IN THE ZONE CAR-T cells

Overview of the CAR-T cell therapy workflow

Blood is harvested from the patient or T cell donor, peripheral blood mononuclear cells (PBMC) are separated and delivered to a GMP laboratory.

Further processing occurs at the laboratory, including isolation of T cells, and depletion of B cells, NK cells and monocytes, which can interfere with T cell activation and expansion.

Autologous (patient) or allogeneic (donor) T cells are purified and activated before transduction of the CAR gene construct via retroviral vectors.

Activation may be cell-based, using native or artificial antigen presenting cells (APCs), or bead-based, employing co-stimulation by CD3 and CD28 antibody-coated magnetic beads that act as artificial dendritic cells.

The activated T cells undergo genetic modification *ex vivo* by viral transduction with a DNA construct encoding the chimeric antigen receptor. Gamma-retroviral vectors have been used in most clinical trials so far, as they deliver high rates of CAR gene expression and offer the flexibility of being available in several different packaging cell lines.

The activated CAR-T cells are expanded outside the body for a period, typically 10–14 days, to reach an appropriate patient-specific therapeutic dose.

Beads are removed, the CAR-T cells are formulated for freezing or adoptive transfer, undergo quality control and are transported to the treatment facility.

Prior to CAR-T cell infusion, the patient receives chemotherapy to reduce tumor load and deplete native lymphocytes that can decrease efficacy of the infused cells. This conditioning may also enhance APC activation and prevent transgene rejection.

The expanded CAR-T cells are infused into the patient where they bind to the tumor cell surface antigen in a non-MHC restricted fashion, proliferate and kill the tumor cells. At this time patients are monitored for adverse effects that may require immediate medical attention, such as cytokine release syndrome (CRS), neurotoxicity, and B cell aplasia.

Ongoing studies aim to determine the most effective dose and schedule of administration for CAR-T cell therapies to obtain optimal efficacy and persistence.

Bioanalysis of CAR-T cell therapies

Development, validation and implementation of robust and accurate methods is vital to monitor the persistence of CAR-T cells and test their safety and efficacy. During CAR-T cell therapy the initial dose is low, followed by *in vivo* cell expansion and decline. Bioanalytical assays need to ascertain whether the transferred cells survive, express the appropriate markers and expand *in vivo*. Specificity, selectivity and sensitivity requirements influence the technology platforms selected, and a secure supply of well-characterized reagents over the lifetime of the study is critical to success.

Challenges

- Purification of T cells
- *Ex vivo* stability of T cells
- Successful gene transfer
- Lot to lot variability of CAR-T cells
- Measuring absolute dose
- Measurement of cellular kinetics
- Availability of CAR-specific and cell phenotyping antibodies
- Lack of patient and control samples for assay development
- Monitoring adverse effects
- Long time period for patient monitoring
- Lack of regulatory guidance

Technologies and reagents

- Quantitative PCR and Droplet Digital™ PCR
  - DNA is stable, assays are sensitive, surrogate measurement for presence of CAR
- High-throughput, multi-parameter flow cytometry
  - Direct measurement, accurate quantification and phenotypic analysis of CAR-T cells, real-time immune cell monitoring *in vivo*, supports qPCR data for PK
- Customized anti-idiotypic antibodies
  - Highly specialized, critical reagent to determine the percentage of transfected T cells prior to administration, and differentiate CAR-T cells from other cells in patient samples
- Multiplex immunoassay analysis of cytokines and chemokines
  - Identification of severe responses, such as cytokine release syndrome, crucial for patient monitoring and treatment

Bioanalysis ZONE

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