A publication of
The Williamsburg BioProcessing Foundation

Winter 2006

BioProcessing JOURNAL

The Most Trusted Source of BioProcess Technology®

Vol. 5 No. 4

www.bioprocessingjournal.com

Considerations for Characterization and Comparability of Recombinant DNA-Transduced Cellular Products

By LILIA BI

Recombinant DNA-transduced cellular products encounter the product development and regulatory issues of both gene therapy and cellular therapy products.[1] The characterization of recombinant DNA-transduced cellular products remains highly challenging for both sponsors and regulatory agencies. The regulatory concerns and product testing for such cellular products are similar to those for all biologicals. These concerns include the demonstration of product safety, identity, purity, and potency; the control of the manufacturing process to ensure the consistency of product manufacturing under a proper quality control program; and the demonstration of reproducibility and consistency of product lots by means of defined product lot release testing criteria.

Approaches to Product Development

Product development begins before the first human use of the product and proceeds throughout clinical trials, licensure, and even post-licensure. The first step in the development of a product is to provide preclinical toxicity data from animal model studies. To limit the risks to human subjects in the next step, Phase I clinical trials, data sufficient to demonstrate product safety and appropriate safety testing must be in place. Product safety testing must continue throughout product development and after the product is licensed. CBER/FDA recommends that product characterization proceed with clinical trials and requires that a product be fully characterized with regards to safety, purity, identity, and potency by the time a Phase III clinical trial is initiated.

A quality control program should also be in place from the initiation of Phase I trials. This program should be separate from manufacturing and should ensure the quality and release of the product. Other elements of current Good Manufacturing Practices (cGMP) include adequate documentation and records, adequate personnel training and certification programs, proper production and process controls, equipment qualification, and an environmental monitoring program. Most of these elements should be in place in a form sufficient to ensure product safety at the time a Phase I clinical trial is initiated. These requirements will need to be implemented and demonstrated to consistently provide a safe, potent, and efficacious product by the time a biologics license application (BLA) is filed.

Characterization of Recombinant DNA-Transduced Cellular Products

Manufacturing of recombinant DNA-transduced cellular products may involve multiple reagents, components, and multi-step manufacturing procedures. All reagents and components used for product manufacturing should be tested for safety and characterized to ensure their integrity, stability, and consistency from lot to lot. Biological reagents such as serum, cytokines, growth factors, and antibodies used for cell selection should be well documented and properly tested.[2] If the final product is an adenoviral-transduced somatic cell product, the adenoviral vector, as well as the final genetically modified cells, will need to be tested for safety and characterized for identity, purity, and potency.[3]

The cell component may be autologous or allogeneic. If cells are obtained from an allogeneic donor, then donor testing and screening must be performed in compliance with 21 CFR 1271 (as of March 25, 2005). If cell banks are used, these banks must also be tested for safety including testing for *in vivo* and *in vitro* adventitious agents.[2] If the cell lines are of human origin, then they should be tested for human pathogens. This pertains to the use of viral cell banks as well.

Characterization of recombinant DNA transduced cellular products includes: demonstration of product safety, testing for adventitious agents, determination of purity and identity, assessment of potency, and demonstration of product stability.

Development of specifications for each parameter is an important part of product development and characterization. CBER/FDA recommends that

Lilia Bi, Ph.D. *(bi@cber.fda.gov), is a chemistry, manufacturing, and controls reviewer in the Division of Cellular and Gene Therapies; Office of Cellular, Tissue, and Gene Therapies; Center for Biologics Evaluation and Research; Food and Drug Administration, Rockville, MD.*

specifications be established early in product development and subsequently tightened according to the data generated. As product characterization proceeds, better defined approaches can and should be developed to evaluate proposed test methods and acceptance criteria for release.

In many cases, in-process product characterization and testing are necessary for these complex products. To ensure the consistency, it is important to characterize the manufacturing process. For this purpose, cGMPs play an important role in the control and regulation of each step of the product manufacturing process. Adherence to cGMP standards provides for quality and safety throughout the process and will lead to consistent performance of product lots.

During product development it is important to accumulate data that demonstrates the purity profile of the product and any effect that impurities may have on the manufacturing process and the final product. It is also important to establish specific characteristics that will ensure product integrity and stability. These types of data are critical to gaining a better understanding of the product, the effect of the manufacturing process on that product, and more importantly, the effect any change will have on the product.

One important question to consider is how to characterize the product and what kind of testing should be performed. Some tests are specified in the *Code of Federal Regulations* (CFR), such as the tests covered in 21 CFR 610 for sterility, general safety, and pyrogenicity. For in-process testing, FDA does not require a specific test method; therefore, any scientifically valid test method may be used. For final product testing, if a test method is not specified by the biological product standards in 21 CFR 610, any scientifically valid test can be used.

Tests for potency and identity are usually product specific, so scientifically valid tests will need to be developed. In some cases, more than one test may be needed to address identity and potency. For all testing, CBER/FDA recommends using or developing the most appropriate test for that particular product and recognizes that the test specified in 21 CFR 610 may not always be the most appropriate. Thus, a provision in 21 CFR, 610.9 allows the use of alternative tests. However, by licensure the sponsor must provide data validating that the alternate method gives equivalent or greater assurance than the specified method.

Tests addressing the safety (*e.g.*, sterility, mycoplasma) and purity (*e.g.*, endotoxin) of the product must be performed before it can be used in a Phase I trial. Assays to assure potency will need to be in place before starting a Phase III study. All assays will need to be validated by licensure.

Manufacturing Changes and Product Comparability

Throughout product development and even post-licensure, manufacturing changes will be made to improve the product or the production process. Changes may occur at various steps in the manufacturing process and it is difficult to predict the potential effects of a given change.[4] Changes may involve reagents, viral banks, plasmid stocks, product formulation, and manufacturing procedures. These changes may alter the cellular phenotypes present and affect product safety, purity, identity, and potency. Other changes may include storage conditions or shipping conditions. It is important to consider the order of a change in the manufacturing process. A change early in the process may affect downstream steps.

Another major category of change involves the manufacturing site. This may include a change from a single manufacturing site to multiple sites or may involve a product transfer from an academic site to a company. Either change may introduce different equipment, new personnel, or a new manufacturing process. When multiple sites are used to manufacture the same product, the potential for variation is always greater and manufacturing processes and testing should be under full control.

The products produced before and after manufacturing changes are implemented must be demonstrated to be comparable to support the use of clinical, safety, and efficacy data obtained prior to the change. How does one assess comparability? FDA's 1996 guidance stated, "*FDA may determine that two products are comparable if the results of the comparability testing demonstrate that the manufacturing change does not affect safety, identity, purity, or potency.*"[5] Although this document was written for changes to approved applications, many of the concepts can be applied to changes made during the investigational stages of product development. These concepts may also serve as guides to thinking about the appropriate product characterization data one needs to collect, and to learning about the product and manufacturing process.

In many cases, a comparability protocol will help with subsequent implementation and reporting of chemistry, manufacturing, and control (CMC) changes, especially for approved products. A comparability protocol is a well-defined, detailed, written plan for assessing the effect of specific CMC changes in the identity, strength, quality, purity, and potency of a specific drug product as these factors relate to the safety and effectiveness of the product.[6] It describes the changes covered under the protocol and specifies the tests and studies to be performed, including analytical procedures and acceptance criteria that are sufficient to demonstrate that specific CMC changes do not adversely affect the product. A formal comparability protocol is not always necessary during investigational stages, especially during early product development. However, data demonstrating that a product is comparable before and after any changes may be needed, depending on the stage of product development. Otherwise, the use of clinical data collected before the change will be questionable.

Factors that affect the feasibility of comparability studies include the time of change, the type of change, the number of changes to the manufacturing process, the extent of change, and the ability of available methods to assess the results of the changes. Before developing comparability studies, a sponsor needs to understand its current manufacturing process and the effect any process change will have on product safety, identity, potency, and stability. To

develop comparability studies, a sponsor must identify product characterization assays that measure the effect of the change, keeping in mind that, in many cases, satisfying lot-release acceptance criteria is not sufficient to demonstrate comparability. In the early product development stages, the lot-release specifications for identity and potency are often given as broad ranges and therefore may not sufficiently measure a change. FDA suggests that a sponsor develop pilot-scale or full-scale test data before implementing any manufacturing changes.

Sponsors should avoid altering approved production specifications or validation parameters and plan ahead for effects that changes may have on the product. If differences are found, sponsors should use valid assessments to determine if the differences are meaningful. When a combination of analytical testing and biological assays (*in vitro* or *in vivo*) fails to establish comparability before and after a change, other comparability studies such as preclinical animal studies (pharmacokinetics and/or pharmacodynamics and toxicity) or clinical studies (clinical pharmacology, safety, efficacy) may be needed.[5]

FDA Considerations

There are several examples of what CBER has observed relating to product characterization and the effects of manufacturing changes on product comparability. We have observed that some manufacturers lack a full understanding of the importance of complete product characterization, and also lack a full understanding of the effect that a manufacturing change may have on the product. We have also seen sponsors intending to make manufacturing changes late in product development without full product characterization and a comparability protocol in place.

How can sponsors and CBER overcome these problems? We suggest the following:

- Both the product and the manufacturing process should be well characterized as early as is feasible during product development.
- Multiple tests should be developed for single complex characteristics, such as product potency. For recombinant DNA-transduced cellular products, potency may be measured by expression of the transgene and the biologic activity of the final product.
- Documentation should be maintained throughout product development when manufacturing changes may occur.
- Sponsors should collect data throughout product development and identify assays that are predictive of product changes.

We encourage sponsors to consult with CBER early and often regarding proposed manufacturing changes before they are implemented, and provide a description of the proposed changes and tests used to demonstrate product comparability. We also strongly encourage sponsors to submit data for analysis as a means to enable more meaningful discussions.

Currently, because only limited data is available to evaluate the potential effect of manufacturing changes on recombinant DNA-transduced cellular products, evaluation of product comparability will be reviewed case-by-case. The review processes will follow the principles of the related CBER guidance documents which can be found at <www.fda.gov/cber/guidelines.htm>.

ACKNOWLEDGEMENTS

The author would like to acknowledge Drs. Stephanie Simek, Maritza McIntyre, and Kimberly Benton for their input and many useful discussions.

REFERENCES

1. Center for Biologics Evaluation and Research (CBER). Guidance for industry: Guidance for human somatic cell therapy and gene therapy. Rockville (MD): CBER; 1998 March.

2. Center for Biologics Evaluation and Research (CBER). Draft guidance for reviewers: Instructions and template for chemistry, manufacturing, and control (CMC) reviewers of human somatic cell therapy investigational new drug applications (INDs). Rockville (MD): CBER; 2003 August.

3. Center for Biologics Evaluation and Research (CBER). Draft 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). Rockville (MD): CBER; 2004 November.

4. Benton K. FDA perspectives on characterization and comparability of cellular therapy products. *BioProcessing Journal* 2003:1;44–46.

5. Center for Biologics Evaluation and Research (CBER). FDA guidance concerning demonstration of comparability of human biological product, including therapeutic biotechnology-derived products. Rockville (MD): CBER; 1996 April.

6. Center for Biologics Evaluation and Research (CBER). Draft guidance for industry: Comparability protocols - chemistry, manufacturing, and controls information. Rockville (MD): CBER; 2003 February.