Teleconference of ARMWG Subgroup investigating the additional peak observed on RP-HPLC assay:

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Participants:

Keith Carson (WBF)
Lois Chandler (Selective Genetics)
Michael Ong (Selective Genetics)
Shawn Gallagher (Introgen)
Beth Hutchins (Canji)
Elisabeth Lehmberg (Berlex)
Marie Printz (Selective Genetics)
Paul Shabram (Canji)
Vikki Sluzky (Onyx)
Gary Vellekamp(SPRI)

Minutes:

Gary Vellekamp and Elisabeth Lehmberg recapped the original observations and data from the RP-HPLC assay analyses. A highly chromogenic material identified by a unique peak at 214 nm in the RP-HPLC assay that is not adenovirus was found. This material consists of more than one peak when scanned at 260 nm. The material has a local maximum at 240 nm. One vial of ARM LN 001503 was found to have a "high" level of the material, approximately 4700 area units @ 214 nm. Other vials of ARM LN 001503 and single vials from ARM LN 001504, LN 001505, and LN 001506 also contain the material but at a lower level generally ranging from 100 to 300 area units @ 214 nm. Vellekamp's lab also found that the material is not associated with the virus because if vials were centrifuged, the material was in the supernatant. Analysis of the 1 vial with the "high" level of material and 1 vial with "low" material were also analyzed by LC-MS. The material showed as very low or no molecular weight. Vials from Maria Croyle's short term stability study were also sent to Vellekamp's lab for analysis; there were 6 labeled aggregated and 3 labeled normal. The "aggregated" vials were centrifuged and the supernatant analyzed. The "aggregated" vials contained 250 to 400 AU peaks while the "normal" vials contained 250 to 300 AU peaks.

The ARMWG data showed that the OD260nm/SDS SOP data was very tight (with the exception of 1 lab) and that the infectious titer SOP data was also reasonably tight. Although Vellekamp's lab found a contribution of approximately 30% to the calculated particle concentration via the OD260nm/SDS SOP for the vial with the "high" level of material, the other vials he examined did not seem to have a readily measured contribution from the peak material. The 1 lab with the unusually high OD260nm data will not be included in the determination of the official ARMWG assigned particle concentration. The lab indicated that they believed the data was suspect, i.e., the protocol was probably not followed correctly. However it was agreed to find out if retain vials were available for additional analysis.

Vikki Sluzky, Marie Printz, Elisabeth Lehmberg, and Paul Shabram all recounted past situations where they had found a similar contaminant peak, low molecular weight and highly chromogenic. In Berlex's case, they were able to perform GC-MS analyses and identified the contaminants as plasticizer chemicals that likely leached from tubing. The other laboratories also confirmed that leachate from plastics appeared to be the source in their cases as well.

From this recap of available data, the subgroup concluded that:

- The peak material observed appears to be a highly chromogenic low molecular weight material that is not associated with the virus itself, and likely a plasticizer chemical leachate.
- The material appears to be a contaminant that is in the ARM but at a low level. However the frequency of high levels is not completely clear. Although vials from all 4 lots of the ARM contain the material, very little analysis has been performed on the 3 lots not used for most of the characterization phase.
- There appears to be no relationship between the short term stability data from Croyle's lab (showing less short term stability as compared to the data from GTI and Transgene) and the presence of the peak material.
- It appears that the average user of the ARM would not be affected by the presence of the peak material in their ability to perform particle determination or infectious titer assays, even for the RP-HPLC assay.
- Everyone agreed that the particle concentration derived from the ARMWG OD260nm/SDS SOP is reliable even with the contaminant in the ARM.
- The product insert will contain information about this contaminant.

The subgroup decided that it was important to do some additional studies to get a better understanding of the frequency of "high" level contaminant-containing vials in all 4 lots. So far only 1 vial from LN 001503 has been found to have the "high" level. However no vials have been found that do NOT contain the contaminant.

The subgroups also proposes to analyze available retains from the process to determine where in the process the contaminant might have arisen. Introgen has retains from all 4 lots available. Introgen will determine what retains exist from the formulation buffer itself (w/o virus).

The subgroup agreed to the following plan for additional analyses:

a. SPRI/Vellekamp will analyze based on the Berlex RP-HPLC assay 10 additional vials of LN 001503 as well as the in process materials from Introgen. At least some vials will also be analyzed by LC-MS.
b. Berlex, Onyx, and Selective Genetics all agreed to analyze 10 vials of a sublot based on the Berlex RP-HPLC assay.
c. Berlex will analyze material via GC-MS after subgroup review of analyses. Vials to be analyzed will be forwarded to Berlex.

The subgroup proposes to have ATCC ship the following ARM vials to allow these analyses:

10 vials of LN 001503 to Gary Vellekamp/SPRI
10 vials of LN 001504 to Elisabeth Lehmberg/Berlex
10 vials of LN 001505 to Vikki Sluzky/ONYX
10 vials of LN 001506 to Marie Printz/Selective Genetics

The subgroup proposes to complete all analyses and report back to the ARMWG at the June ARMWG meeting at ASGT.