Considerations for the Development of Chimeric Antigen Receptor (CAR) T Cell Products

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Guidance for Industry

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Guidance for Industry

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I. INTRODUCTION

Chimeric antigen receptor (CAR) T cell products are human gene therapy[1] products in which the T cell specificity is genetically modified to enable recognition of a desired target antigen for therapeutic purposes. This guidance is intended to assist sponsors, including industry and academic sponsors, developing ex vivo-manufactured CAR T cell products. In this guidance, we, FDA, provide CAR T cell-specific recommendations regarding chemistry, manufacturing, and control (CMC), pharmacology and toxicology, and design of clinical studies for oncology indications (including hematologic malignancies and solid tumors). Recommendations specific to autologous or allogeneic CAR T cell products are noted in this guidance. This guidance also provides recommendations for analytical comparability studies for CAR T cell products. While this guidance specifically focuses on CAR T cell products, some of the information and recommendations provided may also be applicable to other genetically modified lymphocyte products, such as CAR Natural Killer (NK) cells or T cell receptor (TCR)-modified T cells. These related product types can be highly specialized, and in many cases, considerations beyond those recommended in this guidance would depend on the specific product and manufacturing process. To discuss considerations specific to these related products or non-oncology indications, we recommend sponsors communicate with the Office of Therapeutic Products (OTP) in the Center for Biologics Evaluation and Research (CBER) before submitting an Investigational New Drug Application (IND) (e.g., by requesting a pre-IND meeting (Ref. 1)).

[1] Human gene therapy seeks to modify or manipulate the expression of a gene or to alter the biological properties of living cells for therapeutic use. FDA generally considers human gene therapy products to include all products that mediate their effects by transcription or translation of transferred genetic material, or by specifically altering host (human) genetic sequences. Some examples of gene therapy products include nucleic acids, genetically modified microorganisms (e.g., viruses, bacteria, fungi), engineered site-specific nucleases used for human genome editing, and ex vivo genetically modified human cells. Gene therapy products meet the definition of "biological product" in section 351(i) of the Public Health Service (PHS) Act (42 U.S.C. 262(i)) when such products are applicable to the prevention, treatment, or cure of a disease or condition of human beings (see Federal Register Notice: Application of Current Statutory Authorities to Human Somatic Cell Therapy Products and Gene Therapy Products (58 FR 53248, October 14, 1993), https://www.fda.gov/media/76647/download).

In general, FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidance means that something is suggested or recommended, but not required.

II. BACKGROUND

CAR T cells[2] are gene therapy (GT) products that are regulated under FDA's existing framework for biological products. We recognize that the development, manufacture, testing, and clinical assessment of CAR T cells is challenging. Careful design and appropriate testing of the CAR transgene[3] and delivery vector are critical to product safety, specificity, and function. CAR T cell manufacturing involves multiple biological materials and complex multi-step procedures, which are potential sources of variability among product lots. Thus, control of the manufacturing process and appropriate in-process and lot release testing are crucial to ensure CAR T cell safety, quality, and lot-to-lot consistency. In addition, changes to the manufacturing process are common during product development. It is essential to understand the effects of such changes on product quality. Comprehensive product characterization studies are valuable for identifying relevant critical quality attributes (CQAs) that can be assessed during manufacture, at lot release, and in comparability and stability studies to assure safety and efficacy (Ref. 2). Critical process parameters (CPPs) can then be established through process qualification, to ensure consistent CQAs for every manufactured batch. (Ref. 2). FDA's guidance entitled "Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs): Guidance for Industry," January 2020 (Ref. 3) (hereinafter referred to as the "GT CMC Guidance") describes the general considerations for GT product manufacturing and testing.

Nonclinical evaluation of CAR T cells is necessary to support a conclusion that it is reasonably safe to administer the product in a clinical investigation (Title 21 of the Code of Federal Regulations 312.23(a)(8) (21 CFR 312.23(a)(8)). Nonclinical testing of CAR T cells can be challenging due to the inherent biological complexity and variability of this product type and the limited availability of suitable animal models to test safety and activity. A case-by-case nonclinical testing strategy should be applied using in vitro, in silico, and in vivo testing strategies, as appropriate, in conjunction with available nonclinical and clinical data from related products to support use of CAR T cells in a proposed clinical trial.

Well-designed early-phase clinical studies are critical to establish safety of the product, adequacy of risk mitigation measures, dose-response relationship, differences in optimal dose based on differences in indication, preliminary evidence of efficacy, and feasibility of manufacturing. For autologous CAR T cells, early-phase studies also provide information on how long it will take to manufacture the product and whether bridging therapy will or will not be used as an attempt to

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[2] CAR T cell products will be referred to as CAR T cells throughout this guidance.

[3] For the purposes of this guidance, transgene means an exogenous gene that is introduced into a host cell. See also (Ref. 10).

control the active disease while subjects wait for the CAR T cell treatment. For allogeneic CAR T cells, early-phase studies can be informative with regards to the risks of graft versus host disease (GVHD). Information gained from these early-phase studies support the development of CAR T cells in later-phase clinical studies and may expedite the clinical development of CAR T cells.

III. GENERAL CONSIDERATIONS FOR CAR T CELL DESIGN AND DEVELOPMENT

CAR T cells are complex products that may incorporate multiple functional elements. The nature of these functional elements, how the functional elements are introduced into the cells (i.e., vector type), the cellular starting material, and the final drug product formulation are all critical to product safety, specificity, and function. Here, we briefly outline key considerations for CAR T cell design and development.

A. CAR Construct

CARs generally contain two types of domains: antigen recognition and signaling. Antigen recognition domains allow CAR T cells to bind to one or more target antigen(s). We recommend sponsors assess the ability of each antigen recognition domain to specifically bind to its target antigen, as described in section V.B of this guidance. Many antigen recognition domains are derived from murine monoclonal antibodies that may be immunogenic in humans, leading to rejection of the CAR T cells or other safety risks (e.g., anaphylaxis). If approaches to reduce immunogenicity (e.g., "humanization" by Complementarity-Determining Region grafting) are used, we recommend the IND describe these changes and their impact on target binding and biological activity (Refs. 4, 5, 6). When multiple CARs are expressed in a single drug product, the CAR construct design should reduce the risk of recombination events, if feasible.

Signaling domains initiate T cell activation. We recommend that the functionality of signaling domains be well supported by information from previous nonclinical and clinical experience or thoroughly demonstrated, as described in section V.B of this guidance. For example, the contribution of transmembrane domain, hinge, and linker regions used to separate different functional regions of the construct should be evaluated, as these may affect CAR T cell specificity, persistence, and activity (Refs. 7, 8, 9).

B. Vector

A "vector" is a vehicle consisting of, or derived from, biological material that is designed to deliver genetic material. Examples of vectors include plasmids, viruses, and bacteria that have been modified to transfer genetic material (Ref. 10). For CAR T cells, the vector is a critical component that furnishes a pharmacological activity for the treatment of disease (section IV.B of the GT CMC Guidance (Ref. 3)). Vectors that integrate into cellular DNA (e.g., retroviral-based vectors or transposons) can provide long term

transgene expression compared to non-integrating vectors. Long term follow up is recommended for products that include integrating vectors, because integrating vectors may increase the risk of delayed adverse events (Ref. 10). The predicted risk of delayed adverse events is thought to be low for non-integrating vectors, and generally long term follow up would not be needed.

In addition to the CAR, vectors may express additional functional elements. For example, vectors may express additional functional elements that allow for the selection or enrichment of cellular subsets during manufacturing (Ref. 11); that modify T cell persistence and/or activity (Ref. 11); or that allow selective in vivo ablation ("suicide genes") of CAR T cells (Refs. 12, 13, 14).

It should be noted that each additional functional element may affect CAR T cell safety and effectiveness. We recommend sponsors provide justification and relevant data to support incorporation of additional elements. The justification should include an assessment of any impact that these additional elements will have on CAR T cell specificity, functionality, immunogenicity, or safety (see section V.E of this guidance). Transgene sequences that are unnecessary for the biological function of a product may be immunogenic in vivo or have other unanticipated effects on product persistence or activity. As a general guiding principle, we recommend that unnecessary transgenes (e.g., antibiotic resistance genes used for plasmid selection) should not be included in the vector.

C. Cellular Starting Material

The starting material for CAR T cell manufacture is generally obtained by leukapheresis of patients (for autologous products) or healthy donors (for allogeneic products). Safety and regulatory considerations differ for autologous and allogeneic products, as outlined in section IV.B of this guidance.[4]

Particular consideration should be given to patients who have received CAR T cells previously. Such patients may be considered for different CAR T cell clinical studies due to lack of response to the previously administered CAR T cells, relapse of the same condition, or treatment for a different malignancy. CAR T cells manufactured using cellular starting material (e.g., leukapheresis) from patients who have received CAR T cells previously may differ from the same type of CAR T cells manufactured using cellular starting material from patients who have not. Previously administered CAR T cells in the starting material may have unexpected effects on CAR T cell manufacturing (e.g., expansion or transduction rates), potency, in vivo expansion, safety, and efficacy. Therefore, evaluation of the previously administered CAR T cell levels in the cellular starting material may be appropriate. This may be accomplished by detection of common vector or CAR features to evaluate the presence of previously administered CAR T cells. In addition, we recommend you collect retention samples of leukapheresis material in the

[4] See also FDA's guidance entitled "Human Gene Therapy Products Incorporating Human Genome Editing: Guidance for Industry," January 2024 (GE Guidance) (Ref. 15).

event that additional analysis is necessary. If an autologous CAR T cell clinical study will enroll patients who have received CAR T cells previously and patients who have not, the potential differences in the CAR T cells should be evaluated and considered in the clinical study design and analysis. We recommend sponsors discuss these considerations for product characterization, testing, dosing, and clinical study design with OTP prior to the IND submission as part of a pre-IND meeting (Ref. 1).

D. Fresh or Cryopreserved Final Products

CAR T cells may be formulated for fresh infusion or cryopreserved for later administration. The choice of formulation depends on the product development strategy and practical constraints.

Fresh CAR T cells have a limited shelf life before product quality degrades. We recommend that the maximum time between formulation and infusion be defined and supported by stability studies and include considerations for preparation prior to administration. Additionally, the timeframe in which release tests can be performed is limited. Therefore, it is crucial to develop and implement well-designed logistics, which may include: timing for sampling and testing for lot release; reporting Quality Control (QC) testing results and Quality Assurance (QA) review for lot release; scheduling product shipping; and receiving and handling of the fresh product at the clinical site.

On the other hand, cryopreservation allows sufficient time for full release testing and flexibility in scheduling patients for infusion. We generally recommend cryopreservation when CAR T cells are manufactured at a central location and shipped to clinical sites for administration. For cryopreserved CAR T cells, the risks associated with infusion of the cryoprotectant should be assessed, and controlled thawing of the product at the clinical site may be critical to maintain product quality.

Regardless of the formulation, there should be appropriate procedures to ensure adequate control of the CAR T cells during shipping to the clinical site. These procedures should be described in the IND and in place before initiating clinical studies. The procedures to ensure CAR T cell product quality during shipping, receipt, storage, and preparation for infusion should be validated prior to licensure.

IV. CMC RECOMMENDATIONS

This section of the guidance addresses considerations specific to CAR T cell products and is not designed to be a stand-alone CMC guidance. Please refer to the general CMC guidance documents on cell and gene therapies available from FDA's website: https://www.fda.gov/vaccines-blood-biologics/biologics-guidances/cellular-gene-therapy-guidances.

We recommend sponsors organize information in the Common Technical Document (CTD) format with the vector CMC information described in a complete Drug Substance (DS) section

and the CAR T cell information organized into a separate DS section and a separate Drug Product (DP) section, as discussed in section IV.B of the GT CMC Guidance (Ref. 3). When CAR T cells are manufactured using a continuous process where there is no clear division between the DS and DP, we recommend that you provide an explanation to support your DS/DP distinction in the summary information in Module 2 of the CTD submission. The CTD DS sections should follow the format and numbering scheme recommended in Module 3 of FDA's Guidance for Industry: "M4Q: The CTD – Quality," August 2001 (Ref. 16), and the sections should be distinguished from one another by including the DS name and manufacturer in the heading (e.g., section 3.2.S.1 General Information [name, manufacturer]).

The emphasis for CMC in all phases of development is product safety and manufacturing control. We recommend that CAR T cells be developed following a lifecycle approach where information is gathered over the course of product development and submitted in a stage-appropriate manner. The amount of CMC information to be submitted in your IND depends on the phase and the scope of the clinical investigation proposed (21 CFR 312.23(a)(7)). Therefore, you may not need to complete all CTD sections in your original IND submission. Similarly, manufacturing must comply with Current Good Manufacturing Practice (CGMP), as appropriate for the stage of development (section 501(a)(2)(B) of the Federal Food, Drug, and Cosmetic Act (FD&C Act) (21 U.S.C. 351(a)(2)(B)) (see also Ref. 17, and 21 CFR 210.2). Additional CMC information may be needed to align product development with the clinical development, especially when the latter is rapidly progressing under an expedited development program. For example, analytical assays should be fit for purpose to support early phase studies and qualified before initiating clinical studies that are intended to provide the primary evidence of effectiveness to support a marketing application.

For CAR T cells in the early stages of clinical development, very few specifications are finalized, and some tests may still be under development (section V.A.4.a of the GT CMC Guidance (Ref. 3)). Characterization data collected during early studies can inform release criteria used in later development to ensure product and process consistency. Thus, characterization studies are crucial to support product development and comparability assessments. For studies in which a primary objective is to gather meaningful data about product efficacy, we recommend that acceptance criteria be refined to ensure batches are well-defined and consistently manufactured. In the Biologics License Application (BLA), the proposed commercial lot release criteria should be based on data from product lots shown to be safe and effective in clinical studies.

A. Vector Manufacturing and Testing

The GT CMC Guidance (Ref. 3) provides recommendations for manufacturing and testing of the vector. The vector safety and quality should be sufficiently characterized prior to initiation of clinical studies. For later phase studies and for licensure, the vector must be manufactured according to CGMP under section 501(a)(2)(B) of the FD&C Act, and analytical assays should be validated. (Ref. 18). During CAR T cell BLA review, vector manufacturing facilities are subject to inspection.

Vector quality directly contributes to the quality and consistency of the CAR T cells. We recommend that sponsors describe the vector structure, characterization and testing of the Master and Working Cell Banks, characterization of reference materials, and vector manufacture and testing. We also recommend stability studies for vectors be conducted to support hold and storage times as described in section V.A.7 of the GT CMC guidance (Ref. 3). Vector lot release testing should include measures of safety, identity, purity, and biological activity. An assay that assesses the biological activity of the transgene may be developed in coordination with the CAR T cell potency assay (see section IV.C.2 of this guidance). Transgene expression alone as a measure of biological activity may be sufficient to support early-phase IND studies; however, additional measures of biological activity will likely be requested for clinical study(s) intended to provide primary evidence of effectiveness to support a marketing application. Additionally, we recommend that vector strength be determined during lot release testing to normalize the amount of vector used for transduction during CAR T cell manufacturing. For example, we recommend testing viral vectors for transducing units per milliliter (mL) in a suitable cell line or healthy donor cells. This allows determination of the amount of vector that is added per cell to achieve the target percentage of CAR-positive cells in the CAR T cell DP while ensuring that the vector copy number remains within target specifications.

Vector safety testing should include microbiological testing such as sterility, mycoplasma, endotoxin, and adventitious agent testing to ensure that the CAR T cell DP is not contaminated. Additional testing may be recommended depending on the type of transgene vector being used. For example, there are additional safety concerns and testing expectations related to the use of retroviral-based vectors (section V.A.4.b.ii of the GT CMC Guidance (Refs. 3 and 19)). The recommendations for long term follow-up of patients generally depends on the safety concerns associated with the vector and the propensity for the vector to integrate (Ref. 10).

B. Collection, Handling, and Testing of Cellular Starting Material

Here, we describe considerations for cellular starting material, using starting material obtained from leukapheresis (referred to as "leukapheresis starting material") as an example. The recommendations in this section may be applicable to other types of cellular starting material as well. Testing recommendations for cell banks originating from allogeneic cells or tissues are discussed in section V.A.2.c.ii.b of the GT CMC Guidance (Ref. 3).

Collection of the leukapheresis starting material should be conducted in accordance with the regulations in 21 CFR part 1271. Autologous leukapheresis starting material does not require a donor eligibility determination (21 CFR 1271.90(a)(1) and Ref. 20), but you may consider a risk-based approach for screening or testing (Ref. 3). Allogeneic leukapheresis starting material, on the other hand, does require a donor eligibility determination, including screening and testing for relevant communicable disease agents and diseases (21 CFR part 1271, Subpart C).

We recommend that procedures used for handling the leukapheresis starting material from collection to the start of the manufacturing process are described in the IND as discussed in section V.A.2.c.ii of the GT CMC Guidance (Ref. 3). This description should include any wash steps or cryopreservation procedures. We recommend these procedures, including handling of the cells and shipment to the manufacturing site, be in place at all leukapheresis collection sites to ensure quality of the material. You should have appropriate procedures in place to ensure adequate control of the leukapheresis starting material during shipping to the manufacturing facility (e.g., temperature control), and information regarding shipping containers and temperature monitoring should be provided. Validation of the shipping process and any hold or cryopreservation steps, including assessment of leukapheresis starting material stability under the intended conditions, should be included for licensure. Once the leukapheresis starting material has been received by the manufacturing facility, subsequent manufacturing must comply with CGMP as appropriate for the stage of development (see Ref. 17, and 21 CFR 210.2).

Due to patient or donor variability, the cellular starting material can represent a major source of lot-to-lot variability in CAR T cell quality and function. The probability of manufacturing success may be increased by establishing acceptance criteria for the leukapheresis starting material used in CAR T cell manufacturing, as experience is gained throughout product development. For example, you may specify a minimum cell number, viability, and percent CD3+ cells. To aid in manufacturing failure investigations, we recommend that you test the leukapheresis starting material for microbial contamination (e.g., sterility or bioburden) prior to initiating CAR T cell manufacturing or that you retain a sample for post hoc testing in the event of a DP sterility test failure. Additional characterization of the leukapheresis starting material (e.g., for percent and absolute number of CD4+ and CD8+ T cells, NK cells, monocytes, B cells) may inform the CAR T cell manufacturing process as these characteristics may influence T cell selection and expansion and final CAR T cell quality (Refs. 21, 22, 23).

To maintain the Chain of Identity (COI), labeling and tracking of material, from collection all the way through CAR T cell administration, must be documented (21 CFR Part 1271 Subpart D). Additionally, for autologous products, we recommend labeling include at least two unique identifiers with label checks built into the batch record prior to each processing step. The COI should also be maintained at the clinical site with two independent patient and label checks at bedside. Please refer to section IV.C.3 of this guidance for considerations regarding labeling for the CAR T cell DP.

C. CAR T Cell Manufacturing and Testing

CAR T cell manufacturing is a complex process that should be developed to achieve the target product profile (Refs. 24, 25). Recommendations for the manufacture of ex vivo modified cells, including CAR T cells, are noted in the GT CMC Guidance (Ref. 3). We suggest sponsors consider the recommendations in the GT CMC Guidance (Ref. 3), as applicable, for: early product characterization (section IV.A); characterization of impurities (sections V.A.3.b.i and ii); manufacturing process development (sections V.A.2.f and V.B.2.c); and facility considerations (section V.C.1). This guidance provides

specific recommendations and additional details for CAR T cell manufacturing and testing.

1. CAR T cell manufacturing process control

Coupled with donor-to-donor variability inherent to the cellular starting material, multi-step manufacturing processes can be a source of variability. To minimize variability and promote consistency between CAR T cell lots, we recommend the manufacturing process be well-controlled. This can be achieved via the use of quality materials, in-process control of PPs, in-process testing, and testing of intermediates and the final product for CQAs, as appropriate for the phase of product development (Ref. 26).

CAR T cell manufacturing often requires specialized reagents, including selection reagents, activation reagents, antibodies, cytokines, serum, and growth factors. The safety and quality of such materials can vary widely depending on factors such as source or vendors. For example, we recommend that human or animal-derived components are not sourced from geographical areas of concern for potential viral and/or transmissible spongiform encephalopathy (TSE) agent contamination and that components be tested appropriately for adventitious agents. Lot-to-lot variability and stability of reagents can also be a potential risk to CAR T cell product quality and safety. We recommend sponsors qualify reagents for quality, safety, and identity through vendor qualification programs and incoming material qualification programs, including quarantine, Certificate of Analysis (COA) and Certificate of Origin (COO) assessment, visual inspection, and testing, as appropriate according to a risk assessment (Ref. 3).

To assure product safety, CAR T cells should be free of viable contaminating microorganisms; however, the final DP cannot be sterilized by filtration or terminally sterilized because cells need to be fully viable and functional. Therefore, manufacturing should be conducted by using qualified aseptic processing under CGMP requirements (Refs. 17, 27), and aseptic processing must be validated for licensure (21 CFR 211.113, Ref. 28). Product safety is further supported by the use of sterility testing (21 CFR 610.12) per United States Pharmacopeia (USP) Chapter 71 or an appropriately validated alternative test method per USP <1223>.

The IND should contain information documenting the ability to produce CAR T cells according to the proposed manufacturing process through the production of developmental or engineering batches. To support process development, sponsors may cross reference information from highly-related CAR T cell manufacturing (e.g., same manufacturing process but with a different CAR construct) at the same facility. Generally, starting material from a healthy donor is appropriate for manufacturing process developmental batches. However, patient-derived starting material may have intrinsic properties that affect CAR T cell manufacturing because of disease state, prior treatment, or other inherent patient characteristics. Therefore, in some cases, additional manufacturing process development using

patient-derived starting material may be recommended for autologous CAR T cells.

We do not require use of approved or cleared medical devices as equipment in CAR T cell manufacturing after collection of the leukapheresis starting material. Manufacturing equipment (such as centrifugation/washing, selection, or incubation equipment, including automated equipment) should be qualified as suitable for the intended use. This qualification is the responsibility of the IND sponsor, not the medical device or equipment manufacturer. Manufacturing equipment operating parameters should be validated to support the BLA.

If information describing reagents[5], the vector, manufacturing equipment, manufacturing process, or a manufacturing facility has already been submitted to the FDA (e.g., in another IND, investigational device exemption (IDE), or Master File (MF)), a letter from the file holder authorizing FDA to cross-reference the previous submission for CMC or other information may be submitted to support an IND. Sponsors should specify what information is cross-referenced and where the information is contained in the cross-referenced file. Any DS, DS intermediate, and DP information should be included in the BLA and should not be incorporated by reference to a MF. Specific questions regarding information that should be included in the BLA should be discussed during a pre-BLA meeting with FDA.

As experience is gained through product development, CPPs should be identified and used to establish in-process controls. Examples include:

- Using a fixed bead: cell ratio at the activation stage.
- Using a constant amount of vector per cell (e.g., a fixed multiplicity of infection for viral vectors) and a fixed duration at the gene transfer step.
- Using fixed electroporation settings.
- Monitoring cell expansion in culture and maintaining an optimal cell density by addition of media.

Appropriate in-process testing at relevant time points is vital to achieve and maintain control of the manufacturing process. In-process testing regimens for CAR T cells typically assess multiple parameters (e.g., viability, cell number, cell phenotype, CAR expression). Results from in-process tests can be used to guide manufacturing decisions at critical steps, such as when to change culture media or when the CAR T cells are ready to harvest.

[5] For the purposes of this guidance, reagents are those materials used for manufacturing (e.g., cell growth, differentiation, selection, purification, or other critical manufacturing steps) that are not intended to be part of the final product. See also section V.A.2.c.i of the GT CMC guidance (Ref. 3).

We recommend stability studies for CAR T cells be conducted to support hold and storage times as described in sections V.A.7 and V.B.8 of the GT CMC guidance (Ref. 3). In-use stability studies should be conducted to support the intended hold time between final formulation and administration for fresh products, or the time between thaw and administration for cryopreserved products. Products manufactured from healthy donor material may be sufficient to support early stability studies. However, for licensure, additional stability studies including products manufactured from patient-derived starting material should be provided to support the determination of product shelf life.

2. CAR T cell analytical testing

Analytical testing of CAR T cells is necessary to assure product identity, quality, purity, and strength[6] of the investigational product (21 CFR 312.23(a)(7)(i)). Sections V.A.4 and V.B.5 of the GT CMC Guidance (Ref. 3) provide general recommendations on analytical testing of the DS and DP. Section V.A.4.b.i of the GT CMC Guidance (Ref. 3) includes recommendations for alternatives to compendial methods (e.g., for sterility and mycoplasma). Section V.B.5.b.i of the GT CMC Guidance (Ref. 3) includes recommendations for cellular products that are administered fresh, or with limited hold time between final formulation and patient administration.

For allogeneic CAR T cells, where each product lot is meant to treat multiple patients, additional testing beyond what is described in this section may be appropriate. For example, additional adventitious agent testing, stringent acceptance criteria for the number of potentially alloreactive lymphocytes, and absence of aberrant growth should be included in lot release testing. Additional recommendations on analytical testing of CAR T cells incorporating genome editing can be found in FDA's guidance entitled "Human Gene Therapy Products Incorporating Human Genome Editing," (Ref. 15) (hereinafter referred to as the "GE Guidance").

Analytical testing for CAR T cells often requires complex assays and development of product-specific biological assays. Thus, we recommend that sponsors begin assay development in early stages of CAR T cell development and use a variety of assays to characterize their product. In general, scientifically sound principles for assay performance should be applied (i.e., tests should be specific, sensitive, and reliable and include appropriate controls or standards). We recommend compendial methods be used when appropriate, and safety-

[6] For purposes of this guidance, "strength" is the equivalent of "potency." As defined in 21 CFR 600.3(s), the word *potency* is interpreted to mean the specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result. During the IND stage, sponsors must submit data to assure the identity, quality, purity and strength (21 CFR 312.23(a)(7)(i)) as well as stability (21 CFR 312.23(a)(7)(ii)) of products used during all phases of clinical study. Biological products regulated under section 351 of the PHS Act must meet prescribed requirements of safety, purity and potency for BLA approval (42 USC 262(a)(2)(C)(i)); (21 CFR 601.2).

related tests should be qualified prior to initiation of clinical studies. Although validation of analytical procedures is usually not required for IND submissions for Phase 1 studies, we recommend providing information showing appropriate control of the test methods. Each assay used for lot release or stability testing should be qualified prior to initiating studies intended to provide primary evidence of effectiveness to support a marketing application, and assays must be validated to support a BLA[7] (21 CFR 211.165(e)).

When changing an assay, a risk assessment should be performed to determine how the assay change impacts CAR T cell evaluation. If there are major changes to assay methodology, we recommend the assay be requalified to ensure that assay performance characteristics remain acceptable. If an assay is replaced with a new assay that measures the same attribute in the same way (e.g., change to another ELISA kit vendor), the assay should be qualified, and a study may be requested to demonstrate that the new assay yields results that are equivalent to the old assay (Ref. 29). We recommend that these studies include side-by-side analysis of the old and new assays using the same test samples. If an assay is replaced with a new assay that measures an attribute in a fundamentally different way (e.g., potency assay changed from cell killing assay to cytokine release assay), the new assay should be qualified, and data to support any associated changes to assay acceptance criteria or the impact on stability should be provided.

a. Flow cytometry

Flow cytometry allows assessment of multiple CAR T cell attributes throughout the manufacturing process (e.g., cell viability, identity, purity, strength).

i. We recommend that the initial IND submission include:
- A description of the assay, including the flow cytometry antibody panel and the gating strategy used to define each cell population detected. Live/dead stain should be included in the flow cytometry panel. We recommend that information on relevant cell populations in the final product, including those not anticipated to have a therapeutic effect (e.g., residual tumor cells, if applicable), be collected.

[7] Each BLA must include a full description of the manufacturing process, including analytical procedures that demonstrate the manufactured product meets prescribed standards of identity, quality, safety, purity, and potency (21 CFR 601.2(a) and 601.2(c)). Data must be available to establish that the analytical procedures used in testing meet proper standards of accuracy, sensitivity, specificity, and reproducibility and are suitable for their intended purpose (21 CFR 211.165(e) and 211.194(a)(2)).

- A brief summary of instrument calibration and QC activities (e.g., beads used and frequency of calibration runs) to ensure accuracy of the results.
- A list of assay controls. Controls may include: single stained compensation controls for calculating compensation values; Fluorescence Minus One (FMO) controls to determine fluorescence spread and gating boundaries for minor populations; and isotype controls to identify nonspecific binding. Throughout assay development, system suitability specifications for each control should be established.

ii. As part of assay development, we recommend you establish and implement written procedures to ensure proper sample staining, acquisition and data analysis.

iii. We recommend performing antibody titration to determine the optimal antibody dilution.

iv. We recommend direct detection of the CAR to determine the percentage of CAR-positive cells. If the CAR is detected by surrogate protein expression (e.g., detection of a co-expressed gene) or other broad-specificity reagents (e.g., protein L), you should evaluate the correlation with CAR expression. Assessment of the sensitivity and specificity of the surrogate marker should be included as part of the justification for use.

v. A comprehensive validation study for lot release flow cytometry assay(s) must be conducted to support licensure.[8] (21 CFR 211.165(e)). This validation study should be conducted per International Conference on Harmonisation (ICH) Q2 (Ref. 30) and include validation of each fluorescently labeled marker in the flow cytometry panel on the flow cytometer(s) used for CAR T cell release. Robustness studies, including defining the maximum holding time for samples before staining and between staining and acquisition, should be included.

b. Vector Copy Number (VCN)

Vector integration can potentially alter expression of cellular genes and contribute to tumorigenicity (Refs. 31 and 32). Therefore, vector integration in the DP is an important safety attribute to measure for CAR T cell release. For integrating vector systems, the average number of integrations per CAR-positive cell, generally referred to as VCN, should be determined and reported on the Certificate of Analysis (COA) for each lot. Determining VCN as a function of total cells includes CAR-negative cells in the denominator and lowers the reported vector integration rate.

[8] See footnote 6.

Using the percentage of CAR-positive cells, the average VCN per CAR-positive cell can be calculated. VCN as a function of CAR-positive cells will provide a more accurate representation of the VCN in modified cells and thus a more accurate representation of product risk for insertional mutagenesis. We recommend that the manufacturing process be optimized to control VCN while meeting the target CAR-positive cell frequency.

We recommend that the VCN release criterion be justified based on a risk assessment. The risk assessment may include supporting data from studies such as insertion site analysis, clonal dominance, dose, indication, study population, etc. Supporting experimental data may be obtained from developmental and engineering manufacturing runs.

For CAR T cells manufactured without extended culture, determining the stably integrated VCN at the time of lot release testing may be difficult (e.g., due to persistence of episomal copies of non-integrated vectors). In some cases, an interim VCN assessment at the time of lot release, followed by subsequent VCN assessment(s) on cultured CAR T cells, may be needed to determine the stably integrated VCN. The appropriate duration of extended culture for the stably integrated VCN (and other release assays, as applicable) is product-specific and should be determined experimentally.

c. Identity

Identity testing is required at all phases of development (21 CFR 312.23(a)(7)) and must be performed on the contents of a final container of each filling of each lot for licensure (21 CFR 610.14). Identity testing should adequately identify a product and distinguish it from other products in the same facility. Of note, we recommend that identity testing for CAR T cells include an assay to measure the presence of the transgene (e.g., CAR expression by flow cytometry, gene detection by PCR) and an assay specific for the intended cellular composition of the final product (e.g., T cell surface markers) as discussed in section V.B.5.b.ii of the GT CMC Guidance (Ref. 3). HLA typing may be performed; however, HLA typing does not detect the genetic modification and, therefore, is not a sufficient identity test. Additionally, HLA typing will not replace requirements for maintaining chain of identity (section IV.B of this guidance).

d. Potency

The CAR T cell DP must be tested for potency (Ref. 33). Upon antigen engagement, CAR T cells kill target cells using multiple mechanisms. Therefore, the use of orthogonal methods (e.g., cell killing assay, transduction efficiency measurements, and cytokine secretion assays) may

be recommended to measure potency. Characterization of CAR T cell function during product development will support comparability studies and will allow you to determine the most appropriate assays to use for commercial lot release.

If the CAR T cells express multiple transgene elements, each transgene may contribute to product safety and efficacy and therefore should be adequately controlled. A potency assay to measure the intended biological activity of each element may be needed, depending on the contribution of each transgene to the product's activity. Justification for the proposed assays may be supported by characterization studies. For example:

- If the CAR T cell targets multiple antigens (e.g., CD19 and CD22), you should assess the activity of the CAR T cells against each individual target antigen because T cell activation upon engagement with either antigen is required for the product's function.
- If the CAR T cell includes a cytokine transgene to enhance the CAR activity, you should assess the activity of the CAR T cells against the target antigen and production of the transgenic cytokine because the cytokine is not primarily responsible for the CAR T cell activity specific to the target antigen.
- If the CAR T cell includes a transgene conferring drug resistance, you should assess drug resistance and CAR T cell activity because they have independent mechanisms of action.

3. Labeling for CAR T cells

Your IND must contain a copy of all labels and labeling to be provided to each investigator in the clinical study (21 CFR 312.23(a)(7)(iv)(d)). We recommend that you include sample or mock-up labels in Module 1 of the CTD. Please note that IND products must bear a label with the statement, "Caution: New Drug—Limited by Federal (or United States) law to investigational use" (21 CFR 312.6). We recommend that the label include the product name, manufacturer information, and, as applicable, the warnings "Do not filter" and "Do not irradiate". Labeling for licensed CAR T cells must conform to the requirements in 21 CFR Part 201 and 21 CFR Part 610 Subpart G, as well as other applicable provisions in the FD&C Act.[9]

Additional labeling is required for autologous CAR T cells. Specifically, CAR T cells manufactured from autologous starting material must be labeled "FOR AUTOLOGOUS USE ONLY" (21 CFR 1271.90(c)(1)). The label must state "NOT EVALUATED FOR INFECTIOUS SUBSTANCES," unless you have performed all otherwise applicable screening and testing under 21 CFR 1271.75,

[9] See also sections 581 and 582 of the FD&C Act (21 U.S.C. 360eee), as added by the Drug Supply Chain Security Act (DSCSA) (Title II of Public Law 113-54).

21 CFR 1271.80, and 21 CFR 1271.85 (21 CFR 1271.90(c)(2)). In addition to these requirements, it is recommended that the label include at least two unique identifiers to confirm patient identification prior to administration.

CAR T cells must also be labeled with the Biohazard legend shown in 21 CFR 1271.3(h), if the results of any screening or testing performed indicate the presence of relevant communicable disease agents and/or risk factors for or clinical evidence of relevant communicable disease agents or diseases. Labeling must also bear the statement "WARNING: Reactive test results for (name of disease agent or disease)," in the case of reactive test results (21 CFR 1271.90(c)(5)).

D. Managing Manufacturing Changes and Assessing Comparability During the CAR T Cell Product Lifecycle

We recognize there may be changes to the CAR T cell design, manufacturing process, or manufacturing facility during product development or post-approval. Changes during the CAR T cell product lifecycle, including changes to the final container, cytokines used during culture, or duration of cell expansion, may impact product quality, safety, efficacy, or stability[10]. There are some changes (e.g., changes to the CAR construct or changing from an autologous to allogeneic product) which would generally result in a new product that should be submitted in a new IND (Ref. 34).

Each change is assessed on a risk-based, case-by-case basis, and we recommend sponsors communicate with OTP (e.g., through an IND amendment requesting advice or a formal meeting request (Ref.1)) when considering such changes.[11] When planning such changes, we generally recommend sponsors consider the following:

- Substantial changes to the vector manufacturing process (e.g., changing from adherent to suspension culture) should be supported by comparability studies. Due to the essential role of the vector in CAR T cell activity, the impact of such changes should be assessed on both the vector and the CAR T cells. Studies should include side-by-side analyses of the pre- and post-change vector. Additionally, CAR T cells manufactured with pre- and post-change vector should be assessed using side-by-side analysis by using the same cellular starting material (e.g., splitting the leukapheresis starting material from the same donor).

- The complexity of comparability assessments may differ depending on the extent of the change to the vector or CAR T cell manufacturing process.

[10] During the investigational phase, some CMC changes without adequate comparability data may result in the trial being placed on clinical hold (21 CFR 312.42).

[11] See also the Draft Guidance for Industry "Manufacturing Changes and Comparability for Human Cellular and Gene Therapy Products," issued July 2023, available at: https://www.fda.gov/media/170198/download. When finalized, this guidance will represent FDA's current thinking on this topic.

For example, a small change in the volume of culture media to manufacture CAR T cells may generally be supported by cell viability and expansion data. In contrast, a more robust comparability study should be conducted for a change to the concentration or type of growth factors or supplements in the culture media.

- When the CAR T cells or vector manufacturing facility is changed, product comparability from the pre- and post-change manufacturing facilities should be assessed.

1. Change management

Prior to implementation of any change, you should conduct a risk assessment to evaluate the potential impact of the intended change on product quality and safety. Understanding the impact of the change is critical to evaluate the ability to combine clinical data generated pre- and post-change. This risk assessment should be based on empirical data generated using developmental lots not intended for administration to patients. This risk assessment should inform whether an analytical comparability study is warranted. Additionally, the stage of product development may impact whether an analytical comparability study is warranted. For changes to be implemented during early-stage development, the major consideration should be avoiding a negative impact on product safety. However, when considering changes to be made at later stages of product development, the sponsor should evaluate the impact of the change on both safety and efficacy. Depending on the type of change, assessment of product stability should also be considered. You must submit changes to the CMC information as amendments to the IND (21 CFR 312.31(a)(1)). We recommend that details of the proposed change(s), the accompanying risk assessment, and the proposed change management strategy be submitted as an amendment to the IND, prior to initiation of comparability studies or implementation of the change.

Analytical comparability of CAR T cells pre- and post-change may be assessed following the general principles described in ICH Q5E (Ref. 35). Note that the term "comparability" does not necessarily mean that pre- and post-change products are identical, but that they are highly similar and that any differences in product CQAs have no adverse impact on CAR T cell quality, safety, or efficacy (Ref. 36). A key function of demonstrating analytical comparability is to ensure that the clinical data generated pre-change continues to be relevant to the safety and efficacy of the post-change product. If there is insufficient evidence to demonstrate analytical comparability, then new nonclinical or clinical studies may be requested, potentially delaying product licensure. Before initiating analytical comparability studies and data analyses, we recommend that you discuss the study design and acceptance criteria with OTP.

In some cases, a change might alter CQAs that cannot be adequately measured in analytical assays. In such a case, analytical comparability studies will be

inadequate to evaluate comparability. Therefore, we recommend sponsors anticipate changes needed to establish a scalable and robust manufacturing process and make those changes prior to initiating clinical studies that are intended to provide primary evidence of effectiveness to support a marketing application.

Regardless of the product development stage, the IND must be updated to reflect the change in manufacturing process (a change in manufacturing process would be considered new CMC information requiring an information amendment; 21 CFR 312.31(a)). When changes are introduced during late stages of development, and there are no plans for additional clinical studies to support a BLA, the analytical comparability studies should be as comprehensive and thorough as those conducted for a licensed product. Differences in CQAs may warrant new nonclinical or clinical studies.

For a licensed product, manufacturing changes must take place within the context of existing change control procedures (21 CFR Parts 210 and 211 and (Ref. 37)). Such procedures should be designed to ensure that manufacturing changes do not affect CAR T cell quality. If changes to product release criteria are proposed, clinical data generated under an IND may be requested to support the safety and efficacy of the post-change product.

2. Comparability study design

The extent of comparability studies will depend on the change, the ability of analytical methods to detect changes in the product, and the stage of clinical development. We recommend that the comparability study design includes justification that the proposed assays are appropriate to detect potential effects of the change(s) on product safety and efficacy as appropriate for the phase of product development. Demonstrating that product manufactured with the proposed changes can meet current lot release criteria is typically insufficient to establish comparability. Comparability studies should be analyzed using appropriate statistical methods and predefined acceptance criteria based on lots shown to be safe and effective.

Early product characterization to establish CQAs facilitates the design of comparability studies. Using a variety of characterization assays throughout CAR T cell development provides a greater understanding of the product and supports the evaluation of quality attributes that may be affected by proposed manufacturing changes. For example, you may propose to change the cytokines used for CAR T cell culturing to alter the cell expansion rate. However, this change may also affect the cellular subpopulations and activation state. Therefore, a variety of product attributes, including cellular surface markers, should be monitored using reliable analytical methods, in addition to those attributes typically tested for lot release.

Some CAR T cell attributes are intrinsically linked to attributes of the cellular starting material. Due to the inherent variability of the cellular starting material for autologous CAR T cells, using historical lots to assess comparability may not be adequate. We recommend that CAR T cell comparability be assessed by side-by-side testing using the same cellular starting material, when possible. For example, leukapheresis starting material from the same donor can be split into two portions and used to manufacture product using the pre-change process with the other portion used to manufacture product by the post-change process. In some cases, comparability studies may be appropriately conducted using CAR T cells derived from healthy donors. Any potential differences between patient and healthy donor material should be considered when designing comparability studies and interpreting the results. You may consider including patient-derived starting material to supplement studies using healthy donor material if potential differences are identified.

E. Single-Site or Multisite CAR T Cell Manufacturing

1. Single-site manufacturing

CAR T cells may be manufactured at a single, centralized location. In this situation, the cellular starting material is collected (e.g., at apheresis centers for leukapheresis starting material) and shipped to a centralized manufacturing facility where the CAR T cells are manufactured. The CAR T cells are later shipped to local or distant clinical site(s) for administration. Single-site manufacturing may reduce the potential for product variability arising from differences between manufacturing facilities. However, there may be logistical concerns with cryopreservation or shipping of the cellular starting material, the final CAR T cells, and the test samples.

2. Multisite manufacturing

The same type of CAR T cells may be manufactured at several facilities. Multisite manufacturing may shorten the timeline from cellular starting material collection to administration for autologous products; however, differences between manufacturing facilities may contribute to product variability. In this case, you should demonstrate that a comparable product is manufactured at each location to support the analysis of the clinical trial results. Sponsors should also demonstrate that analytical methods are comparable across the different sites, if applicable.

As the IND sponsor, it is your responsibility to confirm that each manufacturing site is following CGMPs (21 CFR 200.10(b), 21 CFR 211.22(a), section V.2.a of the GT CMC guidance (Refs. 3 and 38)). We recommend using the same standard operating procedures (SOPs), training, reagents, and equipment across manufacturing facilities, when possible. We also recommend that the IND

describe any differences in the manufacturing process across the manufacturing sites.

Defined acceptance criteria for product quality attributes will help support production of similar products across manufacturing sites. We recommend you demonstrate analytical comparability of the products manufactured at each site by submitting data from CAR T cells manufactured using the same cellular starting material (e.g., splitting the leukapheresis starting material from the same donor). You should include a list of the methods used for testing and the predefined acceptance criteria used for determining analytical comparability. When assessing analytical comparability among multiple manufacturing facilities, we recommend that you identify a reference site to which all other sites are compared. In addition, demonstration of comparability between product lots produced at different manufacturing sites is critical if the corresponding clinical data are combined for efficacy analyses.

3. Multisite testing

Multisite manufacturing is often associated with the same assay being performed at multiple testing sites. For example, flow cytometry is often performed at the time of DS harvest and, therefore, may need to be performed at an analytical lab associated with each manufacturing facility. In this case, we recommend using an assay transfer protocol to ensure that non-compendial testing performed at each site is suitable for the intended purpose and is reproducible among all testing sites. We recommend that the same SOPs, reference materials, reagents, and equipment be used across testing facilities, when possible. When available, standard materials should be used to calibrate equipment at multiple sites to support instrument harmonization. For compendial assays, reproducibility across testing sites generally does not need to be demonstrated; however, it is important to verify that each site can perform the test as intended.

V. NONCLINICAL RECOMENDATIONS

The objective of a nonclinical program for an investigational product is to support a conclusion that it is reasonably safe to administer the product in a clinical trial. Although the diversity and inherent biological properties of GT products, including CAR T cells, necessitate a case-by-case testing strategy, general considerations for nonclinical testing have been previously communicated (Ref. 39).

A. Nonclinical Considerations for the CAR Construct

The design of the CAR construct and the process by which the transgene is delivered to the T cells are critical in determining product safety and activity. Genetic material encoding the CAR can be delivered to T cells using multiple vector types, such as gammaretroviral and lentiviral vectors, transposons, and mRNA (Ref. 10).

A major determinant of CAR T cell safety and efficacy is the antigen recognition domain used to confer target specificity. The antigen recognition domain may originate from monoclonal antibodies (mAbs), endogenous ligand/receptor pairs, or from other sources. Nonclinical evaluation of the antigen recognition domain should include assessment of the specificity and affinity/avidity for the target antigen/cells to evaluate the potential for on-target/off -tumor and off-target toxicities. Undesired targeting of healthy/normal tissues that express the intended target antigen (on-target/off-tumor), as well as unintended targeting of other antigens expressed on healthy/normal tissue is a safety concern that may be evaluated using in vitro and/or in vivo studies. Examples include: (1) tissue cross-reactivity studies using a monoclonal antibody or fusion protein with the same antigen recognition domain; (2) protein arrays; (3) cytotoxicity/cytokine release testing on panels of human primary cells, cell lines, or induced pluripotent stem cell-derived test systems for various organs/tissues; and (4) relevant animal models[12]. We recommend including information from previous clinical experience with a CAR or monoclonal antibody with an identical antigen recognition domain, if available, which may reduce or eliminate the recommendation to perform additional specificity and affinity testing. Sponsors are encouraged to explore a combination of methods to minimize the risk to study subjects and to inform the design of the clinical trial. Identification of potential on-target/off-tumor and off-target activity can be invaluable in establishing enrollment criteria and specific post-infusion assessments and monitoring plans.

Characterization of the target antigen is also recommended. Existing clinical experience with the target antigen and the tissue expression profile of the target antigen can provide supporting information regarding potential off-tumor targets of the investigational CAR T cells. However, antigen recognition domains targeting the same antigen as previous CAR T cells may have a different safety profile and present different toxicity risks. Antigen recognition domains may vary in their affinity or avidity for the target or recognize a different site on the antigen which should be evaluated nonclinically. Additionally, CAR T cells and monoclonal antibodies that utilize the same single-chain variable fragment (scFv) may differ in their safety profile due to the inherent differences between the products (e.g., capacity for CAR T cells to traffic, expand, produce cytokines, induce cytotoxicity, and persist). These differences and their impact on product safety should be considered when characterizing the safety profile of the CAR T cell product.

A variety of activation and co-stimulatory domains have been incorporated into CAR T cells, including the CD3ζ chain, 4-1BB (CD137), CD28, and CD40. These domains have been used in various combinations. Depending on the cell type, certain combinations of co-stimulatory domains can lead to different biological properties, such as unique

[12] The nonclinical program for any investigational product should be individualized with respect to scope, complexity, and overall design. We support the principles of the "3Rs," to reduce, refine, and replace animal use in testing when feasible. Proposals, with justification for any potential alternative approaches (e.g., in vitro or in silico testing), should be submitted during early communication meetings with FDA. We will consider if such an alternative method could be used in place of an animal test method.

cytokine secretion profiles. This can impact the extent of in vivo cell expansion, persistence, and activation of other immune cell types. Addressing the potential for CAR T cells to undergo stimulation-independent growth, including cytokine and antigen-independent growth, and uncontrolled proliferation is an important aspect of nonclinical evaluation. Furthermore, the capacity of CAR T cells to secrete cytokines and mediate cytolysis should be antigen-dependent, which can be tested by exposure to various cells that vary in their expression of the target antigen. The transmembrane domain and hinge regions can also impact CAR T cell safety and activity. These regions may modify the on-target activity by affecting the flexibility of the antigen recognition domain and impact off-target activation (Ref. 40). Comprehensive assessment and characterization of these product characteristics can be accomplished using in vitro and in vivo testing approaches to evaluate antigen-dependent and antigen-independent activity.

B. Nonclinical Considerations for the Cellular Component of CAR T Cells

The nature of the transduced T cells expressing the CAR can also influence the biological activity of the final investigational product. Examples of various T cell populations used to express the CARs include: (1) purified T cell subsets; (2) pools of unselected T cells containing other contaminant cells (e.g., NK cells, B cells, etc.); (3) T cells specific to viral antigens (e.g., cytomegalovirus (CMV), Epstein-Barr virus (EBV)); and (4) selected stem-like or "young" T cells. The potential for uncontrolled proliferation and toxicity may differ depending on the cell source. Thus, nonclinical evaluation may include examination of uncontrolled proliferation, in vitro and in vivo testing for T cell clonality, karyotypic analysis, TCR repertoire analysis, and specificity for viral antigens through ex vivo stimulation and recognition assays.

The T cells may also be autologous or derived from allogeneic sources. For allogeneic CAR T cells, we recommend providing data to address issues such as the potential for a graft versus host response or host rejection of the CAR T cells. Additional nonclinical testing may be requested if genome editing techniques are used to minimize alloreactivity (see section V.E of this guidance).

C. In Vivo Testing of CAR T Cells

Animal models can be useful in demonstrating proof-of-concept data for CAR T cell functionality. There are several limitations due to species specificity of the CAR T cells and the tumor target(s), xenogeneic graft versus host response, as well as the difficulties in modeling human immune responses in animals. Despite these limitations, in vivo testing in murine xenograft models (i.e., human tumor xenograft-bearing mouse models administered human CAR T cells) can provide information on the trafficking and proliferation profile of CAR T cells.

If a relevant surrogate product is available, syngeneic tumor animal models can provide information regarding the interaction of the surrogate CAR T cells with an intact host immune system and potential on-target/off-tumor toxicities. Data should be provided to support the suitability of the model, such as the binding affinity of the antigen recognition

domain for the human target versus animal target and the expression profile of the target antigen in the species being evaluated. Furthermore, characterization of CAR T cell behavior, such as target-dependent activation and proliferation, and anti-tumor responses (e.g., tumor size, animal survival) can provide supportive rationale for product testing in humans.

Due to the nature of CAR T cells, which are expected to expand in vivo to varying degrees, the selection of a starting dose level is often not determined based solely on animal studies. Previous clinical experience with similar CAR T cells can often inform the starting dose level, dose escalation plan, and dosing regimen in the study population. In vitro and in vivo studies to characterize the effector-to-target ratio, kinetics of cell expansion, and pharmacodynamic/pharmacokinetic anti-tumor responses may be informative to guide dose selection.

D. CAR T Cells with Additional Modifications

CAR T cells can include additional components in the transgene, such as suicide genes, detection/selection genes, or immunomodulatory elements. Genome editing or gene silencing techniques may also be used to modify the CAR T cells to reduce immunogenicity (e.g., for allogeneic CAR T cells) or increase activity or persistence.[13] Additional nonclinical testing may be needed for novel accessory molecules and genetic modifications to evaluate functionality of the specific elements and safety of the investigational product. For example, mixed lymphocyte reactions may be informative to evaluate the immunogenicity of products that are modified to reduce the risk of GVHD and immune responses against allogeneic products. Additional modifications that affect CAR T cell persistence may be assessed by cytokine-independent growth assays or appropriately designed in vivo studies. When suicide genes and cell markers/tags are incorporated, we recommend conducting nonclinical studies to demonstrate their function and to establish dosing of any additional drug or biological product that is critical to induce CAR T cell depletion.

The parameters that define CAR T cell safety and activity are multifactorial. Considerations include: (1) the design of the CAR construct (e.g., antigen recognition domain, signaling domains, transmembrane and hinge domains); (2) ex vivo vector delivery method; (3) T cell source; (4) manipulation processes (e.g., activation, cell selection); (5) biological activities (e.g., cytokine expression profiles, cytotoxicity, proliferation); (6) addition of novel components (e.g., suicide genes, detection/selection genes, immunomodulatory elements, genome editing or gene silencing techniques); and (7) route of administration. A combination of multiple testing strategies should be used for a comprehensive nonclinical testing program (Ref. 39). This information, along with available nonclinical and clinical data for related products, can inform clinical trial design and support the administration of investigational CAR T cells to human subjects.

[13] Sponsors may also wish to refer to the nonclinical section (section IV) of FDA's GE Guidance (Ref. 15) for additional nonclinical considerations.

VI. CLINICAL RECOMMENDATIONS

This section describes the clinical considerations for early-phase development of CAR T cells for patients with cancer (hematologic malignancies and solid tumors). A primary objective of early-phase clinical trials should be an assessment of safety. Other objectives may include determination of optimal dosage, pharmacokinetic/pharmacodynamic (PK/PD) studies, evaluation of clinical activity or efficacy, selecting an appropriate population for further clinical studies to investigate efficacy and safety, and other scientific objectives.

A. Study Population

Selection of the study population should consider the anticipated risks and potential benefits for the study subjects to ensure that the overall study benefits outweigh the risks.

1. Advanced vs. early disease stage

CAR T cells have been associated with considerable toxicities, notably cytokine-release syndrome (CRS) and neurological toxicities. In some cases, these toxicities can be life-threatening and fatal. Therefore, in defining the study population, we recommend you consider these toxicities in the context of the potential benefit, disease stage, and other available therapies.

In early-phase trials, sponsors should consider enrolling subjects with severe or advanced disease who have not had an adequate response to available medical treatment or who have no acceptable treatment options. If designed to enroll these subjects, we recommend the trial include procedures to ensure that each subject's treatment options have been adequately evaluated, and the clinical protocol describe the measures to capture the pertinent information regarding prior therapies and justification for enrollment of these subjects.

In subjects who have early-stage disease and available therapies, the unknown benefits of first-in-human (FIH) CAR T cells may not justify the risks associated with the therapy. For any study, the IND submission should provide your rationale and justification for the proposed study population, and the informed consent document must describe the reasonably foreseeable risks associated with the trial as well as alternative courses of treatment (21 CFR 50.25).

2. Tissue-agnostic approach

CAR T cells target a specific antigen (or antigens) expressed by the cancer cell regardless of cancer type. Early-phase trials that include subjects with different cancer types but share a common target antigen (e.g., tissue-agnostic approach) may face challenges in evaluating the efficacy and extent of toxicities. The disparities in underlying comorbidities of the subjects, the

impact of pre-existing tumor burden on toxicities, and differences in dose response relationship may present challenges to the objectives of an early-phase study in evaluating the toxicities and dosing. If you plan to develop a product for the treatment of more than one cancer type using a tissue-agnostic approach, you may consider an early-phase trial that assigns subjects to separate cohorts by the disease types and evaluate the dose-response relationship and severity of toxicities through parallel dose-escalations in these cohorts. We recommend your IND submission includes your rationale for the proposed study design and analysis.

3. Target identification

The anti-tumor effect of the CAR T cells depends on the binding of the CAR with the cognate antigen expressed on the cancer cell. Therefore, it is essential to enroll patients whose tumors express the antigen targeted by the CAR T cells. If a test for the target antigen is not commercially available, a companion diagnostic test may need to be developed to appropriately select subjects for the study. (Ref. 41). Refer to FDA guidances on using these tests for oncology trials, including the streamlined process for study risk determination (Ref. 42) and principles for co-development of an in vitro companion diagnostic device with a therapeutic product (Ref. 43). In these situations, we recommend the clinical protocol include a detailed description of these tests.

4. Pediatric subjects

Some CAR T cells are developed specifically for pediatric conditions. Sponsors who are developing CAR T cells to treat pediatric diseases should consider how they will incorporate the additional safeguards for pediatric subjects into clinical investigations in the overall development program. Clinical development programs for pediatric indications usually obtain initial safety and tolerability data in adults before beginning studies in children. We recognize that in some situations, it may be appropriate to initiate clinical studies of CAR T cell products in children, and in such situations, adequate justification should be provided in the clinical protocol. Title 21 CFR Part 50, Subpart D, provides the process for additional safeguards required for children in clinical investigations. In addition, see section IV.B.5 of FDA's guidance entitled "Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products; Guidance for Industry," June 2017 (Ref. 44) and the draft guidance entitled "Ethical Considerations for Clinical Investigations of Medical Products Involving Children Draft Guidance for Industry, Sponsors, and IRBs," September 2022 (Ref. 45) for additional recommendations on including pediatric subjects in cell and gene therapy trials.

B. Treatment Plan

1. Dose selection, starting dose, and dose escalation

a. Dose selection

CAR T cell dose selection is complex, necessitating several factors to be considered.

Transduction efficiency can differ from lot to lot, resulting in variation in the percentage of transduced cells. This variation can lead to substantial differences in the active cell dose administered to different subjects, even when the same total cell dose is administered. Ideally, manufacturers should work to control variability in the transduction process. However, even with a consistent manufacturing process, such variations in transduction efficiency are expected to occur. To mitigate this variability in dosing, we recommend CAR T cell dose levels be based on the number of viable transduced CAR T cells in the product, rather than the total cell number. In addition to transduction efficiency, other factors that should be considered in determining the dose include the total number of cells administered to subjects and cell viability. Sponsors can propose a flat dose or calculating dose based on weight or body surface area, with appropriate justification based on factors such as age and disease. We encourage sponsors to discuss their proposed dosing strategy with FDA.

b. Starting dose

The totality of available data should be considered when proposing the starting dose. If available, previous clinical experience with similar CAR T cells, even if for a different condition, may help to justify the clinical starting dose. However, we recommend sponsors be careful when using such an approach to extrapolate the starting dose as the in vivo behavior of CAR T cells may be different depending on the disease, antigen load, study population, and CAR constructs. The choice of pre-conditioning lymphodepletion regimen may influence CAR T cell in vivo proliferation and should be considered when selecting CAR T cell dose.

c. Dose escalation

Clinical development of CAR T cells has often included dose escalation in half-log (approximately three-fold) increments. However, the dosing increments used for dose escalation should consider nonclinical and any available clinical data regarding the risks and activity associated with the change in dose. The clinical protocol should provide specific criteria for dose escalation and de-escalation. Specifically, the clinical protocol should include a detailed definition of dose-limiting toxicities (DLTs) and

justification for exemptions of any toxicities that will not be considered as DLTs. Most CAR T cell toxicities appear related to the rapid release of large amounts of cytokines (resulting in CRS) and may be correlated to the activation status of the CAR T cells, which can be driven by the level of the tumor antigen (tumor load) in vivo. Because the tumor burden differs among subjects, a given dose that may be safe in one subject who has a low tumor burden may cause considerable toxicities at the same dose in another subject who has a higher tumor burden. Therefore, single-patient cohorts and intra-patient dose escalation are typically not suitable for FIH CAR T cell dose-escalation studies. Continual reassessment methods (CRM) may be suitable with appropriate justification.

2. Repeat dosing

CAR T cells can persist in the subject or have an extended duration of activity. In addition, lymphodepleting therapy before CAR T cell infusion is myelosuppressive, and additional lymphodepletion in the context of repeat CAR T cell dosing may pose life-threatening risks to subjects. Therefore, most CAR T cell trials use a single administration or one-time dosing regimen. If proposed, repeat dosing should be based on a preliminary understanding of the product's duration of activity and toxicity. We recommend the sponsor provide justification for, and strategies to mitigate risks of, the proposed dosing strategy.

3. Staggering

When there is no previous human experience with the proposed CAR T cells or related product, treating several subjects simultaneously may represent an unreasonable risk. To address this issue, consider staggered treatment to limit the number of subjects who might be exposed to an unanticipated risk within a cohort, followed by staggering between cohorts. We recommend that the staggering interval, either within a cohort or between cohorts: (1) be long enough to monitor for acute and subacute adverse events prior to treating additional subjects at the same dose or prior to increasing the dose in subsequent subjects; (2) consider the time course of acute and subacute adverse events that were observed in the animal studies and in previous human experience with related products; (3) consider the expected duration of product activity; and (4) be practical in the context of overall development timelines.

4. Consideration for manufacturing delay or failure

Autologous CAR T cells are manufactured separately for each subject in a trial, and this manufacturing process may take many weeks. During this period, the subject might have disease progression or deteriorating condition and no longer meet the eligibility requirements at the time of planned lymphodepletion and product administration. To mitigate this risk that the subject would become ineligible, the enrollment criteria may need to include factors that evaluate

whether the subject will still be eligible for product administration when the manufacturing process is complete. Alternatively, the trial might include separate criteria (i.e., different than the study enrollment criteria) that need to be met at the time of lymphodepletion and product administration.

In some situations, manufacturing failures can happen, leading to unavailability of products for a given subject. It is important to gain an understanding from early-phase trials of the likelihood of manufacturing failure and any subject factors that may relate to such failures (e.g., subject characteristics that might predict a poor cell harvest). This information can facilitate design of subsequent trials by suggesting subject selection criteria to reduce the chance of manufacturing failure, or by prompting the development of a treatment protocol with a formalized manufacturing failure contingency plan.

To mitigate risk to subjects from production-related (i.e., manufacturing) failures, the protocol should be designed so that the subject is not committed to receive high-risk lymphodepleting regimen until it is known that the product is available. The protocol should also clearly specify whether a new attempt for treatment will be made with another round of manufacturing and whether an untreated subject will be replaced by increasing enrollment. Failure-to-treat may be an important trial endpoint that is part of a feasibility evaluation, and there should be plans to analyze the proportion of failure-to-treat subjects to look for factors that may predict failure to administer the product and to evaluate the consequences to the subject if there is a failure-to-treat.

5. Bridging therapy

A manufacturing delay or failure may prompt the investigators to use "bridging therapy" in an attempt to ameliorate the underlying disease while the subject waits for the production of the CAR T cells. However, such bridging therapy could confound the interpretation of treatment effects from the subsequent CAR T cells because it may be difficult to ascertain whether any tumor response observed in these subjects is due to the prior bridging therapy or due to the CAR T cells or both. In addition, lack of bridging therapy standardization can further complicate the interpretation of the CAR T cell clinical trial results. To help understand the impact of any bridging therapy on the interpretation of the overall study results, we recommend that sponsors consider reassessing baseline disease before lymphodepletion and conducting separate pre-specified analyses for: (1) all subjects; (2) subjects who received prior bridging therapy; and (3) subjects who did not receive prior bridging therapy.

C. Clinical Pharmacology Considerations

Clinical pharmacology assessment for CAR T cells includes pharmacokinetic (exposure), pharmacodynamic (response) and immunogenicity studies. PK and PD assessments provide important information for determination of the safety and effectiveness of drug

products. Immunogenicity assessments evaluate potential risks posed by immune responses to CAR T cells.

1. Pharmacokinetics

CAR T cells are living drugs capable of proliferation after administration. Therefore, conventional absorption, distribution, metabolism and elimination (ADME) criteria cannot be applied to model the pharmacokinetics of CAR T cells. After administration, CAR T cells expand and persist in the human body. Samples, such as blood samples, should be collected with a specified schedule to monitor in vivo persistence and proliferation of CAR T cells. To better characterize the kinetic profiles of CAR T cells, additional PK samples may be collected from certain disease-related tissue(s). For systemic exposure, the sponsor should collect blood samples with sufficient sampling time points to derive a CAR T cell concentration-time curve. We recommend the following PK measures pertaining to CAR T cell expansion and persistence: peak exposure (Cmax); time to reach peak exposure (Tmax); partial area under the curve (pAUC); last observed measurable concentration (Clast); and time of Clast. To evaluate the association between CAR T cell expansion and clinical outcomes, CAR T cell expansion rate can be assessed and used for correlative analysis between exposure and efficacy and/or safety. To evaluate factors which may affect CAR T cell in vivo expansion and persistence, patient-related factors, product-related factors, and concomitant therapies should be considered. Patient-related factors include, but are not limited to, age, sex, levels of targeted antigen expression, and tumor burden. Product-related factors include, but are not limited to, CAR T cell composition and differentiation status.

To characterize CAR T cell in vivo kinetics, we recommend that the PK sampling schedule include sufficient time points especially during the expansion phase, which is usually around the first two weeks post-infusion. The persistence of CAR T cells may be monitored by measuring levels of transgene and CAR expression. To explore the relationship between CAR T cell exposure and response, we recommend sponsors perform, if possible, functional analysis (immunophenotyping) of CAR T cells.

2. Pharmacodynamics

Upon binding specifically to antigen-expressing cells, CAR T cells initiate signaling cascades to promote T cell activation, proliferation, acquisition of effector functions, and production of cytokines and chemokines. These events lead to elimination of target cells. CAR T cell pharmacodynamic assessment includes monitoring changes in levels of cytokines, chemokines, effectors, blood immunophenotyping, and clinical endpoints (such as tumor cell killing). We recommend that the sponsor select pharmacodynamic biomarkers based on the CAR T cell mechanism of action, target disease-specific attributes, and clinical

outcomes. The PD sampling scheme should reflect the characteristics of PD biomarkers and anticipated duration of response.

To improve the CAR T cell safety and effectiveness profile, we recommend assessing the following exploratory correlative analyses: (1) the relationship between CAR T cell final product characteristics and CAR T cell pharmacokinetic profiles; and (2) the relationship between CAR T cell exposure and responses using clinical PK and PD data.

3. Immunogenicity

An immunogenicity assessment is important due to the potential impact of immunogenicity on clinical outcomes. We recommend developing assays to detect humoral and/or cellular immune responses against the CAR T cells (CAR and co-expressed transgenes, if applicable) during product development. Both patient-related and product-related factors which may affect CAR T cell immunogenicity should be considered. Patient-related factors include genetics, age, sex, disease status, general immune status, pre-existing antibody(ies) against the CAR T cells, and concomitant medication. Product-related factors include: CAR T cell origin (autologous or allogeneic); CAR molecular structure and posttranslational modifications; co-expressed transgenes; product impurities; formulation excipients; and container closure materials.

For PK, PD, and immunogenicity sample analysis, assays may be developed and refined throughout product development. We recommend using validated bioanalytical methods for clinical studies intended to provide primary evidence of effectiveness to support a marketing application (Ref. 46).

D. Safety Evaluation and Monitoring

CAR T cell safety considerations include the risks associated with: (1) cell procurement; (2) concomitant therapy (e.g., the use of immunosuppressive lymphodepleting regimen prior to CAR T cell administration); and (3) CAR T cells.

1. Clinical monitoring

We recommend the clinical protocol include a detailed monitoring plan that is adequate to protect the safety of subjects. The elements, procedures, and schedules of the monitoring plan should be based upon available information, including nonclinical and prior clinical experience with the proposed product or related products. For a FIH product, or a product with limited prior human experience, to minimize the possibility that subjects are exposed to unacceptable toxicities, staggered enrollment should be considered (see section VI.B.3 of this guidance).

A particular concern of CAR T cell toxicity is CRS (see section VI.D.2 of this guidance). A management plan or algorithm, based on clinical signs and symptoms for administering anti-cytokine therapy (e.g., tocilizumab), should be described.

CAR constructs are engineered genes that are not naturally occurring and, therefore, contain components that are not endogenous to the recipient. When administered, these exogenous components may elicit immune responses with the potential to affect CAR T cell persistence or counteract the effect (anti-tumor activity or toxicities) of CAR T cells. We recommend that CAR-reactive immune responses be monitored. For example, some CAR T cells may include murine-derived sequences and thus may generate human anti-mouse antibody (HAMA). We encourage sponsors to describe their plan and appropriate test(s) for such monitoring, along with a management plan to address the results of such monitoring.

2. Toxicity grading

We recommend the clinical trial protocol include a toxicity grading system to inform decision-making such as dose escalation and patient management. We recommend that sponsors use the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) for grading toxicities, with specific considerations for CRS and neurotoxicity. A management algorithm for these toxicities should be described in detail.

CRS and neurologic adverse reactions are toxicities associated with CAR T cells that can be life-threatening and fatal. Thus, prompt recognition and appropriate management of CRS and neurologic toxicities are integral to clinical trial design. We recommend that sponsors consider using standardized consensus criteria[14] for grading CRS and neurologic toxicities with justification for the grading criteria chosen. Psychiatric toxicities may also be associated with CAR T cells and should be assessed, reported, and managed appropriately.

3. Dose-limiting toxicities (DLTs), stopping rules and attribution

a. DLT definition

We recommend DLTs be well defined in the clinical protocol. The definition should include CRS toxicities. The following are examples of treatment-emergent CAR T cell DLTs:

- Any Grade 4 or 5 CRS;
- Any Grade 3 CRS that does not resolve to ≤ Grade 2 within 7 days;

[14] See, e.g., Ref. 47.

- Any autoimmune toxicity ≥ Grade 3;
- Grade 3 and greater neurotoxicity;
- Grade 3 and greater allergic reactions related to the cell infusion; and
- Grade 3 and greater organ toxicity (cardiac, dermatologic, gastrointestinal, hepatic, pulmonary, or renal/genitourinary) not pre-existing and not due to the underlying malignancy and occurring within 30 days of cell infusion.

The DLT definition may vary depending on many factors, such as the underlying disease, CAR T cell characteristics, and the grading system used to characterize the toxicities. Any exception or exemption of treatment-emergent toxicities from the DLT definition should be clearly described and justified. In addition, the observation period for DLTs should be adequate to capture both acute and delayed toxicities.

b. Attribution

It is often difficult to attribute an observed treatment-emergent toxicity to a specific cause during the clinical study due to confounding factors such as the symptoms of the underlying disease, concomitant treatment, and CAR T cell therapy. Therefore, we recommend DLTs be defined independent of attribution to CAR T cells unless a clear alternative cause can be described.

c. Stopping rules

Stopping rules are criteria for halting the study based on the observed incidence of particular adverse events. The objective of study stopping rules is to limit subject exposure to risk in the event that safety concerns arise. Well-designed stopping rules may allow sponsors to assess and address risks identified as the trial proceeds, and to amend the protocol to mitigate such risks or to assure that human subjects are not exposed to unreasonable and significant risk. Examples of stopping rules for CAR T cell clinical studies may include an increase in the number or frequency of expected severe adverse events, unexpected severe adverse events (e.g., > two (2) Grade 4 CRS for a FIH CAR T product), or any death within the 30 days after CAR T cell administration.

E. CAR T Cell Persistence and Long Term Follow-up

We recommend the clinical protocol describe 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 fluids, and tissues. If an invasive procedure is used to procure the specimen, a separate informed consent is recommended to inform the trial subjects of the risks of the procedure. 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 vector, and for the activity of the CAR T cells, including gene expression or changes in biomarkers.

If death occurs during the trial, planning for postmortem studies to assess the cause of death, including CAR T cell persistence, toxicity, and activity, should be considered.

The duration of follow-up for subjects who have received CAR T cells depends on the underlying disease, persistence of the CAR T cells, and the CAR vector. Subjects should be followed for 15 years after treatment with CAR T cells containing an integrated transgene. FDA recommends that a plan be provided for follow-up, including funding, in the event the sponsor ceases to operate or decides to inactivate, transfer, or withdraw the IND before completion of the long term follow up. For additional information on long term follow-up for CAR T cells, please refer to FDA's guidance entitled "Long Term Follow-Up After Administration of Human Gene Therapy Products; Guidance for Industry," January 2020 (Ref. 10).

F. Allogeneic CAR T Cells

In addition to all of the clinical considerations discussed above, there are additional considerations for CAR T cells derived from allogeneic sources. We recommend the clinical protocol describe whether there is a plan for immunological matching of the donor and recipient, and if so, clearly describe the methods for such matching. In addition, a major concern for recipients of allogenic CAR T cells is GVHD. Clinical monitoring should include plans to collect information regarding the symptoms and signs of GVHD. A grading system used to assess GVHD (Ref. 48) and a corresponding management algorithm should be included in the clinical protocol. Furthermore, DLT and study stopping rules should incorporate GVHD.[15]

[15] FDA's GE Guidance also addresses additional clinical considerations for allogeneic CAR T cells that incorporate human genome editing (Ref. 15).

VII. REFERENCES

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* When finalized, this guidance will represent FDA's current thinking on this topic.