子導入から iPS 細胞の選定、樹立のために長期間の培養を行う可能性が高く、かつ、導入した目的遺伝子の発現は初期化には必要でも iPS 細胞としての樹立時には不要、あるいは遺伝子発現が見かけ上はなくなっている可能性があり、これは従来の ex vivo 遺伝子治療とは大きく異なる点である。すなわち、細胞の初期化時には目的遺伝子の発現の評価が必要と考えられるが、樹立の時点では、むしろその遺伝子発現がどれだけ残存しているかを確認する必要があると想定される。また、導入遺伝子の残存性についても評価が必要となるであろう。従って、ex vivo 遺伝子治療で求められるような目的遺伝子の発現や目的遺伝子の持続性とは異なる視点が必要となる。

参考：EMEA ヒト由来細胞治療薬ガイドライン (EMEA/CHMP/410869/2006　11、Jan. 2007)より抜粋

3.1 遺伝子治療薬と細胞治療薬の複合製品の出発原材料に関する特記事項

細胞を遺伝子改変して用いる場合には、遺伝子治療用ベクターの品質管理、特性解析、非臨床試験に書かれている「遺伝子治療薬の品質、非臨床及び臨床試験に関するガイドライン」を参照すること。形質転換された細胞集団について新たに獲得した特性の適切性や持続性を解析する必要がある。このような細胞に導入された遺伝子から発現される物質の発現量やその品質については特に考慮を払う必要がある。また、実施可能であれば、新たに獲得した発現能について定量するとともに管理するべきである。

日米欧の遺伝子治療薬関連ガイドライン等を参考に、iPS 細胞の樹立に用いるベクターについて明らかにすべきと考えられる事項を以下にまとめた（表２，３参照）。

１．ウイルスベクターの生物学的特徴

ウイルスベクターにより、どのような細胞に遺伝子導入が行えるか、種特異性・組織特異性があるか、静止期の細胞への遺伝子導入は可能かなどについて記載する。遺伝子の導入効率及び導入遺伝子の発現効率について記載する必要がある。導入遺伝子の細胞内での存在様式、安定性についても説明が必要となる。なお、染色体内に組み込まれる場合には、その位置が特定されているか不特定かを明らかにする。

２．ウイルスベクターの製造方法

ウイルスベクターの製造方法について、上記各項における記述をもとに記載する。また、その精製法について説明する必要がある。実用化のためのスケールアップ等の措置を講じた場合は、適切なバリデーションデータを示す必要がある。パッケージング細胞を使用する場合には、その作製手順、選択・同定方法及び種細胞株を確立するまでの単離純化方法、MCB 及び WCB の調製・保存方法、管理法、更新法、特徴及びパッケージング細胞に挿入された DNA 又は RNA の安定性についても記載する必要がある。さらに、培養期間中を通じて、またロット間で細胞のフェノタイプ等が変化していないことの確認試験方法及び試験結果について説明が必要となる。

増殖性ウイルスを含めて、ベクターの品質管理に必要な安全性試験の試験時期、試験方法及び試験結果が求められる。

３．非ウイルスベクターを用いて遺伝子を導入する場合

採用した遺伝子導入法の理論的根拠及び実験的根拠を示す必要がある。また導入に機器等を用いる場合には、機器の性能等についても記載する。導入する DNA/RNA の作製方法、さらに構造分析やその性質に

ついての記載が求められる。導入遺伝子からの生成物の構造及び生物活性について説明すると共に、導入した細胞からの産生能についても解析することが求められる。その他、非ウイルスベクターの製造方法、構造又は組成分析、生物学的特徴についても説明することが必要である。

4．非ウイルスベクターの製造方法

非ウイルスベクターの製造手順、精製法及び管理法について記載する。ベクターの各構成成分（タンパク質、糖質、脂質等）について、由来、調製法、精製法、品質等を詳細に説明する必要がある。タンパク質、糖質、脂質等、生物起原由来の材料を使用する場合には、感染性微生物による汚染の可能性を否定しておくことが必要である。

5．非ウイルスベクターの構造又は組成分析

ベクターの構造又は組成について記載する。ベクターの各構成成分（タンパク質、糖質、脂質等）について、ベクター製造前後の構造又は組成を明らかにしておく必要がある。各構成成分につきロット更新を行う場合には、ロット間の恒常性を明らかにする。例えば、組換えタンパク質やモノクローナル抗体が構成成分の一部である場合には、目的タンパク質生産用の種細胞株の樹立、セルバンクの調製方法、保存方法、管理方法、更新法、生産のための細胞培養方法、目的タンパク質の精製法、構造・組成解析、特性解析、規格及び試験方法並びに保存安定性に関する資料が必要である。また、ベクターの各構成成分について、医薬品としての使用実績があれば記載が必要である。

C-2 遺伝子導入により樹立したiPS細胞の特性解析と安全性評価

遺伝子導入により樹立したiPS細胞の場合、その特性解析と安全性評価に当たり考慮すべき事項については、ex vivo遺伝子治療に関連するガイドラインやICH Q5A等のガイドラインが参考になると考えられる。表4，5に、樹立したiPS細胞で実施すべきと考えられる試験項目を示す。これらの試験項目のうち、ウイルス安全性に関してはICH Q5Aに従い、利用したフィーダー細胞や、用いた血清、増殖因子の由来等を考慮してウイルス等の感染因子の否定試験が必須となる。

iPS細胞の樹立では、ex vivo遺伝子治療と比べてかなりの長期にわたり培養を行うこと、また現時点の技術では、多くの場合、樹立時にフィーダー細胞等の支持細胞との共培養が必要とされており、遺伝子導入細胞の評価には特別な配慮が必要である。iPS細胞の製造過程で、特に遺伝子導入による細胞の初期化時に、フィーダー細胞としてウイルス安全性評価を行っていないマウス胎児繊維芽細胞(MEF)などのプライマリー細胞を用いた場合、MEFに存在するマウスレトロウイルスの安全性評価が必須となるであろう。マウスレトロウイルスは糖鎖抗原α1-3galを持つため、たとえヒトに感染してもヒトの持つα1-3galに対する強い自然抗体で不活化されることが想定されるが、in vitro培養系ではヒトのiPS細胞にマウスレトロウイルスが感染する可能性がある。また、マウスレトロウイルスが感染してiPS細胞の染色体に挿入された場合にはその検出が困難になる可能性がある。従って、原則的にはウイルス安全性に関する試験を行っていない異種フィーダー細胞をiPS細胞の樹立に用いるべきではないと考えられる。

C-3 遺伝子導入により樹立したiPS細胞から分化誘導した機能細胞の特性解析と安全性評価

遺伝子導入により樹立したiPS細胞から分

化誘導した機能細胞の特性解析と安全性評価に当たり実施すべきと考えられる試験項目を表6に示す。遺伝子導入技術により作製されたiPS細胞を再生医療に用いる場合、特に大きな懸念として、挿入変異による造腫瘍性、がん化が挙げられる。iPS細胞はクローニングにより樹立されることから、iPS細胞を樹立した時点で遺伝子の挿入部位の確認を行うと共に、iPS細胞及び分化後の機能細胞について、免疫不全マウスを用いるin vivo造腫瘍試験やin vitro軟寒天コロニー形成試験等により造腫瘍性の評価を実施することが必要となる。しかし、これらの方法を用いても完璧に造腫瘍性、がん化を評価・予測することは現時点の科学では困難であることから、臨床で使用した後の被験者の長期フォローアップをどのように行うかが重要と考えられる。例えば、フランスとイギリスで実施されたX-SCIDに対するex vivo遺伝子治療において、レトロウイルスベクターによる挿入変異が原因となり発症した白血病症状は、遺伝子治療を行ってから3〜5年という長期にわたる体内でのがん化プロセスの後に発症したものと考えられることから、挿入変異によるがん化リスクに関する長期フォローアップについては、この点を考慮した計画を策定することが求められる。

遺伝子治療に用いるex vivo遺伝子導入細胞の場合、遺伝子導入後、細胞のクローニングを行うことなく被験者に投与するため、現時点の技術では投与前に挿入変異の有無、がん化の可能性を確認する方法はない。そのかわりFDA、EMAには遺伝子治療後の長期フォローアップ観察に関するガイドラインがあり、これにより長期リスクを軽減する方策が採られている。遺伝子治療に用いられるex vivo遺伝子導入細胞としてこれまで経験があるのは血液系の細胞に限られており、iPS細胞から分化させた機能細胞とは条件が異なるが、長期フォローアップの方法としては参考になるものと思われる。

D．結論

遺伝子導入により作製したiPS細胞の品質・安全性について、遺伝子治療の観点からどのように評価すべきかを検討し、遺伝子導入によるiPS細胞の樹立と遺伝子導入に用いるベクターについて明らかにすべき事項、遺伝子導入により樹立したiPS細胞及びiPS細胞から分化させた機能細胞の特性解析・安全性評価において遺伝子導入細胞として評価すべき事項を考察した。

F．健康危険情報

該当なし

G．研究発表

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H．知的財産権の出願・登録状況(予定を含む)

1．特許取得　なし
2．実用新案登録　なし
3．その他

なし

表１．遺伝子導入により作製したiPS細胞の品質・安全性評価において参考となる海外ガイドライン

FDA

- Guidance for FDA Reviewers and Sponsors Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs). Center for Biologics Evaluation and Research (April 2008) (資料１)
- Guidance for Industry: Gene Therapy Clinical Trials - Observing Subjects for Delayed Adverse Events. Center for Biologics Evaluation and Research (November 2006) (資料２)
- Guidance for Industry Supplemental Guidance on Testing for Replication Competent Retrovirus in Retroviral Vector Based Gene Therapy Products and During Follow-up of Patients in Clinical Trials Using Retroviral Vectors. Center for Biologics Evaluation and Research (November 2006) (資料３)

EMA

- Concept paper on the development of a guideline on the risk-based approach according to annex I, part IV of DIR. 2001/83/EC applied to advanced therapy medicinal products. EMA/CHMP/CPWP/708420/2009 (December 2009) (Draft) (資料４)
- Guideline on the safety and efficacy follow-up – risk management of advanced therapy medicinal products (EMEA/149995/2008) (資料５)
- Guideline on the quality, pre-clinical and clinical aspects of medicinal products containing genetically modified cells. EMA/CHMP/GTWP/671639/2008 (May 2010) (Draft) (資料６)
- Guideline on follow-up of patients administered with gene therapy medicinal products. EMEA/CHMP/GTWP/60436/2007 (October 2009) (資料７)

ICH ガイドライン

- ヒト又は動物細胞株を用いて製造されるバイオテクノロジー応用医薬品のウイルス安全性評価. ICH Q5A(R1) (資料８)
- 組換えDNA技術を応用したタンパク質生産に用いる細胞中の遺伝子発現構成体の分析. ICH Q5B (資料９)
- 生物薬品（バイオテクノロジー応用医薬品／生物起源由来医薬品）製造用細胞基剤の由来、調製及び特性解析. ICH Q5D (資料１０)

表２．ウイルスベクターを用いて iPS 細胞を作製する場合の考慮事項

- 野生型ウイルスの生物学的特徴
- 導入 DNA 又は RNA の作製方法，構造分析，性質
- 導入遺伝子からの発現タンパク質の生物活性等
- 目的遺伝子以外の DNA あるいは RNA の作製方法、構造及び性質
- パッケージングに用いる細胞の培養方法、生物学的特徴
- パッケージング細胞の培養方法、生物学的特徴
- ウイルスベクターの粒子構造上の特徴
- ウイルスベクターの生物学的特徴
- ウイルスベクターの製造方法
- セルバンクシステムとウイルス安全性

表３．非ウイルスベクターを用いて iPS 細胞を作製する場合の考慮事項

- 遺伝子導入法の理論的根拠及び実験的根拠
- 導入 DNA 又は RNA の作製方法、構造分析、性質
- 導入遺伝子からの発現タンパク質の構造及び生物活性
- その他の DNA の製造方法、構造及び性質
- 非ウイルスベクターの製造方法
- 非ウイルスベクターの構造又は組成分析
- 非ウイルスベクターの生物学的特徴

表４．遺伝子導入により樹立した iPS 細胞（バンク）の試験項目

- ウイルス安全性試験（Q5A を参考に）
- 生存率、倍加時間等
- 確認試験（表面抗原発現等；最終製品の純度解析に利用）
- 遺伝子の挿入部位の確認
- 造腫瘍性の確認（悪性腫瘍の有無）
- 遺伝型や表現型の解析
  （マイクロアレイ解析やエピジェネティックな遺伝子発現を含む）
- 純度試験（無菌試験、マイコプラズマ否定試験、エンドトキシン）
- 機能解析（分化能等）

表５．　iPS 細胞のウイルス安全性評価

●レトロウイルス及び内在性ウイルス試験	
• 感染性試験	＋
• 電子顕微鏡観察	＋
• 逆転写酵素活性	＋*1
• その他細胞種特異ウイルス試験	適宜実施
●非内在性ウイルス又は外来性ウイルス試験	
• in vitro 試験（MRC-5, Vero 細胞等）	＋
• in vivo 試験 （マウス脳内接種等）	＋
• 抗体産生試験 （HAP、MAP 等）	＋*2
• その他細胞種特異ウイルス試験	適宜実施

*1:レトロウイルス感染性試験が陽性のときは不要

*2:例えばマウス，ラット，ハムスターでの抗体産生試験，
通常，げっ歯類由来の細胞に対して適用する．

表６．遺伝子導入 iPS 細胞から分化誘導した機能細胞の特性解析

- 遺伝的安定性評価（染色体解析（G バンド解析、mFISH） や CGH 等）
- 導入遺伝子の解析(導入遺伝子の残存性)
- 造腫瘍性評価（試験の限界：長期フォローアップが必要）
- 感染因子の迷入に関する試験
- 細胞の純度（目的細胞、未分化細胞を含む目的外の細胞）：表面抗原
- 機能細胞の生物学的機能；細胞由来の生理活性物質の産生や有効性に関連する生物活性
- 目的外の望ましくないサイトカインや増殖因子の産生能
- 製造工程由来不純物試験（血清、添加剤、抗生物質等：試験結果等を考慮して）
- 目的外の細胞への分化

Guidance for FDA Reviewers and Sponsors

Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs)

Additional copies of this guidance are available from the Office of Communication, Training, and Manufacturers Assistance (HFM-40), 1401 Rockville Pike, Suite 200N, Rockville, MD 20852-1448, or by calling 1-800-835-4709 or 301-827-1800, or from the Internet at http://www.fda.gov/cber/guidelines.htm.

For questions on the content of this guidance, contact the Office of Cellular, Tissue, and Gene Therapies at 301-827-5102.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
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This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the appropriate FDA staff. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. INTRODUCTION

This guidance document provides to you, sponsors of a human gene therapy investigational new drug application (IND), recommendations on the chemistry, manufacturing, and control (CMC) information to include in an original IND. This guidance also applies to combination products that contain a human gene therapy biological product in combination with a drug or device as part of the final product. Also, this guidance instructs FDA CMC reviewers about the information to record and assess as part of an IND review, taking into consideration the various manufacturing challenges for these products.

In order to deliver a safe and effective product, human gene therapies present many manufacturing challenges. Some of these challenges include the variability and complexity inherent in the components used to generate the final product, such as the source of cells (i.e., autologous or allogeneic), the potential for adventitious agent contamination, the need for aseptic processing, and in the case of ex vivo genetically modified cell therapies the inability to "sterilize" the final product because it contains living cells. Distribution of these products can also be a challenge due to stability issues and the frequently short dating period of many ex vivo genetically modified cell products, which may necessitate release of the final product for administration to a patient before certain test results are available.

This guidance finalizes the draft guidance entitled, "Guidance for FDA Review Staff and Sponsors: Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs)" dated November 2004 (69 FR 64958; November 9, 2004).

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

A. How will FDA Reviewers and Sponsors Use this Guidance?

FDA's primary objectives in the review of INDs are to help ensure the safety and rights of human subjects in all phases of an investigation and, in Phases 2 and 3, to help ensure that the quality of the scientific evaluation of the investigational product is adequate to permit an evaluation of its safety and effectiveness (21 CFR 312.22(a)). This guidance will help sponsors and FDA reviewers to assess, given the phase of the investigation, whether sufficient information is provided to assure the proper identification (identity testing), quality, purity, and strength (one aspect of potency) of the investigational product (21 CFR 312.23(a)(7)(i)). These principles apply to investigational biological products and drugs; however, specific terms, such as safety, identity, purity, and potency, are generally understood to be applicable to biological products and are used throughout this document.

If you are a FDA reviewer, you will use this guidance as you assess the safety, identity, purity, and potency of an investigational product and you will use the format of the human gene therapy CMC review template (Appendix A) in preparing your reviews. Because of the wide variability of the contents of IND amendments, you are only expected to use the attached template during review of original IND submissions. However, you should consult this document for guidance throughout the investigational new drug development process.

The human gene therapies CMC review instructions and template described in this guidance are tools to assist FDA in the review of human gene therapy INDs. They are designed to serve as a guide to help ensure that all applicable regulatory requirements are reviewed at the appropriate stage of product development. In addition to the CMC review instructions and template, some general considerations are discussed in Appendix B that should be helpful in assessing proposed release criteria testing and specifications. Section 10.70 (21 CFR 10.70) provides further instruction to FDA reviewers regarding documentation of review decisions.

If you are a sponsor of a human gene therapy IND, you may use this guidance in developing an IND submission that will be adequate to permit FDA reviewers to make an assessment of the safety, identity, purity, and potency of your investigational product. Other regulatory documents that may be relevant are listed in the references (see Section XII below).

B. How is this Guidance Organized?

This guidance is organized in a format that generally corresponds to the sections in the CMC review template provided in Appendix A. In each section, where necessary, we (FDA) provide recommendations as to the information you may submit in your original IND submission. As necessary throughout this document, we give specific instructions to FDA reviewers concerning their documentation and assessment of an IND submission during the CMC review. Many of the instructions for FDA reviewers provided in this guidance are distinguished by the designation "Note to FDA Reviewers."

II. ADMINISTRATIVE INFORMATION TO BE DOCUMENTED BY FDA REVIEWERS

Note to FDA Reviewers: Document in your review all of the IND information listed below. Most of this information should be available on Form FDA 1571, the sponsor's cover letter, or the reviewer assignment notice from the Regulatory Project Manager (RPM) of the application division.

- BB-IND Number (assigned by Center for Biologics Evaluation and Research (CBER) after receipt);
- Date of submission;
- 30-day review due date;
- Sponsor – name, address, title, phone, fax;
- Sponsor point of contact (sponsor's authorized representative) – name, address, title, phone, fax;
- Title of IND;
- Proposed use;
- Product description;
- Phase of study;
- Cross-referenced INDs, investigational device exemptions (IDEs), and master files (MFs): List all regulatory files (IND, IDE, MF) that the sponsor has obtained permission to cross-reference in support of this file. The file under review must contain a letter signed by the person who submitted the cross-referenced file (21 CFR 312.23(b)), giving FDA permission to cross-reference the file. This letter should identify the nature of the information being cross-referenced (e.g., pre-clinical, product manufacturing, and/or clinical) and where it is located within the file being cross-referenced. You should verify that the cross-referenced information satisfies the IND requirement for which the information is cited. If the letter of cross-reference is absent or inadequate, or the cross-referenced information is inadequate for the purpose cited, the RPM or the reviewer should notify the sponsor to obtain additional information;
- Key words: Include three to four words that can be used to identify the product, indication, and any materials, components, or devices that may be part of the final product or used in the manufacturing process. These key words should be general enough to be used in a database search;
- Introduction/rationale: Summarize relevant information on the development of the product if the sponsor provides this information. In addition, document and assess, as appropriate, the sponsor's scientific rationale and justification for using the product for the indication under review; and
- Study objectives.

III. PRODUCT MANUFACTURING AND CHARACTERIZATION INFORMATION TO BE SUBMITTED BY SPONSORS AND DOCUMENTED BY FDA REVIEWERS

As described in the following sections, you should provide a detailed description of where and how the gene therapy product is manufactured. You should include all of the components and materials used during the manufacture of the gene therapy product, such as the vector, cells, cell bank systems, and any reagents or excipients. In addition, you should describe all procedures used during the manufacturing process. Examples of these procedures may include vector derivation, purification, preparation of cell banks systems and testing, including final formulation of the product. This information will allow us to assess the identity, quality, purity, and potency of your product. For further information, refer to the guidance on "Content and Format of Investigational New Drug Applications (INDs) for Phase 1 Studies of Drugs, Including Well-Characterized, Therapeutic, Biotechnology-Derived Products" (Ref. 1). In addition, you may refer to the other documents listed in the references (see Section XII below).

We also encourage sponsors to use the format and headings described in Appendix A to facilitate an efficient review by FDA.

Note to FDA Reviewers: Document and assess product manufacturing and characterization information in your IND reviews. Organize the CMC review using the format and headings described in Appendix A and below, as appropriate.

A. Product Manufacturing – Components and Materials

Note to FDA Reviewers: Document the source of all materials and components and summarize the testing performed on those materials and components, and review the specific instructions and recommendations set out below.

Your IND must include a list of all components used in manufacturing of your product (21 CFR 312.23(a)(7)(iv)(*b*)). The sections below detail the information on manufacturing components that we recommend you submit in an IND, and that FDA reviewers will document and assess.

1. Vector

You should provide the following information about your vector:

a. Gene Therapy Vector Construct

A description of the history and detailed derivation of the gene therapy vector including:

- The gene map, with relevant restriction sites, and any vector constructs used during generation of the final vector and their sources;
- The gene insert;

- Regulatory elements, such as promoter, enhancer, and poly-adenylation signal; and
- Selection markers.

b. Vector Diagram

A diagram of the vector identifying the gene insert and regulatory regions, and any other relevant elements, such as pertinent restriction endonuclease sites:

Note to FDA Reviewers: Document the vector diagram by scanning it into the review document.

c. Sequence Analysis

Vectors 40 kilobases (kb) or less: We recommend that you fully sequence all vectors under 40 kb, perform sequence analysis, and submit an annotated sequence of the entire vector. You should provide a summary of the sequence analysis, indicating the origin and function of each component of the vector that accounts for all nucleotides such as promoters, known coding sequences, polyadenylation signals, origins of replication and restriction sites used during construction of the vector or for diagnostic tests. You should provide an evaluation of the significance of all discrepancies between the expected sequence and the experimentally determined sequence and an evaluation of the significance of any unexpected sequence elements, including open reading frames. We recommend viral vectors be sequenced from the master viral bank (MVB) when appropriate. Plasmid sequence should be obtained from the master cell bank (MCB), and retroviral vector sequence should be obtained from the MVB/packaging cell line or from DNA obtained after transduction of a stable cell line.

Vectors greater than 40 kb: You should summarize the extent and results of sequence analysis that you have performed including any testing performed by restriction endonuclease analysis. We recommend that you perform sequence analysis of the gene insert, flanking regions, and any regions of the vector that are modified.

2. Cells

a. Allogeneic and/or Autologous Cell Components

You should describe the following information in your IND:

- Cell source: tissue and cell type (e.g., colon, hematopoietic, neuronal, T-cells);
- Mobilization protocol: document whether or not donor cells are mobilized or activated in vivo in the donor;

- Collection or recovery method: state the procedure used to obtain cells (e.g., surgery or leukapheresis indicating the device used if possible), the name and location of the collection facility, and transport conditions if shipped to a processing facility for further manufacturing; and
- Donor screening and testing: the donor screening and testing that is performed to determine donor eligibility. Requirements for screening and testing donors of human cells and tissues are described in 21 CFR Part 1271 (see final rule, "Eligibility Determination for Donors of Human Cells, Tissues and Cellular and Tissue-Based Products (HCT/Ps)") (Ref. 2). When appropriate, you should document the donor safety testing that is performed. In addition, FDA has published a final "Guidance for Industry: Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps)" (Ref. 3). We recommend that you review this guidance to ensure that the donor qualification criteria described in your IND are consistent with current recommendations.

1) Autologous

You are not required to make a donor eligibility determination or to perform donor screening for cells and tissues for autologous use (21 CFR 1271.90(a)(1)). However, you should determine whether your manufacturing procedures increase the risk of propagation of pathogenic agents that may be present in the donor. If so, you should document whether the donor is reactive for specific pathogens. Also, you should describe precautions to prevent the spread of viruses or other adventitious agents to persons other than the autologous recipient (see Ref. 2).

2) Allogeneic

You must perform donor screening and testing as required in 21 CFR Part 1271 for all allogeneic cells or tissues except those that meet the exceptions in 21 CFR 1271.90(a). Donors of all types of cells and tissues must be screened and tested for HIV-1, HIV-2, hepatitis B virus (HBV, surface and core antigen), hepatitis C virus (HCV), *Treponema pallidum* (syphilis), and CJD (screening only). Donors of viable leukocyte-rich cells or tissues should be screened and tested for human T-lymphotropic virus types 1 and 2 (HTLV-1, HTLV-2) and CMV. In addition, you should document whether FDA-licensed, cleared, or approved test kits are used in these detection assays and document which tests are used. Include a description of the type of serological, diagnostic, and clinical history data obtained from the donor. You should consider other issues such as typing for polymorphisms and

human leukocyte antigen (HLA) matching, where appropriate. If cord blood or other maternally derived tissue is used, you should describe testing and screening performed on birth mothers.

Note to FDA Reviewers: Communicate with the clinical reviewer regarding any issues or concerns relating to the screening or testing of the donor cells.

b. Cell Bank System

You should describe pertinent information, as described in Sections 1) through 3) below, relating to the cell bank system (i.e., master cell bank (MCB), and working cell bank (WCB)) used in product manufacture. In addition, you should describe the history, source, derivation, characterization of each cell bank (both MCB and WCB), and the frequency at which testing is performed. For further information, refer to the document on "Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals" (Ref. 4). See also ICH document Q5D, "Derivation and Characterization of Cell Substrates Used for Production of Biotechnological/Biological Products" (Ref. 5), and, where applicable, "Guidance for Industry: Source Animal, Product, Preclinical, and Clinical Issues Concerning the Use of Xenotransplantation Products in Humans" (Ref. 6) and the "PHS Guideline on Infectious Disease Issues in Xenotransplantation" (Ref. 7).[1]

Note to FDA Reviewers: Document and assess the testing that is performed on each cell bank. Determine if the most relevant and critical testing for the particular gene therapy product has been performed. Appropriate tests should be performed depending on the species of origin used to derive the cell bank.

1) Master Cell Bank (MCB)/Packaging Cell Line[2]

You should include in the IND information regarding MCB history, source, derivation and characterization, including testing to adequately establish the safety, identity, purity, and stability of the cells. This section will likely address:

- Product microbiologic characteristics, including sterility, mycoplasma, in vivo and in vitro testing for adventitious viral agents, as appropriate (see Section IV below);
- Freedom from the presence of specific pathogens, including, for human

[1] If a feeder cell line of animal origin is used to propagate human cells (i.e., human and non-human animal cells are co-cultivated), the final product falls within the definition of a xenotransplantation product (see both Refs. 9 and 10).

[2] If an ecotropic cell line was used during the generation of a retroviral producer cell line, we recommend that sponsors test for ecotropic retrovirus (see "Product testing"). Reviewers would assess and document the testing that was performed.

cells, testing for CMV, HIV-1 & 2, HTLV-1 & 2, EBV, B19, HBV, and HCV, as appropriate. For cell lines that are exposed to bovine or porcine components (e.g., serum, serum components, trypsin), appropriate testing would include testing for bovine and/or porcine adventitious agents. See further discussion of bovine components and reagents in Section III.A.2.a;

- Identity of the cells, including tests to distinguish the specified cells through physical or chemical characteristics of the cell line (i.e., phenotype, genotype, or other markers);
- Purity of banked cells, including identification and quantification of any contaminating cells;
- Testing for activity of cells (e.g., activated lymphocytes, dopamine secretion, insulin secretion) and cell maturation (e.g., dendritic cells). This should be performed if activity is relevant to the therapeutic nature of the product;
- End of production cells (EOP). This should be tested on a one time basis to assess genetic stability of the MCB/packaging cell line; and
- Processes critical to product safety, as applicable, including:
    - Culture conditions used, including documentation of all media, and reagents/components used during production, with copies of relevant certificates of analysis (COA);
    - Method of introduction of vector (transfection, transduction, infection) into MCB/parental cells to establish vector producer cell;
    - Analysis and selection of producer cell clone; and
    - Cryopreservation, storage, and recovery of the MCB, including information pertaining to cell density, number of vials frozen, storage temperature, and cell bank location; and
    - Genetic and phenotypic stability of the MCB after multiple passages as well as viability of cells after cryopreservation. We recommend that, while the IND is in effect, you perform a stability assessment EOP as a one-time test. This testing is usually performed later in product development and would be included as part of the biologics license application (BLA).

2) Master Viral Bank (MVB)

You should provide a description of the MVB and the testing that you have performed to ensure safety, purity, and identity. We recommend that you address:

- History and derivation of the MVB;
- Culture conditions used during tissue culture scale up;
- Testing of media and other reagents used during production, including COAs;

- Product microbiologic characterization – including sterility, mycoplasma, in vivo and in vitro testing for adventitious viral agents, as appropriate;
- Freedom from the presence of specific pathogens, such as human viruses if the cell line is of human origin, or pathogens specific to the origin of the production cell line (e.g., murine, non-human primate,;
- Tests to identify presence of replication competent virus;
- Identity testing to establish the presence of gene therapy vector and therapeutic transgene (e.g., Southern blot); and
- Information pertaining to the cryopreservation of the MVB, including condition and storage location(s).

3) Working Cell Bank (WCB)/Working Viral Bank (WVB)

The WCB/WVB may have been derived from one or more vials of the MCB/MVB. As discussed in the guidance documents referenced in III.A2a, the amount of information needed to document characterization of the WCB/WVB (derived from the MCB, and MVB, respectively) is usually less extensive than that needed to document characterization of the MCB/MVB. If there is a two tiered cell bank system in place (MCB, MVB), we recommend that you test the WCB/WVB for:

- In vitro adventitious viral agent testing;
- Replication competent virus;
- Bacterial and fungal sterility;
- Mycoplasma; and
- Limited identity testing (e.g., Southern blot, flow cytometry).

3. Reagents

You must list in your IND any reagents used in manufacturing the product (21 CFR 312.23(a)(7)(iv)(*b*)). For the purpose of this guidance, reagents are those materials that are used for cellular growth, differentiation, selection, purification, or other critical manufacturing steps but are not intended to be part of the final product. Examples include fetal bovine serum, trypsin, digestion enzymes (e.g., collagenase, DNAse) growth factors, cytokines, monoclonal antibodies, antibiotics, cell separation devices, and media and media components. These reagents can affect the safety, potency, and purity of the final product, especially by introducing adventitious agents. For monoclonal antibodies, refer to the guidance on "Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use" (Ref. 8) for further information.

a. Tabulation of Reagents Used in Manufacture

We recommend that you provide the following information on all reagents used during product manufacturing:

- concentration of the reagent at the manufacturing step at which it is used;
- vendor/supplier;
- source:
    - *human*: If human albumin is used, you should have procedures in place to ensure that no recalled lots were used during manufacture or preparation of the product. If using human AB serum, ensure serum is obtained from an approved blood bank and meets all blood donor criteria. For all other reagents that are human derived you should identify whether it is a licensed product, or clinical or research grade, and provide a COA or information regarding testing of the donor and/or reagent.
    - *porcine*: If porcine products are used, a COA or other documentation that the products are free of porcine parvovirus.
    - *bovine*: If a reagent is derived from bovine material, you should identify the bovine material, the source of the material, information on the location where the herd was born, raised, and slaughtered, and any other information relevant to the likelihood that the animal may have ingested animal feed prohibited under 21 CFR 589.2000. It may be that bovine material is introduced at different points in production of a reagent; the information described above should be provided for all bovine materials used. For more information see, "Proposed Rule: Use of Materials Derived from Cattle in Medical Products Intended for Use in Humans and Drugs Intended for Use in Ruminants," (72 FR 1581; January 12, 2007); found at http://www.fda.gov/cber/rules/catruminant.htm. In addition, you should provide a COA to document that bovine materials are compliant with the requirements for the ingredients of animal origin used for production of biologics described in 9 CFR 113.53.

Note to FDA Reviewers: For all animal derived products, enter the following information in the animal components database: source organism, supplier/vendor, country of origin, and stage of manufacture. Additionally, the information provided on bovine materials should be evaluated to determine whether, for informed consent that is adequate under 21 CFR Part 50, the subject should be informed of the potential risk that TSE agents have been introduced into the final product.

- Reagent quality: We recommend that you use FDA-approved or cleared, or clinical grade reagents whenever they are available.

Note to FDA Reviewers: If the reagent is regulated as a biological product, drug, or device, consider whether a consultative review should be obtained. See Section III.A.5.b below for further information about consultative review process.