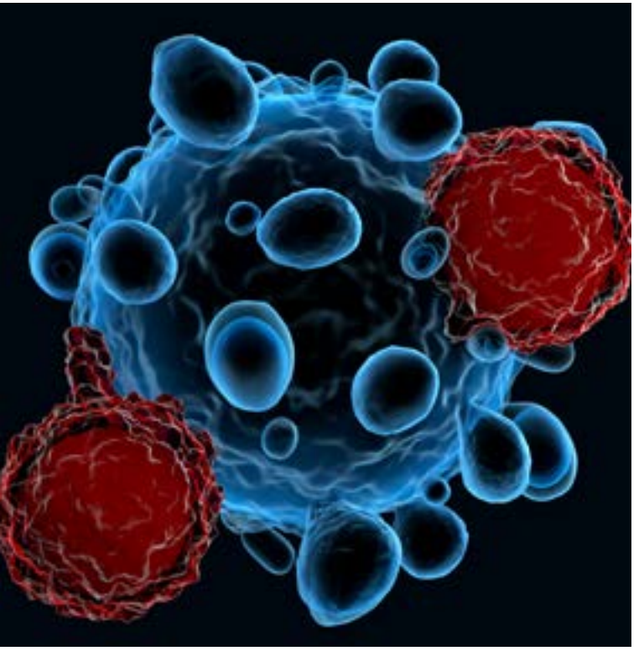
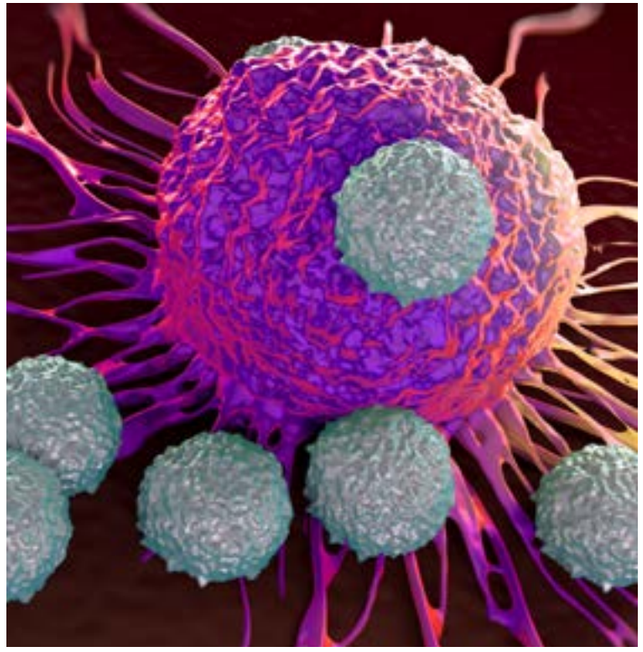
Immuno-Oncology Advances:

Accelerating CAR T Cell Therapy with Flow Cytometry





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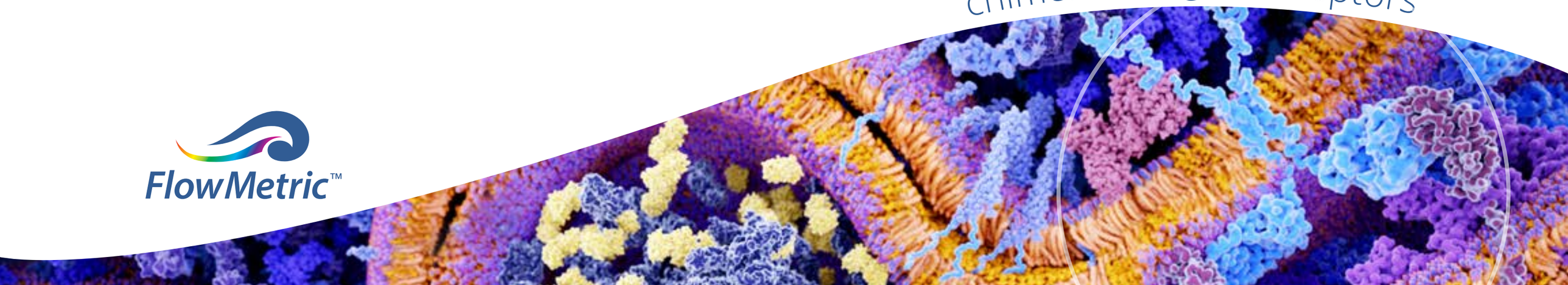
The field of immuno-oncology has changed the way many cancers can be treated through the development of ***customized cell therapies****. Several different types of adoptive T cell therapies are currently being studied in clinical trials and use different strategies to modify a patient's T cells so they can* ***specifically target tumor cells****. One particular form of therapy uses T cells carrying* ***chimeric antigen receptors*** *(CAR T cells) that can treat hematologic malignancies and solid tumors. This white paper reviews the cutting-edge field of CAR T cell therapy and how* ***flow cytometry has been instrumental*** *to the development and implementation of this experimental therapy.*



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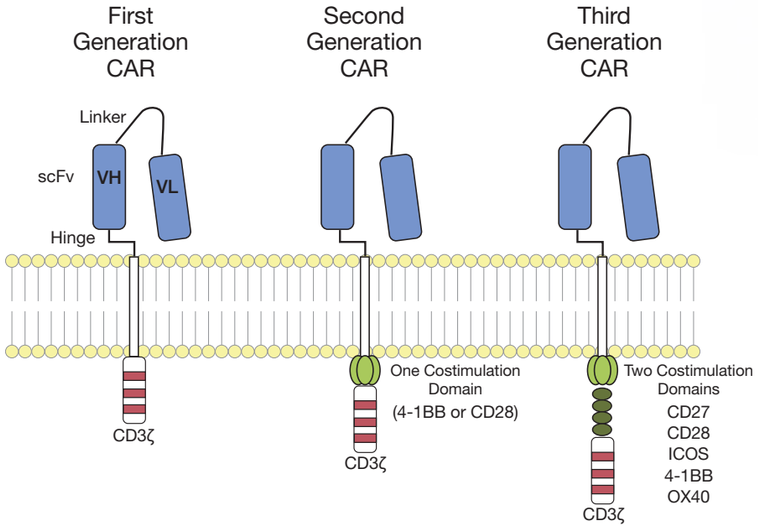
Tinkering with T Cells

CAR T cells are genetically altered T cells that express chimeric T cell receptors (TCRs) that includes the TCR constant domain and a variable domain derived from an antibody that can be engineered to target a specific tumor cell. The first CAR T cell proof-of-concept paper was published in 1989, and demonstrated that a T cell could be engineered to target an antigen with antibody-like specificity and also activate TCR signaling and did not require binding with the major histocompatibility complex (MHC) . Early CAR T cell clinical trials had mixed results, asthese cell infusions showed minimal toxicities, but treatment outcomes were confounded by poor engraftment of CAR T cells in patients and limited reduction in tumor burden.

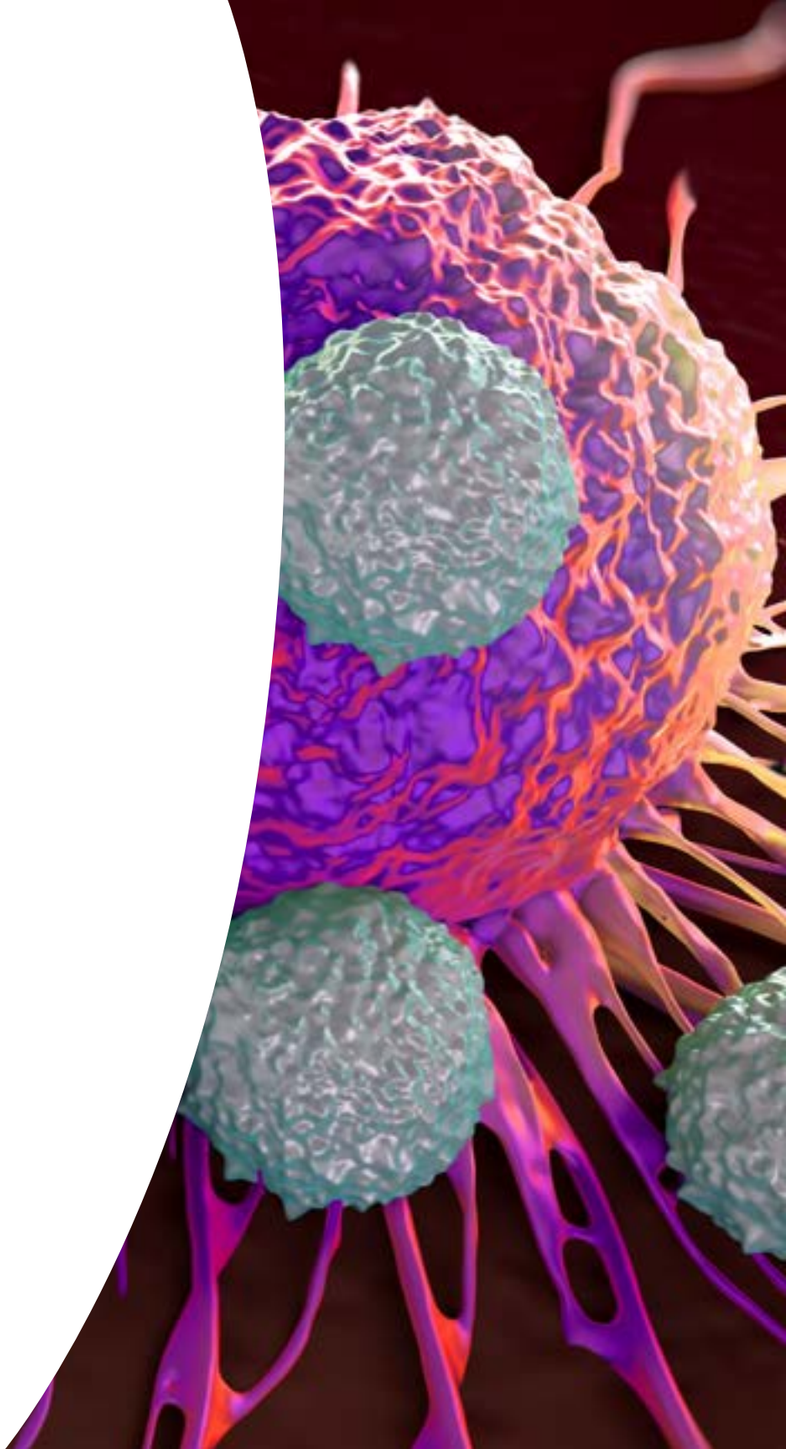
Second generation CAR T cells have included CAR constructs that contain costimulatory molecule domains between the TCR and antibody fragments, including 4-1BB or CD28[4,5,6,7]. The addition of CD28 has been associated with rapid CAR T cell expansion in patients, and 4- 1BB has supported longer persistence of these cells in circulation[8,9,10]. Now third generation CARs are under development that combine multiple costimulatory domains in order to combine benefits of these domains on expansion and persistence of cells in patients[11].



Shannon L. Maude, David T. Teachey, David L. Porter and Stephan A. Grupp, et al. CD19-targeted chimeric antigen receptor T-cell therapy for acute lymphoblastic leukemia. *Blood.* 2015 125:4017-4023; doi: https://doi.org/10.1182/blood-2014-12-580068

Flow Cytometry and CAR T Cells - From Cell Isolation to Patient Monitoring

Flow cytometry has been essential to the preclinical development and clinical monitoring of CAR T cells since these studies were first initiated. CAR T cells must be made from a patient's own T cells, which are obtained through leukapheresis, a blood collection procedure that collects only white blood cells and returns red blood cells and other blood components to a patient's circulation. White blood cells contain a wide range of immune cells, and T cells are separated from these other cell types by binding to antibody-coated beads specific for T cell surface markers. This antibody binding step also activates T cells to proliferate. The purified T cell specimen can be genetically modified using transduction techniques that introduce CAR DNA into T cells. CAR T cells can be evaluated by sequencing or flow cytometry to confirm expression of CAR on the cell surface. Cells are also screened for viability before being prepared for infusion back into the patient. Chemotherapy usually precedes CAR T cell infusion in order to deplete a patient's existing immune cells and give CAR T cells a better chance at proliferating and targeting tumor cells.



Flow Cytometry and CAR T Cells - From Cell Isolation to Patient Monitoring *Continued*

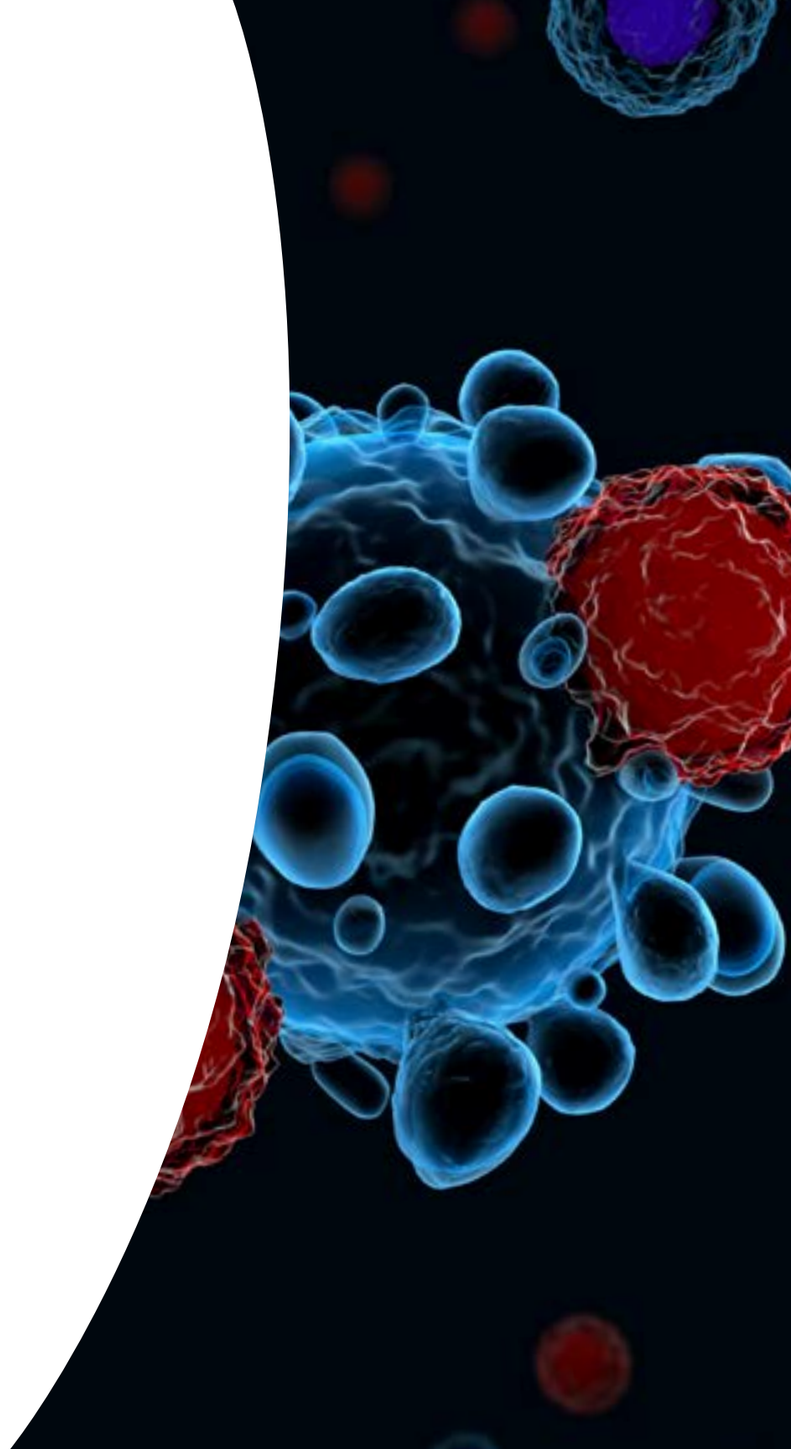
Different validated flow cytometry procedures are used throughout CAR T cell production, including monitoring the purity, fitness and overall quality of the manipulated cell preparation. These validated protocols assure that handling and processing steps result in a CAR T cell product that contains functional and viable CAR T cells. Not only is flow cytometry used to evaluate T cell activation, CAR expression, and viability, but T cell exhaustion is also typically examined. Exhausted T cells express several markers, including PD-L1 and FoxP3, and these cells cannot effectively target and kill tumor cells. Next-generation CAR T cell protocols are being optimized to reduce the fraction of T cells that show an exhausted phenotype, thus ensuring a more consistent and efficacious product.

Flow cytometry is critical to monitoring the frequency and functionality of CAR T cells in a patient's circulation following infusion. In addition to measuring changes in tumor or leukemia/lymphoma parameters, flow cytometric measurements of CAR T cells inform clinicians as to how well the treatment is working. This type of clinical flow cytometry also requires a validated protocol to assure the consistency, accuracy, and sensitivity of this critical test.

CAR T Cells on Trial and in the Clinic

CAR T cells are currently being tested in several hundred clinical trials that target both hematological cancers and solid tumors. CD19-specific CAR T cells were one of the first and best studied forms CAR T cell therapy. CD19 is expressed on B cells and is used to target malignant B cells associated with several forms of leukemia and lymphoma[13]. Stunning results have been seen in several pediatric and adult CD19 CAR T cell clinical trials, including an almost complete response rate in patients with relapsed or refractory acute lymphoblastic leukemia (ALL)[14]. Encouraging response rates have also been seen in patients with chronic lymphocytic leukemia[15] and diffuse large B cell lymphoma[16]. These impressive results led the U.S. Food and Drug Administration to approve the first CAR T cell immunotherapy on August 30, 2017 for the CD19 CAR T cell preparation called tisagenlecleucel (KYMRIAH, Novartis Pharmaceuticals Corp.)[17]. This first-of-its-kind biologic was approved to treat relapsed or refractory B cell ALL in pediatric patients.

Currently, CAR T cell trials are underway or are actively recruiting patients who have different solid tumors, including mesothelioma, pancreatic, ovarian, prostate, breast or lung cancer, or different hematologic malignancies, including ALL, multiple myeloma, acute myeloid leukemia, and non-Hodgkin's lymphoma[18].

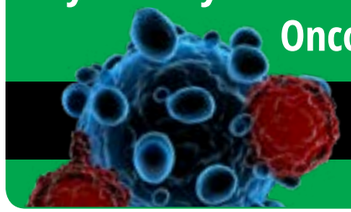


Conclusion

CAR T cell therapy is considered one of the most stunning breakthroughs in cancer treatment and is already transforming how previously fatal cancers can be treated, like relapsed ALL. Flow cytometry is an indispensable tool in the development and application of CAR T cell therapy, and the use of this technique relies on the involvement of experienced flow cytometry experts with both technical and regulatory expertise. Other forms of cancer immunotherapy are also making their way through clinical trial pipelines, and the future landscape of cancer treatment for even the most challenging cancers is looking promising.

ADDITIONAL RESOURCE

T Cell Exhaustion – Using Flow Cytometry to Monitor this Immuno-Oncology Impediment



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ADDITIONAL RESOURCE

Whole Blood Flow Cytometry Assays for Immuno-Oncology

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