Development and Scale-up of a Novel Adenovirus Production Process

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Abstract

Virus-like particles (VLPs) are non-replicating therapeutic vectors devoid of viral genes. VLPs are created by transfection of plasmid DNA containing a therapeutic-related expression cassette (eg transgene, gene editor, etc; flanked by ITRs and an adenovirus packaging signal) into an engineered production cell line. DNA transfection is followed by infection of the production cell line with a "helper" virus (HV) to co-locate both genomes in the nucleus. The HV provides adenovirus genes in *trans* to replicate the VLP genome and create capsid particles. The production cell line then precisely edits the helper viral genome to remove the packaging signal in a highly efficient process (Figure 1). With no viral genes required, the entire VLP genome capacity – 35 kb – is available for therapeutic designs that are more than 7x AAV and 4x Lenti viral or LNP capacities. VLP production in an academic setting utilized an adherent engineered production cell line with minimal process optimization. Ensoma's process development strategy focused on adaptation of this adherent cell line to serum-free, suspension cell culture. Suspension processing is ideal to achieve cell culture consistency and ability to scale the process. The adherent cell line was adapted by stepwise removal of serum into chemically defined media followed by adaptation to suspension culture. The adapted suspension cell line was compared to the adherent cell line to ensure product quality was not altered in the VLP product. With a suspension cell line in hand, the next stage of development focused on bioreactor parameter screening at small scale in both AMBR15 and 2L bioreactors. In bioreactors, VLPs are produced by co-infection of the production cell line with existing "helper" and VLP viral stocks. A DOE strategy in AMBR15 and 2L bioreactors identified key parameters to further optimize in subsequent experiments. The finalized small-scale process was scaled to 20L bioreactors internally for process confirmation and then transferred to a CDMO for a clinical scale production. At the clinical scale, cell expansion operations required changes that differed from small scale, including cell culture expansion through a rocker reactor. The first clinical scale production saw an increase in host cell protein (HCP) impurities not observed at small scale. Experiments were performed that focused on a deeper understanding of key cell culture parameters to reduce HCPs. When these parameters were implemented in subsequent clinical scale batches, final HCPs impurities were acceptable.

In summary, VLP process development utilized a DOE strategy to quickly develop a clinical scale production process. This strategy identified cell expansion and production parameters that demonstrated linear scalability from AMBR15 to 20L to clinical scales, which should allow for consistent scaling beyond the clinical scale. The timeline from successfully adapted production cell line to a clinical scale batch was <6 months and was aided by both DOE and QbD principles.

Ensoma's Virus-like Particle (VLP)

Therapeutic Advantages of Virus-like Particle (VLP)

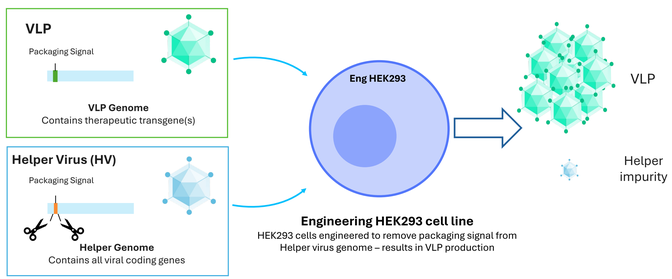
"Gutless" vector
Contains no viral genes, resulting in low immunogenicity & high payload capacity

Large Payload Capacity
35 kB Capacity enables multiplexed gene insertion controlled by distinct regulatory elements

Hematopoietic Tropism (HSCs)
Highly preferential transduction of HSCs & derived lineages

Evolved capsid
Virus-like capsid built on evolved, high efficiency gene delivery vectors

Precise molecular production of Virus-like Particle (VLP)



Please see additional Ensoma posters:

- Acute Safety and Biodistribution Profile of Hematopoietic Stem Cell (HSC) Targeting Virus-like Particles Based on Helper-dependent Adenovirus Serotype 5/35++ in Non-human Primates – ***poster 1779***
- Novel In Vivo Gene Therapy Approach to Hematopoietic Stem Cell (HSC) Engineering Creates Durable HSC-Derived Neutrophils to Treat X-Linked Chronic Granulomatous Disease – ***poster 1780***
- In Vivo Engineering of Hematopoietic Stem Cells with Virus-like Particles to Generate Multi-Lineage CAR Immune Cell Therapy for Cancer – ***poster 1783***

Suspension Adaptation of Production Cell Line

Adherent cells expressing CRE were adapted to sequentially reduced serum levels mixed with seven chemically defined media. After serum reduction, clones were successfully adapted to two media in suspension cell culture. Growth rate, viability, and cell clumping level were assessed. One clone was selected, and a pre-Master cell bank was created in serum-free media for subsequent process development.

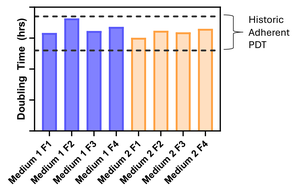


Figure 1. Doubling time of cells adapted to suspension and chemically defined media.

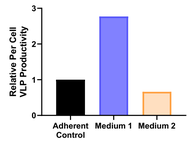


Figure 2. Per cell productivity of suspension adapted cells normalized to adherent control.

Early VLP Process Development with AMBR15

AMBR15 screening experiments identified process parameters for further optimization. A DOE strategy was implemented to evaluate control parameters including pH, DO, and agitation. Clear dependencies were observed for pH and sparging which would be further refined at the 2L bench-scale (Figure 3). VLP productivity parameters were also evaluated including MOIs and infection cell density. Additional dependencies were established around MOI ratio (Figure 4). Using DOE with the AMBR15, the operating parameters were able to be established, and key dependencies were identified to further investigate at the bench-scale.

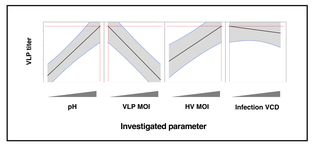


Figure 3. Ambr15 screening DOEs identify critical parameters for further studies.

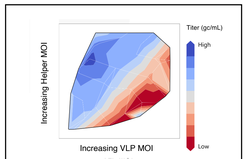


Figure 4. Combination of VLP and Helper MOI are key parameters for productivity.

Bench-scale (2L) Process Development Studies

With the baseline upstream process developed, additional upstream optimization and downstream process development followed at the 2L bench-scale. Increasing VLP titer at harvest was established by studying MOI ratios between VLP and helper viruses. A baseline downstream process was established and purified VLP products were shown to have variable levels of the process related impurities host cell protein (HCP) and DNA (hcDNA). Continued development identified three operations that could contribute to these variable outcomes: consistent cell counting, harvest additives and chromatography operations.

For cell counting, cell clumps were thought to contribute to inconsistent cells counts and subsequently, uninfected cells during VLP production. An anti-clumping reagent was added during cell counting and resulted in better dispersion and more consistent counts (Figure 5).

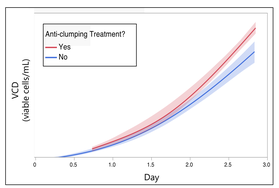


Figure 5. Anti-clumping treatment of samples increased cell counts, indicating some clumping.

Harvest additives (e.g. nuclease, lysis reagents) were investigated and resulted in more consistent reduction in HCP and hcDNA. This was coupled with further optimization of washes and elution steps during chromatography. This resulted in more consistent clearance of host cell impurities (Figures 6).

Lane #	Sample
1	Molecular Weight Marker
2	Development run #1
3	Development run #2
4	Tech transfer process

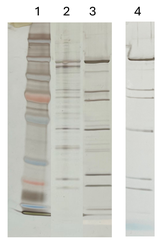


Figure 6. Representative VLP batches examined by silver stain gel electrophoresis

Clinical Scale Productions

The optimized bench-scale process was transferred to a CDMO for production scale development. This transfer included productions at 2L and 10L to confirm process performance. Once process transfer was confirmed, a clinical scale production run was performed for scale-up confirmation. The clinical scale batch was successful in confirming cell culture expansion, production in clinical scale bioreactors, downstream operations and drug substance aliquoting.

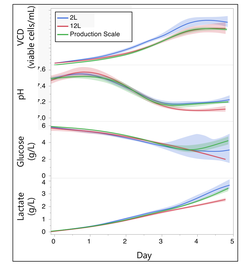


Figure 7. Consistent parameter trends between 2L, 10L, and production scale demonstrate successful process scaling.

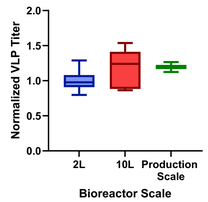
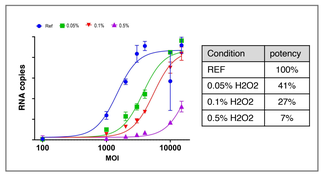


Figure 8. Productivity conserved at production scale. 2L and 10L bioreactors are representative scale down models.

Analytical Characterization of VLPs

VLP characterization included benchmark techniques for studying strength, identity, safety and quality. Potency methods can compare purified VLP from various batch to ensure process development or scale-up activities do not alter VLP function. A VLP potency method was developed to evaluate cell infection, trafficking to the nucleus and expression of transgene RNA. The RNA potency method was shown to detect reduction in activity when VLPs were exposed to various denaturants (e.g. H2O2 and temperature). This provided confidence the RNA expression method could detect functional differences between batches.

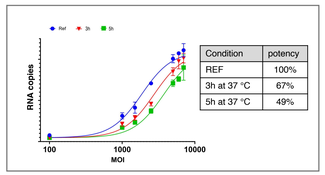
VLP function in presence of H2O2



Condition	potency
REF	100%
0.05% H2O2	41%
0.1% H2O2	27%
0.5% H2O2	7%

Figure 9. Potency results for the same VLP batch treated with various amounts of hydrogen peroxide

VLP function in presence of temperature

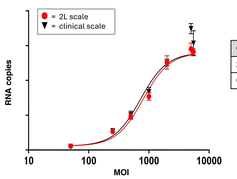


Condition	potency
REF	100%
3h at 37 °C	67%
5h at 37 °C	49%

Figure 9. Potency results for the same VLP batch held at 37 °C for various times

Function of clinical scale VLP compared to bench-scale VLP

With an effective potency method in place, we confirmed that material produced at clinical scale was comparable to development material (2-10L scale). This established that our clinical scale VLP production process can support GMP productions



Condition	potency
2L (REF)	100%
Clinical Scale	117%

Figure 10. Potency comparison of VLPs produced at development scale (2L) and clinical scale