

Maximizing Vector Production - Targeting Antiviral Defences Using Small Molecule VSEs™



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Introduction

High Cost and Low Supply in Vector Manufacturing

- There are currently approximately 800 ongoing clinical trials involving virus products, which all require significant manufacturing capacity. This number is expected to grow 10x by 2026 (Figure 1).
- There is a gap between current demand and available manufacturing capacity, even if additional planned capacity comes online (Figure 2).

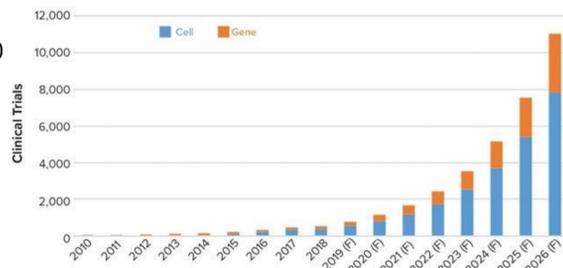


Figure 1. Cell and Gene Therapy Clinical Trials

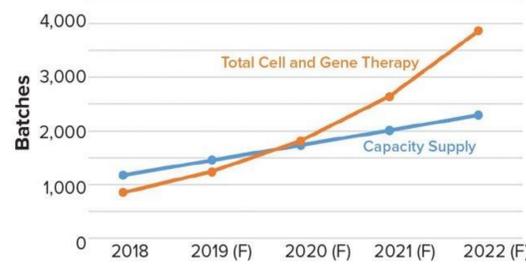


Figure 2. Cell and Gene Therapy Supply and Demand

Addressing Manufacturing Bottlenecks

- Current approaches to improve virus manufacturing focus on optimizing:
 - Producer growth properties
 - Culture media nutrient composition
 - Vector design
 - Bioreactor configuration
 - Downstream purification process

These do not address antiviral defense pathways

The Complex Intracellular Antiviral Response

Vector Production Triggers the Complex Antiviral Network

- The intracellular antiviral defense response is a complex interconnected network
- Multiple facets of the cascade are triggered via the detection of foreign nucleic acid and/or molecular bi-products of viral replication/assembly
- **Antiviral defenses remain partially, if not fully, intact in producer cells**

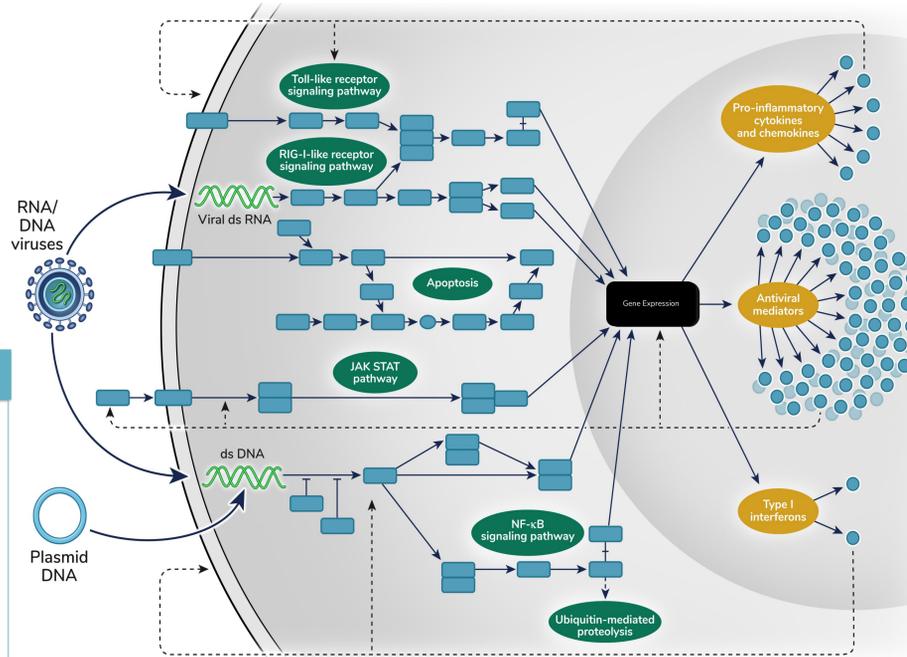


Figure 3. Simplified figure of antiviral responses

Current Approaches to Overcoming Antiviral Defenses

- **Knock-out cell lines** → good for limited targets but not broad targeting. Some antiviral targets overlap with cell proliferation and survival. The novel cell lines need to be qualified.
- **Transient gene knockouts (shRNA)** → good for limited targets but not broad targeting. Typically results in incomplete expression. Costly and complex.
- **Virica's VSE™ Technology**
 - Virica's Viral Sensitizer™ technology, also known as VSEs™, are proprietary small molecules that easily incorporate into manufacturing processes as a simple media additive
 - **Virica's VSE™ technology transiently and broadly inhibit multiple components of the antiviral response to effectively overcome this barrier to vector manufacturing**

Did You Know?

- Many manufacturing cell lines genetically modified to attenuate antiviral defenses still have many underlying antiviral pathways intact due to their important contribution to cell survival and other integral functions
- **For example:** Vero cells do not produce IFNβ but can activate IRF-3, NF-κB, and type III IFN production / response in reaction to viral triggers

VSE™ Technology and Use in Lentivirus Manufacturing

Scalability of VSE™ Formulation

- Various scale-up experiments have demonstrated the ability of the VSE™ formulation to consistently enhance lentivirus production by up to 7.4-fold (Figure 5).

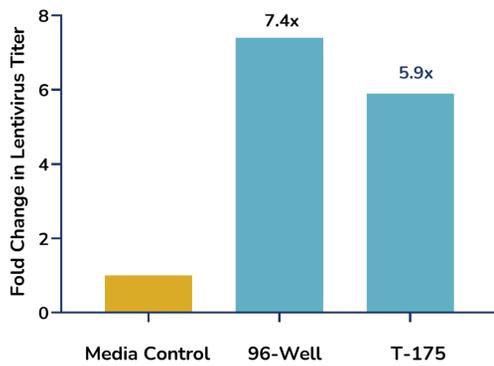


Figure 5: Scalability of VSE™ formulation

Versatility of VSE™ Formulation

- The lentivirus VSE™ formulation is adaptable to a variety of different media, including combinations with common manufacturing additives such as sodium butyrate (Figure 6).

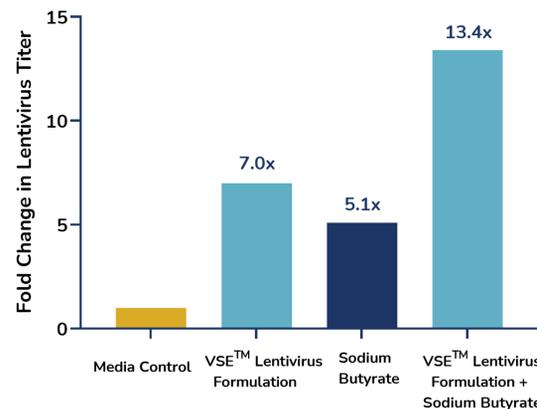
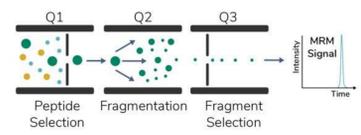


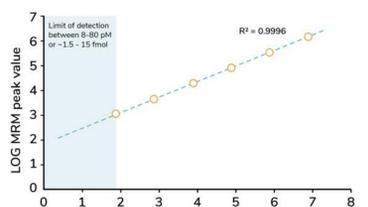
Figure 6: Versatility of VSE™ formulation

Regulatory Considerations

Virica has developed sensitive assays to track VSEs during the manufacturing process



MRM peak signal as a function of VSE concentration. **LOD in pM range**



VSE spiked-in final product readily detected by MRM

VSE used in USP undetectable by MRM after purification (pM LOD)

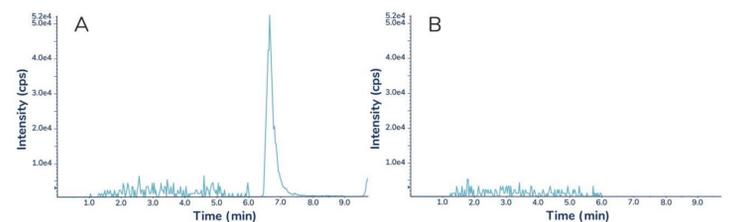


Figure 9: Analytical Assays for tracking of VSE molecules during manufacturing

VSEs™ Enhance AAV and MVA Manufacturing

AAV Manufacturing

- 8 VSEs™ were screened for the ability to improve production of AAV2 in HEK293 SF suspension cells (3rd party collaboration).
- Unoptimized shaker flask formulation development data indicates significant enhancement in yield with 6/8 compounds (Figure 7).

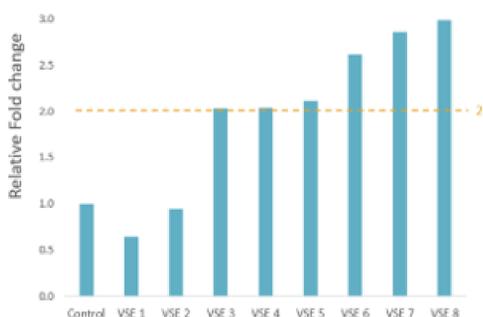


Figure 7: Multiple VSEs™ enhance AAV2 production

MVA Manufacturing

- Addition of VSE™ to small-scale culture improved MVA yield by 10x (results not shown).
- Addition of VSE™ formulation to scale-up model (iCELLis® Nano Bioreactor) improved yield of MVA by 4x (Figure 8).

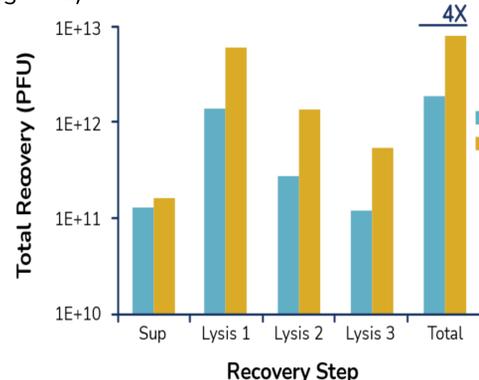
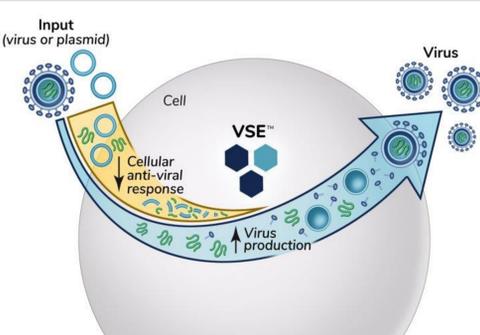


Figure 8: VSEs™ enhance MVA production

Summary

- Each manufacturing platform will have its own unique set of anti-viral pathways that result from the complex interaction of various platform components (e.g. viral product, cell substrate etc.)
- Virica's VSEs™ address these often-neglected cellular antiviral defenses to uniquely enhance viral vector manufacturing yields.



Virica offers partners an opportunity to customize VSE™ formulations that best suit their specific manufacturing platforms.

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