Q² Solutions®

CAR-T Cell Therapy

Cell & gene therapies are now used to treat both rare and common diseases. Gene therapy involves the transfer of genetic material, usually within a viral vector for uptake into diseased cells. Cell therapy involves the transfer of genetically engineered cells into a patient. For oncology treatments, CAR-T, or Chimeric Antigen Receptor T Cell, is a type of cell therapy which involves the introduction of T cells that have been genetically modified to recognize specific antigens that are expressed on tumor cells.

CAR-T therapeutic development is now commonplace following recent approvals of drugs in CD19 expressing disease. These CAR-T products have been approved by the US FDA for certain types of leukemia and lymphoma, and both pharmaceutical and biotech companies are investing in this approach to treating cancer, with an estimate of more than 500 active clinical trials globally[1].

CAR-T clinical development and associated laboratory analysis for safety and efficacy are different to traditional small molecule and antibody therapeutic approaches, due to genetically modified cells being used. Accordingly, specific laboratory approaches are required to derive pharmacokinetic profiles utilizing innovative flow cytometry and molecular assays. For safety, immunogenicity testing is required for the measurement of humoral antibodies and cellular host response against autologous CAR-T constructs. Additionally, assays or a panel of assays covering cytokines, pro-inflammatory markers and chemokines are required to safety events due to cytokine storms.

The requirement for high-quality timely laboratory data is required to assist management of patients in CAR-T clinical trials, but also to provide pharmaceutical and biotech companies with important information for regulatory filing, in addition for their own internal tollgate decision points. As these trials become global, sponsors will need laboratories that can provide test results in their geographic region, as the cells tend to be genetically engineered and processed for infusion locally in that geographical region. A global central laboratory is required to provide services from China, in which CAR-T clinical development programs are increasingly abundant. The laboratory in China can validate locally for clinical trial testing, or receive the T cells as part of a technology transfer from its other global locations.

As a laboratory services organization supporting CAR-T clinical development programs, we are able to provide Flow Cytometry, Cytokine, Genomic, BioAnalytical & Immunohistochemistry (IHC) testing for different intended uses including Expansion/Persistence (Pseudo PK), Efficacy, Prediction, Resistance, Safety, and Proof of Mechanism/Pharmacodynamics. Below is a table with our lab testing capabilities, with the factors to consider.

CAR-T Clinical Trial Lab Testing Capabilities

	Flow Cytometry	IHC	ELISpot	PCR/ddPCR	NGS	Immunoassays	LCMS/MS	Immunogenicity
Immunophenotyping	✓							
Tumor Infiltrating Lymphocytes	✓	✓						
PBMC	✓							
T Cell Stimulation Assay	✓		✓					
Minimal Residual Disease (PCR)	✓			✓	✓			
Microsatellite Instability (PCR)		✓		✓	✓			
Cytokines						✓		
Protein Expression	✓	✓					✓	
cfDNA (Digital Droplet PCR)				✓	✓			
Vector Integration site/ Persistence (PCR)				✓				
Anti-Drug Anitbody								✓

Cell Expansion & Prolonged Treatment Persistence

This is used to determine if the CAR-T is present and what the absolute cell numbers are over the course of time.

Flow Cytometry and droplet digital PCR (ddPCR) are applicable technologies to measure the construct over time and provide a "pseudo" pharmacokinetic profile. ddPCR assays have been implemented in our laboratories in the US, UK and China to provide information to support local CAR-T studies. Flow cytometry-based assays have been implemented across our global locations.

Flow Cytometry can be used to test the product itself prior to infusion into the patient. While this testing can be performed locally, a central laboratory provides a globally harmonized approach for determining both the integrity and phenotype of the product.

[1]Source: https://clinicaltrials.gov

Efficacy

Is the CAR-T stimulating, infiltrating, persisting and proliferating?

Flow Cytometry and NGS (next generation sequencing) approaches are being used for MRD (Minimal Residual Disease) determination in B-cell disease. CAR-TIL (tumor-infiltrating lymphocyte) measurement is a useful efficacy tool to monitor resultant migration of Effector cells. Also, ddPCR can be used to monitor the presence and abundance of the CAR-T and Flow Cytometry utilized to determine phenotype of the CAR-T on administration to the patient and to measure persistence throughout the treatment.

Resistance

Is there evidence of lack of target engagement or is their evidence of residual disease?

Antigen loss on the tumor cells can indicate resistance. CAR-T immune checkpoint expression with typical markers (for example, PD-L1, FoxP3, Lag-3) using Flow Cytometry and IHC technologies can also indicate resistance. Unwanted immunogenicity to the CAR-T may also impact the efficacy of the therapy. Detection of the presence of anti-drug antibodies (ADA) and neutralization properties of the response is possible via conventional ADA and/or cell-based methods, including flow cytometry assays.

Safety

What is the biological evidence behind Cytokine Release Syndrome (CRS) and Neurotoxicity?

Immunogenicity testing in CAR-T clinical trials is commonly performed also from the safety perspective. Assays that detect antibodies to the CAR-T construct can determine cellular and/or humoral responses. Typically, these assays are run under GLP quality systems, for which test data is provided back early within the clinical trial.

Cytokines, Chemokine and Pro-Inflammatory panels of assays using MSD or Luminex technology are used extensively to explain Cytokine Release syndrome – a common safety event with CAR-T. In addition, many of the next generation cell therapies have been engineered to synthesize and release cytokines, chemokines and other factors. Depending on the context and risk profile, the concentrations and immunogenicity of these products may require analytical support.

Proof of Mechanism/Pharmacodynamics

Evidence that the target is engaged to result in a biological change of the target and or downstream effect.

There are several approaches to demonstrating proof of mechanism. One is by measuring cytokine release. Another way is with use of immunophenotyping by Flow Cytometry, which can provide evidence of both immune status modulation as well as CAR-T persistence and differentiation status through the treatment cycles.

Our CAR-T Solutions

At Q^2 Solutions, we are providing this high level of laboratory testing to support cell & gene therapy approaches including CAR-T. More information on the validation and testing approaches that we have adopted to evaluate safety and efficacy of this Immuno-Oncology therapeutic approach is available upon request.

As CAR-T therapies journey through clinical development, the requirement for clinical trial testing from multiple clinical trial centers increases and so does the need for central laboratory testing. Harmonized testing and proficiency provide added assurance for reproducible and accurate data.

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