Exploring the Changing Bioanalytical Solutions in Gene and Cellular Therapies

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CHANGING THERAPIES

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Agenda

- Overview
- Study Based Perspective
- Analytical Technologies Overview
- Case Study
- Summary

Overview

Overview

Therapeutic Platforms

+ Cellular modifications
+ Viral/gene based therapeutics
+ Non-viral therapeutics (e.g., oligonucleotides)

Types of Analysis

+ Traditional PK only for non-viral therapies (e.g., oligonucleotides)
+ Vector Analysis – Vector Copy Number (VCN)
+ Immunogenicity
+ Biomarkers
+ Activity assays

Overview

Technologies

+ Cell based assays, LBA, LC, LC/MS, Flow cytometry, ELISpot, PCR, IHC, FISH

Study Related Considerations

+ Pre-clinical, Early Development, Clinical
+ Study Design and Implementation
+ Logistics – Drug, Patients & Samples
+ Geography
+ Analytical Technologies
+ Data Management and Reporting
+ Compliance
+ Duration/Durability

Study Based Aspects

Study Based Aspects

- Pre-clinical, Early Development, Clinical
  - Accelerated & Abbreviated
  - Research challenges
    - In house, Hospital, Academia, CRO
    - Regional to global transition
- Study Design and Implementation
  - Global
  - Sites and Patient Recruitment
  - Patient, therapeutic agent/drug and study sample logistics
- Sample Logistics
  - Single patient and timepoints
  - “Linked samples”
  - Matrices
  - Lab Concierge

Sample Logistics: Perspective

"Focus on the Patient"

"Focus on the Individual Sample"

Study Based Aspects

- Laboratories
  - Types of laboratories
  - Locations of laboratories
  - Collaboration between laboratories, pharma, CRO’s, sites
- Data Management and Reporting
  - Multiple laboratories, tests, databases, formats
  - Sparse sampling over long periods of time
  - Organizational changes
- Compliance
  - CLIA
  - Bioanalytical Guidance's
  - Gene & Cellular Therapy Specific Guidance's
  - Exploratory/Fit for purpose

Study Based Aspects

- Duration/Durability
  - Continuation of analysis over time
    - Within Study
    - Within Development
    - Post Approval
  - Analytical methods, instrumentation, technology, compliance, ownership
- Cross Laboratory Opportunities
  - Relationship between cGMP/CMC and Bioanalytical
  - Relationship between Central Labs and Biomarkers/Bioanalytical
  - Methods & Technology
  - Broader understanding of gene therapy characterization

Analytical Technologies Overview

Study Based Perspective: Analytical Technology

Phase of Development

- R&D/Early Development & Preclinical
- Enrollment
  - Immunogenicity/nAb
  - Disease/Health Status - IHC
- Treatment
  - VCN & Vector Shedding
    - qPCR, ddPCR
  - Immunogenicity
    - Cell based assays
  - Biomarkers
    - LBA, LC, LC/MS, Flow cytometry, ELISpot, IHC, FISH
  - Activity Assays
    - LBA, LC, LC/MS

Key Technologies: Molecular Genomics

Molecular assays uses

+ Detect replication-competent viral vectors (e.g., AAV, adenovirus, retrovirus, and lentivirus).
+ Gene Expression, Gene Quantification and Genetics Variation Analysis
+ Toxicology studies, biodistribution programs

Technologies- qPCR technology, ddPCR, Sanger Sequencing

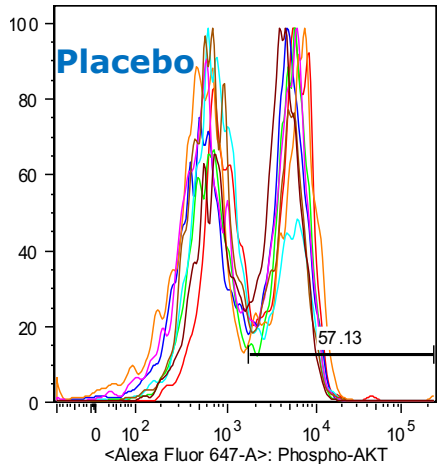
+ Appropriate tools for quantifying the copy number of a DNA or RNA target in a defined set of organs and tissues.
+ DNA sequencing can be used to confirm the integrity of a viral vectors used in the delivery of gene therapy

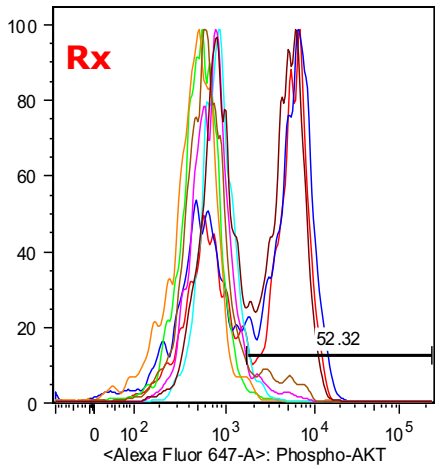
Challenges:

+ Incurred samples for assay performance/control samples
+ Regulatory guidance's
+ Long follow up periods (assay maintenance)

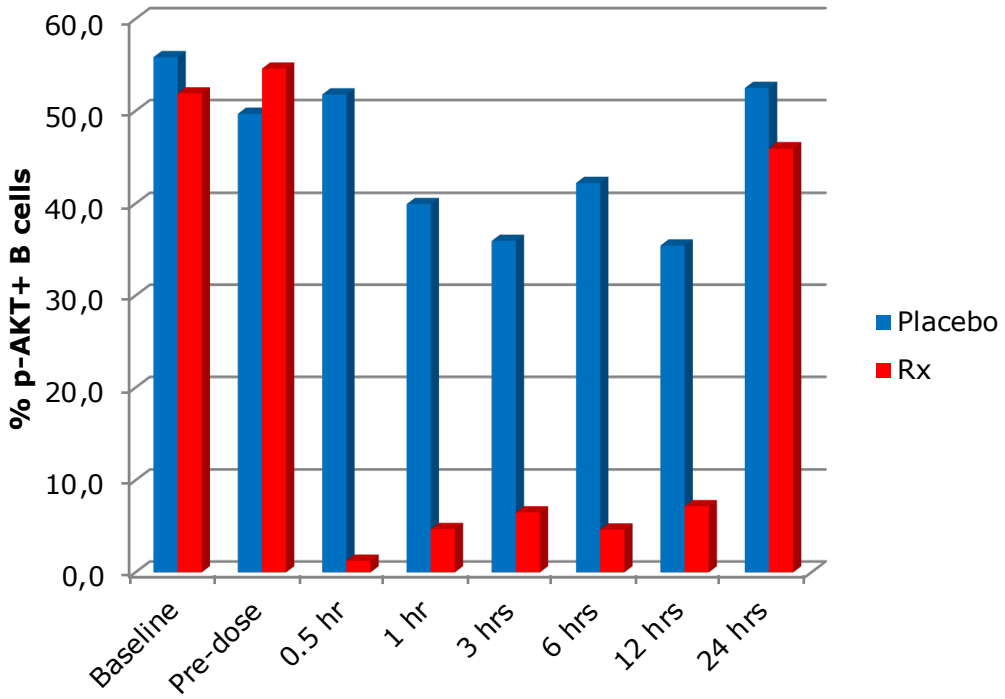
Key Technologies : Flow Cytometry

Case Study-2: p-AKT expression on B cells





p-AKT+ B cells



Key Technologies : Flow Cytometry

Flow cytometry uses

+ Measurement of expression of genetically modified product (e.g. CAR expression)
+ Monitoring the cellular kinetics of infused cells (e.g. CAR-T cell numbers over period of time)
+ Immune cell profiling

Challenges:

+ Samples for assay development - not available (e.g. Spike normal samples with CAR-T cells)
+ Sample stability
+ Incurred samples for assay performance/control samples
+ Regulatory guidance
+ Long follow up periods (assay performance)

Case Study & Summary

Gene Therapy in BCMA-Expressing Multiple Myeloma

Inclusion:

Evidence of cell membrane BCMA expression, as determined by a validated immunohistochemistry of formalin-fixed, paraffin-embedded (FFPE) tumor tissue (e.g., bone marrow biopsies or plasmacytoma)

Exploratory Objectives to be Evaluated:

- Persistence, immune phenotype, and function of Gene Therapy in the blood, bone marrow and/or tumor tissue
- Cytokine/chemokine induction in the blood of subjects after infusion of Gene Therapy
- Level of BCMA-expressing (BCMA+) cells in blood and bone marrow, and the level of circulating soluble BCMA
- Measures of tumor sensitivity/resistance to Gene Therapy
- Minimal residual disease (MRD) in subjects achieving a complete response
- Development of an anti-CAR immune response
- Utility of the IHC BCMA expression assay

Gene Therapy in BCMA-Expressing Multiple Myeloma

Companion Diagnostic:

- A separate consent form to allow BCMA pre-screening by immunohistochemistry
- A BCMA IHC assay was performed as an investigational laboratory screen that may eventually be developed for a companion diagnostic if efficacy and safety is observed

Gene Therapy in BCMA-Expressing Multiple Myeloma

Sample Type	Biomarkers/Analyte & Technology	Lab Location/Type
FFPE tissue	IHC: BCMA Expression	Performed in a Central Lab
• Blood (plasma) • Bone Marrow and/or tumor tissue	• LBA: Cytokine Panel • LBA: Chemokine Panel (e.g, IFNg, IL2Ra, IL6, IL10, TNFa, IL1Ra, IL1B, MIP1a, and MIP1B)	Performed in a Central Lab and Biomarker Lab
Blood (serum) and Urine	LBA: Immunoglobulin levels (e.g., IgG, IgA, IgM) & kappa and lambda free light chains	Performed in a Central Lab
Blood (plasma)	LBA: soluble BCMA	Performed in a Biomarker Lab
Blood (peripheral blood mononuclear cells) and tumor cells	Flow Cytometry: CAR+ T Cells and BCMA+ Cells	Performed in Biomarker Lab (rapid turnaround)
• Blood (plasma) • Bone Marrow and/or tumor tissue	PCR: Vector Copy Number	Performed in Biomarker Lab
Immunogenicity	LBA: Evaluate anti-CAR antibodies	Performed at Bioanalytical Labs
Bone Marrow	Flow cytometry, FISH, cytogenetics, morphology	Performed in Central Labs, Biomarker Labs and 3PL

Summary

Individual gene therapy assays are typically not more complex than traditional bioanalytical assays or technologies. However, other consideration might contribute to the potentially more complex measurement of gene products:

+ Study design, patient and site enrollment, duration of testing and overall logistics make overall projects complex and challenging.
+ Unclear regulatory or different compliance guidance for gene therapeutic products. This can slow down or hinder the bioanalytical process of the method development/validation during if various issues, questions, options arise.
+ Very efficient but very expensive studies: patients/clients/bioanalytical parties are limited in funds, resources & time. However, most studies require short turn-around time. This combined with potential regulatory questions can create issues during the method developed & validation. And, even more complications with multiple matrices within tight timelines.
+ Technologies include traditional bioanalytical techniques, but not in the same importance. Focus is in nAb, VCN and biomarkers. PK is limited to oligonucleotide based therapies.
+ Gene products provide a dramatic treatment. The biological responses can be complex. Cytokine Release Syndrome, interference with various biomarker measurements, long term oversight.
+ Can in-vitro assays developed using relevant surrogate matrix, reference standards and quality controls, etc., really measure the real expressed products and biomarkers?

Thank you

Abstract

This talk will discuss the various types of gene/cellular therapies, the technologies used and challenges that bioanalytical scientists will face. There have been recent successes shown in cellular and gene therapy products and a growing interest of biotechnology companies on these therapies.

From a bioanalytical perspective, these projects are vastly different from other types of drug development projects. They are not necessarily more challenging from an analytical perspective, but they are very challenging from a logistical, study and compliance complexity standpoint. Often, oligonucleotides are thought of as the major gene therapy. However, this is just one segment of gene and cellular therapies. There are three main therapeutic platforms including cellular modifications, viral/gene based therapeutics as well as non-viral which include oligonucleotides. These can range from the use of genetically engineered, enucleated red cells, to modified AAV (adeno-associated virus) gene vectors including the CRISPR/Cas9 genome editing system.

From an analytical perspective, the analytical technologies used during the pre-clinical and clinical development of these therapies include traditional as well as non-traditional bioanalytical technologies. By the nature of the therapies themselves, the types of experiments and data required to move from pre-clinical to humans require different approaches. Significant research into the efficacy, route of administration, formulation, biodistribution of the delivery vector and the protein(s) being expressed and potential immunogenicity is required. In addition to LBA for biomarkers and immunogenicity, PCR, sequencing, flow cytometry and ELISPOT technologies are needed to monitor vector shedding, vector copy number, sequences, activity, etc. Activity assays are also required in both-GMP and bioanalytical development.