LVV reference standard initiative

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Notes by Janet Chung, bluebird bio

Goal: global availability of reference standard material permits standardization of quantification techniques across research and manufacturing organizations
Use in calibration of internal (product-specific) reference materials

Approach: follow establishing protocol for other viral vector reference materials

Example:

ad5 ATCC VR1516
AAV2 ATCC VR1616

Expected workflow:

1. Establish working group
2. Manufacture reference standard material / generate aliquots
3. Test standard material aliquot globally / report results

There still seems to be some confusion regarding to the use of this material -> a survey may be needed to ensure all needs are met

Survey result

18 respondents:
Statistics:

- Half EU, half USA
- Academic, gene therapy company, CMO (1/3 each)

Some agreement from the survey:

1. 3rd generation, VSVG with GFP LVV was proposed as choice of construct (95% agreed)

    a. Enhanced GFP construct is proposed, although no construct is known for now (harder to keep enhanced gfp LVV stable, but histidine formulation buffer may help with stability)
    b. Promoter choice (to drive GFP expression?
    c. What is the process that will be used for purification?
2. Recommendations: 5000 vials @0.5ml per vial, titer 5e7 to 1e8 Tu/ml
    a. Boro says 5000 is feasible but aiming for 1e8 tu/ml titer
    b. Concerns that the proposed number of vials is to high
3. Support offered:
    a. Manufacturing
    b. Characterization testing in lab
    c. Safety testing
    d. Stability testing
    e. Writing of various SOP
    f. Raw material supply

Biggest cost is probably manufacturing, but optimistic on number of responses to donate products and services.

Process steps, raw materials and other manufacturing details required to understand LVV RSM characteristics need to be fully disclosed.

Testing will be performed by various academic and industry organizations with established SOP -> compile result for public view
Well characterization cell bank and plasmid will be made available

Next step -

1. Consolidate LVV RSM working group
    a. Email lvwg@isobiotech.org if interested
    b. No limit on participants

2. Obtain alignment for working groups on points of discussion
3. Meeting time and date for next meeting
4. Comments -> lvwg@isobiotech.org

Points of discussion (lead by Boro)

1. Manufacturing
    - Mercedes: how to manufacture it
    - Boro: suggested standard process to avoid IP: adherent process and standard finish fill
    - Cell line: 293 type cells- can we buy them or have them donated
    - Keith: cannot have proprietary process (what if we run out of 5,000 vials and have to re-manufacture)
    - Boro: everything should be made publically available
    - Addgene: typically will only distribute material to academic institutions but now working on industry distribution
    - Addgene could re-sequence plasmids if they need to be modified
    - Boro: cellegesis IP
    - Boro: traditional production method: CF, adherent, CaPO4 transfection
    - Someone mentioned lipofection but will dramatically increase cost of manufacturing
    - Mercedes: titer can be achieved with concentration
    - Boro: what other reagents are people thinking of other than CaPO4? PEI can also be used it is cheaper. Someone offer donations of Lipofectamine
    - Question about QC plasmid: plasmids should be fully sequenced [addgene sequenced all dna deposited routinely] CaPO4 also is not the more reproducible method.
    - Scale: 24CST= 1 lot, 2 Harvests, 25 to 2.5L of 0.5 - 1e8 Tu/ml; Vladimir mentioned FP is 2.5L so harvest should be much more

depending on the harvest titer (2 harvest x 24L scale) ; one lot of manufacturing

- Suspension process may have IP issues; adherent process is more available to everyone -> survey to see if anyone can offer a process?
- TT vs producer cell -> TT agreed
- Hand voting -> majority voted for adherent LVV, although it was noted that suspension, serum free process is the future of LVV manufacturing and PrCL still has a long trajectory to go.
- Suspension LVV DSP is not standardized and not easily made available to everyone. Otto- For adherent process there are available purification protocols.
- **Summary on USP:**
  - **Construct: 3rd generation SIN vector pseudotyped with VSVG with GFP transgene (the actual construct sequence (i.e., promoter, GFP have not been fully determined)**
  - **Adherent process with transient transfection (not sure if it would be performed with CaPO4, PEI, or lipofectamine. Lipofectamine is expensive but will be considered if donated)**
  - **Serum containing process**
  - **Cell factory type process**
  - **Media change will be performed to get rid of plasmid**
  - **2x harvest x 24L scale**
  - **Cell line, plasmids need to be fully disclosed to public and made available**

2. Purification, formulation, fill /finish
   - Purification could be basic.
   - TFF will be used to concentrate the harvest to make final titer 5e7 to 1e8 TU/ml -> aggregate formation? TFF should be optimized to avoid aggregates. Sterile filtration should be added as final step.

- Is it sufficient to just to do TFF, or also add chromatography? Chromatography should be considered to increase purify, but adding concerns for IP - maybe add diafiltration, sterile filtration
- Same organization should do the DSP as well to avoid shipping USP material
- Formation: HEPES can be used (already published) to increase stability
- Fill finish: cryovials (not glass). Boro envisions the same organization will do USP, DSP, finish/fill (manual fill). 4C is stable for LVV to fill. M1 filler can do 1,500 vials x 2 machines = 3000 vials per day. Should be filled on the same day to ensure this is one single lot
- Purity is determined by DSP process -> typical experience with TFF/chromatography DSP showed that LVV made this way is suitable for ex vivo applications. Concern was raised about the presence of host cell dna -> proposed benzonase treatment; can do qPCR +/- benzonase treatment to see how much residual dna is there. The problem is not necessary host cell, but more about residual plasmid. Impurity should not inhibit TDX as this is the main readout. Some level of impurity would also be present and expected, but should be fully characterized. When to add benzonase? One or two steps?
- Otto: tfx -> add benzonase at media exchange -> need to be discussed and agree on the process in order to be fully transparent
- CME should be included to get rid of plasmid that has not gone into cells; absolutely essential for CaPo4 process
- Re-state of the purpose of standard material; **each organization should only have access to limited # of vials to quality your in-house standard for assays**
- Purity is important factor to determine transduction ability

- **Survey to address needs of reference material should be conducted**
- Request drafts for proposal of manufacturing (from start -> finish/fill) but afraid it will take another year before things start rolling.
- Specify purity standard may be difficult and unreasonable
- **Summary:**
  - **DSP process: TFF -> chromatography -> diafiltration (into formulation buffer) -> sterile filtration [benzonase treatment is considered but not sure if it is agreed upon 1 vs 2 steps and the timing of addition (beginning or end of process)**
  - **Formulation: HEPES formulation can be used to extend LVV stability @4C**
  - **The 5,000 vials need to filled on the same day to be considered the same lot**
  - **The manufacturer will need to be able to perform USP/DSP/finish/fill (5,000 vials) in the same facility -> To Do: request drafts of proposal**
  - **A lot of concerns on purity standard on the reference material**
  - **The full manufacturing process including associated reagents will be fully disclosed and made available to public**
  - **Reference material lot info agreed: titer is 0.5e7 to 1e8 Tu/ml, 5,000 vials@0.5ml each….5000 vials may be too many**

3. Characterization and safety testing
   - Titer
     - What cell type - Hela, HT1080, Jurkat (T cell like cell); HCT116 could also be a choice. -> HT1080 is probably most universal

        - Infectious titer by Flow vs PCR (copy number) on transduced cells. qPCR vs digital PCR. Primer sequence should be made available. qPCR should be sufficient. qPCR is more standard; ddPCR needs to optimize (sometimes 2-log discrepancy to qPCR)
    - P24 ELISA is absolutely essential on viral stock - total p24, not viral associated
    - Others: viral RNA by RT-qPCR (Vladimir) on virus stock, residual plasmid DNA, residual host protein and DNA, sterility (myco), RCL
    - Characterization will be performed in multiple labs and compiled data made available to public
    - Titer protocol should be very detailed (what cell, how to dilute, incubation, etc)
    - GFP has IP issue? MTA may grant access to third party use for a specific purpose. Cyan(?) may be open (without having to pay royalty, Addgene suggestion)
    - Reagents and SOP will be made available and standardized
    - **Summary:**
        - **Reagents and SOPs for characterization assays (titer, p24 ELISA to determine total viral particle) will be made available**
        - **Titer (by GFP flow and copy number by standard qPCR) should be performed by multiple labs (academic and industry) with compiled data made available**
        - **Other characterization assays suggested include viral genome RNA (RT-qPCR), residual plasmid DNA (qPCR), residual host protein (SDS-PAGE), residual host DNA**
4. Vector safety testing: - one time test per lot
    - Mycoplasma
    - Sterility
    - Endotoxin
    - Bioburden

- RCL
- Adv virus may be too expensive
- Others? Adventitious agent test (expensive!)
- S**ummary:**
  - **Mycoplasma, sterility, endotoxin, bioburden, RCL are common assays to test LVV safety**
  - **Some debate on adventitious virus testing - some people say required, some disagree. This testing is also very expensive**

5. Drafting request for donation
   - Cell line
   - Plasmid
   - Production
   - Purification
   - Formulation and short term stability testing
   - Safety testing
   - Filling
   - Characterization
   - Long term stability and sterility test
   - Comment:
   - **Summary:**
     - **Email distribution list to facilitate donation of above items**
6. Stability study (short vs long term)
   - **Stability testing should be done on the same site (multiple organization if possible).**
   - **Should also include shipping stability**
   - **Boro shared his experience in long term (5-7 years) stability for LVV stored at -80C - no change in titers**
7. Distribution
   - **Addgene could help with virus distribution (they are currently distributing virus stocks in their catalog)**
   - **NIBSC could help with storage and distribution**

- **Shouldn’t be a company but an organization as ATCC, NIBSC. ATCC cost has gone up.**
- **Should have a limit on # of vials that be requested by the same institution per year**
- **Certain # of vials needs to be reserved as "bridging material" for the next batch of reference material - this # still needs to be determined**
- **Reference material will be stored in -80C (not liquid nitrogen)**
- **A point of contact is needed to keep track of user request and inventory**

8. Cost
    - Storage (-80C) cost
    - Distribution cost-> point of contact to keep inventory (should be a limit # per year, for example)
    - Shipping
    - Manufacturing
    - Testing
    - **The reference material will be available to everyone but with a cost to recover costs not limited to 8a-8e**
9. Other points
    a. What happens when inventory is low and new batch is needed? How to bridge material? This will depend on stability of the product. Boro has not seen titer drop in -80c for 5-7 years storage. Need to define # of retains needed for bridging (cannot be distributed).
    b. Victor Lu. Need to ensure continuity. Depends on stability. 7+ years shown already
    c. FDA said WG is good to discuss preparation.

Other reference material, e.g. VCN, is also important but not scope of this meeting