Assays For An AAV Vectored HIV Vaccine

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Targeted Genetics

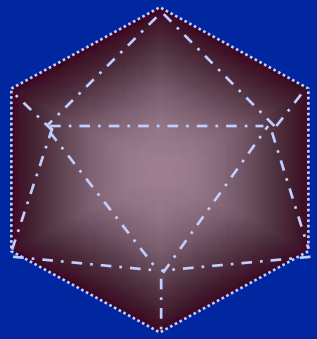
AAV-based HIV-1 Vaccine tgAAC09

- Collaborative development program
  - Targeted Genetics
  - International AIDS Vaccine Initiative (IAVI)
  - Phil Johnson (CCRI/CHOP)

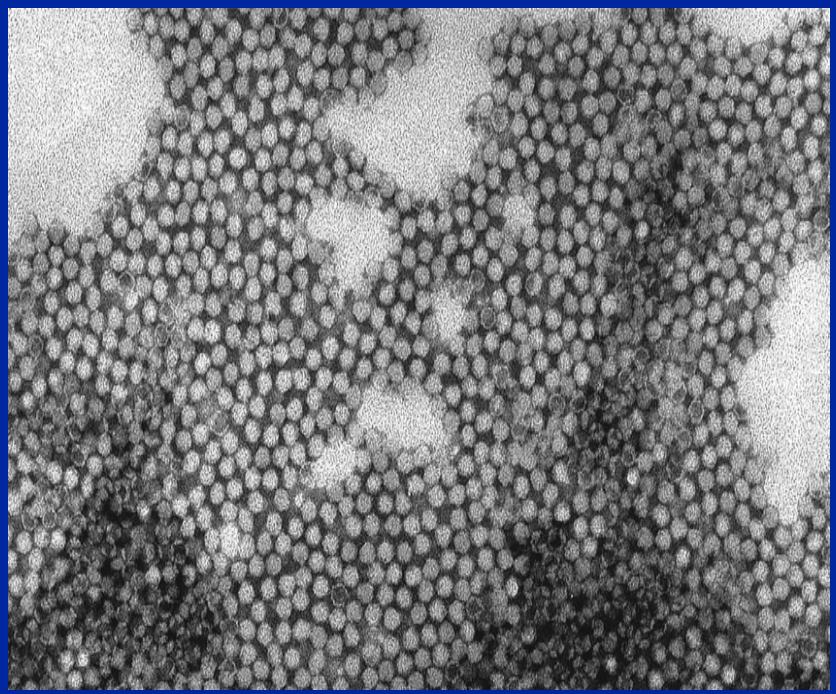
Rationale For AAV As A Vaccine

- rAAV vaccine is simple, non-replicating
- Elicits robust and durable immune response after single dose in monkeys
- Both antibody and T-cell responses are induced
- rAAV vaccine protects monkeys against virulent SIV challenge (plasma load ↓; slow disease)
- Highly purified and well characterized product
- Extensive pre-clinical testing confirms safety
- Good safety profile in humans as gene therapy vector

- Phase 1 trial underway in Belgium, Germany & India



The AAV Particle



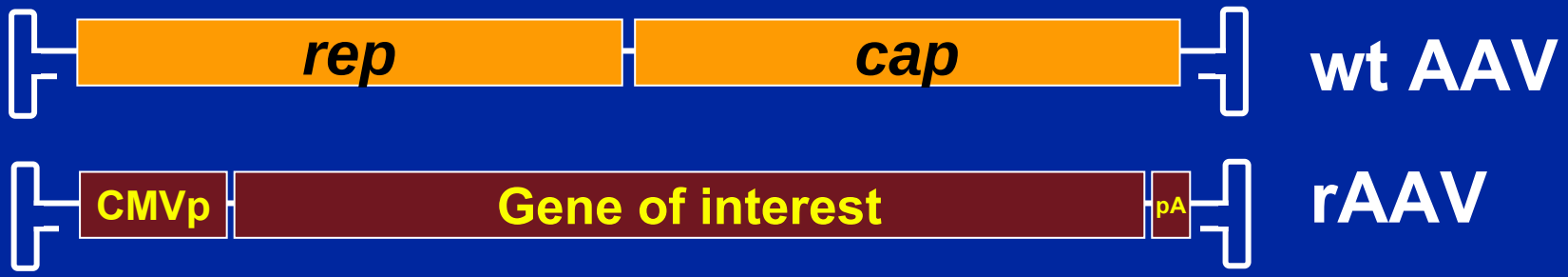
- Parvovirus
- 25 nm virion
- Non-enveloped
- Icosahedral capsid
- VP1, VP2, VP3

Biology of Wild-Type AAV

- Replication defective
- Requires helper virus (ad, herpes)
- Site-specific integration in cultured cells
- *No association with any disease, tumor, or other pathologic condition*

rAAV Vectors Are Simple

- Based on wild-type AAV
- Devoid of any AAV genes

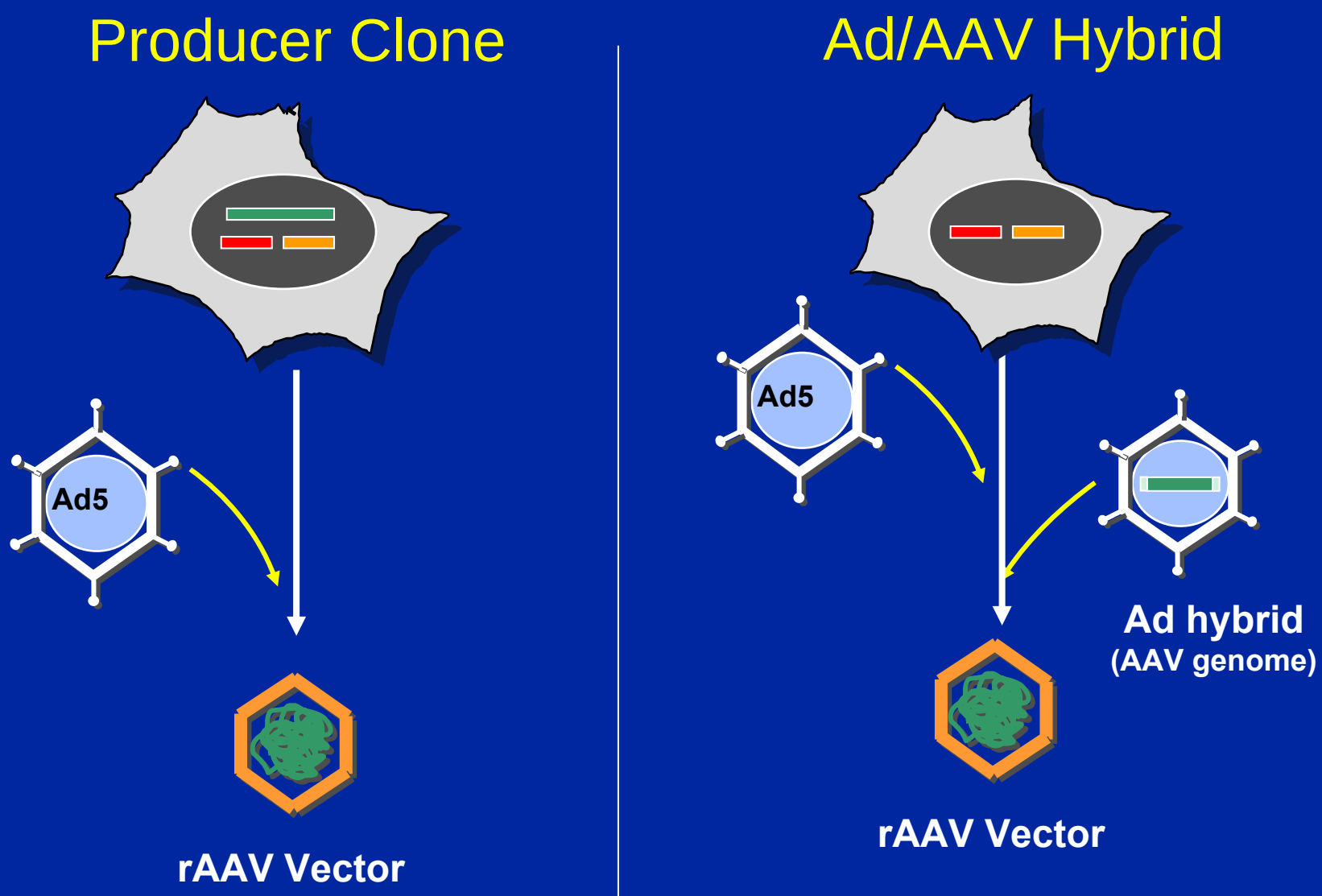


Recombinant genome contains
< 400 nt of wild-type AAV sequence

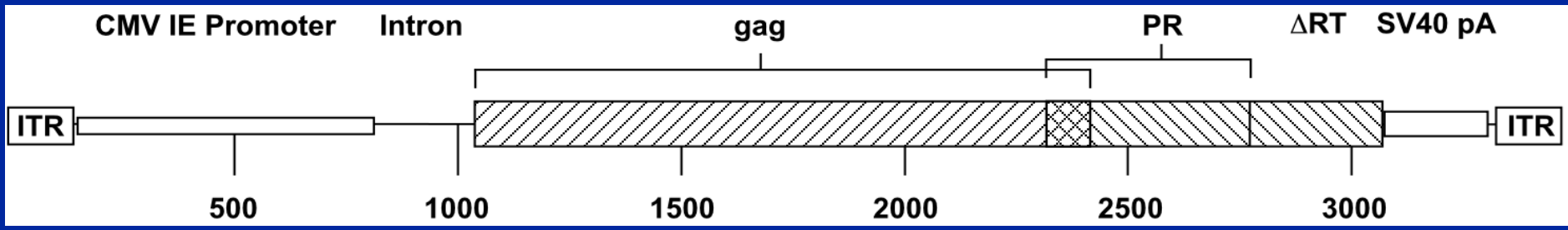
Biology of rAAV Vectors

- "Doubly" replication defective
  - > Requires rep/cap + helper
- Behave like other DNA transfer vectors in cultured cells
- Genomes persist as episomal concatamers in vivo
- Numerous ongoing and proposed clinical trials

Scalable Approaches for rAAV Production



tgAAC09 Vaccine Genome

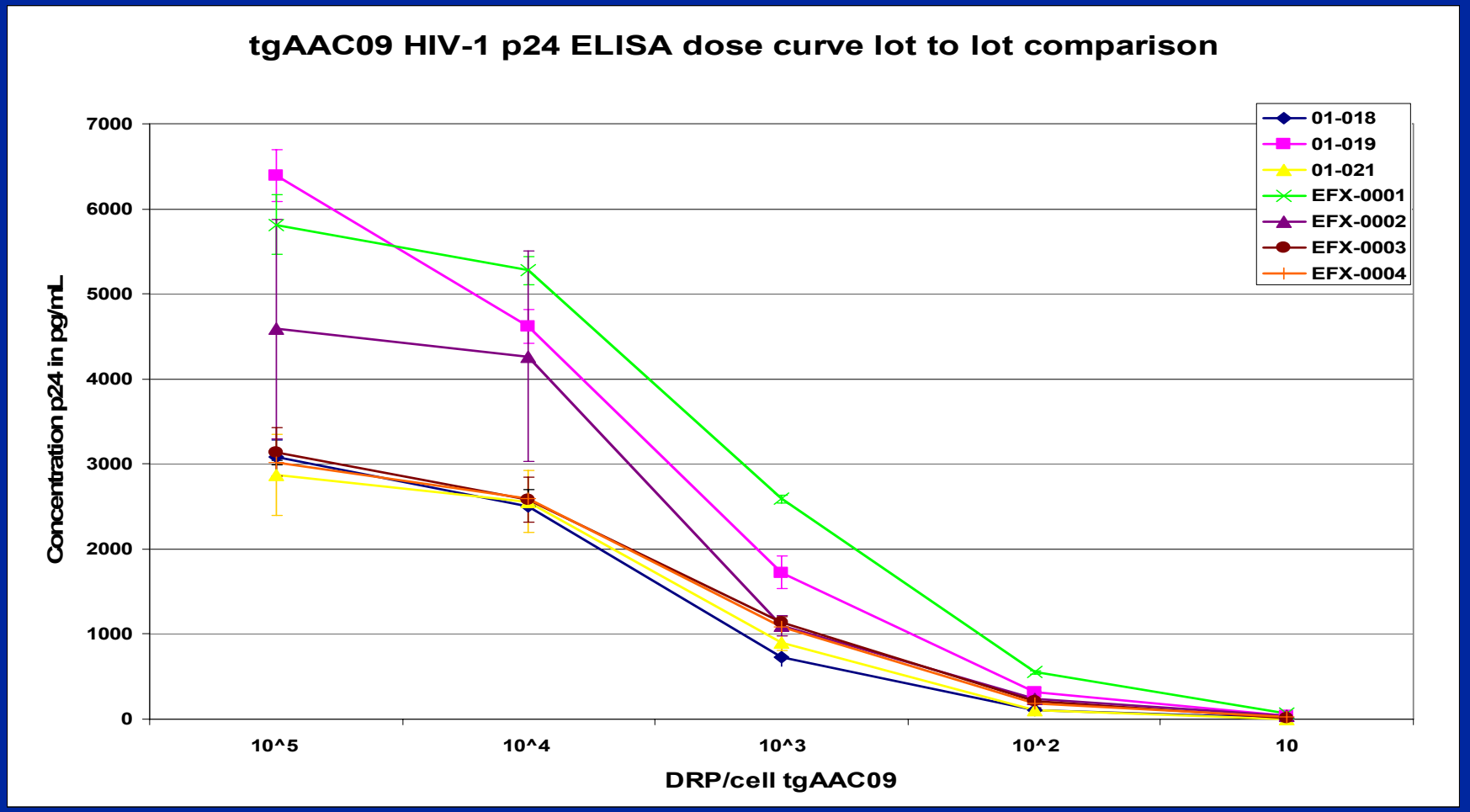


- Clade C
- Circulating virus, near South Africa consensus
- Humanized codons
- Packaged in AAV-2 capsid
- Prototype design

Assays For Characterizing rAAV

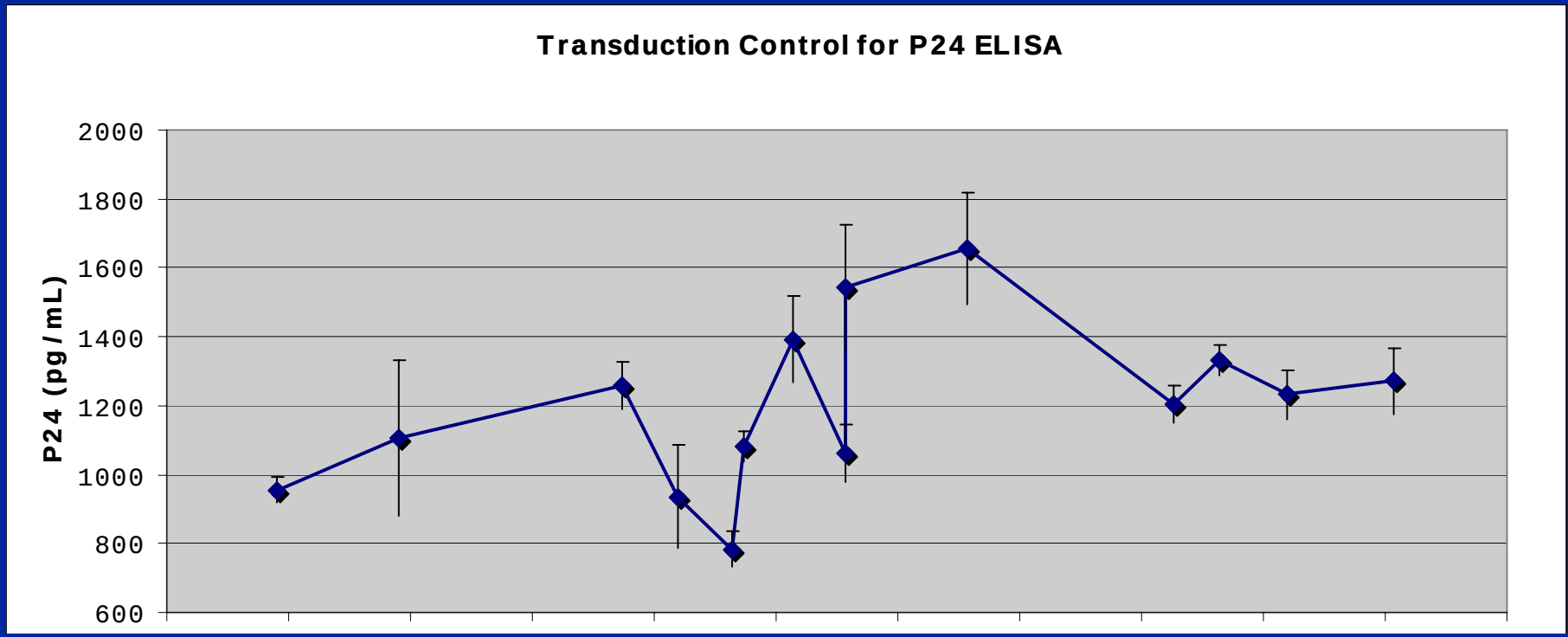
- In vitro (strength)
  - Particles containing DNA (QPCR, DRP); high precision
  - Infectivity (cell-based, $TCID_{50}$ format, DNA replication); lower precision
    - P:I useful for lot-lot consistency, quality
    - P:I in vitro does not necessarily correlate with in vivo efficacy
      - AAV2 lower P:I than AAV1 but AAV1 more potent in vivo
- In vitro (potency), transgene expression
  - Transduction (transgene-specific)
    - Transduction portion (cell-based) less precise
    - Read-out=ELISA for p24; high precision
- In vivo (potency)
  - Anti-transgene titers; ELISA
  - Anti-transgene cellular response; ELIspot, etc
  - Dose and time variables

Dose-response of p24 Expression in Cell Culture



- In vitro potency assay
- 7 vector lots
- Dose-responsive expression, similar curves

In vitro Potency Assay
Reference Control Performance



- single-MOI transduction , 14 assays, CV of 20%

Pre-clinical Evaluation in Two NHP Experiments

- dose-ranging in rhesus macaques
- AAV2 vs AAV1

Dose Response Study

tgAAC09 Vaccine administration IM

3.3×10^9 DRP n = 6
3.3×10^{10} DRP n = 6
3.3×10^{11} DRP n = 6
3.3×10^{12} DRP n = 6

Immune Assays

anti-gag ELISA

IFN-γ ELIspot

Same Vaccine DNA in AAV-1 capsid

gag-PR in AAV-1 capsid
3.3×10^{8} DRP n = 6
3.3×10^{9} DRP n = 6
3.3×10^{10} DRP n = 6
3.3×10^{11} DRP n = 6
AAV-2
3.3×10^{11} DRP n = 6

Immune Assays

anti-gag ELISA

IFN-γ ELIspot

Conclusions

- A single administration of tgAAC09 [AAV-2] induces:
  - ✓ Dose-dependent, long-lasting antibody responses
  - ✓ Robust and dose-dependent IFN-gamma SFC
  - ✓ Pools of cells that can rapidly expand to produce both antibody and IFN-gamma

- AAV-1 is more efficient than AAV-2
  - reverse is true in vitro
  - cell substrate receptor/trafficking issue

Conclusions

- Have in vitro vector characterization assays, vector quality & consistency
- Have quantitative in vitro transgene expression assays, potency
- Have quantitative assays to measure anti-transgene antibody responses and cell-based anti-transgene immune responses in vivo, potency
- In vitro transgene expression correlates with animal models
  - dose-responsive transgene expression in vitro & anti-transgene responses in vivo, humoral and cellular
- Current focus- in vitro assays (vector characterization and consistency, quantitative transgene expression) for phase I/early phase II