Overview of the CAR-T cell therapy workflow

Blood is harvested from the patient in a cell donor, peripheral blood mononuclear cells (PBMCs) are isolated and separated and delivered to a sterile environment.

Antigen-specific programming of donor T cells are purified and activated before transduction of the CAR gene code. Production of CAR-T cells is critical.

Activation may be cell based, using native or artificial antigen presenting cells (APCs) or employing either transfection or electroporation using CD3 and CD28 antibodies which can help target the T cells that act as artificial antigen receptor.

The activated CAR T cells undergo genetic modification and transduction of the CAR gene into the T cell which is performed in a specialized environment.

Prior to CAR-T cell infusion, the patient receives chemotherapy to prepare the bone marrow to induce tumor lysis and prepare immune system for the CAR-T cells to combat the infused cells. The CAR-T cells are then infused into the bone marrow and in most clinical trials for as much as eleven days with CAR gene expression and after the infusion has finished in available in several different packaging cell lines.

Challenges

- Purification of T cells
- In vivo stability of T cells
- Functional population of CAR-T cells
- Measuring absolute dose
- Measurement of qualitative isotype
- Availability of CAR-specific and cell phenotyping antibodies
- Lack of patient and product monitoring for biomarkers
- Monitoring adverse effects
- Error prevention for cell sorting
- Lack of regulatory guidance

Technologies and reagents

- Quantitative FACS and Digital PCR
- Offline culture, assays are sensitive, surrogate markers are used

- High throughput, multi-parametrer flow cytometry
- Standardized parameters for quantitative and phenotypic analysis of CAR-T cells, near to immune system from which lymphocytes had been harvested.

- Customized anti-apoptotic antibodies
- High-specialist, critical respect to determine the percentage of transduced T cells over total T cells and efficiency of CAR-T cells from patient plasma

- Multiparametric analysis of cytokines and chemokines
- Identification of severe responses, such as cytokine release syndrome toxic for patients monitoring and treatment.