Manufacturing of Gene Therapies: Ensuring Product Safety and Quality

SLIDE 1
This presentation will discuss the manufacturing of gene therapy products to ensure product safety and quality.

SLIDE 2
This slide shows the FDA's published definition of gene therapy products. Gene therapy products are defined as all products that mediate their effects by transcription and/or translation of transferred genetic material, and/or by integrating into the host genome, and that are administered as nucleic acids, plasmids, viruses, such as adenoviral vectors, or genetically engineered micro-organisms, such as bacteria. These products can be used to modify cells in vivo, by a direct administration of a gene therapy product to a patient, or by transfer to cells ex vivo prior to patient administration. The definition shown here comes from the FDA 2006 guidance on long-term follow-up.

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This chart gives you an idea of the number and type of active gene therapy Investigational New Drug, or IND, applications as of July 2009.

Depicted here are primarily replication defective gene therapy products, the most common being plasmid-based vectors. Plasmids can be delivered as naked DNA or they may be encapsulated in a liposome for enhanced delivery.

In terms of viral vectors, adenoviral products are the most commonly seen vector. They are primarily used in cancer indications.

Retroviruses have the inherent property of integrating into the host's genome and allow for stable and long-term integration of therapeutic genes.

AAV stands for adeno-associated virus. AAV-vectors do not integrate into host genomes but they do allow for long-term gene expression in non-dividing cells. AAV products under IND are primarily used to treat neurological disorders that involve direct administration of the viral vector into the brain.

Pox viruses include vaccinia, fowlpox, and canarypox. They are primarily used as cancer vaccines, because the product itself is somewhat immunogenic and can act as an adjuvant in the vaccine therapy.

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There are a number of other products reviewed by the gene therapy branch that are difficult to categorize. Some are gene therapy products, and some actually are not considered gene therapy products. But most in this chart are replicating

products.

At the time this presentation was written, CBER had approximately 32 INDs using oncolytic viruses. These are live viruses that replicate preferentially in tumor tissue and through the process of replication. They are able to destroy tumor tissue. In theory, they do not replicate in the normal surrounding tissue, so, they do not destroy the neighboring normal tissue.

Some oncolytic viruses are natural, wild type viruses. They have the inherent ability to replicate in dividing cells, such as tumor cells. Others carry modifications that serve to attenuate the wild type virus and make it less pathogenic. Some may also carry a therapeutic gene to enhance targeted tumor cell killing.

FDA is seeing a growing number of live microbial products that are live bacteria or yeast. Many are anaerobic bacteria that preferentially replicate in the necrotic tumor environment in cancer patients. Most of these carry a therapeutic gene, such as a cytokine, to enhance tumor cell killing.

CBER also has about eight INDs using herpes simplex virus vectors. Some of these are replication defective vectors that carry a therapeutic gene. Several are actually just wild type attenuated herpes virus that are being used as oncolytic viruses. It is a little confusing how they are categorized, but in the end they are all regulated in much the same manner.

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Let's talk about how FDA regulates gene therapy product manufacturing. As illustrated in this slide, the manufacturing process can be quite complex. This slide shows an example of a product made of patient cells transduced ex vivo with a retroviral vector. There are a number of components used in the manufacturing process that need to be overseen. You have allogeneic cells as a starting material where there would be donor screening requirements. These cells may be selected for a certain subtype, so FDA has oversight to make sure that monoclonal antibody use in the cell selection is of appropriate quality. You then have the selection device which is reviewed. Of course there is oversight of the process of manufacturing a gene therapy vector, and then the culturing process which may utilize materials that are research-grade reagents. And of course the oversight of the final product.

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This is the basic outline on how FDA oversees the manufacturing process for gene therapy products to ensure product safety and quality. The evaluation of these products will involve assessing the components used in product manufacture, and final product testing and characterization as well as an evaluation of the control of the manufacturing process.

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The components used in the manufacturing of gene therapy products that would be assessed include the vector, the cells which can be allogeneic or autologous used for ex vivo transduction, the cell bank systems used for the production of gene therapy products, and the ancillary reagents used for producing the vector or culturing the patient cells.

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In terms of assessment of the vector, manufacturers are asked to submit a description of the history of the vector and details on the derivation of the vector construct. The sponsor is asked to submit a vector diagram outlining the essential elements of the vector. Currently, FDA requires a full sequence analysis of gene therapy vectors that are less than 40 kilobases in size prior to initiating a clinical trial.

For some of the larger vectors, FDA encourages full sequencing prior to phase 3, but prior to phase 1, sequencing of the major regions, such as the inserts, flanking regions, and modified regions is expected. This is done to ensure the absence of unexpected sequences in the gene therapy product.

FDA found some surprises in a few of the gene therapy products once full sequencing was done. For example, a large fragment of salmon DNA was found in a commonly used adenoviral vector. The vector originated from the 1950s, when salmon DNA was used to help precipitate the original vector construct. This DNA sequence would not have been found without full sequencing of the vector.

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For cells and cell substrates used in the production of gene therapy products, the requirements are similar to those that would be asked for any biologic production systems. Examples are those used to make a vaccine, recombinant protein or monoclonal antibody, where the FDA would want information on the substrate to include the history, source, and general characteristics of the cell substrate.

In terms of autologous or allogeneic cells used to make ex vivo modified cell-based products, FDA would ask for information about the source of cells, the collection procedure, and compliance with donor eligibility requirements.

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Cell bank and virus bank testing is consistent with the way FDA in general regulates biological products. Safety testing includes sterility, mycoplasma, and the various adventitious virus tests, including the two general virus screens, in vitro and in vivo adventitious agent testing. Additional testing is required for bovine and porcine viruses, if the fetal bovine serum and trip-sin used in manufacturing have not been tested.FDA may ask for species-specific viral tests that are specific for the type of cell lines being used.

For gene therapy products that are designed to be replication incompetent, one sometimes sees recombination events during manufacturing that will generate a replication-competent virus. This can be a safety concern. So, at every stage of manufacturing, the sponsor is asked to test for replication-competent virus. FDA has guidance on specific assays and limits.

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Master cell bank characterization would involve relatively basic testing for identity, isoenzyme analysis, purity, contaminating cell analysis, tumorigenicity, and other tests, such as viability.

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For master viral bank testing FDA would require a sequence of the vector and a restriction map. Transgene-specific protein expression, titer and stability data for the viral bank would be looked at.

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For the working cell banks and viral banks, safety testing would involve sterility, mycoplasma, in vitro adventitious agents, and testing for replication-competent virus. For characterization, FDA again would expect testing for identity, stability, and other appropriate assays.

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For the reagents used in manufacturing, FDA asks for a tabulation of the reagents used, including the source of the reagents used. If the reagent is a human-derived material or an animal-derived material, additional tests may be required. FDA would also need to know if the reagent is research grade or for human use. For the manufacturing of gene therapy products, it is often seen that research-grade products are being used, so FDA asks that a certificate of analysis be supplied for the reagent. If there is a regulatory master file available, the manufacturer is asked to supply a letter from the master file holder to allow cross reference to that master file. A qualification program should be in place to assess the quality of reagents used in the manufacturing process, especially if research grade reagents are used.

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In terms of product manufacturing, overall, the scheme for vector production and purification is assessed. If the process involves ex vivo transduced cells, then FDA would assess the method of collection, processing, culture, transduction procedures, and any other modification, such as irradiation, and, of course, the conditions for final harvest of the cells.

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For the formulation of the final product, FDA would assess the formulation of the buffer and excipients, the final concentration of the product to be administered, and the storage conditions after final formulation.

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Final product testing for complex biologics, such as cell and gene therapies, is quite a challenge. You do not always have a well-defined active ingredient, and there is a lot of variability in the manufacturing process. Therefore, final product testing is focused on product characterization, and lot to lot consistency. FDA asks the manufacturer to provide a listing of release test methods and acceptance criteria for the final product in their IND.

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Safety tests are common to all biologics, and are outlined in the regulations. The basic requirements for safety testing include sterility, mycoplasma, and endotoxin testing. For gene therapy products, FDA asks for additional testing for adventitious agents, due to the nature of these products.

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As mentioned earlier, final product characterization is a challenge for regulating complex biologics. General requirements for testing the final product for purity, identity, and potency are described in the regulations. Of course, FDA would also want to assess the stability of the final product and cell viability, if the product is cell-based. Our approach to product characterization for complex biologics is that as the clinical trial progresses, the sponsor should continue to develop test methods, to better characterize the product, and better define acceptance criteria.

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This slide shows more detail in terms of final product characterization. Identity can be relatively straightforward for a gene therapy product; a restriction map or structural characterization should be done. For purity, the main concerns are impurities that can be process-related, and include residual DNA, protein, or culture reagents. For viral vectors, there should be an assay in place to measure the ratio of infectious to non-infectious particles.

Stability is something that should be tested throughout all clinical phases of development and beyond.

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A potency assay should indicate biological activity relevant to the product, and should be quantitative. This is something FDA requires prior to initiation of phase 3. But for early clinical development, manufacturers are asked to assess the viral titer for viral product, and have an assay in place to assess the expression of the therapeutic gene. This is typically done by ELISA, if it is a protein-based therapeutic.

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There seems to be a lot of confusion about Good Manufacturing Practices, also

known as GMPs, and the relationship between product characterization and GMP.

Current Good Manufacturing Practice, or cGMP, is defined as a set of current scientifically sound methods, practices, or principals that are implemented and documented during product development and production, to ensure consistent manufacturing of safe, pure, and potent products.

Many manufacturers think that GMPs are just having in place a number of Standard Operating Procedures, or SOPs, writing everything down, and having a second group sign off on a certificate of analysis. But GMPs are about controlling your manufacturing process. If you do not accurately identify the purity and potency of your product, it will be difficult to ensure that the manufacturing process can produce a consistent product. Gene therapy products, as well as many other biologics, are in general quite complex and difficult to characterize. Therefore, FDA has allowed phasing in of GMP and characterization requirements during clinical development, with the expectation that the manufacturer demonstrate an increase in the control of the manufacturing process with clinical trial advancement.

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Optimizations of manufacturing procedures generally evolve as the clinical trial progresses.

In the U.S., phase 1 clinical trials are in general exempt from GMP requirements. This is described in the guidance document for phase 1 clinical trials. FDA utilizes a stepwise approach to implementing cGMPs and product characterization requirements that is illustrated on this slide.

Safety testing is required prior to initiation of phase 1. This includes sterility, mycoplasma, endotoxin, and viral tests. Basic characterization prior to phase 1 is expected, but FDA does allow a phasing in of lot release tests, both in-process and final release tests. Initially, lot release testing is primarily based on pre-clinical data. The acceptance criteria may be somewhat broad. Characterization may be somewhat limited, but FDA expects a development, refinement, and a tightening of acceptance criteria based on data throughout clinical development.

All analytical test procedures need to be based on what is outlined in the regulations, or equivalent tests, and validated by licensure. FDA does not require full validation of test procedures prior to licensure. Basic validation is certainly expected. This is called assay qualification during clinical development to show that all tests and processes have shown to be specific, sensitive, and reproducible.

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In summary, FDA uses a stepwise approach to enforce the regulatory

requirements. Safety testing is the priority, starting from phase 1 and continuing throughout development. Product characterization for complex biologics is a challenge, and is something the FDA expects will increase during clinical development. It is also expected that the sponsor demonstrate control of the manufacturing process through GMP practices, with an overall goal of ensuring a safe and quality product.

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This concludes the presentation, "Manufacturing of Gene Therapies: Ensuring Product Safety and Quality".

We would like to acknowledge those who contributed to its development. Thank you.