ISBioTech 5th Spring Meeting - March 10, 2015

Reference Standard Meeting – Minutes by Suzi Melotti, bluebird bio

Keith Carson (ISBioTech) opened the meeting at 12:45 who stated that:

- ISBioTech has experience with helping groups set up reference standards for use in industry.
- The process is well defined upfront.
- That a working group is formed, and that anyone who has a valid interest can be part of the working group.
- Starting materials, cell lines, virus/ plasmid have to be well characterized. The final product and every lot thereof must be well characterized.
- 13 companies and labs were involved in the characterization of AAV5: it may not be possible to do that for LVV.
- Not talking about GMP manufacturing, talking about well documented manufacturing.
- ISBioTech will maintain the documentation of any reference standard created.
- Purpose: To validate an internal reference material and to validate the assays.
- Can we agree on what would be made that could become a reference material?
- A wildtype virus was used for Ad5 much to Keith's surprise: he didn't think anyone would use it in their labs but they have been.
- Keith also made clear that after FDA and other comments, once a protocol was settled on the working group would vote on them and there was not a single protocol or assay that went ahead with that didn't have unanimous agreement from the working group.

Harry Malech (NIH) made clear that:

- If there emerges a consensus, a space would be reserved with ASCGT for this group to meet again.
- ASCGT sponsored a standardized pathways conference where the goals were to standardize the tests that might be needed for AAV and integrated vectors. It was more focused on AAV and biodistribution issues, what was available, what companies would share.
- If one were to generate reference materials it might have value in biodistribution studies. In terms of genotoxicity issues and testing before going to the clinic if it's useful or needed based on the diversity of LVV.

Jakob Reiser (FDA/CBER) commented:

- They (FDA) had internal discussions.
- He's agnostic, but sense was that the main issues that the FDA is aware of is safety and effectiveness of transduced cell product.
- Any standards that can help along that line would be recommended highly. The FDA won't drive the effort but will look at whatever the working group decided to be called a standard and make comments.

- Sponsors should have internal standards in place; this kind of standard would help to broaden the views and gain information if it's done more broadly.
- We are all for it but we would like to know what it is all about.
- He was told that internal retroviral standards were made available before through ATCC but not sure how frequently that has been used.

Keith Carson (ISBioTech) commented that he is not familiar with that (internal standards for AAV) and was not sure if they exist in the community

Boro Dropulić (Lentigen) commented:

- LVV standard is problematic.
- Manufacturing methods are evolving, serum free medium is being used, and pseudo types are evolving. Product may change with medium, etc.
- More useful may be a cell line containing a single copy of the vector. Bora.

Harry Malech (NIH) commented:

- Suggested a standard with a specific use could be beneficial.
- Standards don't sit alone and must be used in context.
- If you want to show there is no RCL (Replication Competent Lentivirus) you must run an assay and rule out RCL.
- Big thing with testing, you do need negative and positive standard and show where your material sits relative to those standards.
- Posed the question: Could the field use positive and negative standards for certain testing?

Jakob Reiser (FDA/CBER) commented:

- A standard for RCL would be good.

Harry Malech (NIH) commented:

- A standard in genotoxicity testing could also be good.
- It's evolving, but an immortalization assay in mice by default most groups doing that have used FFD type.
- It's difficult to set up yourself: the field might benefit from choosing a certain control.

Jakob Reiser (FDA/CBER) commented:

- FDA can't comment on a specific control.

Harry Malech (NIH) commented:

- The test may change but standard control will remain the same. May be able to find a standard positive control moving forward.

Keith Carson (ISBioTech) commented:

- Keeping in mind the purpose of a reference or control is for 2 companies presenting data on their products to be compared to the same numbers. Some assays were problematic due to repeatability issues with the assays.

Mercedes Segura (bluebird bio) commented:

- If you want to standardize quantitation techniques, a reference standard would be useful.
- Depends on the goal of what the standards to be used for and then which virus could be used.

Keith Carson (ISBioTech) commented:

- It needs to be something that others can compare their own materials to and use to validate their own assay.

Yuan Zhao (NIBSC) commented:

- Instead of vector you're comparing product to, maybe a series of standards.
- What are the safety assays, what do you need conceptually for each one?
- Perhaps a panel of different types of viruses for each of those.

Keith Carson (ISBioTech) commented:

- We could ask supply/manufacturing companies to donate the materials involved.
- This was done with AAV5 but still cost main sponsor company $100K and it still took a year.

Yuan Zho (NIBSC) commented:

- If it's a control for cell based assays, control could be supernatant or lysate which is genetically defined which would be less expensive to manufacture.
- Overall in regulatory we have a concern more for safety.
- Have been thinking of RCL where the problem is to generate a generic one, and it would be based on cells and envelope.

Mercedes Segura (bluebird bio) commented:

- One idea was to have it (a reference material) for the quantitation assays.
- Based on the AAV publication the purpose of a Reference standard would be to standardize quantitation.
- It looked like something that was done, material is available, would be valuable for LVV.
- Many things could be done, we need to focus on one at a time.

Arifa Khan (FDA/CBER) commented:

- Sounds like people feel it could be useful but not sure how and in what capacity. Perhaps to make a list of what people would like to see in a reference material, what characteristics, and what, then, would be the use.
- How do people think a reference material could be used in their own situation?
- Controls for assays are important and useful for people to use the same system for their assays; it not just the vector, titer depends on cell line.

Keith Carson (ISBioTech) commented:

- Thinks more LVV applications will be seen.

Joe Hughes (Wuxi AppTec) commented:

- Not replicating is interesting, but RCL one would be interesting, people require this for patients and for FDA.
- What's the level of sensitivity of the assays?
- Agreed that we lack good positive and negative controls.

Boro Dropulić (Lentigen) commented:

- Thinks the variability is great.
- Have a lentiviral vector to monitor copy number.
- Having a defined cell that has one copy could be useful.
- Would rather use a vector that is not replicating.

Keith Carson (ISBioTech) commented:

- There are a few ways to proceed.
- Set up a linkedin group for comments.
- Or we can let if fall off and talk about it next year.

Arifa Khan (FDA/CBER) commented:

- Collective thinking of what are people aware that might already be available rather than just creating something from scratch. Knowledge sharing can be helpful.

Keith Carson (ISBioTech) commented:

- Maintaining sterility and stability testing on Ad5 is being maintained at a cost to Lonza Houston. Bluebird bio deserves credit for getting the discussion started.

Mercedes Segura (bluebird bio) commented:

- Is anyone interested in generating a specific standard with a specific purpose?

Vladimir Slepushkin (Novartis) commented:

- people who want to contribute can contribute.
- The positive control for RCL is important, but only (about) 2 labs can develop that.
- Can BREL can make positive control available for sale?
- A cell line with one copy of the vector is very useful but if every company can do it on their own we don't need a cell line that works for everybody.
- For LVV vector field the need for this standard may not be as crucial as it's used *ex vivo*.

Jakob Reiser (FDA/CBER) commented:

- There are *in vivo* applications on going.
- We are in favor of addressing safety issues and will incorporate it into guidelines if/when they become available.

Otto Merten (Généthon) commented:

- We need to pay attention to 2 different vector types, HIV and equine based virus.
- They are not the same. Equine used in brain and eye, HIV is *ex vivo* but in the future they may both be used for both.
- Most people today use HIV 1 vector types.

Mercedes Segura (bluebird bio) commented:

- Most people use HIV 1, HEK cells, VSVG and transient transfection.

Victor Lu (FDA/CBER)

- Favor a general standard, guidance recommends a reference standard.
- It is not absolutely required if there is an in house standard.
- If you have a standard, you have to verify the suitability of it.
- In addition to well characterized reference standard, suitability must be demonstrated.
- Right now no comparison can be made between companies making products.

Marci (ISBioTech) commented:

- What made Ad5 reference standard important at the time it was made, what were the drivers for that?

Keith Carson (ISBioTech) commented:

- At that time, 1999, all the adenovirus trials were stopped.
- RAC (Recombinant DNA Advisory Committee) or NIH said 'you're not doing this anymore' until we have a better understanding of the numbers you're giving us.
- Hasn't happened with LVV, we haven't killed anyone yet. People were previously dosing on infectious units or titer, and what happened was every adenovirus you put in a patient was

introducing antigens, but in meetings since everyone in the meeting the data was back to talking about infectious units, instead of total particles.

- The percent of empty capsids is not being considered anymore. Dosing based on infectious units.

Victor Lu (FDA/CBER) commented:

- Functional titer and chemico physical titers must be done; must characterize the reference standard.

Vladimir Slepushkin (Novartis) commented:–

- Agree that it's important and it will be more important with *in vivo* applications.
- The ratios of infectious to non-infectious will become more important for direct injection too.

Yuan Zho (NIBSC) commented:

- There are a lot of people here (at the reference standard meeting), so there must be interest in having the discussion.

Otto Merten (Généthon) commented:

- This has been done for AAV and has shown it's important.

Victor Lu (FDA/CBER) commented:

- If you create a reference standard it must come with specific methodologies.
- When we look at comparability, we look at final product and the critical reagent of the vector itself. How would that impact the transducer cells? If we have a different vector, it may pose problems.

Joe Hughes (Wuxi AppTec) commented:

- What came out of adenovirus was a reference standard and a few assays that we all agreed to; TCID50 infectivity assay.
- There was also an HPLC method.
- A reference standard may not be important for *ex vivo*, but it will be important for *in vivo*.

Mercedes Segura (bluebird bio) commented:

- Provided a summary AAV8 study, noting that the same methods used with high variability.

Keith Carson (ISBioTech) commented:

- It sounds like there is interest especially with more *in vivo* applications.
- Anyone doing anything would like to compare products and testing materials to reference to demonstrate control.

- Have to work to define what would make sense.
- Keith will send out an email to all who attended and arrange for a Linkedin group to be formed for further discussion.

Meeting attendees:

First Name	Preferred Name	Last Name	Organization
Kari		Airenne	University of Eastern Finland, A.I. Virtanen Institute
Wei Xia		Ang	A*STAR - Institute of Bioengineering and Nanotechnology
Thomas	Tom	Brieva	Celgene Cellular Therapeutics
Kevin		Briggs	SAFC Carlsbad Inc.
Keith		Carson	ISBioTech
Henry		Chiou	Thermo Fisher Scientific
Amitabha		Deb	Novartis Pharma
Boro		Dropulić	Lentigen Technology Inc., a Miltenyi Biotec Company
Sha		Ha	Merck Research Laboratories
Jing		Han	FDA CBER
Joseph	Joe	Hughes	WuXi AppTec Inc.
Syed	Rafat	Husain	FDA CBER
Peter		Jones	Oxford BioMedica plc
Bernadette		Keane	bluebird bio Inc.
Claudia		Kloth	Benitec Biopharma Ltd.
Kassim		Kolia	FinVector Vision Therapies OY
Nikolay		Korokhov	BioReliance Corporation
Olli		Laitinen	Karolinska Institutet
Christine		Le Bec	Généthon
Hanna		Lesch	FKD Therapies Oy
Xiaobin	Victor	Lu	FDA CBER
Harry		Malech	National Institutes of Health
Suzanna	Suzi	Melotti	bluebird bio Inc.
Otto-Wilhelm	Otto	Merten	Généthon
Sean		O'Bryan	bluebird bio Inc.
Wu		Ou	FDA CBER
Brian		Paszkiet	Thermo Fisher Scientific
Jakob		Reiser	FDA CBER
Isabelle		Rivière	Memorial Sloan Kettering Cancer Center
María Mercedes	Mercedes	Segura	bluebird bio Inc.
Anandita	Ana	Seth	Lonza Houston, Inc.
Vladimir		Slepushkin	Novartis Institutes for Biomedical Research
Xin		Swanson	Lonza Houston, Inc.
Ana Margarida Palma	Ana	Teixeira	Instituto de Biologia Experimental e Tecnológica
Barbara	Barb	Thorne	Celladon Corporation
Kim		Yang	Lonza Walkersville, Inc.
Yuan		Zhao	National Institute for Biological Standards & Control