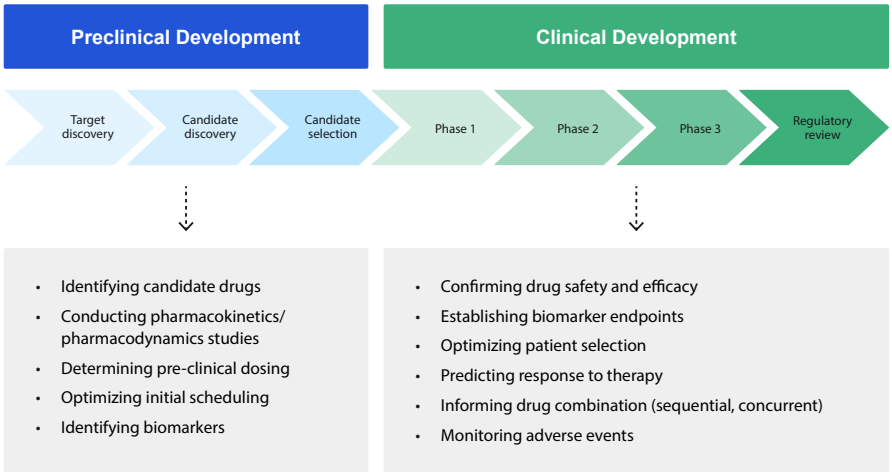
CAR T-Cell Therapy Development: Guidance for Safety, Efficacy, and Consistency

Background

March 2022 draft guidance from the U.S. Food and Drug Administration, "Considerations for the Development of Chimeric Antigen Receptor (CAR) T Cell Products," includes recommendations for the various phases of CAR T-cell development, including sequencing at different stages of development — 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Help CAR T developers follow the recent FDA draft guidance, and support development of more effective CAR T therapies.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 Immunosequencing, Adaptive's T-cell receptor (TCR) and B-cell receptor (BCR) sequencing assay, provides a quantitative end-to-end immunosequencing solution that helps pharma partners discover the breadth and depth of the adaptive immune repertoire. It has been used for characterization of CAR T products and monitoring at different stages of development for more than a decade.

Adaptive can help CAR T developers:
- Streamline the development and commercialization of CAR T therapies
- Quicken the path to market
- Mitigate 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 life cycle — including IND submission, manufacturing and clinical development, and post-marketing long-term follow-up commitments.

Questions about the FDA draft guidance on CAR T development?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

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

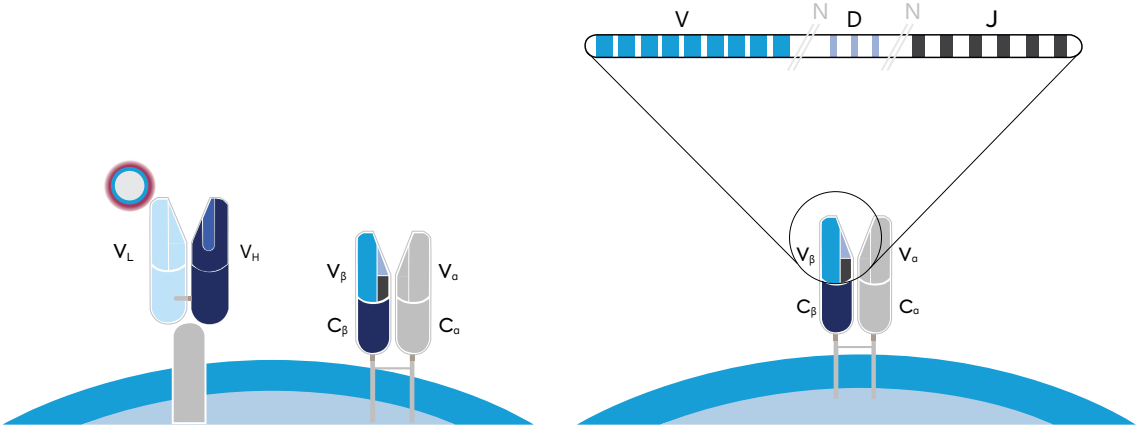
Adaptive Immunosequencing can help CAR T-cell developers follow recent FDA draft guidance, and support development of more effective CAR T therapies

In March 2022, the U.S. Food and Drug Administration (FDA) issued draft guidance for institutions developing chimeric antigen receptor (CAR) T-cell therapies. This guidance includes specific recommendations for the various phases of CAR T-cell development, as well as sequencing at different stages of development of CAR T cells — from preclinical testing through clinical manufacturing to tracking patients for 15 years post-infusion.

This paper is intended to help institutions follow the recent FDA draft guidance, and to ultimately develop more effective CAR T therapies. 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, mitigate safety concerns, and reduce costs.

Tracking Therapeutic T Cells: CAR vs. TCR

Immunosequencing tracks CAR T cells by specifically sequencing T cells' complementarity-determining region 3 (CDR3). The CDR3 region is the unique location within the endogenous TCR that allows us to track individual T-cell clones through DNA sequencing.



CAR Staining

Traditionally, cell therapy products are tracked by fluorescent staining of the CAR expressed on the surface of living cells, followed by measurement via flow cytometry.

TCR Sequencing

Adaptive Immunosequencing utilizes the rearranged VDJ sequence in the CDR3 of the TCR beta chain as a natural barcode to track individual T cells.

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 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.

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Immunosequencing 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%

Validation data summary available on request.

* 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 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 confirms the identity of the infusion product, based on human leukocyte antigen (HLA) type and repertoire overlap with the leukapheresis product. • Immunosequencing allows researchers to compare the quality of the starting material repertoire and CAR T product against Adaptive's database of thousands of healthy controls.	**Improved clinical outcomes:** • Identity testing through TCR repertoire analysis and HLA typing provides continuous quality control in the manufacturing process. • Donor screening or characterizing the starting material repertoire can have a significant impact on infusion product quality and clinical outcomes.

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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 that 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 Immunosequencing can be used to support preclinical evaluation of the cellular component of cell therapies. • With our mouse and human assays, Adaptive can support all types of preclinical studies, including syngeneic and xenogeneic mouse models.	**Improved clinical development:** • By using the same Immunosequencing 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 between exposure and safety.	**T-cell fraction:** • To explore the relationship between CAR T-cell exposure and response, Immunosequencing can be used to count CAR T cells in each sample based on TCR sequencing reads.	**Improved pharmacokinetics:** • TCR repertoire analysis using Immunosequencing has the potential to become the gold standard for PK monitoring. • Immunosequencing is more sensitive than flow cytometry and more accurate than PCR testing, as vector copy numbers can be off 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 PK 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 Immunosequencing provides valuable information on the endogenous repertoire and response to treatment.	**Improved pharmacodynamics:** • TCR repertoire analysis using Immunosequencing 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 not associated with the infusion product expand over time.

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Clinical Recommendations: Persistence		
FDA Recommendation	**Adaptive's Solution**	**Benefits to CAR T Development**
The FDA recommends that the clinical protocol describes the plan 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 or tissue. Subjects should be followed for 15 years after treatment with CAR T cells that contain an integrated transgene. The FDA also recommends that analytical methods for assessing CAR T-cell persistence are 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 Immunosequencing for **long-term monitoring** to detect the occurence of potentially malignant clones expanding after Cmax.	**Improved safety monitoring:** • TCR repertoire analysis using Immunosequencing meets the FDA requirements for both persistence and clonality monitoring with one assay. • Flow cytometry: (1) does not track clonality; and (2) may miss CAR T cells that no longer express 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 measured by transgene copy number may be overestimated if the top clones have many transgene copies.

Therapeutic Response Assessment

Adaptive Immunosequencing 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, when reduction or elimination of minimal residual disease (MRD) following treatment is recognized as one of the best prognostic indicators for improved outcomes in patients. Adaptive's MRD assay for lymphoid malignancies has been used in more than 30 CAR T clinical trials to demonstrate deep therapeutic responses associated with newer investigational agents.

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 repertoire.

Our state-of-the-art T-cell and B-cell sequencing capabilities can support sponsors in multiple phases of CAR T-cell therapy development — from product construct analysis to long-term therapeutic response monitoring.

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