

# MINUTES

## Adenovirus Reference Material Working Group Meeting

March 22, 2001

8:30 AM – 3:30 PM, Life Technologies, Gaithersburg, MD

### *Summary*

The Adenovirus Reference Material Working Group met March 22, 2001 to review proposals made in response to their posted call for bids on different stages of production, vialing, and the repository of a purified Adenovirus Type 5 Wild-type Reference Material. The group also determined the requirements for participation in two key aspects of characterization of the reference material, determination of particle concentration and of infectious titer. The call for proposals to participate in the characterization phase will be publicized in May and at the American Society of Gene Therapy Annual Meeting May 29 to June 3, 2001 in Seattle to encourage participation from all sectors of adenoviral gene therapy, including academia. The group still needs to determine how stability monitoring of the reference material will be handled. The working group plans to meet to discuss this phase during the ASGT conference.

### *Details*

The working group briefly overviewed all proposals received (summary table) including two that were not widely available prior to the meeting. There were multiple bids for all RFPs. The group also determined that bids would be awarded by simple majority votes with bidders abstaining from voting on their own proposals. FDA representatives announced that they would abstain from voting on all bid proposals but would provide their assessment of the strengths and weaknesses of proposals.

The Working Group also received offers of services or supplies under RFP 3.0. None of the RFP 3.0 service/supply offers were specifically discussed. The Williamsburg BioProcessing Foundation will ensure that all institutions awarded bids are made aware of the RFP 3.0 service and supply offers so that they may take advantage of these proposals if they wish.

RFP 1.0 Cell Bank Donations and RFP 2.0 Virus Material Donations were reviewed together. **RFP 1.0 Cell Bank Donors** included PER.C6 cell bank vials from Crucell, 293 cell bank vials from the University of Alabama at Birmingham (UAB), and 293 cell bank vials from Q-One Biotech (Glasgow, Scotland). All bids supplied characterization information and offered at least the 20 requested 1 mL vials. FDA indicated that due to limited US clinical experience with the PER.C6 cell line at present and to the fact that the 293 cell line was still the most commonly used for adenovirus production that they preferred use of the 293 cell line for production of the Ad5WT reference material. The Q-One Biotech cell bank donation was considered complete but included the use of horse

serum (and appropriate adventitious agent testing). The UAB cell bank donation was complete with the exception of one test, Hepatitis C, of either the Master or Working Cell Bank. A motion was made and seconded to select the donor from one of the two 293 cell bank donations based on the commonality of the experience base with 293 cells. The motion was approved with 19 Yes, 2 No, and 2 abstentions.

Discussion then focused on the pros and cons of the two 293 cell bank donation proposals. Q-One Biotech is located in Scotland and although they have a Massachusetts subsidiary, the cell bank vials would have to be transferred from Scotland to the US for production of the reference material. The concern was raised that the USDA is currently delaying entry of goods containing animal serum into the U.S. due to the foot-and-mouth disease problem in the U.K. (even though this is irrelevant to a cell bank made several years ago) that this might impede transfer of the cell bank vials to the US. Q-One Biotech representatives did not participate in the meeting so their input to this concern could not be addressed. The UAB working cell bank is derived from one of the pre-made 293 Master Cell Banks made and characterized by BioReliance which have been widely used for clinical trial materials. A motion was made and seconded that the Working Group award the bid to UAB on the basis of the commonality of usage of their cell bank with the proviso that the missing HCV test be performed. The motion was approved 22 Yes, 0 No, and 4 abstentions.

**RFP 2.0 Virus Material Donation.** Three proposals were submitted. One proposal from Crucell was donation of a plasmid that could only be used with the PER.C6 system. Because a 293 cell bank was selected, the Crucell proposal was not reviewed or considered further. The other two proposals were for Ad5 WT virus materials, one from Canji and one from UAB. FDA representatives indicated that the Canji proposal was considered complete and acceptable to them. The UAB proposal appeared to be for a purified material that was not characterized as called for in the bid request (AAV, sterility, and mycoplasma information were missing), was not from a plaque-purified material, and contained slightly less viral particles than called for. This purified material was from a lysate material that was fully characterized per the bid request. However UAB indicated that there was not enough lysate to fulfill the bid request requirements for viral particles. On that basis a motion was made and seconded that the bid be awarded to Canji. The motion was approved 22 Yes, 0 No, and 5 abstentions.

RFPs 4.0, 5.0 and 6.0 were discussed together because certain institutions provided bids in more than one of these categories and the group felt that there might be advantages to coupling awards. **RFP 4.0 Virus Bank Production Donation** proposals were sent in by Canji, Introgen, and UAB. FDA representatives indicated that with the exception of a missing identity test in the Introgen proposal, that they considered both the Canji and Introgen proposals complete and comparable. There was concern that the UAB proposal would not result in enough material.

For **RFP 5.0, Purified Formulated Bulk Material Donation**, the FDA representatives indicated that while the Canji proposal did not include an in vitro test for adventitious agents they found the proposal acceptable because the proposal did include an in vivo test for adventitious agents and met all other requirements of the bid request. The FDA representatives also noted that the Introgen proposal was complete but also did not include an in vitro adventitious agent test on the purified virus bulk. The Introgen proposal did include such a test on the cell culture prior to the infection step. There was no mention of an in vivo adventitious agent test in their proposal. FDA representatives indicated that at this stage of gene therapy development they were willing to accept the inability to perform in vitro adventitious agent testing as currently practiced on a wild-type adenovirus, however they hoped that this issue could be addressed for future batches.

**RFP 6.0, Vialing and Freezing Donation** bidders included the American Tissue Culture Collection (ATCC), Introgen, and the National Institute for Biological Standards and Control (NIBSC). FDA representatives noted that while ATCC would not perform the vialing under CGMP that they included media fills before and after vialing. FDA indicated that endotoxin testing was missing from the ATCC proposal. They also questioned whether the proposed Nunc vial was adequate. ATCC indicated they would include endotoxin testing and would change to any vial the group recommended. FDA representatives indicated that the Introgen proposal was complete as submitted and included all testing as well as additional testing not required by the bid request and would be performed under CGMP. The NIBSC proposal was not proposed to be performed under CGMP and it was also missing endotoxin testing. NIBSC indicated that they would add the endotoxin testing but had not planned on media fills.

After the FDA review of the RFP 4.0, 5.0, and 6.0 bid proposals, the group discussed the advantages of grouping the votes on the awards of RFP 5.0 and 6.0, or of grouping the votes on the RFPs 4.0, 5.0, and 6.0. Additional questions were asked of the bidders to clarify freeze-down methods and stability information on the container/closures in the RFP 6.0 proposals.

A motion was made and seconded that the RFP 4.0 Virus Bank Production donation be awarded to Canji. One rationale for this was that the group felt that production of the virus bank did not need to be coupled to production of the purified bulk directly. Another rationale was that since Canji was selected to provide the virus source material that there could be some advantage in having Canji produce the virus bank on the basis of familiarity with the material. The motion was passed with 22 Yes, 0 No, and 5 Abstentions.

The Working Group felt it attractive to pair the production of the purified bulk and the vialing of the bulk in one location to prevent problems that might be encountered in transferring the bulk from one country or location to another. The Working Group also suggested that the labels used for the vials be coordinated with the repository institution to incorporate any requirements they might have.

Introgen indicated their ability to customize the label per the Working Group's requirements. Given the discussion of gas permeability of the closure system, Introgen also indicated that they had performed stability studies with the closure/container proposed, including both shipping studies (using dry ice) and a laboratory-based dry ice storage study, without any stability problems. A motion was made and seconded that RFP 5.0 and 6.0 be awarded to Introgen. The motion was approved with 20 Yes, 0 No, and 6 Abstentions.

**RFP 7.0 Repository Donation.** FDA representatives reviewed the four different repository donation bids from ATCC, MDS PharmaServices, NIBSC, and Q-Biogene. ATCC's proposal contained a complete description addressing all issues. Additionally they have already in place overseas distributors located in Europe, Japan, and South Korea. The MDS PharmaServices proposal also contained a complete description addressing all issues. They are part of a larger organization that may be able to assist the US operation with distribution in Europe; however this would have to be confirmed. The NIBSC proposal contained a complete description addressing all issues and included a willingness to partner with a US distributor or repository to ensure easy access for US requestors. The NIBSC proposal also raised an issue, however, in that they were uncertain of funding for an additionally required freezer for storage of the reference material. The Q-Biogene proposal also contained a complete description addressing all issues in the bid request. Q-Biogene's proposal also included distribution out of both their Carlsbad, California and their French locations.

The Working Group discussed the pros and cons of awarding the bid to more than one repository institution and determined that this would be disadvantageous to ensuring best use of a precious resource. The Working Group reviewed the pros and cons of each proposal addressing the issues of multi-site storage (available through ATCC and Q-Biogene), web-based ordering capability (ATCC, Q-Biogene), history and familiarity of the field on both sides of the Atlantic Ocean with the different institutions and their distributors as repositories (ATCC and its UK partner LGC, NIBSC), and whether it was worthwhile keeping the reference material with a non-profit institution (ATCC, NIBSC). The Working Group agreed to defer discussion and decisions on the proposed price per vial and what limitations if any on the number of vials allowed per customer request to after a decision on award of the repository bid. After discussion, a motion was made and seconded that the repository bid be awarded to ATCC. The rationale included the non-profit status of the ATCC, its partnership with LGC in the UK to serve Europe, and its long history and sole reason for existence as a repository. The motion was approved as 21 Yes, 0 No, and 6 Abstentions.

The Working Group then discussed the need for restrictions on orders. The group determined that they would prefer not to deal with theoretical problems but rather to focus on data. With that, a motion was made and seconded that there be a limit of 10 vials per order and that distribution of the first 500 allotment of vials be closely monitored. A report should be made to the Working Group in 6 months (after release to the public of the reference material) or after distribution of the 500 vials, whichever came first, of the distribution pattern for the reference material. This report would include

numbers of vials requested by country and by customer type (industrial, academic) and the average vial number requested per customer or per order. The motion also included the recommendation that information on how an institution could use the reference material to set up an internal institutional reference material be made available to customers. The Working Group will assist the repository in creating this information. The motion was passed with 26 Yes and 0 No votes, and 2 Abstentions.

The Working Group next discussed whether the price per vial should include a premium. The possible uses for a premium could be [1] for funds for production of the next lot of the reference material, [2] reimbursement of some costs involved in production of the first lot of the reference material, and/or [3] application of funds towards development of a replication-deficient adenovirus standard. The group determined that such a fund could be held by either ATCC or the Williamsburg BioProcessing Foundation if required. The discussion focused on the cooperative spirit of the donations thus far and the fact that money considerations could be perceived as tainting the process. One consideration was that this issue could be deferred and revisited when distribution of the first 500 vials was reviewed. However after discussion the motion, seconded, to provide within the price per vial a premium to serve as a funding mechanism was not approved with a vote of 0 Yes, 25 No, and 2 Abstentions. No price premium will be included at this time and the suggested price per vial of \$162 was accepted as contained within the ATCC proposal.

**Characterization Phase RFPs.** The Working Group turned to consideration of two new requests for proposals, RFP 8.0, Participation in Establishing the Particle Concentration of the Adenovirus Reference Material, and RFP 9.0, Participation in Establishing the Infectious Titer of the Adenovirus Reference Material. Two proposals were presented for discussion.

*Proposal #1 (FDA)*

At least 6 testing laboratories participate.

Each testing laboratory will test reference material for virus particle count and infectivity.

All 6 labs will determine virus particle concentration via the OD260 nm method.

Infectivity assay will be done by plaque assay using one agreed upon cell line and SOP.

Each testing lab can submit other assay for VP and infectivity with SOPs.

*Proposal #2 (Alternative)*

At least 6 testing laboratories participate for particle count determination.

At least 6 testing laboratories participate for infectious titer determination.

Laboratories do not have to do both types of analyses.

Particle count:

All 6 labs will test virus particles by the same OD260 nm method/SDS

Labs may also make proposals to perform other methods to establish particle number, submitting detailed information on the SOP for the method. Purpose is to establish particle count via orthogonal approach. Methods could include PN via RP-HPLC, AE-HPLC, EM, DNA Pico Green, DNA qPCR, etc.

Objective is to establish a more accurate extinction coefficient for the OD260 nm assay.

Infectivity assay:

All 6 labs will test virus particles by same method using 1 cell line. SOP to be provided.

All 6 labs to perform NAS titer calculation to determine infectious titer.

Each testing lab may submit other assay data for infectivity with detailed SOPs.

FDA representatives saw no major differences between the two proposals. They indicated that the FDA is not wedded to the plaque assay, considering it quite variable. The FDA just wants a simple infectious titer assay that everyone can do and perform in the same way.

Discussion of the particle count proposal included whether it was important to include the orthogonal methods and whether it should be required or optional for those participating in particle count characterization. It was decided it should be optional but that labs proposing orthogonal methods must also perform the OD260/SDS method. It was pointed out that the accuracy of the particle number would be improved by taking the orthogonal approach but that some orthogonal methods may not truly correlate with particle number. Additionally there was concern that too few data points would come in using the orthogonal methods (N's of one or two labs per method vs. minimum of 6 for OD260 nm method). It was also suggested that the label could indicate "Viral Particle Concentration by OD260/SDS". While the group found it attractive to gain data from orthogonal methods they hoped that laboratories could propose cooperative bids that allowed for data from the same SOPs by multiple laboratories. It was also decided that the bid request would include a request for historical information on the proposed orthogonal method. For the OD260/SDS SOP, the composition of the formulation buffer for the reference material would be included so that laboratories could use an appropriate blank for the assay. Selective Genetics, who is providing the formulation as part of the Introgen RFP 5.0 proposal had no objections to providing those details for analytical lab use.

The Working Group agreed on the need to include the correction for diffusion in the calculation of infectious titer (NAS calculation). To ensure that the calculation was correctly performed a well annotated spreadsheet would be provided to analytical groups allowed to participate in characterization of the infectious titer so that the raw data variables required were properly included in the calculation. The Working Group discussed the common elements of infectious titer methods and agreed that the method should measure the most common definition of infection, that the virus enters the cell and reproduces itself, spreading to another cell. It was felt that plaque assay variability was a problem and that the variability could frequently be due to interpretation/definition of "plaques" and inherent issues such as controlling the agarose overlay step or plating cells to achieve appropriate 'cell lawns'.

A readout based on cytopathic effect (CPE) seemed more reasonable and easier for training. Because the UAB cell bank donation is for 100 vials the Working Group agreed that WCB cell vials should be provided to groups accepted to participate in characterization of the reference material infectious titer to lessen sources of variability. After much discussion the Working Group determined that offering an SOP for a CPE-based infectious titer assay was desirable but also providing one for plaque assay determination of infectious titer would not be necessary. When the issue was raised as to whether the reference material should be assayed in a way to allow its use as a standard or control in RCA bioassays, the FDA representatives commented that the agency is re-considering its reliance on infectivity-based assessments of RCA and may move to particle-based assessments of RCA including PCR methods. Thus the Working Group should not concern itself with characterization of the reference material with regard to RCA bioassay usage.

The Working Group also considered the need to build into the SOPs some instructions to eliminate bias in the analyses since all groups would know the nominal particle concentration provided by Introgen. It was suggested that the SOPs provided include instructions that one person make a series of dilutions that are provided to the analyst in a blinded fashion for analysis. The group also reached consensus that multiple replicate analyses would be important for both particle and infectious titer determination. For particle determination via OD260/SDS it was suggested that the reference material be brought to a total volume of 1 mL and analyzed as is, with two readings per vial and 3 vials analyzed per laboratory participating. For the infectious titer SOP, the suggestion was that there be at least two vials with 3 replicate analyses per laboratory using a dilution scheme with a history of better precision [NOTE: BioReliance will provide information on precision vs. dilution scheme for a 96-well version of this method.].

For orthogonal methods that are proposed for particle determination or alternative methods proposed for determination of infectious titer, the bid must include information on the amount of material (number of vials) requested. The SOPs are attached separately.

The Working Group decided that they would also request proposals for additional unspecified characterization of the reference material. However depending on the nature of proposals received in response to RFP 10.0, Establishment of Other Characteristics of the Adenovirus Reference Material, that the Working Group reserved the right not to accept any proposals made.

RFP 11.0 Participation in Stability Study of the Adenovirus Reference Material. The Working Group discussed the need for some concrete stability test schemes to discuss prior to fixing the requirements for the bid request. Several group members (D'Andrea, Croyle) volunteered to come up with something for the next working group meeting, addressing which tests and the test intervals as well as the number of vials and replicates per test point and test. It was discussed that sterility or bioburden recertification should be included in the assessment of stability of the reference material.

The Working Group decided that it would finish and publish the request for bids for RFPs 8.0, 9.0, and 10.0 prior to the ASGT Conference so that the conference could be used as a means to publicize them and encourage participation proposals from academic laboratories.

The Working Group agreed to meet during the ASGT conference to finish their discussion of RFP 11.0, the stability phase.

The Working Group asked the Williamsburg BioProcessing Foundation to formally contact the bidders to let them know which groups were awarded the bids for RFPs 1.0, 2.0, 4.0, 5.0, 6.0, and 7.0.

Attendees:

Aguilar-Cordova, Estuardo (Harvard)  
Beecham, Jeff (Harvard)  
Borellini, Flavia (Cell Genesys)  
Bowe, Mark (Novartis, GTI)  
Buck, Charles (ATCC)  
Butman, Bryan (GenVec)  
Byrnes, Andrew (FDA)  
Carson, Keith (Williamsburg BioProcessing Foundation)  
Croyle, Maria (Univ Texas)  
D'Andrea, Mark (Selective Genetics)  
Flagge, Frank (Introgen)  
Gilbert, Jim (MDS PharmaServices)  
Gruber, Dale (Invitrogen Corp/Life Technologies)  
Hutchins, Beth (Canji, USP)  
Keegan, Jesse (Genzyme)  
Koehl, Michel (Transgene)  
Lardenoije, Rene (Crucell)  
Lehmberg, Elisabeth (Berlex)  
Meager, Tony (NIBSC)  
Nesbit, Heike (Cell Genesys)  
Cornavaca, Eric (Invitrogen Corp/Life Technologies)  
Shabram, Paul (Canji)  
Sharpe, Geoffrey (Cobra Therapeutics)  
Simek, Stephanie (FDA)  
Sublett, Richard (Introgen)  
Vacante, Dominick (BioReliance)  
Vellekamp, Gary (Schering Plough)  
Venables, David (Covance)

Guests:

Kotov, Alexander (Univ Alabama at Birmingham)  
Jolicoeur, Pierre (Q-Biogene)  
Leduc, Annie (Q-Biogene)

Submitted by Beth Hutchins, 3/23/2001