Table 6: Characterization of CAR-T Cell Products – Challenges and Opportunities

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Scope:

Cell and gene therapy products (CGTP) are the most complex biologics with regards to their structure, molecular size, and mode of action. This level of complexity poses multiple challenges for adequate characterization and assessment of critical quality attributes based on structure and product functional properties. The limitations of the currently used analytical tools further complicates characterization studies needed to demonstrate comparability per recommendations of ICH Q5E guidance needed during the development and product life cycle.

Participants, using CAR-T products as a representative model for CGTP but not limited to it, will discuss state-of-art characterization tools, developing suitable analytical methods, and sharing unique challenges in characterization of CGTP.

Suggested Discussion Topics:

1. Cellular starting material characterization
2. Discuss various currently used tools for products characterization including the selection of functional assay and interpretation of results with regards to potential impact on clinical safety and efficacy for:
    a. Vector
    b. Cell product
3. Characterization of product- and process-related impurities
4. Applicability of data obtained from small scale process/developmental facility
5. Necessity of animal models to characterize product/demonstrate comparability
6. New analytical tools that may increase level of characterization and knowledge about the process and products
    a. Cell imaging
    b. Improved phenotyping and functional assessment
    c. Single cell NGS
    d. Metabolic analysis
    e. Other methods?

Discussion Notes:

Introductions:

CAR Ts are complex products, which makes characterization a challenge; there is a need to improve the identification of quality attributes linked to clinical outcome. The goal of the discussion is to touch on currently used methods, and potentially new ones

Discussion:

The phenotype of apheresis starting materials is important to understand, and whether it will be linked to a given impurity in the final drug product and/or clinical outcome. There is a need to collect as much characterization data as possible.
How can we use information that is going to come out of starting material characterization? We need to understand and collect the data related to clearance of impurities and impact of starting material composition on final product.

Industry is moving from autologous to allogeneic products that may result in more homogenous starting material. That has impact on characterization needs.
For allogenic cells starting material and multiple vector lots it is understood that there is variability of the starting materials. This requires certain flexibility of the manufacturing process parameters and controls, but it has to be a single process rather than variations of thereof (product is the process).

The specifications and their numerical limits should be implemented during the products' development phases, particularly for parameters impacting the mechanism of action and product safety. It is understood that manufacturing experience is limited but scientific reasoning should be applied for this process.
Early established release attributes seem to be rather at high level – which are the areas to be further explored for meaningful specifications at developmental stages? For example, vector titer and vector copy number, and number of positive CAR cells in drug product should be a release parameter with a limit established based on product prior knowledge and scientific justification. .

Cells derived from healthy donors are frequently used during the product development and comparability characterization studies cells. However, they may differ in their properties. Knowledge has to be built from this experience in addition to the products manufactured in the clinical space. There are several avenues to build understanding of product and process. Can novel technologies allow to build the broader knowledge to reduce the risk of deviations and lot failure? What critical pieces of information are missing for a more complete characterization?
The depth of single cell investigation may be helpful, but it varies between the companies in what they determine as relevant for their product; like need for single transduction assay or multiplex CRISPR/Cas9 editing.

Manufactures feel that there should be a balance between effort made in product characterization in development and information provided to regulators. The industry concerns are related to submitting the characterization information that may be used to establish new policies by regulatory agencies that in turn may become overburden for product development. The limit on VCN number may be a good example. Nature of the current method is an average of VCN. The agencies tend to further ask questions on the distribution of VCNs across the population which is technically difficult to establish lot-to-lot consistent values.

Single cell NGS techniques may be used for characterizing vector transduction, chromosomal instability and protein expression, but it would be challenging to use for batch release testing; There is an ongoing debate on the potential application of this methodology for manufacturing process control.

It is a natural progression – to obtain more granular genetic information on the unit, like single product cells. These measurements when combined with extracellular and intracellular phenotyping are powerful characterization tools. The challenge is to generate enough data and understand their significance particularly if it is a statistically rare event (e.g. 4-10.000 cells). The single method that could also be used for durability of expression, development of cells over time.

Determination by NGS of efficiency of the CRISPR/Cas9 edits – many cells will not have all edits, difference in copies of the transgene. What is the utility and regulators acceptance for multiplex assays? Would regulators get to see it?

Metabolic analysis – would that trigger selection of a different starting cell population? There is expectation for the assessment of exhaustion, but that can also be measurement of activation. The methods are not standardized yet and some cells are harder to activate and other cells are harder to transduce. The metabolic data related to exhaustion are almost a product/patient specific, i.e. our understanding is still limited.

The outcome of characterization studies is crucial for decisions related to progress in clinical development, establishing adequate release specifications and understanding/ investigation of manufacturing deviations. However, the boundary for introduction of a novel method, beyond the characterization studies, e.g. for routine release testing, is challenging to establish. The main concern is within the limited understanding of tested quality attribute and complex analytical method vs. setting specifications for routine lot release. This poses risk for frequent lot rejections. Especially since method optimization is an ongoing process that may affect outcome of analytical data over time. It was concluded that regulators, in most cases, do not raise significant concerns with observed small differences due to method variability, particularly if well explained.

The responsibility on development of novel characterization methods for CGTP products lies within the Industry and Regulators. The nature of the product determines the characterization need. A novel method, when broadly accepted, may reduce regulatory burden and increase our knowledge about CGTP products.