Adenoviral Reference Material Working Group
Bid Submission Form
Long-term Stability Studies
RFP 12.0

Please complete the following fields: This is a group submission; Beth Hutchins is the group coordinator.

Contact Information – RFP 12.0

*Contact Individual:	Beth Hutchins, Ph.D.
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***If laboratories are submitting a proposal as a group, a main contact should be provided along with contact information for each participating laboratory (attach additional copies of this form).**

Please indicate if your institution is also submitting proposals for the other activities:

- [X] Determination of Particle Concentration
- [X] Determination of Infectious Titer
- [ ] Short-term Stability Study
- [X] Other Characterization
- [ ] Donation of Supplies/Other Services for Characterization Phase

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Please complete the following fields:

Contact Information – RFP 12.0

*Contact Individual:	Michael Grace, Ph.D.
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***If laboratories are submitting a proposal as a group, a main contact should be provided along with contact information for each participating laboratory (attach additional copies of this form).**

Please indicate if your institution is also submitting proposals for the other activities:

- [X] Determination of Particle Concentration
- [X] Determination of Infectious Titer
- [ ] Short-term Stability Study
- [X] Other Characterization
- [ ] Donation of Supplies/Other Services for Characterization Phase

Experience and Capacity:

Canji, Inc. is a wholly owned subsidiary of Schering Plough Corp., specializing in adenoviral vector gene therapy including pharmacology, analytical method, and process development. Canji's Process Sciences' personnel have a well-established reputation for expertise in adenoviral vector technology, evidenced by publications and issued patents related to production and characterization methods. The BioAnalytical Group in Biotechnology Development Operations at Schering Plough Research Institute (SPRI) is the group receiving methods from Canji to support clinical development of adenoviral gene therapy products

The BioAnalytical/QC Group in Process Sciences would perform the long-term stability study under the leadership of Dr. Barry Sugarman and Paul Shabram. Additionally, Dr. Michael Grace (SPRI) would oversee performance of the electron microscopy analyses and Dr. Peter Ihnat (SPRI, Development Operations) would review final protocols and data. Dr. Grace has been involved in transfer and validation of methods from Canji to SPRI supporting clinical development of SCH 585800 (rAd-p53) and SCH 412499 (rAd-p21). Dr. Ihnat has been responsible for developing formulations for adenoviral vectors at SPRI, and developed the formulations for SCH 58500 (rAd-p53) and SCH 412499 (rAd-p21).

Although no company can forecast with certainty what lies in their future, Canji believes that we can reasonably commit to the five year program outlined below.

Experimental Plan:

All equipment to be used for storing or analyzing the Adenovirus 5 WT Reference Material is part of Canji's calibration & preventive maintenance program. Freezers are monitored 24-hr/day with alert notification via paging; these items are also on emergency back-up power.

The attached charts indicate the timepoints and methods to be used, along with replicate and vial numbers. Vials will be stored at two temperatures, -80°C and –20°C. All tests for T=0 will be initiated within 30 days of the first date of manufacture/filtration by Introgen. Testing for all other timepoints will take place within a ±2 weeks of the scheduled date.

Method Descriptions and replicates proposed per method:

- Particle number by the OD 260nm/SDS method supplied by the Working Group (see RFP 8.0). A total of 4 replicates will be performed per time point, utilizing a total of 2 vials per time point.
- Infectious titer by the method supplied by the Working Group (see RFP 9.0). A total of 3 replicates will be performed per time point, utilizing a total of 2 vials per time point.
- Resource Q HPLC Assay. A total of 4 replicates will be performed per time point, utilizing a total of 2 vials per time point.
  - *Reference*: **P.W. Shabram,** D.D. Giroux, A.M. Goudreau, R.J. Gregory, M.T. Horn, B.G. Huyghe, X.D. Liu, M.H. Nunnally, B.J. Sugarman, and S. Sutjipto. Analytical anion-exchange HPLC of recombinant type-5 adenoviral particles. *Human Gene Therapy* 8, 453-465 (1997)]
  - *SOP previously supplied to WBF and ARMWG*
- Particle Size Analysis via Coulter N4 instrument – Particle size determined a total of 2 replicates per time point, utilizing a total of 2 vials per time point.

- Light scattering at OD 320 nm (reported as the ratio of UV absorbance at OD 320 nm to OD 260 nm w/o SDS) provides a qualitative assessment of aggregation status. A total of 2 replicates will be performed per time point utilizing a total of 2 vials per time point.
- Electron microscopy will be used by the SPRI BioAnalytical Group to assess particle integrity and the presence of micro-aggregates. This analysis will be performed using 1 vial per time point listed.
- Sterility will be performed as a surrogate for container integrity via the direct inoculation method. A total of 10 vials will be utilized for each time point. This number of vials may represent only 0.2% of the total vials manufactured (if 5000 vials are manufactured).

An additional method may also be applied at a limited number of time points, *i.e.*, Scanning Calorimetry. This strictly research method is currently being examined at SPRI for its ability to provide information related to adenoviral vector stability. If SPRI finds the method useful, the analysis would be performed by SPRI Development Operations. Two vials per time point would be required; with a maximum of 4 time points anticipated (0 mos, 12 mos, 36 mos, and 60 mos).

The amount of material required for each test is listed in the following tables. Replicates will be performed in order to provide better assurance of measurements taken. A **total of 236 vials** of the purified Adenovirus 5 WT Reference Material are requested to monitor stability at both –80°C and –20°C. Additionally we would request 2 vials of the 293 Test Cell Bank be made available to support the infectious titer assay. Canji would expand a vial of the 293 Test Cell Bank, banking that to support the 5 year schedule of infectious titer assays.

At each time point, vials will be allowed to thaw at room temperature and will be aseptically mixed during the thaw process by trituration using a micropipette. Containers will then be utilized for their assigned analytical method immediately, or stored at 2-8°C for up to 4 hours until analysis begins.

Data will be submitted to the Working Group after the analyses at T=0 and annually thereafter, unless data at 6 or 18 months warrants earlier disclosure. Canji and SPRI will be ready to begin the T=0 analyses in early September.

Vials Monitored For Stability at –80°C Storage

-80°C	***Method***							
Time Point	*Particle Conc'n*	*Particle Conc'n & Quality*	*Bioactivity*	*Aggregation*	*Aggregation*	*Aggregation*	*Aggregation (if found useful)*	*Container Integrity*
	OD260 nm SDS	Resource Q HPLC	Infectious Titer	Particle Size Analysis	Light Scattering	Electron Microscopy	Scanning Calorimetry	Sterility
T=0	X	X	X	X	X	X	X	nd
T= 6 mos	X	X	X	X	nd	nd	nd	nd
T=12 mos	X	X	X	X	X	X	X	X
T=18 mos	X	X	X	X	nd	nd	nd	nd
T=24 mos	X	X	X	X	nd	X	nd	nd
T=36 mos	X	X	X	X	X	X	X	X
T=48 mos	X	X	X	X	X	X	nd	nd
T=60 mos	X	X	X	X	X	X	X	X

-80°C	***Vial Numbers per Method***								
Time Point	*Particle Conc'n*	*Particle Conc'n & Quality*	*Bioactivity*	*Aggregation*	*Aggregation*	*Aggregation*	*Aggregation (if found useful)*	*Container Integrity*	***Total No. of Vials***
	OD260 nm SDS	Resource Q HPLC	Infectious Titer	Particle Size Analysis	Light Scattering	Electron Microscopy	Scanning Calorimetry	Sterility	
T=0	2	2	2	2	2	1	2	nd	13
T= 6 mos	2	2	2	2	nd	nd	nd	nd	8
T=12 mos	2	2	2	2	2	1	2	10	23
T=18 mos	2	2	2	2	nd	nd	nd	nd	8
T=24 mos	2	2	2	2	nd	1	nd	nd	9
T=36 mos	2	2	2	2	2	1	2	10	23
T=48 mos	2	2	2	2	2	1	nd	nd	11
T=60 mos	2	2	2	2	2	1	2	10	23
Total No. of Vials	*16*	*16*	*16*	*16*	*10*	*6*	*8*	*30*	***118***

Vials Monitored For Stability at –20°C Storage

-20°C	***Method***							
Time Point	*Particle Conc'n*	*Particle Conc'n & Quality*	*Bioactivity*	*Aggregation*	*Aggregation*	*Aggregation*	*Aggregation (if found useful)*	*Container Integrity*
	OD260 nm SDS	Resource Q HPLC	Infectious Titer	Particle Size Analysis	Light Scattering	Electron Microscopy	Scanning Calorimetry	Sterility
T=0	X	X	X	X	X	X	X	nd
T= 6 mos	X	X	X	X	nd	nd	nd	nd
T=12 mos	X	X	X	X	X	X	X	X
T=18 mos	X	X	X	X	nd	nd	nd	nd
T=24 mos	X	X	X	X	nd	X	nd	nd
T=36 mos	X	X	X	X	X	X	X	X
T=48 mos	X	X	X	X	X	X	nd	nd
T=60 mos	X	X	X	X	X	X	X	X

-20°C	***Vial Numbers per Method***								
Time Point	*Particle Conc'n*	*Particle Conc'n & Quality*	*Bioactivity*	*Aggregation*	*Aggregation*	*Aggregation*	*Aggregation (if found useful)*	*Container Integrity*	***Total No. of Vials***
	OD260 nm SDS	Resource Q HPLC	Infectious Titer	Particle Size Analysis	Light Scattering	Electron Microscopy	Scanning Calorimetry	Sterility	
T=0	2	2	2	2	2	1	2	nd	13
T= 6 mos	2	2	2	2	nd	nd	nd	nd	8
T=12 mos	2	2	2	2	2	1	2	10	23
T=18 mos	2	2	2	2	nd	nd	nd	nd	8
T=24 mos	2	2	2	2	nd	1	nd	nd	9
T=36 mos	2	2	2	2	2	1	2	10	23
T=48 mos	2	2	2	2	2	1	nd	nd	11
T=60 mos	2	2	2	2	2	1	2	10	23
Total No. of Vials	*16*	*16*	*16*	*16*	*10*	*6*	*8*	*30*	***118***