# Bioanalytical Solutions for CAR-T and NK Cell Therapies

## Introduction

Synexa Life Sciences is a bioanalytical and biomarker services laboratory specializing in the development and validation of complex and custom designed assays with progression into clinical trial sample analysis. Our depth of services enable us to support our customers throughout their clinical journey, from translational studies into late phase clinical trials.

## Our expertise

We recognize the need for science-driven solutions in the field of CAR-T and NK cell therapies. Our experienced flow cytometry and pharmacogenetic scientists have developed tailored solutions for our customers to critically assess CAR-T cell persistence. This includes multi-parameter, functional flow cytometry with up to 28-colour resolution and highly sensitive and customizable digital PCR solutions for cell therapies.

## Translational analysis

Our Prototrials® platform bridges an important gap between drug development activities and clinical studies. In light of draft FDA guidelines for CAR-T cell manufacturers, our customers can better understand and validate cellular potency and target engagement.

### Multiparameter flow cytometry to demonstrate CAR-T cell functionality and confirmation of CAR-T cell construct:

- **Immunophenotyping of CAR-T cell and associated cell subsets:** Up to 28 parameter flow cytometry panels
- **CAR-T cell function:** Markers of cytokine and granzyme b production
- **CAR-T cell exhaustion**
- **CAR-T cell memory**
- **Targeted cell killing (potency):** Immunophenotyping and co-culture with target cells



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### Additional analyses ensuring confidence in CAR-T cell safety:

- **Assessment of cytokine release syndrome:** multiplexed quantification of cytokine release
- **Tissue cross-reactivity**
- **Cytotoxicity**
- **Biodistribution**

## Clinical analysis

Regulatory guidelines for the development, validation and implementation of bioanalytical assays for CAR-T cell and NK cell therapy products are still under development. With over 20 years of experience in the biopharma industry, Synexa will adopt an integrative approach using best practices for all validation assessments depending on context of use.

### Pharmacokinetics (PK):

Cellular therapies have unique PK profiles due to rapid *in vivo* expansion and long-term persistence. Therefore, there is a highly variable relationship between dose and exposure.

Measurement of CAR-T cell and NK cellular kinetics can be accomplished via quantitative or digital PCR or flow cytometry.

### qPCR/dPCR:

- **Detection of integrated CAR-T cell trans-gene copies/cell**
- Indirect measurement of CAR-T cell therapy and cannot distinguish functional CAR-T cell products
- Superior assay sensitivity
- High throughput and variable matrix types
- Can permit distinguishment of autologous vs native cells in NK cell therapies

### Flow cytometry:

- Direct measurement of CAR-T cell therapy via antibody specific fluorescent CAR labelling
- **Characterizes and phenotypes functional CAR-T cell and associated cell populations**
- Reduced sensitivity and restrictive sample matrix

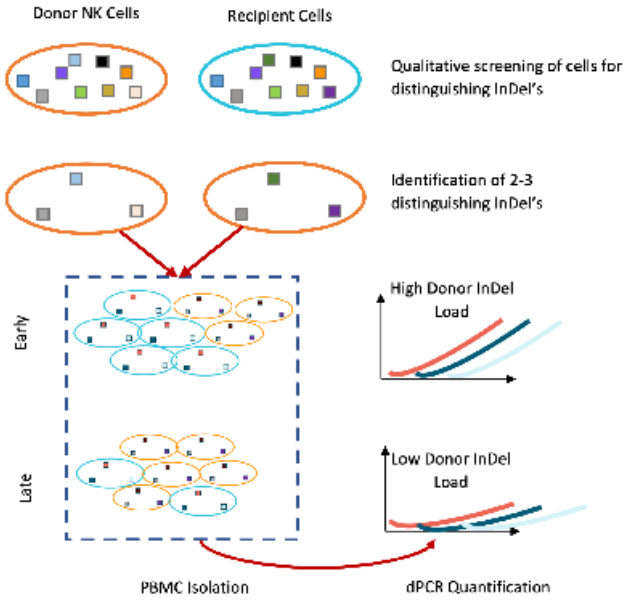
### Immunogenicity:

- Immunogenicity is a critical factor for the evaluation of the safety and efficacy of CAR-T cell products. The FDA recommends the development of assays to detect humoral and cellular immune responses against CAR-T cell.
- B-cell driven humoral Immunogenicity can be measured using ELISA based ligand binding assays that detect anti-drug antibodies (ADA) against the CAR construct. Flow cytometry-based cellular assays, employing appropriate positive controls and reporter cell lines can permit detection of ADAs which have clinical relevance. These assays will be validated according to the available FDA guidelines.
- T-cell driven cellular Immunogenicity can be assessed by ELISpot assays using CAR-T cell peptides and the patients PBMCs or cytotoxicity assays which monitor the potential of a patient's PBMCs to lyse CAR-T cell expressing reporter cell lines.

# Case Studies/Data

## Persistence of immune-cell therapy

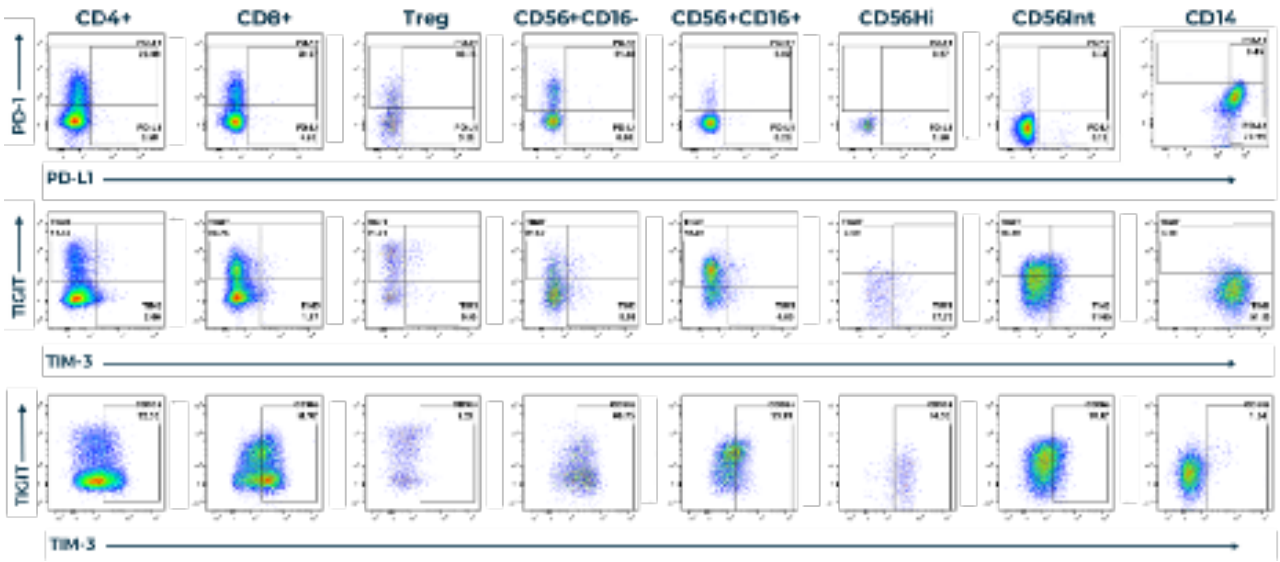
- The Sponsor developed a technology for *ex vivo* immune-cell expansion.
- Donor immune-cells were administered to HLA matched patients.
- By repurposing a qPCR (and converting to the dPCR platform) chimerism assay used in bone marrow and blood stem cell transplantation, we were able to distinguish donor from patient cells.
- Deletion-Insertion-Polymorphism (DIP) analysis was used to identify genetic markers suitable to distinguish between donor and recipient cells.




### Flow cytometry: Co-inhibitory expression gating strategy

Depending on the application, Synexa employs a rigorous approach to flow cytometry validation, including development of a gating strategy for appropriate cell types.

**Co-inhibitory Receptor Expression on Various Cell Subsets**




For more information **contactus@synexagroup.com** to see if we can find a solution to your bioanalytical challenges.

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