Viral Design:
Impact of Early Integration Across R&D and Process Development Workflows to Accelerate Development

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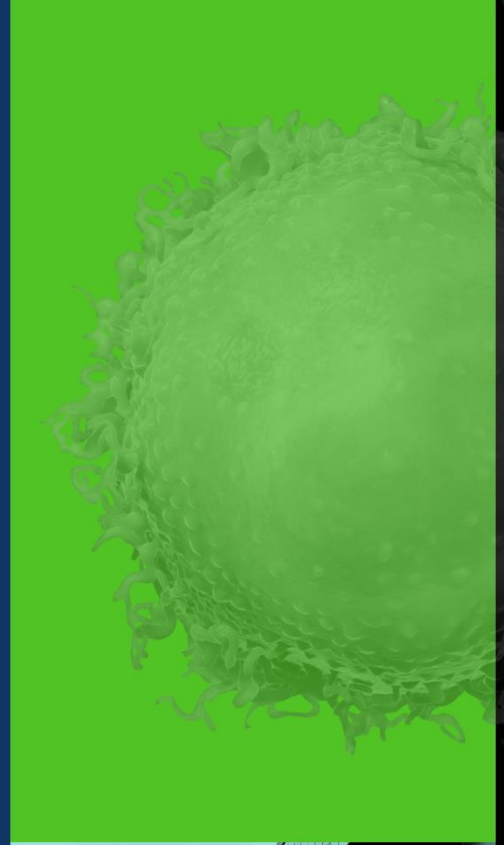

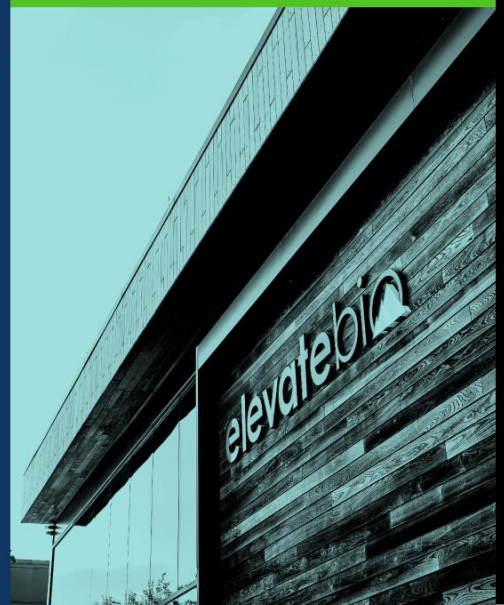

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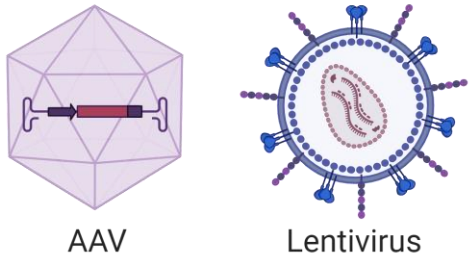
Outline of Today's Presentation:

- **Challenge: Many cell & gene therapy companies struggle to find the right balance between screening an adequate number of novel viral vector construct designs and moving quickly through development to obtain a candidate with necessary vector metrics**
- Transition from R&D to PD can be bumpy with delayed timelines- how to improve?
- What vector metrics are critical to incorporate in R&D?
- What factors should be considered in viral vector design?
- Case Study #1- Improving AAV Packaging
- Case Study #2- Lenti: Improving TCR Expression
- Key Learnings & Takeaways



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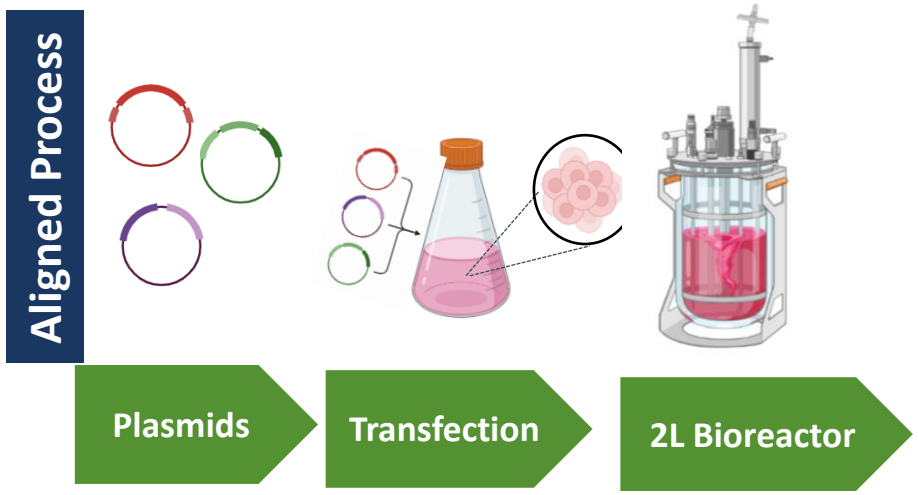
Aligned R&D and PD Process Aids in Generating Reliable Data Quickly



- Key elements to an aligned process:
  - **Same plasmids**
    - Allows for consistency on size/plasmid ratios
  - **Same transfection conditions**
    - Transfection reagent
    - Cell seeding
  - **Same cell line (recommend suspension)**
    - Removes additional factor of adherent vs suspension production

- Suspension cells for the win!
  - Start early with aligned process that PD and manufacturing will be using
  - Why optimize twice or make a decision in a less that equivalent system?
  - **This is a huge risk**, if not aligned!

- Aligned upstream protocols provide early read on data that's **reproducible** in the PD team
  - Helps kick off early scale-up work faster
  - R&D can do preliminary DoE/optimization testing in shake-flasks

Vector Metrics to Incorporate Early in Development

- **Understand early what stage-gates are needed to drive a successful process development campaign to IND!**
  - **This means obtaining feedback beyond the R&D team: CMC reg, Process Development, TechOps, etc.**
  - This could be a draft target product profile (TPP)
  - Perhaps an early conversation with PD lead on key metrics they need for development
  - Or metrics a potential CDMO is looking to de-risk early
- **TITER, TITER, TITER!!**
  - If you can't hit a minimum titer that PD can work with…that's a red flag
- **Scalability**
  - Small-scale flask data is great early on, but what really matters is your exact scaled down process- typically 2L
  - Reproducibility matters as you look to kick-start development

AAV special considerations:

% Full: Understanding Fulls, Partials, Empties

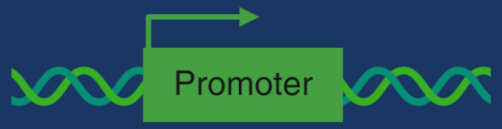
-Analytical Ultracentrifugation (AUC): gold standard for testing, but need specialized equipment and expert to test
-Mass Photometry: method can be leveraged earlier in the process to quickly assess fulls

3rd Generation Sequencing:
Is the vector packaging the therapeutic or is it junk?

-Confirm full therapeutic cassette is accurately packaged
-AAV genome can be tricky given the secondary structure of the DNA

Factors for Design Consideration in Viral Vector Space:

Promoters



- Short, long, contains intron?
- Custom for application / tissue
- Size critical in AAV
- IP considerations

Codon Optimization

- No algorithm is perfect
- Must be tested
- Need to consider whether low/high expression best

PRE Elements

- Regulatory elements may vary by application
- Does the seq fit into the design?
- FDA safety considerations

Size/Orientation

- Ideal size for your therapeutic?
- Insert size depends on what the viral vector can handle
- Options for orientation

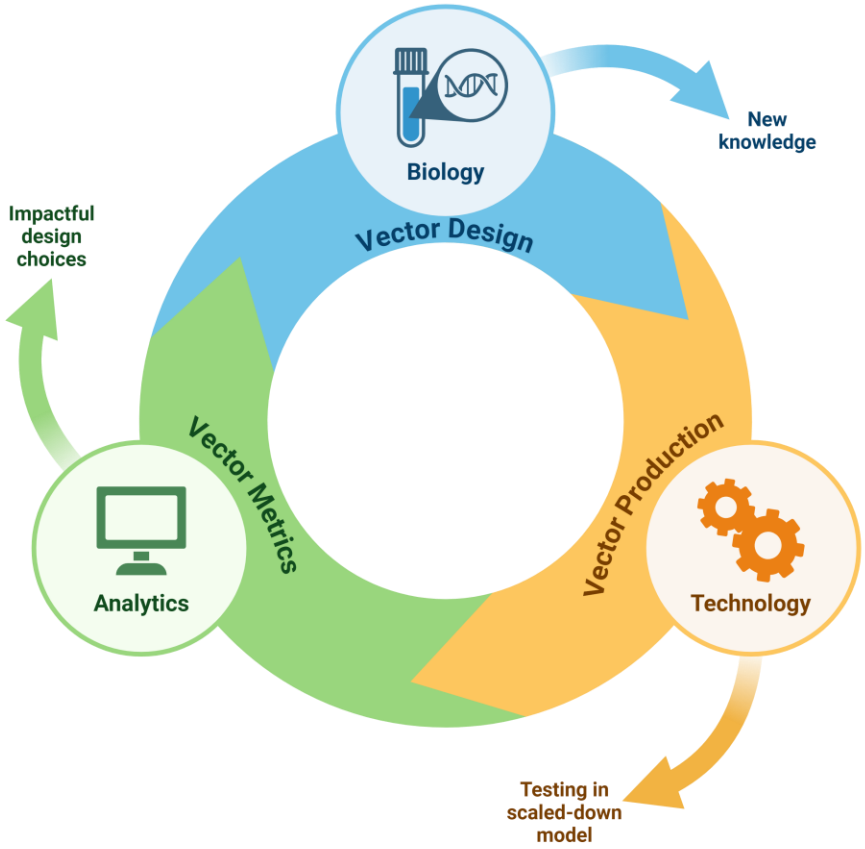
Engineering

- Increase correct pairing for TCRs
- Point mutations for increased performance
- Leveraging concepts from literature

Plasmid Stability

- Concern for AAV plasmids
- Consistent yield and scale-up
- Need to achieve QC metrics: no ITR truncations, full sequencing

Vector Design and Metrics are Interconnected but Take Time to Vet



- Must empirically test designs- cast a wide net on initial designs
- Take designs through production to test for efficacy and key vector metrics
- Build a workflow with the right stage gates to find your top candidate
- If multiple teams are involved- clearly define expectations and timelines

Take home:
Build in enough time for this process so you can screen multiple candidates and select the best candidate with acceptable vector metrics

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Pain Early or Pain Later: Decision on When to Push for Vector Metrics in Development

Research/Early CMC | Process Development / Late-Stage CMC

IND

OR

IND

Goal: Arrive at the <u>BEST</u> candidate nomination, which might not be the first candidate….

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Finding the Right Balance to Deliver the Right Candidate:

Speed

-Nominate a lead candidate
- GMP plasmid manufacturing (6-9 months)
-Viral vector CDMO (16 months+)

Quality

-Premature commitment to the wrong candidate long-term impact
-Need to hit key vector metrics for success

Cost

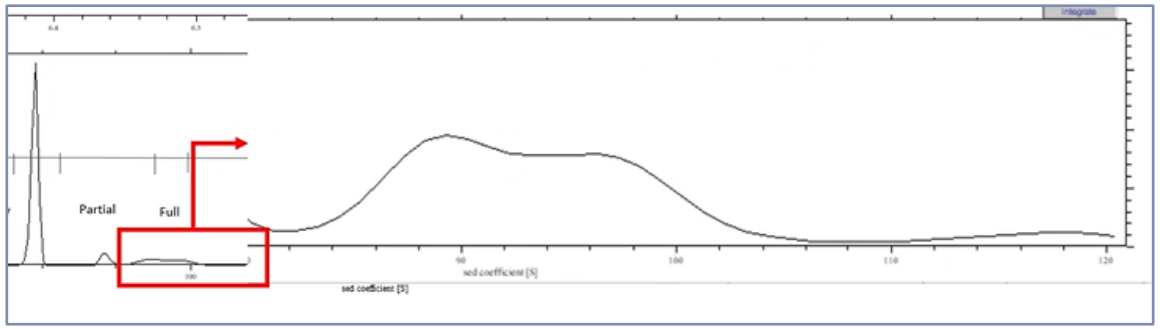
-Development costs $$ PD> R&D to characterize candidate
- Candidate not hitting key metrics, means higher COGs to long-term

Efficiency

-Don't want the program to stall in later stages
-Sunk costs
-Frustrating for the teams
-Major let down for PATIENTS!

Case Study: #1 Improving AAV Packaging

- **Challenge: One of top AAV candidates coming out of *in vitro* and early *in vivo* studies has less than desirable vector metrics- how do we de-risk this before committing to a lead candidate for development?**
- Metrics on track from R&D testing:
  - ☑ Titer
  - ☑ Efficacy
- But some of the initial vector metrics were not ideal
  - ☒ AUC profile showed a **bi-modal peak in the full peak (should be 1 single peak)**
    - **This could be a huge CMC risk later in development**



Decision: From initial data we decided to further pursue additional testing & candidates to de-risk later development work

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Case Study: #1 Improving AAV Packaging

What's causing this issue? How to de-risk?

VS

1) **Hypothesis #1 -Production Issue: Are we creating less than ideal environment for vector packaging?**
    1) Transfection conditions- run small DoE to evaluate different conditions
    2) Repeat the data in multiple hands: R&D and PD
    3) Testing with multiple analytics- including AUC and Mass Photometry

    Results: Repeat testing produced similar results, and no transfection conditions improved the packaging

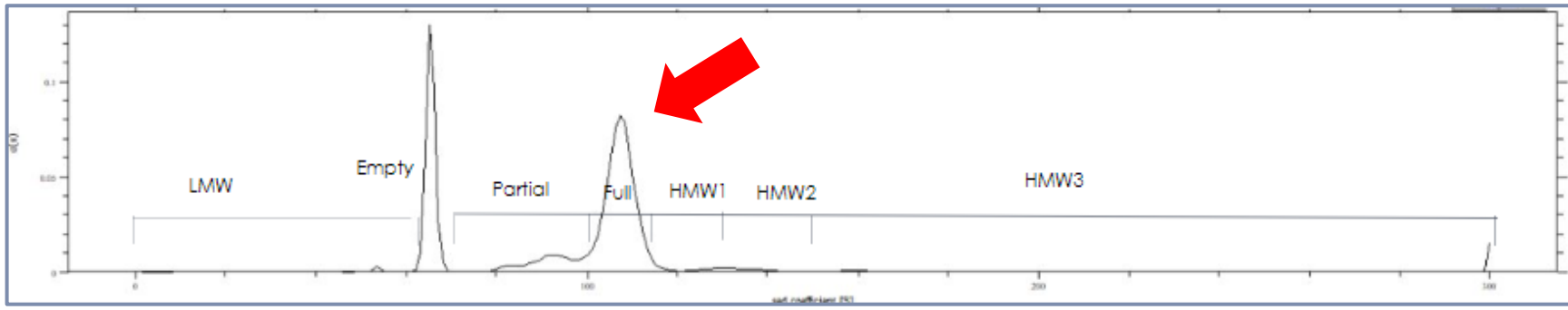
2) **Hypothesis #2- Design Issue: Candidate #1 is large (>4.7 kb) and may be less than ideal packager**
    1) Explore additional constructs with other design choices
        - Other candidates already had strong *in vitro* and *in vivo* data package
    2) Includes variations in size and promoters
    3) Include full panel of analytics on additional candidates

    Results: Alternative candidate produced better metrics

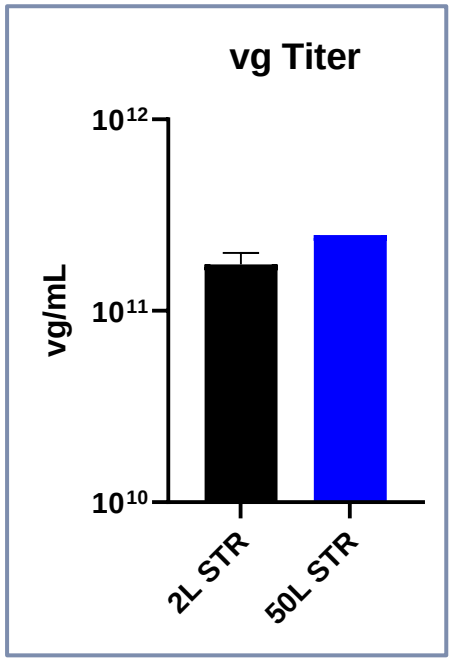
Case Study: #1 Improving AAV Packaging

>5 Candidates were further evaluated, and a lead emerged with improved vector metrics

AUC
-Improved % full profile
-Single peak



Titer
-Hits required titer requirements
-Scalability to 2L and 50L confirmed



3rd Gen Sequencing
-No red flags upon analysis
(data not shown)

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Case Study: #1 Improving AAV Packaging

Lead candidate was selected with improved vector metrics, compared to the original candidate

- ☑ Titer acceptable
- ☑ Scalability confirmed (2L + 50L)
- ☑ Improved AUC profile (now a single peak, more % fulls)
- ☑ No red flags on packaging with 3rd gen sequencing

What was the impactful factor? Size & Final Sequence!

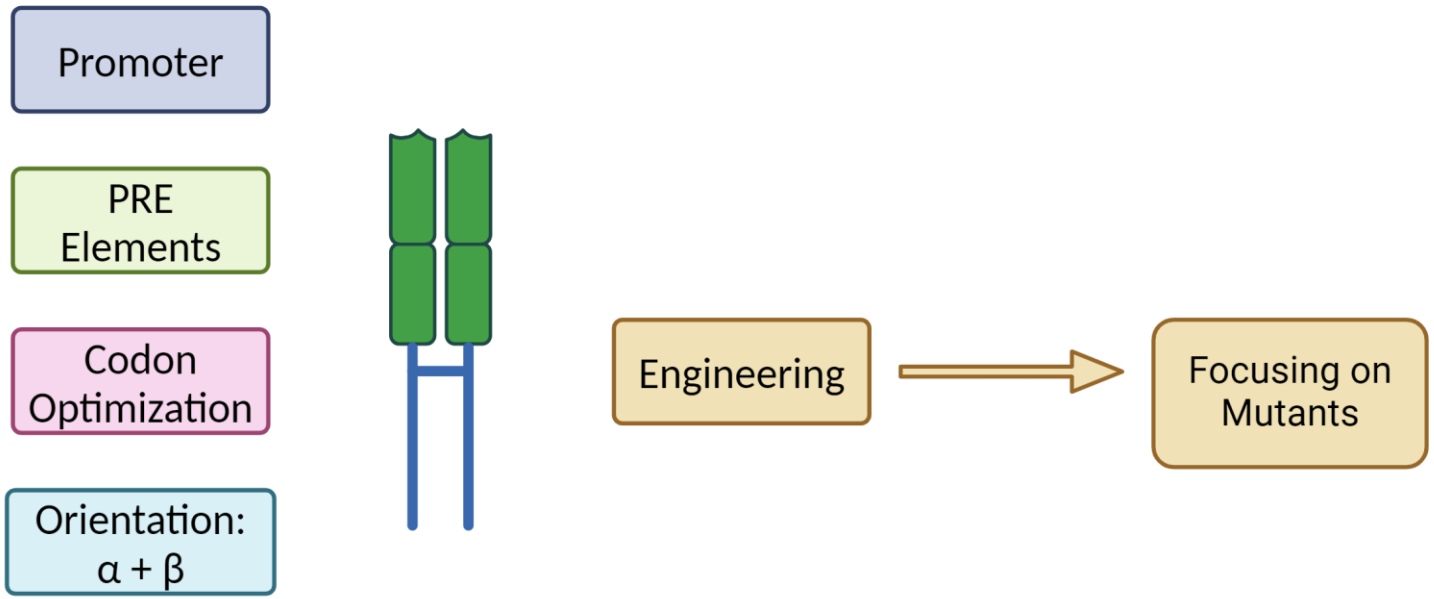
- New lead candidate removed >100 bp to obtain design under 4.7 kb
- Focused on cleaning up 'extra' sequences
- Explored different smaller promoters and polyA sequences
- NO changes to the sequence of the therapeutic portion of the insert
- 96.7% sequence homology from initial candidate to official lead candidate

Case Study: #2 Lenti Improving TCR Expression

Challenge: Quickly assess impact of design on titer, expression, and mispairing with a complex TCR design

Experimental approach:

Generate 60+ plasmids to cast a wide net on designs choices
Metric #1 (Titer): Produce vector and test for vector titer
Metric #2 (Expression): Test Lenti Transduced T-cells for improved TCR expression
Metric# 3 (Mispairing): Test Lenti Transduced T-cells for improved mispairing % compared to lead binder



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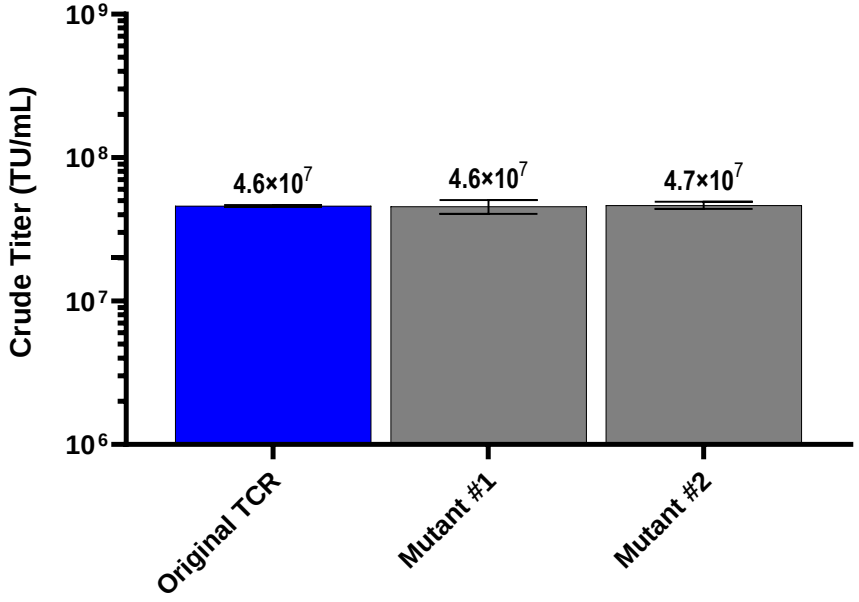
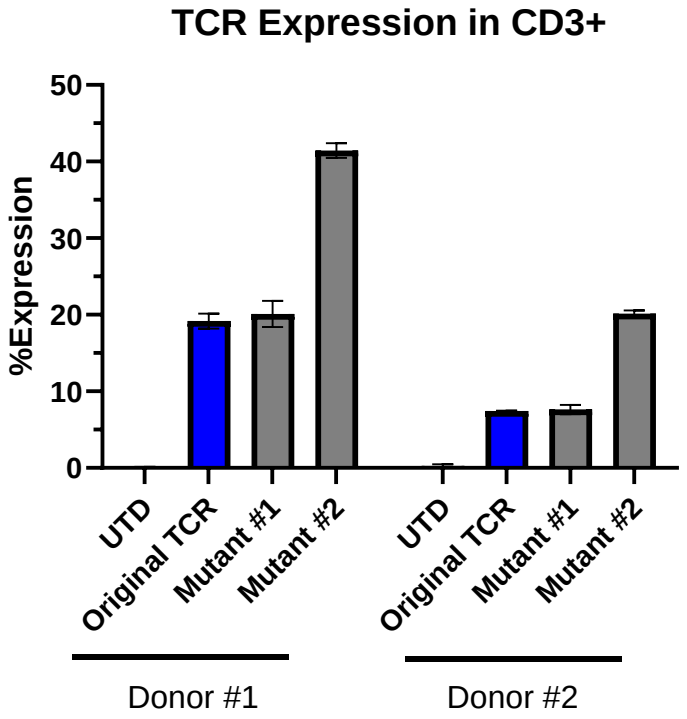
Case Study: #2 Lenti Improving TCR Expression

- **Summary: Mutant #2 (along with other design elements) shows favorable improvements in expression and reducing mispairing to select for continued development**

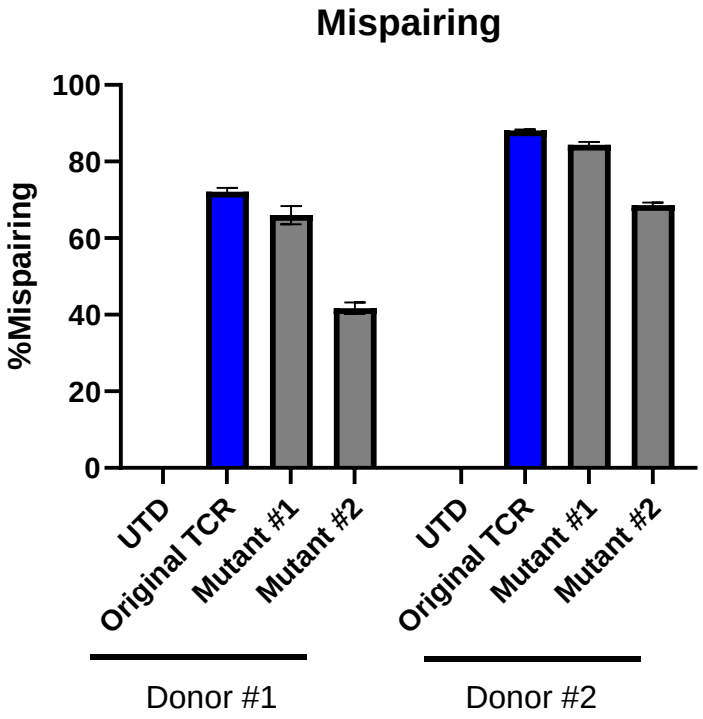
Comparable Titer



Improved Expression



Reduced Mispairing



What Drove Success of the Two Case Studies?

Case Study #1: Improving AAV Packaging

- Size and promoters were key elements
- Having multiple candidates in the pipeline allowed selection of the best candidate
- Aligned processes across R&D and PD speed up candidate selection

Case Study #2: Lenti Improving TCR Expression

- Wide net of designs were screened
- Empirically tested vector on relevant cells to evaluate TCRs
- Focused on key metrics to improve

Summary of Viral Vector Design Lessons Learned:

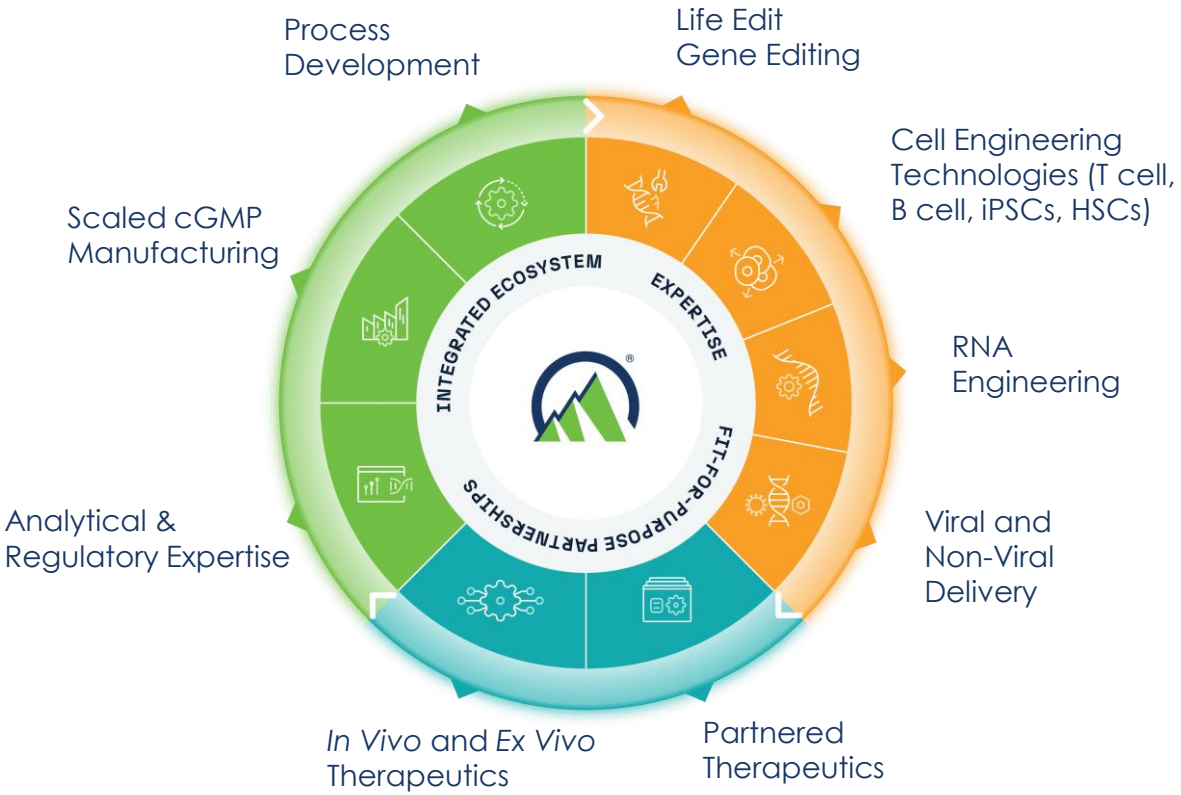
Accelerating Development for New Viral Vectors:
- Cast a wide net of designs early
- Empirically test new viral vectors
- Obtain buy-in from key stake-holders early

How to Improve Transition from R&D to PD?
- Use an aligned process
- Have multiple candidates in the pipeline
- Use suspension cells across R&D and PD

What Vector Metrics are Critical to Review Early On?
- Titer
- Scalability
- % Full and Packaging

What Factors Should be Considered in Viral Design?
- Size
- Promoter
- Codon Optimization & more

ElevateBio's integrated ecosystem provides biopharma companies a new approach to design, manufacture and develop genomic medicines



- ✓ Cutting-edge technology platforms
- ✓ End-to-end process development and cGMP manufacturing
- ✓ Integrated business model
- ✓ Industry-leading talent

Acknowledgements to Highly Cross-Functional Team Delivering Results

Cellular Engineering R&D Team

Viral Vector Process Development

Life Edit AAV Team

Analytical Development

Project Management

TechOps-CMC

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