June 14th, 2022

Dockets Management Staff (HFA-305)
Food and Drug Administration
5630 Fishers Lane, Rm. 1061
Rockville, MD 20852

Re: Docket No. FDA-2021-D-0404: Considerations for the Development of Chimeric Antigen Receptor (CAR) T Cell Products; Draft Guidance for Industry

Dear Sir/Madam:

The Biotechnology Innovation Organization (BIO) thanks the Food and Drug Administration (FDA or Agency) for the opportunity to submit comments regarding the *Considerations for the Development of Chimeric Antigen Receptor (CAR) T Cell Products; Draft Guidance for Industry* (Draft Guidance or Guidance).

BIO is the world's largest trade association representing biotechnology companies, academic institutions, state biotechnology centers and related organizations across the United States and in more than 30 other nations. BIO's members develop medical products and technologies to treat patients afflicted with serious diseases, to delay the onset of these diseases, or to prevent them in the first place.

BIO thanks the Agency for release of this Draft Guidance which provides its current thinking on important issues related to the development of (CAR) T Cell products. We appreciate FDA's statement that recommendations in the guidance will be applicable to other genetically modified lymphocytes. However, as the title of the guidance is limited to (CAR) T cells and there is little mention of therapies beyond (CAR) T, we request that FDA clarify where principles in the guidance would or would not apply more broadly to other applications. We would suggest that FDA expressly expand the scope of this guidance and note more specifically where different cell types may raise unique scientific considerations.

We also recommend adding an Appendix of Abbreviations and Acronyms at the end of the guidance, similar to the Appendix provided in the *Human Gene Therapy Products Incorporating Human Genome Editing* Draft Guidance.

In the letter that follows, BIO provides high level policy recommendations and comments. We also provide detailed, specific comments in the chart that follows this letter.

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I. General Considerations for (CAR) T Cell Design and Development

Cellular Starting Material

The guidance states that starting material from patients who have previously received (CAR) T may differ from the starting material from patients who have not, leading to unexpected effects on manufacturing, potency, in vivo expansion, safety, and efficacy. FDA therefore recommends evaluation of previously administered (CAR) T cell levels in the starting material. But without product-specific assays available to evaluate (CAR) T cell levels from previous therapy, this may be a difficult task. Further, BIO notes that evaluation of previously administered (CAR) T products can be challenging because the associated vector and its specific sequence may not be known or available. There may also be considerations associated with previous treatment with other cell therapies other than (CAR) T.

We believe that FDA should acknowledge these challenges and discuss this potential barrier to development and how sponsors might address the issue. BIO requests that FDA provide clarification on the number and types of evaluations which may be appropriate -- there may be more than one (CAR), each requiring a separate assay. We ask that FDA consider a risk-based framework upon which we can base the need for these assays that is sufficiently flexible and allows for consideration on a case-by-case basis. We also note that such requirements could unintentionally introduce barriers to recruitment of patients if patients must be cell-therapy naïve. In future this may become increasing difficult and seriously limit the eligible patient population.

II. Preclinical Recommendations

Preclinical Safety Assays

The draft guidance discusses the need for comprehensive preclinical assessment of both the vector and cellular components of (CAR) T cells, and evaluation of (CAR) T cells with additional modifications. We recommend that FDA further describe how comprehensive assessments provide the totality of evidence through the use of multiple assays and orthogonal methods. We further request that FDA clarify that of the types of assays used for preclinical assessment are determined on a case-by-case basis.

Certain assays may not be appropriate or informative for all settings. For example, the cytokine independent assay is a potential means of determining the risk of transformation of the (CAR) T cells based on the premise that cytokine independent proliferation is indicative of undesired T cell population. However, there are examples in the literature of transformed T cells (for example, transfected with viral oncoprotein) and adult T-cell leukemia cell lines that do not proliferate in cytokine independent assays. These examples suggest that the cytokine

independent assay is a blunt tool at best and in the preclinical setting is unlikely to fully characterize the risk for transformation under all conditions.

We recommend a wholistic weight-of-evidence (WOE) approach to predicting risk for transformation that takes into account the totality of the preclinical data including integration site analyses, starting cell genomic status, etc., rather than indexing on a single assay. We encourage the agency to consider expanding the language in the guidance to take into account this broader perspective to determining risk for transformation for patients.

III. Clinical Recommendations

Role of Early Clinical Trials

The initial discussion of clinical development in the guidance explains that "well-designed" early-phase clinical trials of CAR-T and other cell therapies "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." (Lines 70-71). We are concerned that, as drafted, the guidance expects too much of these early trials given the complexity and novelty of these C&GT products and often small patient populations (e.g., dosing is challenging based on wide manufacturing ranges resulting from starting materials). For example, we would not expect early phase clinical trials would, in and of themselves, *establish* "the safety of the product" or "differences in optimal dose based on differences in indication." Early phase clinical trials are an important tool in developing information on a range of key aspects of dosing, safety, risk mitigation, and efficacy, but these early studies would not be expected to resolve those issues.

We expect that FDA does not intend that early phase trials fully resolve these complicated issues; therefore, we recommend that the agency clarify the language to acknowledge the limited utility of early phase trials and instead focus on the way that well-designed early phase trials allow further characterization of the product throughout development. For example, Lines 70-71 might be revised as follows - "Well designed early-phase clinical studies are critical to begin to establish, where possible: safety of the product, effectiveness of safety risk mitigation measures, any relationship between dose and response, and any differences in dosing where different indications are being tested."

Dose Selection

BIO recognizes the complexities of (CAR) T cell dosing and appreciates that this guidance includes recommendations on dose selection, starting dose, and dose escalation. We agree that variability in the transduction process should be minimized to the extent possible.

We note that when release testing that determines what dose is in the container (cell count, viability, (CAR) expression) is performed on a frozen drug product, it is difficult to ensure the right dose is in the container. The Guidance does not specify if the release test can be performed before the product is filled and frozen so that the intended dose can be filled. BIO requests that FDA clarify if the release testing that determines what dose is in the container (cell count, viability, (CAR) expression) can be performed before the product is filled and frozen so that the intended dose can be filled.

We recommend defining (CAR) T cell dose in terms of (CAR) positive viable T cells, taking into account both transduction efficiency and viability. While we agree to the principle underlying dose levels based on number of transduced cells, we caution that as (CAR) T products become more complex, with potentially multiple (CAR)s per cell and/or other knock-ins/knock outs, it may not always be clear if dosing should be based on a fully transgenic product, or just the elements that dominate/drive the response. In such cases, dose levels may need to be based on factors other than the number of transduced (CAR) T cells in the product.

In the guidance, FDA states that "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." In contrast, the experience of BIO members does not suggest that body weight or body surface area dosing is always appropriate. In fact, body weight and body surface area may be one of many confounding factors on PK, efficacy, or safety. Highlighting only these two factors may lead to bias or false correlation. Factors that may contribute to this observation is that the number of active cells in the (CAR) T product can vary depending on the manufacturing process and resulting (CAR) T cell levels that vary from product to product. Also, the efficacy and safety of the drug may depend on the target antigen expression and tumor burden which may further contribute to absence of relation between dose and body weight.

In our experience, in adult patients, we have not observed that safety and efficacy are necessarily impacted by BW or BSA. However, in pediatric patients, based on clinical experience, body weight-based dose is more commonly applied due to safety concerns. We recommend that the agency consider revising this language as suggested and adding references to published data that support the correlation between body weight and safety/efficacy. Additionally, if there are relevant data in the public domain where the safety and efficacy of (CAR) Ts were influenced by body weight or BSA, it would be helpful to reference them in the guidance.

Further, we emphasize that manufacturing processes may not always reasonably allow for tuning of dosing to weight or BSA. There are some therapies where weight-adjusted dose is not strongly associated with the area under the curve (AUC) of the transgenic cells after infusion. In these cases, body weight-based dosing would have negligible impact on safety or efficacy

absent an association with exposure. It appears likely that other product or patient factors drive responses in such cases. Given these findings, for some therapies, especially more homogeneous products such as allogeneic (CAR) T, it may therefore be too soon to generalize that dose or body weight dosing might be a key driver of safety and effectiveness. We therefore request the Agency give greater consideration to these potential manufacturing challenges, acknowledge the limitations of body weight-based dosing, and provide further guidance on the appropriate use of other modes of dose calculation.

IV. Considerations for Future Guidance

While outside the scope of this Guidance, BIO believes there are a number of related topics that should be considered for future guidances. These include:

- Expectations regarding capacity studies throughout the lifecycle of the product, especially as it relates to approved products. The scope of these studies, the frequency of the studies, and the regulatory pathways are not clear and can be difficult to navigate while ensuring patient demand is met.
- Guidance on PPQ strategy and validation, including shipping qualification expectations (e.g., the appropriateness of using surrogate material for hold studies and the expectation for product quality assessment of shipments of frozen material
- Devices and combination products. For example, we request that the Agency be explicitly clear that bags used for cryopreservation area containers and not devices (and thus do not need to be filed as a combination product).
- Best practices for sponsors to address import/export hurdles relating to both apheresed starting material and final drug product

BIO appreciates this opportunity to submit comments regarding the Draft Guidance *Considerations for the Development of Chimeric Antigen Receptor (CAR) T Cell Products*. We would be pleased to provide further input or clarification of our comments, as needed and we look forward to future opportunities to collaborate with the Agency on this important topic.

Sincerely,

/s/
Katherine Donigan, Ph.D.
Senior Director, Science and Regulatory Affairs
Biotechnology Innovation Organization

SPECIFIC COMMENTS

SECTION	ISSUE	PROPOSED CHANGE
I. INTRODUCTION		
II. BACKGROUND		
Lines 65- 68	Clarification on the role of in vitro and in vivo testing in development. The term "related products" should be further delineated.	BIO suggests the following edit: A case-by-case preclinical testing strategy should be applied using in vivo and/ or, in vitro, and in silico testing strategies, as appropriate, in conjunction with available clinical and preclinical data from related ~~products~~ CAR T cells with the same manufacturing process but with a different (CAR) construct to support use of (CAR) T cells in a proposed clinical trial
Lines 75-77	Role of GVHD for autologous cells.	The guidance suggests that GVHD is only a factor for allogeneic cells. BIO would welcome clarification as to whether FDA would consider this issue relevant for autologous products.
III. GENERAL CONSIDERATIONS FOR (CAR) T CELL DESIGN AND DEVELOPMENT		
Line 81	It would be helpful for the Agency to address process/considerations for a product that does not meet release specifications and exceptional release. This includes the agency's expectations on what data package is typically needed to change a product release specification for autologous (CAR) Ts in the commercial setting. If clinical data is needed, what is the appropriate scope of evidence needed (i.e., EAP, clinical trial, number of subjects, follow-up).	In the "General Considerations" section, consider adding a section on the process/considerations for a product that does not meet specifications and exceptional release.
A. (CAR) Construct		
Lines 94-95	"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."	BIO requests that FDA clarify if the domains refer to "antigen recognition domains" or "signaling domains", or both.

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B. Vector		
Lines 103-104	Functionality of signaling domains may be selected based on previous nonclinical/clinical experience	BIO recommends the following edit: "We recommend that functionality of signaling domains be well supported including information from previous nonclinical and clinical experience or thoroughly demonstrated, as described in section V.B of this guidance"
Lines 105-107	The language of contribution of effect is reminiscent of the requirement for combination drugs or biologics, so we recommend clarification that the signaling elements are part of the product and do not require clinical testing.	BIO recommends the following edit: "For example, the contribution of transmembrane domain, hinge, and linker regions used to separate different functional regions of the construct should be evaluated in preclinical studies, as these may affect (CAR) T cell specificity and activity (Refs. 7, 8, 9)."
Lines 117-118	"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)."	As more safety-related data becomes available based on real-time clinical experience, we recommend that FDA remains flexible in its definition of "long term follow up".
Lines 130-131	"We recommend sponsors provide justification and relevant data to support incorporation of additional elements."	BIO requests that FDA please provide examples of "relevant data" and "additional elements" required at each phase of development including in vivo and in vitro information where relevant. BIO also requests that FDA include recommendations on associated characterization/release testing for additional elements. BIO suggests that the justification should include an assessment of any impact that these additional elements will have on (CAR) T cell specificity, functionality, immunogenicity, or safety and describe characterization and testing of these elements (see sections IV.C.2.d and V.E of this guidance).

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C. Cellular Starting Material		
Line 139	Cellular starting materials	We suggest the agency adds a paragraph to this section to acknowledge the possibility of using fresh or frozen apheresis material for autologous products (as is described in the Final Product section starting on Line 171).
Lines 155 - 159	Characterization studies may be required to assess tumorgenicity of the final product derived from patients who have received (CAR) T cells previously.	BIO requests that FDA please clarify if IL-2 independent growth evaluation is also expected for (CAR)-T products derived from previously treated patients. Additionally, it would be helpful for the guidance to discuss how a sponsor can design an assay to detect VCN if the previous CAR-T was made by a different manufacturer and has an unknown/proprietary sequence unknown to the secondary sponsor.
Lines 160-162	The potential differences in the (CAR) T cells should be evaluated and considered in the clinical study design, and the cumulative VCN target be determined through experience and justified based on a risk assessment (see section IV.C.2.b of this guidance).	BIO requests that FDA please provide recommendations on cumulative VCN limits in the final product.
D. Fresh or Cryopreserved Final Products		
Lines 177-184	For fresh products, the need to define the maximum time to infusion and the importance of addressing testing/release logistics are noted, but the potential need for lot release considerations prior to full product specification testing is not addressed. There is some coverage of this in the main GT CMC guidance (Ref. 3), but identifying the issue in this guidance would be valuable.	BIO recommends the following sentences to help address this issue: "For products with shelf life shorter than the time required for full end product testing or the full evaluation of manufacturing deviations, a two-stage batch release may be appropriate. The specification should address requirements for release for infusion, including use of in-process data (Stage 1), and final batch release (Stage 2). (Ref. 3). Procedures should cover the minimum evaluation of deviations prior to release for infusion

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		and describe actions to be taken if out-of-specification data are obtained after batch infusion."</u>
Lines 186-194	Cryopreserved products also have limited shelf-life upon thawing; however this section reads as though in-use stability is only a concern for products which are not cryopreserved.	BIO recommends revision of text in line 189-191 as follows: "For cryopreserved (CAR) T cells, the risks associated with infusion of the cryoprotectant should be assessed <u>and we recommend that the maximum time between thawing and infusion be defined and supported by stability studies</u>. Controlled thawing of the product at the clinical site may be critical to maintain product quality." We would suggest that additional language at line 412 be considered to provide valuable guidance on the appropriate stability testing for cryopreserved product. For example, "<u>Similarly, provision of stability information for the intended hold time between thawing and administration is recommended for cryopreserved products</u>."
IV. CMC RECOMMENDATIONS		
Lines 193-194	Unclear of only description of shipping study is required for IND. Need additional information on the extent of data required.	BIO requests that FDA include information on the data required for the shipping studies i.e., description of shipping procedure for initial clinical studies; preliminary data from a pilot shipping study prior to initiation of pivotal trial and validated shipping study prior to licensure
Lines 199-202	Identifying the vector CMC information as a Drug Substance is different than the EMA's position to describe the vector as a starting material.	We suggest the Agency consider opportunities for global harmonization in this area.
Lines 202-205	When (CAR) T cells are manufactured in a continuous process with no clear division between DS and DP, sponsors may find that making a distinction between them can be arbitrary. The designation of a process	BIO suggests that FDA adopt the language used in Section IV.B of the FDA's "CMC Information for Human Gene Therapy IND" guidance, which acknowledges that "some gene therapy products may not have a distinct DS."

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	intermediate as a de facto "DS" can lead to additional regulatory complexity: does the intermediate require release and stability specifications, what are the ramifications for global development, etc.	
Lines 204-205		BIO requests that FDA please consider providing brief example of a (CAR) T process that would require DS/DP sections.
Lines 226 - 229	Cellular characterization data collected during early studies, outside of specification testing, can inform release criteria used in later development to ensure product and process consistency.	BIO requests that FDA please clarify if characterization, in this context, is equivalent to FIO.
A. Vector Manufacturing and Testing		
Entire section	Consider including recommendations for vector stability data needed at IND submission (as per lines 408-410)	We recommend stability studies for vector be conducted to support hold and storage times as described in sections V.A.7 of the GT CMC 410 guidance (Ref. 3).
Lines 236-237	The vector should be well-characterized prior to initiation of clinical studies, although specifications may still be under development	BIO requests that FDA please provide further requirements for well-characterized vector. Viral Vector may still be under phase-appropriate development at FIH.
Lines 237-239	"For licensure, the vector must be manufactured according to CGMP standards…" Does this imply that vector used earlier in development doesn't have to be GMP?	BIO requests that FDA please clarify GMP requirements for early development vectors.
Lines 245-254	The guidance states that a biological potency assay is likely required for clinical study(s) intended to provide primary evidence of effectiveness to support a marketing application. The value of a biological potency assay for viral vectors used in CAR T manufacturing is unclear given the overlap between the biological potency assay for the viral vector, and the potency assay developed for the (CAR) T product itself. In other words, any biological assay used as a potency assay	BIO recommends the following edit: "Vector lot release testing should include measures of safety, identity, purity, and potency…. Transgene expression alone as a measure of potency ~~may~~ should 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."

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	for vector release will likely be redundant with the biological potency assay developed for release of a (CAR) T product. Thus, for (CAR) T products a potency assay that measures transgene expression is likely sufficient for vector release and should be the general recommendation for all stages of product development.	
Lines 245-249	The guidance suggests that a vector potency assay assessing biological activity is required for vector release regardless of the phase of the study. Vector concentration in terms of transduction units/mL is only mentioned in lines 251-255. There is a lack of clarity regarding expectations for vector potency assay development relative to phase, specifically when "additional functional elements" are components of the transgene. It is unclear if potency assays specific to additional functional elements must be developed to demonstrate their biological function, i.e., function of elements present separate from that of TCR/(CAR) T, if these are expected or required for licensure, and if so, at what point they are introduced, and with what format, i.e., qualitative vs quantitative.	BIO recommends clarification that a functional potency assay is not required for early INDs. BIO also recommends differentiating between "transgene" and "(CAR) T" with regard to expected demonstration of vector potency. The following language may help clarify the distinction and the relevance of that distinction for assay development: "A potency assay that assesses the biological activity of the transgene may be developed in coordination with the (CAR) T cell potency assay and used for characterization during early phase trials. Vector potency may be demonstrated by transgene expression (early-phase studies), demonstration of (CAR) T biological function (late-phase studies), or other biologically relevant functional assays. Where the vector transgene contains additional functional elements, the expectation(s) is that…The potency assay format may be qualitative during early-phase studies…"
Lines 251-255	FDA discusses the development of assays regarding vector potency that would be used to "normalize the amount of vector used for transduction during (CAR) T cell manufacturing." We have specific concerns regarding the timing of the development of potency assessments and request additional guidance on that point.	BIO requests clarification about the timing of development of such a potency assessment would be valuable, particularly as it relates to early phases of clinical development and the timing of other potency characterization of (CAR) T products.

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Lines 255 - 257	For commercial lot manufacturing, the T cell transduction volume can then be determined by the amount of vector Transducing Units that is added per cell to achieve the target percentage of (CAR)-positive cells in the (CAR) T cell DP.	BIO requests that FDA please clarify if/when MOI approach is expected.
B. Collection, Handling, and Testing of Cellular Starting Material		
Lines 277-282	"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."	The current drafted language could be interpreted as all collection sites being required to have the same exact collection protocol, however, there may be differences in institutional practices that may be acceptable. We recommend the Agency adjust this language to focus on processing conditions that are expected to impact the quality of the leukapheresis starting material.
Lines 293-295	"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." For (CAR) T with a short a manufacturing process, the time needed to acquire results from sterility testing prior to initiation of (CAR) T cell manufacture could delay the start of manufacturing. Do you need to justify not testing leukapheresis starting material? When both starting material and DP are tested for microbial contamination what will failure of the	BIO suggests that a risk-based approach to implement Sterility testing post hoc but prior to release would allow for a quicker overall manufacturing process without compromising patient safety. BIO requests that the Agency clarify the expectation to specify acceptance criteria for leukapheresis. BIO recommends removing the recommendation to test on starting material and keep the recommendation to retain a sample for post hoc testing. BIO recommends the following edit: "retain a sample of leukapheresis starting material for post hoc testing in the event of a DP sterility test failure to ensure starting material contamination rather than manufacturing process induced contamination."

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	test on the starting material inform if the final product is tested negative? What is the expectation to specify acceptance criteria for leukapheresis? Addition of in-process controls or release criteria at apheresis sites?	
Lines 301-307	Is donor eligibility, screening, or testing necessary for autologous leukapheresis starting material? While it is understandable that screening, testing and donor eligibility may not be needed to address risk of transmission of infectious agents, there may yet be considerations around retro/lentivirus present in starting material from autologous donors that might lead to complementation/recombination of the retro/lentiviral vector.	BIO requests additional clarity about screening of HIV/ HTLV or other relevant retro/lentivirus in autologous donors.
Lines 310-313	It is unclear if the described requirement of two unique identifiers and label checks is for all (CAR) T products or only for autologous. Line 593 implies this is only required for autologous.	BIO requests the following addition: "Additionally for autologous leukapheresis, 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."
C. CAR T Cell Manufacturing and Testing		
1. CAR T cell manufacturing process control		
Entire Section	There is a focus in the guidance on assessments of stability associated with the use of "fresh" (CAR)-Ts. This focus leaves the suggestion that stability issues are limited for cryopreserved product.	BIO requests additional guidance regarding these cryopreserved products given that processes around controlled thawing at the clinical site may be critical to maintaining product quality. BIO requests that FDA provide additional clarity around thawing of cryopreserved product, timing of use after controlled thawing, and appropriate stability data that should be provided to address these cryopreservation stability issues.
Lines 332-334	While in-process control of process parameters and testing of intermediates and final product for quality	BIO requests that FDA include an acknowledgment of phase-appropriate control strategies.

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	attributes is essential, it should be acknowledged that the identification of CPPs and CQAs is something that evolves over the course of product development. Thus, process controls should be expected to be implemented in a phase-appropriate fashion.	
Lines 343-348	"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."	We appreciate the flexibility and recommend FDA allow qualification of ancillary materials for quality, safety, and potency during process/method development. We recommend the agency address novel or custom materials and research grade only available materials from vendors. It would be helpful for this discussion to elaborate on: • How sponsors qualify ancillary materials for what stage of development, and grade of use (i.e., GMP, research only) • Expectations of single use materials and their qualifications for lifecycle management and • Expectations for particulates and testing for particulates for single use materials
Line 345	Not every ancillary component may need a potency assay.	BIO suggests the following edit: "We recommend sponsors qualify ancillary materials for quality, safety, and ~~potency~~ concentration or strength 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."
Line 349	While we agree that rigid microbial controls should be in place for (CAR) T development we believe the	BIO suggests the following edit:

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	language should be modified in order to not inhibit the development of future therapies that may utilize microbial pathways during manufacturing.	"To assure product safety, (CAR) T cells should have defined microbiological quality levels as deemed appropriate ~~be free of viable contaminating microorganisms"~~
Lines 358-360	Is information regarding the use of developmental or engineering batches to support vector manufacturing processes valuable for FDA?	The Guidance makes specific reference to use of developmental and engineering batches to support demonstration of ability to manufacture transduced cell product. BIO requests similar clarification about the value of this information to support conclusions about the ability to manufacture vector according to proposed manufacturing processes.
Lines 364-366	"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." Until more experience is gained from a large number of patients, it is difficult to understand *a priori* how individual patient-derived starting material based on an (often unique) combination of disease state, prior treatment, or other inherent patient characteristics may impact the manufacturing process.	BIO requests flexibility on this issue until this greater experience is obtained.
Line 371	The guidance does not discuss the use of devices which may be used for further preparation and/or manipulation of the cells at the clinical site before administration of the final drug product.	BIO requests that the Guidance include a section on the preparation and/or manipulation of cells before administration at the clinical site.
Lines 380-388	It is not clear if information on ancillary materials of facility information can still be submitted to a BLA via reference to a master file.	BIO requests that FDA clarify that ancillary materials, facility information, critical reagents or drug substance starting material can still be submitted to a BLA via reference to a master file.

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		BIO also requests that the Agency clarify whether information regarding any components of DS, DS intermediate, and DP can be incorporated in the BLA by reference to a MF? For example, can an excipient MF be cross-referenced within the BLA for (CAR) T products?
Lines 394-396	Can a fixed multiplicity of infection for viral vectors be established as a CPP?	BIO would recommend that reference to a range of multiplicity of infection for viral vectors would reflect appropriate flexibility and feasibility.
Lines 402-406	Expectations regarding IPCs and acceptance criteria, regarding guiding manufacturing decisions and/or termination especially in reference to cell counting. Is it the expectation that if IPCAC are not met, batches be terminated?	BIO requests additional clarity on FDA's expectations regarding IPCs and acceptance criteria, regarding guiding manufacturing decisions and/or termination especially in reference to cell counting.
Lines 412-415	"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." While we understand the Agency's concerns, we note that there is limited availability of patient-derived materials for purposes beyond treatment. The limited patient-derived material is therefore prioritized to manufacture the (CAR)-T product. While sponsors can make every effort to meet the Agency's request, we note it may not always be possible because of the limited amount of patient-derived material available. Since material from patients may be limited, a stability study for material from patients may be impractical, therefore, more guidance is needed for stability studies in these situations.	Instead of conducting stability studies on all patient-derived materials, we suggest that the Agency could instead emphasize proper storage conditions, along with the proper handling of the (CAR) T product during thawing and administration to the patient. BIO requests additional guidance on extent of stability studies from patient material where material from patients is limited, (e.g., conducting stability study with material from one patient as supporting data).

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2. CAR T cell analytical testing		
Line 428	General recommendations around testing of allogeneic products, including a specific reference to allogeneic T cell products, are included in Lines 337-347 of the draft guidance titled "Human Gene Therapy Products Incorporating Human Genome Editing" (March 2022). There is no cross-reference or mention of these recommendations in this guidance document.	BIO recommends including a reference at line 428 to the draft guidance titled "Human Gene Therapy Products Incorporating Human Genome Editing" as follows: Additional recommendations on analytical testing of allogeneic (CAR) T products can be found in the GE Draft Guidance (Ref. 15).
Lines 440-443	"Each assay should be qualified prior to initiating studies intended to provide primary evidence of effectiveness to support a marketing application…" Does this suggest that early Phase 1 study materials wouldn't have to be tested by qualified assays?	BIO requests that FDA please clarify.
Lines 440 - 443	In general, and for Ph1, scientifically sound principles for assay performance should be applied through assay qualification (i.e., tests should be specific, sensitive, and reproducible and include appropriate controls or standards). It is unclear if assay qualification is required at FIH/IND submission	BIO requests that FDA clarify if assay qualification is required at FIH/IND submission.
a. Flow cytometry		
Lines 475-476	Additional guidance on the "instrument calibration and QC" information on flow cytometry methods should be included in the IND would be valuable.	BIO suggests that examples of relevant supporting documentation (e.g., instrument calibration reports) could be helpful for sponsors.
Line 507	Training records would have to be available for anybody who performs manufacturing and/or testing in a GMP environment, not just for flow cytometry.	BIO suggests that FDA delete this specific statement regarding training records.
b. Vector Copy Number (VCN)		
Lines 517-522	"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	BIO suggests that the correlation between VCN and (CAR) expression should be calculated following *in-vitro* culture of the final product to not prolong manufacturing timelines and

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	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." In the case of rapidly- manufactured (CAR) T drug products with no or minimal *in-vitro* culturing steps, the result of the bulk assessment for VCN when normalized against total (CAR) expression may result in an unrealistically high percentage of VCN per transduced cells.	compromise development of a rapid manufacturing process that is essential in delivery of drug products to patients as soon as possible.
Lines 530 - 532	Development material may be used to determine VCN.	BIO suggests the following edit: "Supporting experimental data may be obtained from representative development material and multiple engineering manufacturing runs."
534-540	How long to culture (CAR) T cells before doing reassessment of VCN? Perform extended culture on sample of (CAR) T cells not harvested for DP? Is stably integrated VCN a lot release criteria?	BIO requests further clarification.
Lines 537-538	"In this case, an interim VCN assessment at the time of lot release, followed by subsequent VCN assessment on cultured (CAR) T cells, may be needed to determine the stably integrated VCN."	BIO requests that the Agency clarify expectations for "interim assessment at time of lot release."
	c. Identity	
Entire Section	For autologous use, where all final product containers are intended for patient administration, the requirement	BIO believes that added flexibility can be added to the guidance. It should be possible to perform identity testing on a

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	for identity testing to be 'performed on the final labelled product' can create challenges with cryopreserved product and adds risks associated with sampling at this stage of manufacture.	sample taken at the time of finished product filling into the final labelled product container. As such, we recommend wording reflect this flexibility, e.g., "Identity testing must be clearly associated with the final labelled product."
Lines 548 - 552	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, or VCN gene detection by PCR).	BIO requests that the Agency please clarify that either method is acceptable.
Lines 548-552	"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)."	BIO requests that the Agency please clarify "Cellular composition of the final product". A (CAR)-T normally comprises a highly pure T cell population, where other cell types, including B cells, could be considered as impurities.
Lines 552-556	What does HLA typing provide for autologous (CAR) T cells? This would be more useful for allogeneic (CAR) T cells. If this is really meant for autologous, more description on benefit of HLA typing for autologous cells is needed.	BIO recommends the following edit: "HLA typing may be performed for allogeneic (CAR) T cells…"
	d. Potency	
Lines 560-568	As (CAR) T cells kill target cells using multiple mechanisms, one potency assay may not be enough to show full activity of (CAR) T cell.	BIO requests that the Agency please clarify the approach for Vector Potency assay development. BIO notes that a potency assay should demonstrate mechanism of action and activation of the (CAR) T cell. We request that FDA specify that if one assay is not enough to show full MOA or activity, a matrix of assays is recommended. We also suggest that the inclusion of other assays focused on

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		early phenotypical/transcriptional changes of the (CAR) T cells may be warranted.
Lines 570-571	"If the (CAR) T cells express multiple transgene elements, there should be a potency assay to measure activity of each functional element."	BIO would recommend additional clarity from FDA regarding the measurement of additional transgene elements, particularly where there may be challenges in assessing additional elements depending upon the type of protein. BIO further requests that FDA please clarify if two potency assays would be needed for two different (CAR)s. Finally, additional guidance and clarity would be valuable regarding the details of potency assay development, for example, guidance on quantitative vs. qualitative assays and the appropriate phase for assay development.
Line 574	There is no guidance on TCR/CAR T product RCL testing and strategy of management given use of SIN vectors, testing on vector release, early-phase vs late-phase agency expectations and long-term TCR/CAR T product RCL testing requirements.	BIO recommends that FDA include guidance regarding RCL testing following the "Potency" discussion.
3. Labeling for CAR T cells		
Line 577	Further clarity needed.	BIO requests that the Agency be more descript regarding commercial labels and the expectations of disclosure of components in formulation, especially when differentiating between an off the shelf formulation buffer and an in-house proprietary buffer.
D. Managing Manufacturing Changes and Assessing Comparability During the CAR T Cell Product Life Cycle		
Lines 616-617	"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."	BIO suggests the following replacement for these lines:

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	It would be helpful if FDA could be more direct and explicit with this sentence.	"Changes to the (CAR) construct or an autologous to allogeneic product which would result in a new product, should be submitted in a new IND." BIO requests that FDA please provide specific and relevant examples to demonstrate changes where a separate IND would be needed.
Lines 643-648	We find this paragraph confusing as written.	BIO suggests the following edit: "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~~ support using preclinical data to support the clinical study or combining the clinical data resulting from the product produced at each manufacturing facility."
Entire section, lines 650-741	The repeated emphasis on "analytical comparability" throughout this section is remarkable, given that (CAR) T is such a complex cellular product. As currently written, a user of this guidance will receive mixed messages about whether or not their (CAR) T product could actually achieve comparability through assays alone. References to analytical comparability are qualified by statements such as, "If there is insufficient evidence to demonstrate analytical comparability" (lines 678-679) and "In some cases, a change might alter CQAs that cannot be adequately measured in analytical assays" (lines 684-685).	BIO requests that FDA please clarify: what is the Agency's position on the likelihood of demonstrating (CAR) T product comparability through the use of analytical methodologies alone? If analytical comparability will generally suffice only when process changes are minor (i.e., changes to non-critical raw materials), this should be made clear. Otherwise, the sponsor is led to believe that analytical comparability alone is the base case, and that the need to supplement with nonclinical or clinical data is highly exceptional.

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Lines 630 - 633	Please clarify if (CAR) T paired-arm analysis is required for vector changes at any phase of product development. Could vector analytical comparability be sufficient for early-phase changes?	BIO suggests the following edit: "Additionally for vector changes made during clinical study(s) intended to provide primary evidence of effectiveness to support a marketing application, (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…"
1. Change management		
Lines 654-655	"Understanding the impact of the change is critical to evaluate the ability to combine clinical data generated pre- and post-change."	While we note that this directive is needed given the current state of the (CAR)-T field, we also envision a future where this may not be necessary, i.e., at a future date when we have a better understanding of change and how it may impact clinical outcomes. It would therefore be helpful for the Agency to include accompanying statements that are forward-looking about improving the understanding of the product and the manufacturing process (including how process steps impact the product) so that there is the possibility of focusing a comparability assessment on the manufacturing process itself. For allogeneic (CAR) T cell products, it is reasonable to approach a comparability assessment by using split manufacturing of healthy donor starting material. In addition, it would be helpful for the Agency to provide greater detail about how sponsors can continue to improve understanding of the (CAR)-T product and its mode of action as well as the product's fate upon administration to the patient to enable more meaningful assessments of product comparability. Otherwise, manufacturing changes, which are inevitable and often necessary to ensure supply to patients, potentially trigger

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		the formation of a new product or entail the use of additional clinical data.
Lines 666-669	"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."	BIO requests guidance regarding what kinds of proposed process changes FDA would like to have submitted to the IND for their review prior to initiating comparability studies. BIO also requests that the Agency clarify the definition of "later stages of product development" as included in Line 662-3.
Lines 676-678	"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." Due to lack of or availability of patient material, it is often difficult to fully develop a robust analytical comparability protocol to evaluate the impact to the post-change product.	We recommend evaluation on a case-by-case basis based on the magnitude of the manufacturing change taking an overall risk-based approach.
2. Comparability study design		
Lines 738-741	"However, if product manufactured from healthy donors is not adequate to assess product comparability for autologous (CAR) T cells, the comparability study should include evaluation of (CAR) T cells manufactured from patient cellular starting material."	BIO agrees that, where feasible, inclusion of patient material could be an important element in the assessment of a manufacturing change. However, based on the manufacturing change, inclusion of patient material may not always be possible and therefore should not be required but be a recommended element for a comparability study.
E. Managing Manufacturing Changes and Assessing Comparability During the CAR T Cell Product Life Cycle		
1. Single-site manufacturing		

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Lines 743		For the multisite manufacturing considerations, we recommend adding import/export considerations for manufacturing of subjects outside the US. Specifically, when apheresed materials collected ex-US are imported they need to be linked to an IND number which may be difficult with an ex-US investigator. In general, import/export issues pose a significant challenge for sponsors given the extremely time-sensitive nature of transportation and temperature controls.
2. Multisite manufacturing		
Lines 777-782	Similar to our comment in Section IV.D: the reader is left with the impression that analytical comparability alone will usually be adequate to establish comparable product between manufacturing sites.	BIO requests that the Agency clarify whether it is the Agency's position that analytical comparability will usually be adequate to demonstrate comparability between sites (and by extension, the need for nonclinical or clinical comparability data would be exceptional)?
V. PRECLINICAL RECOMENDATIONS		
A. General Preclinical Considerations for Cell and Gene Therapies		
Lines 809-813	The General Considerations are too vague and would benefit from a clear statement that (CAR) T cells could be made in two different ways i) ex vivo for autologous or allogenic transplantation or ii) in vivo from direct delivery to the target cells. The preclinical program then needs to take into account the unique pharmacological and toxicological risks related to the mode and method of delivery. The general guidance from 2013, REF 36, then primarily refers to the second mode of (CAR) T cell development.	BIO requests further clarity on the two broad ways in which (CAR) T cells might be made to clarify the uniqueness of the PharmTox approach for each.
B. Preclinical Considerations for the Vector Component of CAR T Cells		
Lines 822-823	The focus on the (CAR) antigen recognition domain as the major determinant of preclinical safety assumes that all (CAR) T cells are made ex-vivo. The guidance doesn't take into account that viral vectors delivered	BIO requests that FDA clarify that toxicities from (CAR) T cells need to take into consideration the mode and method of delivery and that different toxicities may arise from in vivo

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	directly in vivo to express a (CAR) may have their own safety issues.	generation of (CAR) T cells that may be related to either i) the vector used or ii) the (CAR).
Lines 830-833	"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..."	BIO requests that FDA include examples of cell microarray technology to evaluate (CAR) specificity. Please also include relevant species for conducting these assays.
Lines 838-839	It is unclear if the agency considers all methods listed – tissue cross reactivity, cytotoxicity on cell lines, protein arrays, or in vivo models – as being necessary or if some carry a greater weight than others. The statement is that "Sponsors are encouraged to explore a combination of methods"	BIO requests that FDA clarify if it is the Agency's position that all methods need to be conducted or if some carry greater relevance than others. In some instances, there may be no relevant assay – perhaps clarify that relevant assays be applied on a 'case-by-case' basis.
Lines 843-853	An antibody is not always available for the characterization of the target antigen.	BIO requests further clarity on the relevance of the application of e.g., *in situ* hybridization instead of immunohistochemistry in those instances when an antibody is unavailable. Perhaps clarify that relevant methods are applied to characterize the target antigen as available.
Lines 846-848	"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." Antigen recognition domains, even if identical to a previous (CAR) T cell, may have a different toxicity profile due to differences in affinity. This is acknowledged for different antigen recognition domains, but not for identical ones.	BIO requests that the Agency to allow sponsors to account for these differences in the final guidance.

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Lines 851 to 853	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). No information given if the stated difference between mAb and (CAR) T should be specifically addressed	BIO requests the addition of text to clarify if the stated difference between mAb and (CAR) T should be specifically addressed.
Lines 862-865	"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." Depending upon the antigen being considered, evaluating the (CAR) T cells potency in Ag-expressing versus Ag-knockdown target cells should be considered (without requiring the generation of preclinical cell killing assay across an array of cells expressing the antigen at different levels).	BIO requests that FDA consider evaluating (CAR) T cell potency in Ag-expressing versus Ag-knockdown cells, when appropriate.
Lines 868-870	"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." What is meant by a comprehensive assessment of the product characteristics to evaluate antigen-dependent and antigen-independent activity?	BIO requests that the Agency provide clarity regarding the instances where there may be no relevant model to full evaluate the consequences of off-target activation by co-stimulatory domains in the construct. In many instances this may require an immune competent model. Given that some co-stimulatory domains may be human specific a comprehensive evaluation may not be achievable. We also request the Agency provide examples of acceptable in vitro and in vivo testing strategies or provide an opportunity to evaluate on a 'case-by-case' basis.
C. Preclinical Considerations for the Cellular Component of CAR T Cells		

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Lines 880-883	"Thus, preclinical evaluation may include examination of cytokine-independent cell growth, in vitro and in vivo testing for T cell clonality, karyotypic analyses, TCR repertoire analysis, and specificity for viral antigens through ex vivo stimulation and recognition assays." The reader is left with the impression that there are straightforward in vitro and in vivo ways to assess clonality for (CAR) T cells. In some instances, clones might bring clinical benefit and in other instances they might confer risk. In vitro transduced T-cells can only be kept in culture for a short period of time, typically not more than 14-days. In vivo humanized mice given human (CAR) T cells, with time, succumb to graft vs host disease and therefore cannot be maintained for an extended period of time, typically 30-50 days. These assays are not sufficiently sensitive to detect the slow emergence of a clone that may occur in a patient. Additionally, it may be appropriate to restrict the karyotype analyses on genome edited (CAR) T cells only, but not for every (CAR) T product. It is unlikely to expect karyotype abnormalities by viral or non-viral insertions.	BIO requests that FDA clarify how a clonal assay might help differentiate between benefit versus risk as existing methods have limited predictive power. Clonality would be well suited to the plan for long term follow in patients and could be part of the ongoing genomic analyses to (CAR)e for these patients. BIO requests additional clarification on the utility of the assay and encourage the introduction of a 'weight-of-evidence' (WOE) approach that takes into account the totality of preclinical and CMC packages to predict risk. BIO suggests that the Agency consider that it may be appropriate to restrict the karyotype analyses on genome edited (CAR) T cells only, but not for every (CAR) T product.
D. In Vivo Testing of CAR T Cells		
General comment	It is difficult to provide specific guidance of what kind of non-clinical PK and safety should be collected and how to use such information, we suggest specifying the role of nonclinical PK and safety in this section.	BIO suggests specifying the role of nonclinical PK and safety in this section. The role of nonclinical PK can be further discussed in informing PK characteristics such as expansion in human, while limitation in such translation could be added.
Lines 893-899	Appropriate consideration of role of in vivo toxicity studies.	Keeping in mind the 3R principles, BIO proposes the addition of text suggesting that in vivo efficacy studies can be utilized to

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		determine the toxicity profile (if cross reactivity in the murine model is demonstrated) by designing a Hybrid pharmtox study with limited safety endpoints.
Lines 893-914	Within the context of In Vivo testing of (CAR) T cells the Guidance defers solely to prior clinical experience to guide dosing or dose extrapolation from the preclinical to clinical testing strategy. Novel (CAR) T's or (CAR) T's made by in vivo transduction will have no *a priori* evidence on which to base dosing. The reader is left with the impression that well established or new methods for dosing are not available or considered.	BIO requests that FDA clarify that investigators can apply a variety of methods to help guide dosing beyond prior clinical experience and may include in vitro and in vivo studies to characterize: the Effector to Target Ratio, kinetics of cell expansion, pharmacodynamic/pharmacokinetic anti-tumor responses etc.
Lines 896-899	"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." Mouse xenograft models can also provide information on on-target/on-tumor effect (pharmacological proof of concept) in an in vivo context despite limitations.	BIO requests that the Agency to include this testing strategy as an acceptable approach in the final guidance. BIO requests that FDA provide examples that show that preclinical animals can provide reasonably accurate estimates of (CAR) T cell trafficking and proliferation.
Lines 901-903	"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." The use of surrogate product to support the licensing for (CAR) T may not always be appropriate.	We recommend a case-by-case evaluation as FDA is considering the need for such information.
Lines 913-914		It would be helpful for the agency to provide more detail on how a sponsor would characterize a "similar (CAR) Ts". For example, does it refer to a (CAR) T intended for the same

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		target or patient population, or with same (CAR) design elements such as antigen recognition or signaling domains, etc.
E. CAR T Cells with Additional Modifications		
Lines 928-930	"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."	We request the Agency provide examples/suggestions of acceptable preclinical studies to determine functionality and dosing in this case.
Lines 932-941	In the list of parameters provided Route of Administration is clearly missing. The list only takes into account the parameters associated with Ex Vivo (CAR)-T cells. Vector delivery method accounts for the vector under consideration – viral or non-viral. Route of administration is missing and is an important parameter for In Vivo (CAR) T's where the encoding vector could be delivered directly into a lymphoid tissue, body organ or intravenously.	BIO requests that FDA please add 'Route of Administration' to the list of parameters.
VI. CLINICAL RECOMENDATIONS		
A. Study Population		
2. Tissue-agnostic approach		

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Lines 991-997	The draft guidance document discourages tissue-agnostic approaches for (CAR) T in early development, specifically calling out the need to evaluate dose-response and parallel dose escalation in different tumor types. This guidance seems inconsistent with Lines 1059-1061 where previously clinical experience, even for a different condition, can be used to justify the clinical starting dose. Is this guidance also being provided to tissue agnostic study design for traditional therapies? What evidence suggests dosing of (CAR)-Ts is more specific to a disease type than traditional small/large molecules?	BIO recommends allowing more flexibility for tissue agnostic study design that is at least commensurate with traditional therapies.
4. *Pediatric subjects*		
Lines 1020-1021	For certain cancers, there may not be evaluable adults, or the disease is of a certain nature or stage that safety or proof of concept would not be applicable to targeting children.	Clinical development programs for pediatric indications usually obtain initial safety and tolerability data in adults before beginning studies in children, unless this is unethical or unfeasible.
2. Treatment Plan		
a. *Dose selection, starting dose, and dose escalation*		
b. *Starting dose*		
Line 1057-1066	For (CAR) T therapies in oncology, the preclinical studies are used to demonstrate proof-of-concept (as also highlighted by the draft guidance, line 893) and usually not directly used to support dose selection.	It would be helpful to elaborate and clarify how preclinical studies can support starting dose selection and in determining the associated risk.
Line 1058-1063	The guidance indicates that prior experience with (CAR) T should be used with care in identifying the starting dose for another (CAR) T.	BIO recommends that FDA provide clarification about the value of prior experience with earlier generations of the same (CAR) T therapy in identifying an appropriate starting dose.
b. *Repeat dosing*		

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Lines 1091-1099	"(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."	BIO requests that FDA clarify the terms of repeating vs splitting dose. To our understanding splitting dose is to split single infusion into multiple ones within a short period (in days) to mitigate acute safety risk; while repeated dosing is to give an additional dose after patient relapses, which usually happens in months. Lymphodepletion is more common for repeated dosing, and therefore more likely to pose safety risk is for repeating dosing, and not the case for splitting dosing. We would further request clarification of repeat sequential dosing (in proximity) vs repeat dosing (i.e., after failure post initial response for example).
4. *Consideration for manufacturing delay or failure*		
Lines 1118-1127	The guidance suggests that the protocol eligibility 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. In the alternative, sponsors may consider separate criteria at time of administration vs the time of enrolment	BIO suggests that the Agency provide examples of approaches to eligibility criteria that would improve the likelihood of subject eligibility at the time of product administration or the use of separate criteria at time of administration and time of enrolment. Examples would be particularly helpful in this context in considering real-world application of the appropriate principles.
Lines 1129-1147	The guidance acknowledges that manufacturing failures can occur and discusses appropriate mitigation, particularly around study enrollment. The guidance does not discuss whether non-conforming therapy may still be viable for treatment (including data/justification to be provided by the sponsors) and how those subjects should be considered in study data (e.g. captured in a separate cohort).	BIO notes that additional guidance on these issues would be valuable for sponsors and patients.
5. *Bridging therapy*		

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Entire Section	The guidance focuses on whether bridging will or will not be used to control active disease while waiting for (CAR) T cell treatment. Additional flexibility may be appropriate.	The guidance assumes or implies that patients are not eligible for treatment with the therapy in development until they have progressive disease and therefore active disease management may need to be addressed via bridging. BIO requests clarification on the acceptability of treatment prior to progression from prior line and how the agency might consider approaches to efficacy outcomes in that setting.
Entire Section	The Guidance suggests that bridging approaches be limited or standardized. However, where there are not very short manufacturing times, and there are a diversity of bridging approaches depending on tumor type, questions remain about acceptable approaches to addressing efficacy and safety analyses. Subgroup analyses may be challenging if sample sizes are small within any given bridging approach. Further bridging is often necessitated due to need for patients to have progressed from prior line of therapy prior to receiving cell therapy. Can the Agency comment on acceptability of dosing patients prior to confirmed RECIST progression from prior line?	BIO recommends that FDA provide additional guidance regarding the best approach to account for variations in bridging strategy, including bridging that may result from progression from an earlier line of therapy.
C. Clinical Pharmacology Considerations		
Lines 1180-1182	"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." If samples are routinely collected this would pose operational challenge and may not always be clinically relevant (e.g., different tumor).	BIO recommends the following edit: "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.

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	PK samples may be collected for exploratory purpose when clinically feasible and available if certain tissue (e.g., bone marrow) is involvement in the disease state.	
Lines 1184	"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)."	It would be helpful for the guidance to provide examples of preferred analyte/PK measure in relation to (CAR) T cell expansion and persistence (e.g., (CAR) T cell flow, cytometry level, transgene level) We suggest replacing last observed concentration (Clast) with last observed measurable (non-BQL) concentration (Clast) which may be more specific: "…last observed measurable (non-BQL) concentration (Clast)…"
Lines 1187	"Partial exposure (pAUC) can be used for correlative analysis between exposure and efficacy and/or safety." Reference to pAUC may be limited and could be cited as example while adding Cmax also as parameter explored in analysis.	BIO recommends the following edit: Cmax and partial exposure (pAUC) correlative analysis between exposure and efficacy and/or safety.
Lines 1188	This statement may be too limited and suggest expanding to concomitant therapy (e.g., used for treatment of CRS like corticosteroids or IL 6 antagonist)	BIO recommends the following edit: "To evaluate factors which may affect (CAR) T cell in vivo expansion and persistence, both patient-related, and product-related factors ~~and~~ concomitant therapy and concomitant therapy should be considered."
Lines 1225	We recommend developing assays to detect humoral and cellular immune responses against the (CAR) T	It would be helpful for the draft guidance to include examples of analyses that should be performed at a minimum.

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	cells (CAR and co-expressed transgenes, if applicable) during product development.	
Lines 1228-1229	"Both patient-related and product-related factors which may affect (CAR) T cell immunogenicity **should** be considered." IG impact on clinical outcomes (such as efficacy and safety endpoints) should be assessed.	BIO recommends the following edit: "If IG impact on safety or efficacy is observed clinically, patient-related and product-related factors which may affect (CAR) T cell immunogenicity ~~should~~ may be considered and assessed."
Lines 1237-1239	"We recommend using validated bioanalytical methods for clinical studies intended to provide primary evidence of effectiveness to support a marketing application (Ref.40)." Other qualified assays can also be used.	BIO recommends the following edit: "We recommend using validated bioanalytical methods for primary/secondary endpoints in clinical studies intended to provide primary evidence of effectiveness to support a marketing application."
D. Safety Evaluation and Monitoring		
Lines 1257-1260	Monitoring for cytokine levels may not be the best mechanism for deriving a management plan or algorithm for CRS. Clinical presentation is most relevant for monitoring.	BIO recommends the following edit: "A particular concern of (CAR) T cell toxicity is CRS (see section VI.D.2 of this 1257 guidance). In addition to monitoring for clinical signs and symptoms of CRS, 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."
3. *Dose-limiting toxicities (DLTs), stopping rules and attribution*		
a. *DLT definition*		

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Lines 1299-1308	FDA gives examples of DLTs, but does not provide clarity that these examples are not minimal or recommended DLTs.	BIO recommends additional clarification on this point to avoid confusion. We would also ask the Agency to comment on tumor burden and patient characteristics as considerations for patient specific determination of DLT.
b. *Attribution*		
Lines 1319-1323	It can often be difficult to ascertain the attribution of a treatment emergent adverse event to a specific cause, but not always. If an adverse event is clearly not related to study treatment (for example, clearly related to underlying disease), the adverse event is not a toxicity, although it is still an adverse event.	We recommend editing to read either: "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." Or "...Therefore, we recommend DLTs be defined independent of attribution to (CAR) T cells. Any exception or exemption of treatment-emergent toxicities from the DLT definition should be clearly described and justified."
Lines 1322-1323	"Therefore, we recommend DLTs be defined independent of attribution to (CAR) T cells."	BIO requests that FDA please consider other possible approaches. This is the most conservative approach and may not be appropriate in all situations.
E. CAR T Cell Persistence and Long Term Follow-up		
Entire Section	We believe that FDA should acknowledge some of the challenges associated with LTFU for C&GT products. Patients may not be willing to consent to the details of a LTFU study before actually receiving treatment.	BIO suggests that FDA should provide additional clarity on whether consent (beyond notification of LTFU before treatment consent) to LTFU procedures can occur following completion of treatment phase, particularly where LTFU occurs in a separate study.

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