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

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Draft Guidance for Industry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 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 clinical study design. 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, much of the information and recommendations provided will 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, we recommend sponsors communicate with the Office of Tissues and Advanced Therapies (OTAT) 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).

The contents of this document do not have the force and effect of law and are not meant to bind the public in any way, unless specifically incorporated into a contract. This document is intended only to provide clarity to the public regarding existing requirements under the law. FDA guidance documents, including this guidance, should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word should in FDA guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

CAR T cells[2] are regulated as a gene therapy (GT) product 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 and at lot release, and in comparability and stability studies (Ref. 2). Critical process parameters (CPPs) can then be established through process qualification to ensure that manufactured batches consistently meet CQAs (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.

Preclinical 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 21of the Code of Federal Regulations 312.23(a)(8) (21 CFR 312.23(a)(8)). Preclinical 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 preclinical testing strategy should be applied using in vivo, in vitro, and in silico testing strategies, as appropriate, in conjunction with available clinical and preclinical 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, response to risk mitigation measures, dose-response relationship, differences in optimal dose based on differences in indication, and preliminary evidence of efficacy and feasibility of manufacturing. For autologous CAR T cells, early-phase studies also provide information on how long it will

[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).

take to make the product and whether bridging therapy will or will not be used as an attempt to control the active disease while subjects wait for the CAR T cell treatment. For allogeneic CAR T cells, early-phase studies can also inform 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 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).

Signaling domains initiate T cell activation. We recommend that the functionality of signaling domains be 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 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 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]

Patients who have received CAR T cells previously 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 produced 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 produced 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. Additionally, due to the risks associated with increased vector integration frequencies, CAR T cell testing should include evaluation of the vector copy number (VCN) in the final product both for the newly introduced and previously administered CAR T cells, if the previously administered CAR T cells are detectable. If an autologous CAR T cell clinical study will enroll patients who

[4] See also FDA's draft guidance entitled "Human Gene Therapy Products Incorporating Human Genome Editing: Draft Guidance for Industry," March 2022 (GE Draft Guidance) (Ref. 15). When finalized, this guidance will represent FDA's current thinking on these issues.

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. Sponsors should also consider whether any given patient would still be eligible to receive the adjunctive therapy necessary for the administration of an additional CAR T cell treatment including another non-myeloablation preparative regimen, such as chemotherapy or total body radiation, which may pose life-threatening risk of myeloablation to patients who have been previously extensively treated. We recommend sponsors discuss these considerations for product characterization, testing, dosing, and clinical study design with OTAT 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. 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, in place before initiating clinical studies, and validated prior to licensure.

IV. CMC RECOMMENDATIONS

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 life cycle approach where information may be 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, CAR T cells and vectors are to be manufactured under Good Manufacturing Practice (GMP) conditions that are 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). 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 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)). Cellular 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.

A. Vector Manufacturing and Testing

The GT CMC Guidance (Ref. 3) provides recommendations for manufacturing and testing of the vector. The vector should be well-characterized prior to initiation of clinical studies. For licensure, the vector must be manufactured according to CGMP standards (21 CFR Parts 210 and 211) and analytical assays must be validated (21 CFR 211.165(e), Ref. 18). During CAR T cell Biologics License Application (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, vector manufacture and testing, and vector stability. Vector lot release testing should include measures of safety, identity, purity, and potency. A potency assay that assesses the biological activity of the transgene may be developed in coordination with the CAR T cell potency assay. Transgene expression alone as a measure of potency may be sufficient to support early-phase IND studies; however, additional measures of biological

potency will likely be requested for clinical study(s) intended to provide primary evidence of effectiveness to support a marketing application. Additionally, we recommend vector lot release testing include assays to determine the vector concentration that can be used 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. Subsequently, T cell transduction can then be optimized to determine the amount of vector that is added per cell to achieve the target percentage of CAR-positive cells in the CAR T cell DP.

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 compromised. Additional testing may be recommended depending on the type of transgene vector being used. For example, there are additional safety concerns related to the use of retroviral-based vectors and additional testing expectations (section V.A.4.b.ii of the GT CMC Guidance (Ref. 3) and (Ref. 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

The nature of the cells used as starting material may be critical for CAR T cell quality and function. Due to patient or donor variability, the cellular starting material can represent a major source of lot-to-lot variability. 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.

We recommend that procedures used for handling the leukapheresis starting material from collection to the start of the manufacturing process are described 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 be in place at all leukapheresis collection sites to ensure quality of the process, including handling of the cells and shipment to the manufacturing site. 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.

The probability of manufacturing success may be increased by establishing acceptance criteria for the leukapheresis starting material used in CAR T cell manufacturing. For example, you may specify a minimum cell number, viability, and percent CD3+ cells. 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. 20, 21, 22).

Autologous leukapheresis starting material does not require donor eligibility determination (Ref. 23), screening or testing (21 CFR 1271.90(a)(1)). Allogeneic leukapheresis starting material, on the other hand, does require donor eligibility determination and screening and testing for relevant communicable disease agents under 21 CFR Part 1271, Subpart C. 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).

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, 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 tailored to achieve the target product profile (Refs. 24, 25). Recommendations for the manufacture of ex vivo modified cells, which would include 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 CPPs, in-process testing, and testing of intermediates and the final product for CQAs (Ref. 26).

CAR T cell manufacturing often requires specialized ancillary materials, 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 problematic. We recommend sponsors qualify ancillary materials for quality, safety, and potency 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.

To assure product safety, CAR T cells should be free of viable contaminating microorganisms; however, the final DP cannot be terminally sterilized as cells need to be fully viable and functional. Therefore, manufacturing should be conducted by using validated aseptic processing under current good manufacturing practice (CGMP) conditions (Ref. 27). 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 qualified and validated test method.

The IND should contain information demonstrating 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, when using patient-derived starting material, additional manufacturing process development 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 cellular starting material. The suitability of manufacturing equipment (such as centrifugation/washing, selection, or incubation equipment, including automated equipment) should be qualified by assessing the CQAs of the product under the chosen mode of operation and specific equipment settings. 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 ancillary materials[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.

Throughout 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 to determine when the CAR T cells are ready to harvest.

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). Please note that if you plan to administer fresh CAR T cells, we recommend providing stability information for the intended hold time between final formulation and administration. Products manufactured from healthy donor material may not accurately represent the stability profile for autologous CAR T cells; therefore, we recommend that products manufactured from patient material be included in stability studies.

[5] For the purposes of this guidance, ancillary materials 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).

2. CAR T cell analytical testing

Analytical testing of CAR T cells is necessary to assure product safety, identity, quality, purity, and strength (including potency) 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 alternative methods to compendial assays. 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.

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. Validation of analytical procedures is usually not required for IND submissions for Phase 1 studies; however, we recommend providing information that demonstrates appropriate control of the test methods. In general, scientifically sound principles for assay performance should be applied (i.e., tests should be specific, sensitive, and reproducible and include appropriate controls or standards). We recommend compendial methods be used when appropriate, and safety-related tests should be qualified prior to initiation of clinical studies. Each assay 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.[6] (21 CFR 211.165(e)).

When changing an assay, a risk assessment should be performed to determine how the assay change impacts evaluation of the CAR T cells. 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. We recommend that these studies include 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

[6] 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)).

be qualified, and justification for any associated changes to assay acceptance criteria 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). The flow cytometry assays used during development should be scientifically sound and provide results that are reliable and reproducible.

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.
- Information regarding instrument calibration and QC 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 the nonspecific binding. Throughout assay development, system suitability criteria for each control should be identified.

ii. As part of assay development, we recommend you establish and implement written procedures to ensure proper sample staining, acquisition and data analysis. Additionally, we recommend performing antibody titration to determine the optimal antibody dilution.

iii. 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 demonstrate a correlation with CAR expression. Demonstration of the sensitivity and specificity of the surrogate marker should be included as part of the justification for use.

iv. A comprehensive validation study for lot release flow cytometry assay(s) must be conducted to support licensure.[7] (21 CFR 211.165(e)). This validation study should be conducted per International Conference on Harmonisation (ICH) Q2 (Ref. 28) 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. Training records for all users who performed the validation studies should be available.

b. Vector Copy Number (VCN)

Transgene integration can potentially alter expression of cellular genes and contribute to tumorigenicity (Refs. 29, 30). Therefore, transgene integration in the DP is an important safety parameter to measure for CAR T cell release. If the vector system directs transgene integration, 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 non-transduced cells in the denominator and lowers the reported vector integration rate. Using the percentage of CAR-positive cells, the average VCN per CAR-expressing cell can be calculated. VCN as a function of CAR-expressing cells will provide a more accurate representation of the VCN in transduced cells and thus a more accurate representation of product risk for insertional mutagenesis. We recommend that the transduction process be optimized to control VCN while meeting target transduction frequency.

We recommend that the VCN release criterion be determined through experience and 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 multiple engineering manufacturing runs.

In some cases, such as 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 this case, an interim VCN assessment at the time of lot release, followed by subsequent VCN assessment(s) on

[7] *See* footnote 6.

cultured CAR T cells, may be needed to determine the stably integrated VCN.

c. Identity

Identity testing is required at all phases of development (21 CFR 312.23(a)(7)) and must be performed on the final labeled product 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 cellular composition of the final product (e.g., cell surface markers) as discussed in section V.B.5.b.ii of the GT CMC Guidance (Ref. 3). HLA typing may be performed for autologous CAR T cells; 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

Both the vector and the CAR T cell DP must be tested for potency[8] (Ref. 31). Upon antigen engagement, CAR T cells kill target cells using multiple mechanisms. Therefore, a matrix approach may be recommended to measure potency (e.g., cell killing assay, transduction efficiency measure, and cytokine secretion assays). We recommend using orthogonal methods to characterize CAR T cell function during product development. This approach will support comparability studies and will allow you to determine the best matrix of assays to use for commercial lot release.

If the CAR T cells express multiple transgene elements, there should be a potency assay to measure activity of each functional element. For example, if the CAR T cell includes a cytokine transgene in addition to the CAR, you should develop a potency assay to assess the activity of that cytokine, in addition to the potency assay(s) to assess CAR activity.

[8] For purposes of this guidance, "strength" is the equivalent of "potency." 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 (21 CFR 601.2).

577 3. Labeling for CAR T cells
578
579 Your IND must contain a copy of all labels and labeling to be provided to each
580 investigator in the clinical study (21 CFR 312.23(a)(7)(iv)(d)). We recommend
581 that you include sample or mock-up labels in Module 1 of the CTD. Please note
582 that IND products must bear a label with the statement, "Caution: New
583 Drug—Limited by Federal (or United States) law to investigational use" (21 CFR
584 312.6). We recommend that the label include the product name, manufacturer
585 information, and the warnings "Do not filter" and "Do not irradiate". Labeling
586 for licensed CAR T cells must conform to the requirements in 21 CFR Part 201
587 and 21 CFR Part 610 Subpart G, as well as other applicable provisions in the
588 FD&C Act.[9]
589
590 Additional labeling is critical for autologous CAR T cells. CAR T cells
591 manufactured from autologous starting material must be labeled "FOR
592 AUTOLOGOUS USE ONLY" (21 CFR 1271.90(c)(1)). The label should also
593 include at least two unique identifiers to confirm patient identification prior to
594 administration.
595
596 Depending on the donor testing and screening performed for autologous starting
597 materials (see section IV.B of this guidance), the label for autologous CAR T
598 cells must state "NOT EVALUATED FOR INFECTIOUS SUBSTANCES,"
599 unless you have performed all otherwise applicable screening and testing under
600 21 CFR 1271.75, 21 CFR 1271.80, and 21 CFR 1271.85 (21 CFR 1271.90(c)(2)).
601 CAR T cells must also be labeled with the Biohazard legend shown in 21 CFR
602 1271.3(h), if the results of any screening or testing performed indicate the
603 presence of relevant communicable disease agents and/or risk factors for or
604 clinical evidence of relevant communicable disease agents or diseases. Labeling
605 must also bear the statement "WARNING: Reactive test results for (name of
606 disease agent or disease)," in the case of reactive test results (21 CFR
607 1271.90(c)(5)).
608
609 **D. Managing Manufacturing Changes and Assessing Comparability During the**
610 **CAR T Cell Product Life Cycle**
611
612 We recognize there may be changes to the CAR T cell design, manufacturing process, or
613 manufacturing facility during product development or post-approval. Changes during the
614 CAR T cell product lifecycle, including changes to the final container, cytokines used
615 during culture, or duration of cell expansion, may impact product quality, safety,
616 efficacy, or stability[10]. There are some changes (e.g., changes to the CAR construct or

[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).

[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).

changing from an autologous to allogeneic product) which would generally result in a new product that should be submitted in a new IND.

Each change is assessed on a case-by-case basis, and we recommend sponsors communicate with OTAT (e.g., through an IND amendment requesting advice or a formal meeting request (Ref.1)) while considering such changes. 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 CAR T cell manufacturing process. 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, comparability between manufacturing facilities should be established to ensure that the properties of the investigational product are not altered in a manner that would prohibit using preclinical data to support the clinical study or combining the clinical data resulting from the product produced at each manufacturing facility.

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. 32). 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. 33). 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 OTAT.

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 chemistry 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. 34)). 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.

707
708 2. Comparability study design
709
710 We recommend that the comparability study design includes justification that the
711 proposed assays are appropriate to detect potential effects of the change(s) on
712 product safety and efficacy. Demonstrating that product manufactured with the
713 proposed changes can meet current lot release criteria is typically insufficient to
714 establish comparability. Comparability studies should be analyzed using
715 appropriate statistical methods and predefined acceptance criteria based on lots
716 shown to be safe and effective.
717
718 Early product characterization to establish CQAs facilitates the design of
719 comparability studies. Using a variety of characterization assays throughout CAR
720 T cell development provides a greater understanding of the product and supports
721 the evaluation of quality attributes that may be affected by proposed
722 manufacturing changes. For example, you may propose to change the cytokines
723 used for CAR T cell culturing to alter the cell expansion rate. However, this
724 change may also affect the cellular subpopulations and activation state.
725 Therefore, a variety of product attributes, including cellular surface markers,
726 should be monitored using reliable analytical methods, in addition to those
727 attributes typically tested for lot release.
728
729 Some CAR T cell attributes are intrinsically linked to attributes of the cellular
730 starting material. Due to the inherent variability of the cellular starting material
731 for autologous CAR T cells, using historical lots to assess comparability may not
732 be adequate. We recommend that CAR T cell comparability be assessed by
733 side-by-side testing using the same cellular starting material, when possible. For
734 example, leukapheresis starting material from the same donor can be split into
735 two portions and used to manufacture product using the pre-change process with
736 the other portion used to manufacture product by the post-change process. In
737 some cases, comparability studies may be appropriately conducted using CAR T
738 cells derived from healthy donors. However, if product manufactured from
739 healthy donors is not adequate to assess product comparability for autologous
740 CAR T cells, the comparability study should include evaluation of CAR T cells
741 manufactured from patient cellular starting material.
742
743 **E. Single-Site or Multisite Manufacturing**
744
745 1. Single-site manufacturing
746
747 CAR T cells may be manufactured at a single, centralized location. In this
748 situation, the cellular starting material is collected (e.g., at apheresis centers for
749 leukapheresis starting material) and shipped to a centralized manufacturing
750 facility where the CAR T cells are manufactured. The CAR T cells are later
751 shipped to local or distant clinical site(s) for administration. Single-site

manufacturing may reduce the potential for product variability arising from differences between 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 (Ref. 3), and (Ref. 35)). 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 submit data, ideally from qualification runs using the same cellular starting material, performed at each site to demonstrate analytical comparability of the products manufactured at each site, including 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 sites are compared. In addition, demonstration of comparability between products produced at different manufacturing sites is critical if the corresponding clinical data are combined for efficacy analyses, as discussed above.

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, 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. PRECLINICAL RECOMENDATIONS

A. General Preclinical Considerations for Cell and Gene Therapies

The objective of a preclinical 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 preclinical testing have been previously communicated (Ref. 36).

B. Preclinical Considerations for the Vector Component of CAR T Cells

The design of the CAR vector 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 has been delivered to T cells using multiple vector types, including gammaretroviral and lentiviral vectors, transposons, and naked 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. Preclinical evaluation of the antigen recognition domain should include assessment of the specificity and affinity for the target antigen to evaluate the potential for on-target/off-tumor and off-target toxicities. Undesired targeting of healthy/normal tissue 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 both in vitro and in vivo studies. Examples include: (1) tissue cross-reactivity studies using a monoclonal antibody or fusion protein with the same antigen recognition domain; (2) cytotoxicity testing on panels of human primary cells, cell lines, induced pluripotent stem cell-derived test systems, etc., for various organs/tissues; (3) protein arrays; and (4) relevant animal models[11]. We recommend including information from previous clinical experience with a CAR or monoclonal

[11] The preclinical 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.

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 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 if the recognition domains are not identical. Different antigen recognition domains may vary in their affinity for the target or recognize a different site on the antigen which should be evaluated preclinically. 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).

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 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 cytokine-independent growth and uncontrolled proliferation is an important aspect of preclinical evaluation. Furthermore, capacity of CAR T cells to secrete cytokines and mediate cytolysis should be restricted in an antigen-dependent manner, 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. 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.

C. Preclinical Considerations for the Cellular Component of CAR T Cells

The nature of the transduced 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, preclinical evaluation may include

examination of cytokine-independent cell growth, in vitro and in vivo testing for T cell clonality, karyotypic analysis, TCR repertoire analysis, 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 (e.g., mixed lymphocyte reactions, HLA typing). Additional preclinical testing may be requested if genome editing techniques are used to minimize alloreactivity (see section V.E of this guidance).

D. 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.

E. 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. Gene 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. Additional preclinical 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 are incorporated, we recommend conducting preclinical studies to demonstrate their function and to establish dosing of any additional drug or biologic that is critical to induce CAR T cell depletion.[12]

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

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.

[12] Sponsors may also wish to refer to the preclinical section (section IV) of FDA's GE Draft Guidance (Ref. 15) for additional preclinical considerations. When finalized, this guidance will represent FDA's current thinking on these issues.

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.

However, 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 risks associated with the trial (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. Unless the antigen is expressed in nearly all tumor cells, such as CD19 expression in B cell malignancies, and can be detected by commercially available, marketed tests, such tests to detect an antigen will generally be considered a companion diagnostic test (Ref. 37). Refer to FDA guidances on using these tests for oncology trials, including the streamlined process for study risk determination (Ref. 38) and principles for co-development of an in vitro companion diagnostic device with a therapeutic product (Ref. 37). In these situations, we recommend the clinical protocol includes 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. 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. 39) 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 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. In our experience, the safety and effectiveness of CAR T cells are strongly influenced by body weight (or body surface area (BSA)); therefore, we recommend calculating the cell dose based on weight or BSA rather than using a flat dose.

b. Starting dose

If animal or in vitro data are available, there might be sufficient information to determine if a specific starting dose has an acceptable level of risk. If available, previous clinical experience with CAR T cells, even if for a different condition, may also 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, intra-patient dose escalation, and continual reassessment methods (CRM) are typically not suitable for FIH CAR T cell dose-escalation studies.

2. Repeat dosing

CAR T cells can persist in the subject or have an extended duration of activity. Consequently, repeated dosing might be unnecessary or not be an acceptable risk until there is a preliminary understanding of the product's duration of activity and toxicity. In addition, lymphodepleting therapy before CAR T cell infusion is myelosuppressive, and additional lymphodepletion in the context of repeat or split CAR T cell dosing may pose life-threatening risk of myeloablation to subjects. Therefore, most CAR T cell trials use a single administration or one-time dosing regimen. We recommend the sponsor provide justification for, and strategies to mitigate risks of, any repeat or split dosing.

3. Staggering

When there is no previous human experience with the specific 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 product administration. To mitigate this risk that the subject would become ineligible, the enrollment criteria may need to include factors that improve the likelihood that 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 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 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 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. Although sponsors should optimize the manufacturing process to avoid a delay in administering the CAR T cells, there may be situations where a bridging therapy is given. To help understand the impact of any bridging therapy on the interpretation of the overall study results, we recommend that sponsors consider 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 and bone marrow samples, should be collected with a specified schedule to monitor in vivo persistence and proliferation of CAR T cells. 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 concentration (Clast); time of Clast; and terminal half-life (t1/2). Partial exposure (pAUC) can be 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, both patient-related and product-related factors 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) and clonality analysis 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 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. 40).

D. Safety Evaluation and Monitoring

CAR T cell safety considerations include the risks associated with: (1) cell procurement in an autologous setting; (2) concomitant therapy (e.g., the use of immunosuppressive nonmyeloablative 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 plan should be described to monitor cytokine levels in patients who have received CAR T cells at baseline and pre-specified time points to capture the dynamics of the cytokine release. Methods for measuring the cytokines should be provided. A management plan or algorithm, based on the cytokine level as an adjunct to the clinical decision 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 re-infused 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. A management algorithm for these toxicities should be described in detail.

CRS and neuropsychiatric adverse reactions are major toxicities associated with CAR T cells. These reactions can be life-threatening and fatal. Thus, prompt recognition and appropriate management of CRS are integral to clinical trial design. We recommend that sponsors consider using consensus criteria for grading CRS and neurologic toxicities or provide justifications for the grading criteria chosen.

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 CAR T cell DLTs:

- Any treatment-emergent Grade 4 or 5 CRS;
- Any treatment-emergent Grade 3 CRS that does not resolve to ≤ Grade 2 within 7 days;
- Any treatment-emergent autoimmune toxicity ≥ Grade 3;
- Grade 3 and greater allergic reactions related to the cell infusion; and
- Grade 3 and greater organ toxicity (cardiac, dermatologic, gastrointestinal, hepatic, pulmonary, renal/genitourinary, or neurologic) not pre-existing or 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 and CAR T cell characteristics. 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.

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., > 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 the 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 CAR T cell persistence 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. 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. 41) and a corresponding management algorithm should be included in the clinical protocol. Furthermore, DLT and study stopping rules should incorporate GVHD.[13]

[13] FDA's GE Draft Guidance also addresses additional clinical considerations for allogeneic CART T cells that incorporate human genome editing (Ref. 15). When finalized, this guidance will represent FDA's current thinking on these issues.

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