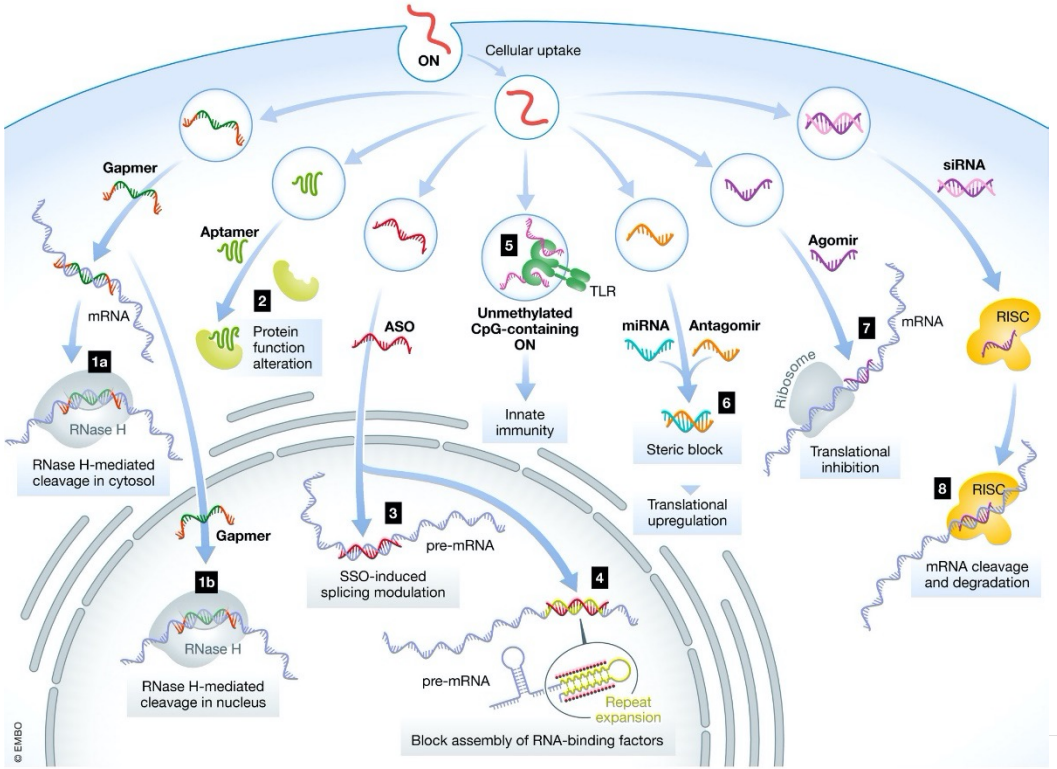


Images: Created with BioRender.com

The growth in oligonucleotide therapeutics

Increasing number of designs

- Antisense oligonucletotide (ASO)
- siRNA
- mRNA
- miRNA
- Aptamer
- Gapmer
- Antagomir
- Agomir
- Carrier proteins, antibodies, nanoparticles



Source: Hammond et al. Delivery of oligonucleotide-based therapeutics: challenges and opportunities EMBO Mol Med (2021)13:e13243
https://doi.org/10.15252/emmm.202013243

The growth in oligonucleotide therapeutics

- **Many options (and challenges) to bioanalytical approaches**

TABLE 1

Considerations for selection of a suitable bioanalytical method for ON analysis.

Method	Sensitivity	Selective quantification of metabolites	Selective quantification of conjugates	Assay complexity (sample preparation/method development)	Comments	Refs
LCMS and LCMS/MS	Medium	Yes	Yes	High/Low	Needs dedicated LCMS for ONs	43
hELISA	Medium/ High	No	No	Low/Medium	Requires specific probe(s)	65
ECL	Very high	No	No	Low/High	Requires specific probe(s)	68
LC-FI	High	Yes	Yes	Low/Medium	Requires specific probe(s)	71
LC-UV	Low	Yes	Yes	Low/Low		50

  - **PCR also feasible?**

Source: Weidolf et al 2021

The rise in oligonucleotide therapeutics

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EBF Spring Focus Workshop 2023

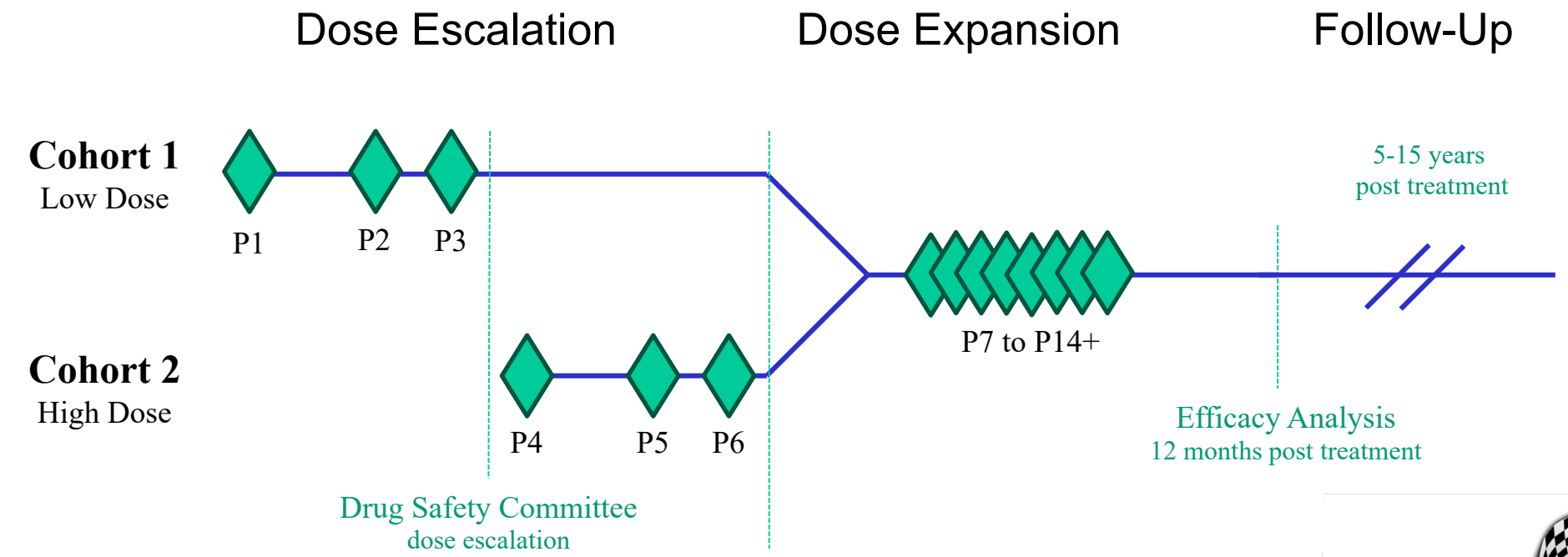
Scientific, Regulatory and Technology Challenges in the Development of Oligonucleotide and Peptide Drugs

08-09 June 2023, NH Málaga, Spain

- https://e-b-f.eu/conferences/past-conferences-2/

Bioanalytical Challenges for Cell & Gene Therapies

An example phase I/II clinical trial design for a gene therapy in a rare disease



Gene therapy: bioanalytical requirements?

- **Pre-existing antibodies to viral capsid**
  - Presences of anti-capsid antibodies may be inhibitory to viral transduction
  - Consider factors such as route of administration, dose, etc.
  - Inclusion/exclusion criteria
    - How quickly are data needed?
    - IVDR/CLIA/IDE/CDx considerations

- **Assay formats**
  - Total antibody (TAb) assay
    - Typically an immunoassay to detect binding antibodies
  - Neutralising antibody (NAb) assay
    - Typically a cell-based transduction inhibition assay
  - TAb only? NAb only? Both?

IVDR – in vitro diagnostic medical device regulations
CLIA – Clinical laboratory improvement amendments
IDE – investigational device exemption
CDx – companion diagnostic

Gene therapy: bioanalytical requirements?

- **Transgene expression**
  - RNA level – qPCR/dPCR
  - Protein level – immunoassay, flow cytometry, other…
  - Clinically, can we get appropriate samples? Target is most often a tissue or cell type, where systemic monitoring via plasma/serum will not be relevant

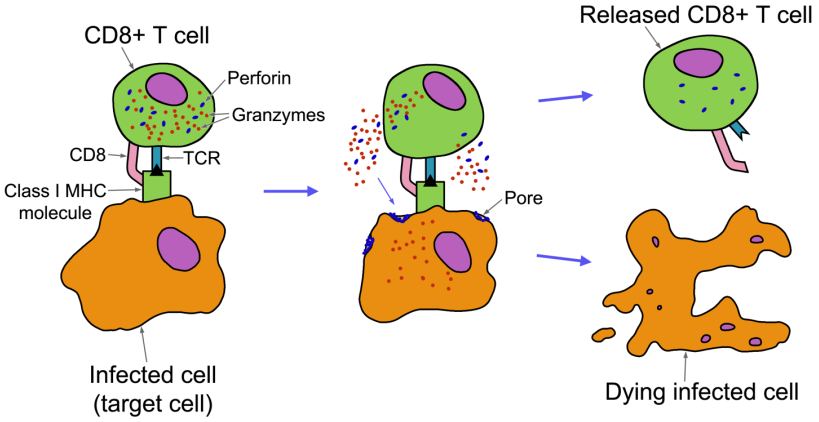
- **Surrogate biomarkers**
  - Downstream indicators of efficacy

- **Inflammatory biomarkers**
  - Often assess panels of proinflammatory cytokines, etc.

Gene therapy: bioanalytical requirements?

- **Humoral immunogenicity to viral capsid?**
  - ADA and/or NAb
  - Assess whether dosing of a virus induces an antibody immune response
  - **Spoiler alert…**

- **Cellular immunity to viral capsid**
  - Typically ELISpot or Intracellular Cytokine Staining (ICS) by Flow Cytometry to measure whether CD8+ T-cell response is elicited
    - Requires 5-10mL blood to isolate sufficient PBMCs for assay



Source: How T-cell mediated cytotoxicity works. Illustration by: Dananguyen. Wikimedia Commons

Gene therapy: bioanalytical requirements?

- **Humoral immunogenicity to transgene protein**
  - ADA and/or NAb
  - Endogenous counterpart may be defective or absent
  - Will transgene protein be exposed to humoral system?

No onasemnogene abeparvovec-treated patient demonstrated an immune response to the transgene.

Source: Zolgensma EPAR Summary of Product Characteristics

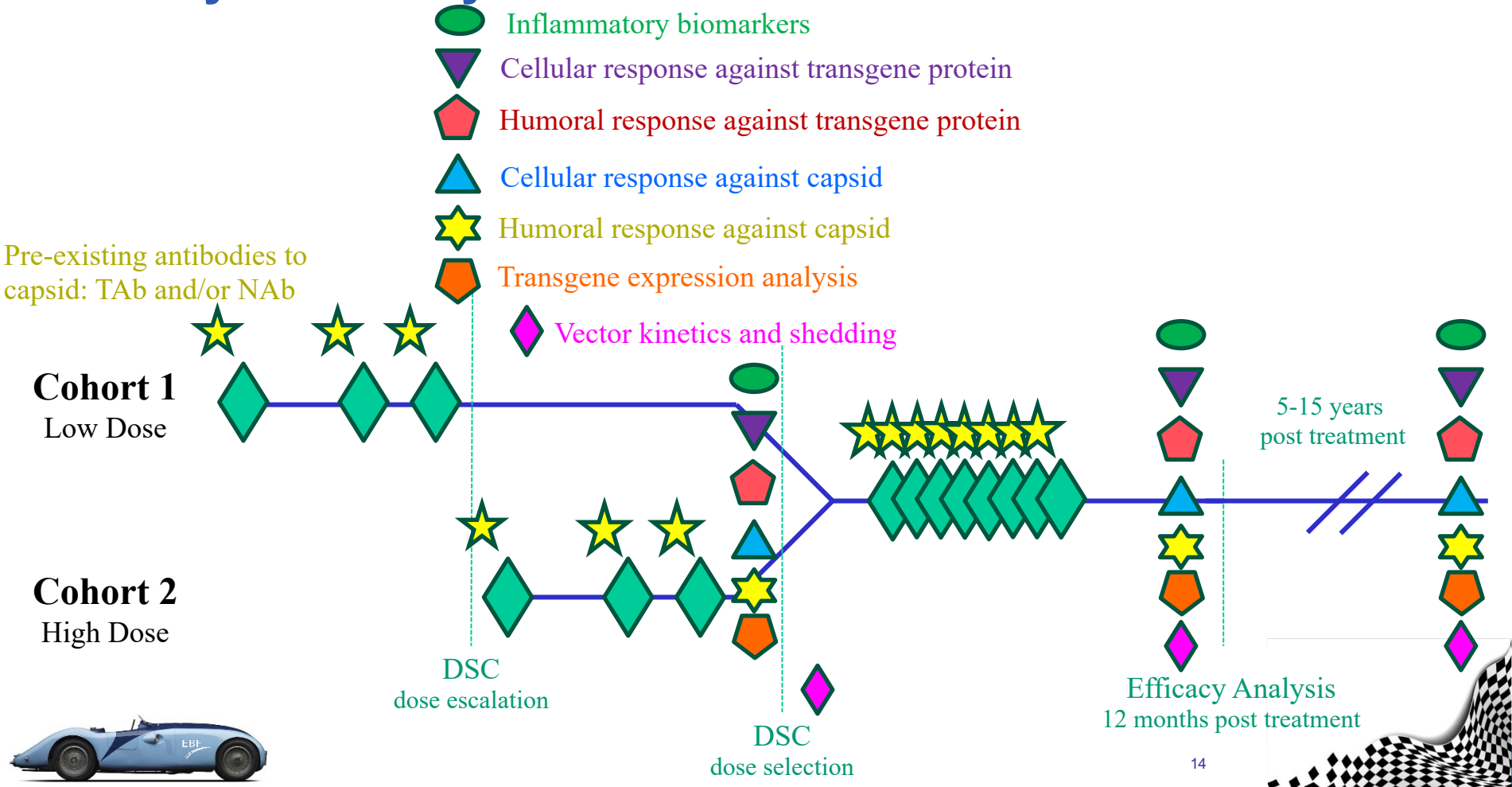
- **Cellular immunity to transgene protein**
  - ELISpot or ICS for CD8+ T-cell response to transgene protein

Gene therapy: bioanalytical requirements?

- **Vector shedding**
  - Investigate whether there is release of virus through excreta (faeces) or secreta (urine, saliva, nasopharyngeal fluids, etc.)
  - Typically qPCR or dPCR

- **Virus kinetics**
  - Kinetics and durability of viral genome copies in serum
  - Typically qPCR or dPCR

Bioanalytical assays

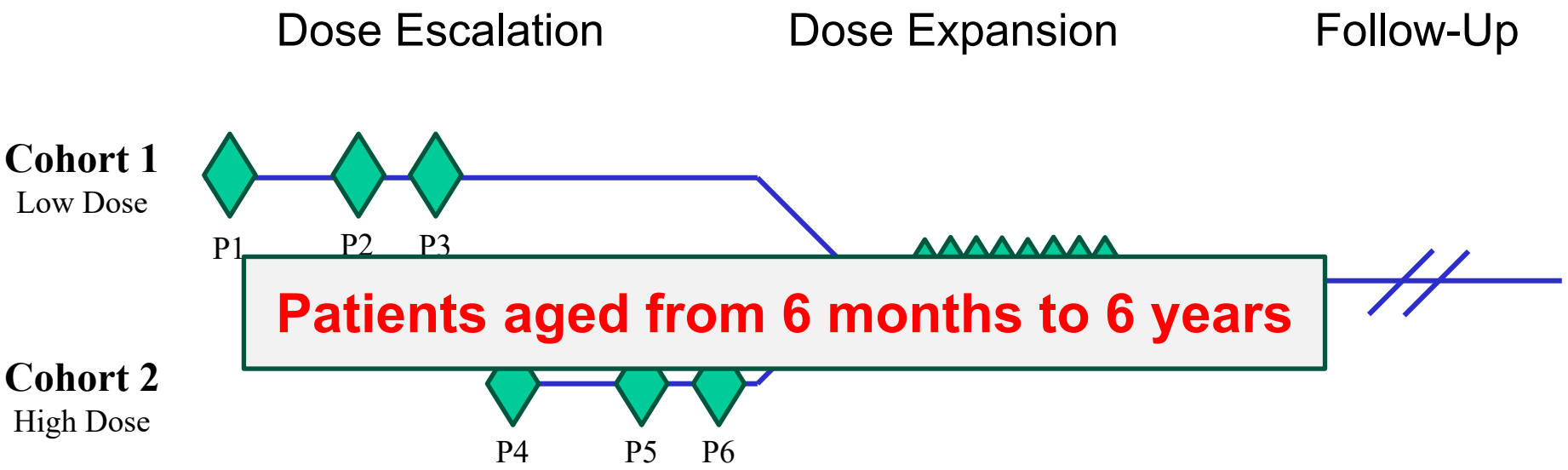


Challenges for bioanalytical support in gene therapy trials

– What is ‘PK’?
– More expansive assessment of immunogenicity
– Large number of assays
– Numerous platforms

– Small number of samples
– Fast turnaround for many data

An example phase I/II clinical trial design for a gene therapy in a rare disease



Behind every sample is a patient

– Is your bioanalysis added value to the patient?

Conclusions

- Measure what adds value to the understanding of the safety and efficacy of the therapeutic, and most importantly, for the patient
- Don’t force fit assay development and validation into a framework that wasn’t intended for it…

Acknowledgements

- EBF Steering Committee and Chair
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- Open Symposium delegates

Contact Information

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