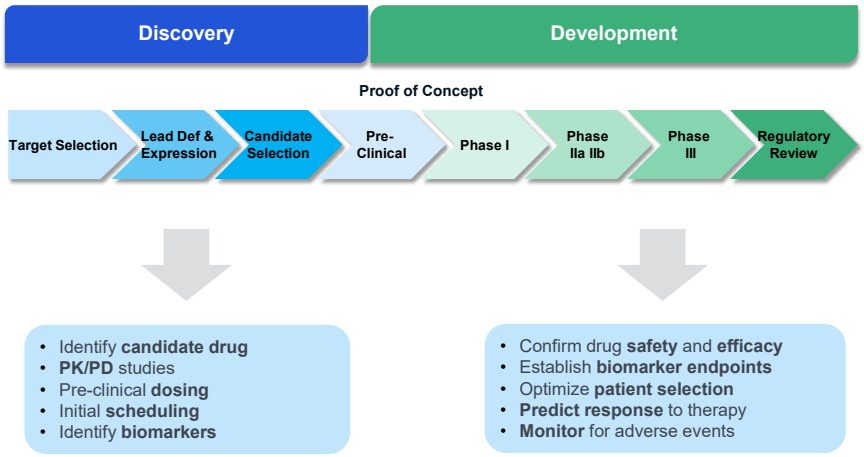
CAR T Cell Therapy Development: Guidance for Safety, Efficacy, and Consistency

Background

March 2022 draft guidance from U.S. Food and Drug Administration (FDA) for CAR T cell therapy developers includes recommendations for the various phases of CAR T cell development, as well as sequencing at different stages of development of CAR Ts – from preclinical testing, through clinical manufacturing, to tracking patients for 15 years post-infusion.

Adaptive's assays can be used as a standard throughout the R&D process



Objective of this white paper:

Help CAR T developers follow the recent FDA draft guidance and support development of more effective CAR T therapies.

Why Adaptive

Adaptive Biotechnologies has been involved in CAR T development from its earliest days. Our technology has been used for characterization of CAR T products and monitoring at different stages of development since 2012.

Adaptive can help companies:

- Streamline the development and commercialization of CAR T therapies
- Quicken path to market
- Moderate safety concerns
- Reduce costs

The immune medicine experts at Adaptive Biotechnologies can assist and provide unique insights to both industry and academic sponsors developing CAR-T cell products in the use of TCR sequencing as the "gold standard" of CAR T monitoring throughout a product's lifecycle – from IND submission, manufacturing, and clinical development through post-marketing long-term followup commitments.

CAR T Cell Therapy Development: Guidance for Safety, Efficacy, and Consistency

In March 2022, the FDA issued draft guidance for institutions developing CAR T cell therapies. This new guidance includes specific recommendations for the various phases of CAR T cell development, as well as sequencing at different stages of development of CAR Ts—from preclinical testing, through clinical manufacturing, to tracking patients for 15 years post-infusion.

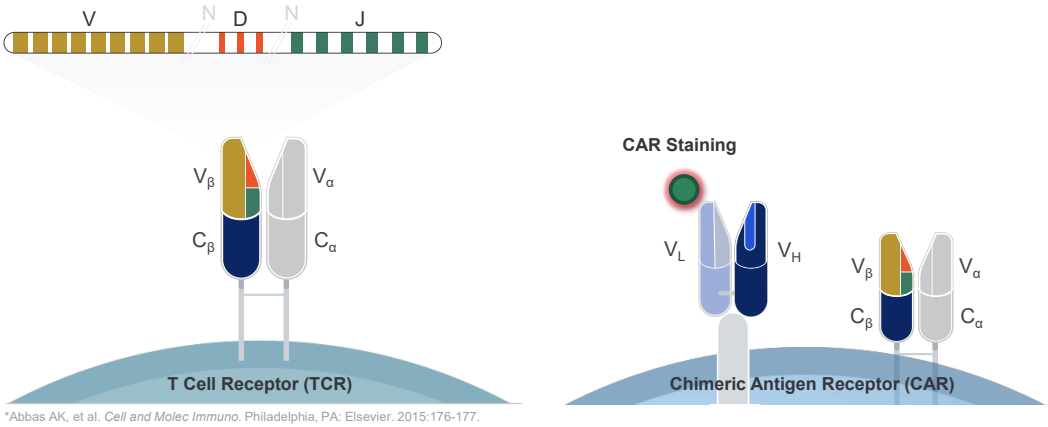
This paper is not only intended to help institutions follow the recent FDA draft guidance, but ultimately to develop more effective CAR T therapies. By guiding organizations through the research and development (R&D) process and following the FDA's draft guidance, Adaptive can help companies streamline the development and commercialization of CAR T therapies, get a drug to market faster, moderate safety concerns, and reduce costs.

Adaptive Biotechnologies: A Long History with CAR T Therapies

The immune medicine experts at Adaptive have partnered with pharmaceutical and biotechnology companies to advance CAR T therapy since the approach was first developed. By guiding organizations through the R&D process and following the FDA's draft guidance, Adaptive can help companies streamline the development and commercialization of CAR T therapies, get a drug to market faster, moderate safety concerns, and reduce cost.

Tracking Therapeutic T Cells: TCR vs CAR

The CDR3 region is the unique region within the endogenous T cell receptor (TCR) that allows us to track individual T cell clones through DNA sequencing.



*Abbas AK, et al. *Cell and Molec Immuno.* Philadelphia, PA: Elsevier. 2015:176-177.

The FDA recommends monitoring the persistence of CAR T cells containing an integrated transgene. The following chart compares published performance metrics across all four monitoring assays included in the FDA's draft guidance. TCR sequencing of the infusion product at the end of manufacturing generates a library of engineered T cell clones that can be tracked longitudinally with unparalleled sensitivity and accuracy.

Our assay tracks CAR T cells by specifically sequencing the complementarity-determining region 3 (CDR3) of T cells.

Performance	Description	Vector Copy Number (PCR)	Integration Site Analysis (NGS)	CAR Staining (FACS)	TCR Sequencing (NGS)
Viable Cells	Assay requirement for viable cells	No	No	Yes	No
Lower Limit of Detection	Lower limit of detecting engineered T cells in the sample (frequency of cell)	.0001% (1 / 1,000,000)	1% (1 / 100)	.02% (1 / 5,000)	.0001% (1 / 1,000,000)
Lower Limit of Quantification	Lower limit of quantifying engineered T cells in the sample (frequency of cell)	.01% (1 / 10,000)	5% (1 / 20)	.05% (1 / 2,000)	.001% (1 / 100,000)
Accuracy (T Cell Count)	Quantitative accuracy of engineered T cell count in the sample (deviation from true value)	>100%	N/A	>20%	<20%
Accuracy (Clonality)	Quantitative accuracy of engineered T cell clones in the sample (deviation from true value)	N/A	N/A	>50%*	<20%

*Clonality analysis by flow cytometry (FACS) requires a separate panel of antibodies specific for Vbeta genes in the TCR locus. This requires the sample to be split into eight additional aliquots for separate staining, testing, and analysis.

Following the FDA Draft Guidance

Chemistry, Manufacturing and Controls (CMC) Guidance

FDA Recommendation	Adaptive's Solution	Benefits to CAR T Development
The FDA recommends identity testing at all phases of chemistry, manufacturing and control (CMC) development to adequately identify a product and distinguish it from other products in the same facility.	**Confirm infusion product** • TCR repertoire analysis provides confirmation of the identity of the infusion product based on HLA type and repertoire overlap with the leukapheresis product. • immunoSEQ allows researchers to compare the quality of the repertoire of the starting material and CAR T product to Adaptive's database of thousands of healthy controls.	**Improved clinical outcomes** • Identity testing through TCR repertoire analysis and HLA typing provides continuous quality control of the manufacturing process. • Donor screening or characterizing of the starting material repertoire can have a significant impact on infusion product quality and clinical outcomes.

Adaptive

Preclinical Recommendations

FDA Recommendation	Adaptive's Solution	Benefits to CAR T Development
According to the FDA, the potential for uncontrolled proliferation and toxicity may differ depending on the cell source. Thus, the draft guidance states preclinical evaluation may include: • Examination of cytokine-independent cell growth • In vitro and in vivo testing for T cell clonality • Karyotypic analysis • TCR repertoire analysis • Specificity for viral antigens through ex vivo stimulation and recognition assays	**Mouse and human TCR Assays** • TCR repertoire analysis with immunoSEQ can be used to support preclinical evaluation of the cellular component of cell therapies. • With mouse and human versions of our immunoSEQ assay, Adaptive can support all types of preclinical studies, including syngeneic and xenogeneic mouse models.	**Improved clinical development** • By using the same immunoSEQ assay in manufacturing, preclinical studies, and clinical trials, sponsors can better understand their cell therapy at each stage of development without additional risk assessments, assay requalification, or comparison studies.

Clinical Recommendations: Pharmacokinetics (PK)

FDA Recommendation	Adaptive's Solution	Benefits to CAR T Development
After administration, CAR T cells expand and persist in the human body. The FDA's draft guidance states that samples, such as blood and bone marrow, should be collected on a specified schedule to monitor in-vivo proliferation and persistence of CAR T cells. Partial exposure (pAUC) can be used for correlative analysis between exposure and efficacy and/or safety.	**T cell fraction** • To explore the relationship between CAR T cell exposure and response. ImmunoSEQ can be used to count CAR T cells in each sample based on TCR sequencing reads.	**Improved pharmacokinetics** • TCR repertoire analysis using immunoSEQ has the potential to become the gold standard for PK monitoring. • TCR sequencing is more sensitive than flow cytometry and more accurate than PCR testing, as vector copy number can be by orders of magnitude based on transgene frequency in the top clones. • TCR repertoire analysis also provides valuable clonality information, unlike transgene levels and CAR expression.

Clinical Recommendations: Pharmacodynamics (PD)

FDA Recommendation	Adaptive's Solution	Benefits to CAR T Development
The FDA recommends assessing the following exploratory correlative analyses: • The relationship between CAR T cell final product characteristics and CAR T cell pharmacokinetic profiles • The relationship between CAR T cell exposure and responses using clinical PK and PD data.	**Endogenous T cell response** • In addition to characterizing and monitoring the infusion product, TCR repertoire analysis using immunoSEQ provides valuable information on the endogenous repertoire and response to treatment.	**Improved PD** • TCR repertoire analysis using immunoSEQ enables correlative analyses between the infusion product characteristics, the PK profile, and PD biomarkers related to the endogenous immune response. • Antigen spreading can be detected as new T cell clones that expand over time and are not associated with the infusion product.

Clinical Recommendations: Persistence

FDA Recommendation	Adaptive's Solution	Benefits to CAR T Development
The FDA recommends that the clinical protocol describes the plans to determine the duration or persistence of the administered CAR T cells in trial subjects. The specimens for such a determination may include blood, body fluid, and tissue. Subjects should be followed for 15 years after treatment with CAR T cells containing an integrated transgene. The FDA also recommends that analytical methods for assessing CAR T cell persistence should be described in detail. Such methods could include tests for the presence of CAR T cells or vectors, and for the activity of the CAR T cells—including gene expression or changes in biomarkers.	Sponsors can use immunoSEQ for **long-term monitoring** using immunoSEQ to detect the occurence of potentially malignant clones expanding after Cmax.	**Improved safety monitoring** • TCR repertoire analysis using immunoSEQ meets the FDA requirements for both persistence and clonality monitoring with one assay. • Flow cytometry does not track clonality and may miss detection of a malignant CAR T cell downregulating surface expression of the CAR. • PCR does not track clonality and may conflate the temporary expansion of multiple clones in a secondary response with the expansion of a single malignant clone. • A secondary response may also look much larger if the top clones have many transgene copies.

Therapeutic Response Assessment

Adaptive's TCR sequencing technology has been implemented to longitudinally monitor T cell therapy products while also tracking a patient's own immune response to these potentially life-saving therapies over time.

In addition to assessing product and host T cell immune responses, monitoring disease burden is a critical component of cancer patient care.

Adaptive's technology can be used to directly monitor disease burden in lymphoid malignancies, for example, where reduction or elimination of MRD following treatment is recognized as one of the most prognostic factors for improved patient outcomes in patients. Adaptive's minimal residual disease (MRD) assay for lymphoid malignancies has been used extensively to demonstrate deep therapeutic responses associated with newer investigational agents, including in more than 30 CAR T clinical trials.

Work with Adaptive Biotechnologies

Adaptive Biotechnologies is a commercial-stage biotechnology company that aims to translate the genetics of the adaptive immune system into clinical products to diagnose and treat disease. Our Immune Medicine platform allows us to tap into the massive diversity of T cells and B cells to be able to read and quantify the adaptive immune system.

Our state-of-the-art T and B cell technologies support sponsors in the multiple phases of CAR T cell therapy development – from infusion product analysis to long-term therapeutic response monitoring.

Adaptive's portfolio is broad and we have developed and advanced specific T and B cell technologies that can support multiple phases of CAR T cell therapy development through both product construct analysis and therapeutic response monitoring.

Questions about the FDA draft guidance on CAR T development? Contact us at busdev@adaptivebiotech.com.

(C) Adaptive Biotechnologies, 2022.