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Molecular Therapy

Overcoming Challenges in CAR T-cell Product CGMP Release

A growing number of phase I/II clinical trials of chimeric antigen receptor (CAR) T-cell therapies are being conducted, including many outside of the United States. Such therapies are also reaching the market, and as such there is an urgent need to reach global consensus on the definition of quality control (QC) release criteria, in particular with respect to product potency. Comparison of the outcomes for patients enrolled in CAR T-cell clinical trials poses challenges due to differences in CAR and vector design, effector T-cell selection, CAR T-cell production, and choice of treatment cohorts. The rational design of bioassays capable of comparing the potency of similar but distinct CAR T-cell products should facilitate a better understanding of the clinical results.

The definition of therapeutic potency is important both as a parameter measuring quality, consistency, and stability between lots according to Current Good Manufacturing Practice (CGMP) regulations, and as a quantitative measure of the actual dose administered to the patient that can be correlated with clinical efficacy. The traditional approach for assessing the potency of biological products is to develop a quantitative *in vitro* and/or *in vivo* bioassay(s) that measure(s) the activity of the product in the context of its relevant mechanism(s) of action (MoA). This process requires a detailed understanding of the product features predicting clinical effects. The MoA might not be fully elucidated, but even a partial understanding will help to define critical quality attributes of the product so as to develop appropriate potency tests.

As antitumor activity is the most relevant MoA for a CAR T-cell product, preclinical studies may help to predict a correlation between this MoA and several product characteristics upon which potency could be based, including T-cell subset product composition, transgene expression, effector function (*in vitro* cytotoxicity, degranulation and cytokine production), *in vivo* persistence, homing and activity in the presence of a hostile tumor microenvironment.

The complexity of CAR T-cell products can present significant challenges in establishing potency assays. Issues include inherent variability of the starting materials (including cell donor variability), limited lot size, limited material available for testing (each lot often represents a patient-specific preparation), limited stability and viability of cellular products, lack of appropriate reference standards, and interactions between complex MoAs. A progressive plan for the implementation of potency assays should be designed that allows evolution of the nature of the potency measurement during product development, as the MoA and other product characteristics become better defined.

Our own preclinical data indicate that *in vitro* potency assays designed to quantify CAR T-cell cytotoxic activity against antigen-specific tumor targets strongly correlate with *in vivo* potency assays performed in a xenogenic mouse model bearing the same antigen-specific tumor cell lines, irrespective of CAR identity or design. Although *in vivo* potency assays can be performed, animal models are not easy bioassays to validate for QC testing for patient-specific lot release (including the issue of a limited lot size). On the other hand, we have found that *in vivo* potency assays are fundamental for verifying other product characteristics, such as the representation of different T-cell subsets and the level of CAR expression in the infused T-cell population, which correlate with the most relevant MoA, i.e., antitumor activity.

In an attempt to define a test compatible with the requirements of pre-release QC, it is intuitive that a single assay is not sufficient to assess all product attributes that measure potency. An alternative approach is to develop multiple complementary assays that measure different product attributes associated with quality, each constituting a bioassay relevant to the definition of release criteria under CGMP manufacturing. Such an assay matrix should provide an adequate measure of potency for lot release when the results are correlated with a relevant biological activity.

Although the majority of CAR T-cell clinical trials are conducted in the setting of hematological malignancies, solid-tumor oncology represents an urgent clinical need. Several CAR T-cell approaches have been designed and are under development for patients afflicted by different solid tumors. Potency assays for CAR T-cell products intended for the

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treatment of solid tumors are even more challenging than those for leukemia, because these tumors tend to be located in anatomical sites to which T cells do not preferentially migrate. In this case, the assay matrix should also include bioassays that can verify and quantify CAR T-cell migration to the tumor site, as well as their activity in the context of the tumor microenvironment.

A progressive implementation approach, whereby the first potency assay for a specific CAR T-cell product is identified during the preclinical phase, will allow researchers to define and justify a strategy of potency assay during product development. Global guidelines must be detailed, because potency assays often represent an important reason for product withdrawal during evaluation by regulatory authorities, and several CAR T-cell products could be shared between different countries with the intent to minimize costs and optimize expertise. Easy and well-validated bioassays (comprising phenotype characterization, cytokine release, and cytotoxicity), performed as potency assays for CAR T-cell product release for phase I/II clinical trials, may help corroborate their relevance and correlation with clinical outcome, leading to the definition of a more complex and sophisticated matrix of tests when the product reaches the market.

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