Chimeric Antigen Receptors, T Cells and Flow Cytometry

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Personalized medicine and immuno-oncology (I/O) have changed the way cancers are being treated. I/O therapies stimulate the immune system to target tumor cells which are often invisible to, or inhibit the immune response. I/O therapies include the use of antibodies to checkpoint inhibitors, or adoptive cell therapies in conjunction with chemotherapy. One adoptive cell therapy being studied in clinical trials uses autologous T cells engineered to express chimeric antigen receptors (CAR-Ts). Two CD19-expressing autologous CAR-Ts (KYMIRAH® and YESCARTA®) have been approved for use in the United States and Europe. This presentation will provide an overview of the application of flow cytometry to the development and clinical monitoring of CAR-Ts.

Chimeric antigen receptors (CARs) are synthetic receptors engineered to bind specific ligands expressed on the surface of tumor cells. Chimeric T cell receptors with anti-tumor specificity were first introduced by Gross, Waks and Eshhar (PNAS, 86:10024) in 1989. Since that time there have been many technical developments that modify the specificity, persistence and expansion of these cells. These modifications include adding intracellular signaling domains, suicide genes to eliminate the CAR-Ts in the case of severe toxicities, tunable CARs where the level of CAR expression can be modified in vivo, and using other cell types such as regulatory T cells, natural killer (NK) T cells or allogenic T cells. The use of allogenic T cells involves deleting cell surface receptors that recognize non-self to remove the possibility of graft-vs-host disease.

Flow cytometry is an important technology used in monitoring all aspects of CAR-T production, clinical testing and use. Assessing cell purity and quality after fractionation of the T cells from blood, the percentage of cells expressing CAR and the level of CAR expression on those cells, and clonal expansion are procedures done by flow cytometry during the generation of CAR-Ts. It is also used to monitor lympho-depletion prior to treatment and the persistence and function of the cells after infusion into the patient and therefore flow cytometry has been instrumental in the development and implementation of CAR-T therapy in immuno-oncology.