AnelloBricks: a novel, cell-free, in vitro assembled viral vector system based on commensal anelloviruses offers a highly modular, versatile, low COGs and scalable manufacturing platform solution to genetic medicines

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Abstract

Viral vectors show great promise for efficient gene transfer and therapeutic drug delivery. However, safety concerns arising from immunogenic and cytotoxic effects as well as difficulties in manufacturing scalability and productivity remain key challenges for their use. Here we report the development of a cell-free, in vitro assembled genetic medicines platform offering a safer and modular approach to treating a broad range of diseases across diverse therapeutic modalities based on human-derived anelloviruses. Anelloviruses are ssDNA viruses that are ubiquitously commensal in human populations and are not known to be the etiological agents of any disease in humans. Anelloviruses are extremely genetically diverse and appear to evade the immune system. The non-pathogenic and commensal nature of anelloviruses make them a prime candidate for use in next generation genetic medicines. This novel in vitro assembled genetic medicines platform is comprised principally of two components: recombinant protein and nucleic acid payload (e.g., DNA, RNA), offering payload versatility while also reducing the complexity of manufacture to conventional and readily scalable production systems. This modular, versatile, and scalable approach has the potential to address the limitations of current cell-based viral production systems and improve access to patients in need by dramatically reducing the cost to manufacture.

AnelloBricks: Ring's platform to unlock genetic medicines

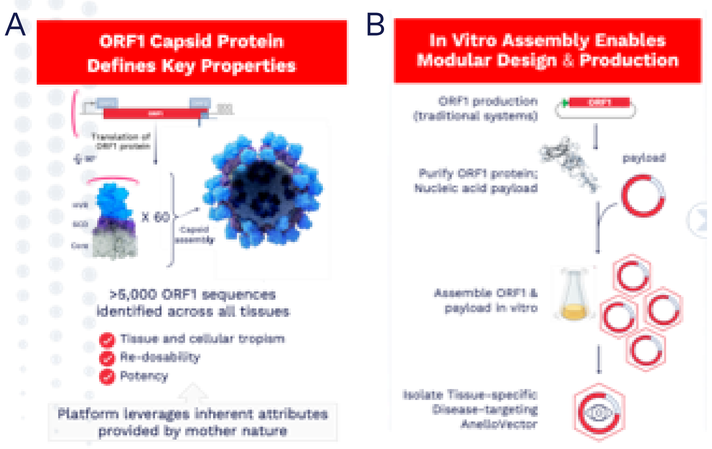


Figure 1. Modular, versatile and scalable. A) Manufacturing viral vectors is typically a complex, variable, and lengthy process. With our platform, however, our vectors can be deconstructed into building blocks. AND Unlike AAV where 3 viral proteins are needed to form the capsid, our technology utilizes a singular capsid protein that can self-assemble under the right conditions. B) One of our largest advantages of our system is that this capsid protein building block can be produced through a well-established recombinant protein bioprocess; gives optionality and increases our accessibility to many recombinant protein CDMOs, where there is much more global capacity and expertise.

Structural understanding provides long-term platform and portfolio value

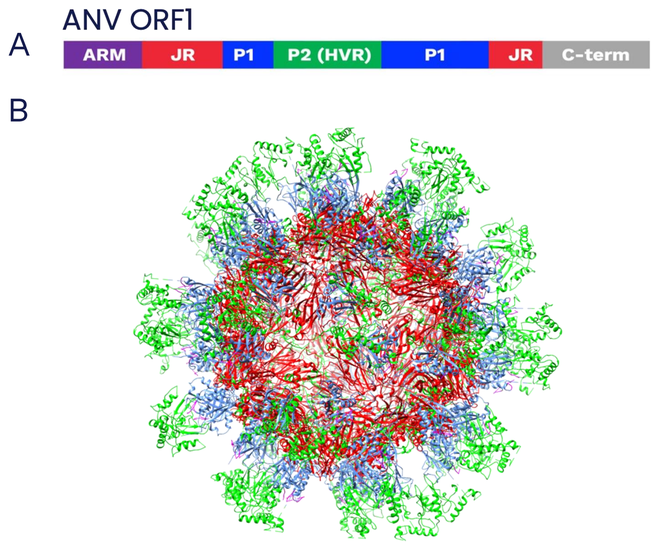


Figure 2. Enabling structure-guided design for capsid/ vector engineering. A) A schematic representation of full length ORF1 is shown as a cartoon labeled and colored by domains. The arginine-rich motif (ARM) is shown in purple, the jelly roll (JR) domains is shown in red, the spike P1 domain is shown in blue, the spike P2 domain is shown in green, and the C-terminal domain is shown in grey. B) Cryo-EM of ANV VLPs produced in mammalian cells reveals that the ORF1 core structure is comprised of 60 JR domains pack in icosahedral symmetry (red), with the spike P1 and P2 domains colored as in A).

In vitro ANV assembly enables modular and flexible therapeutic design

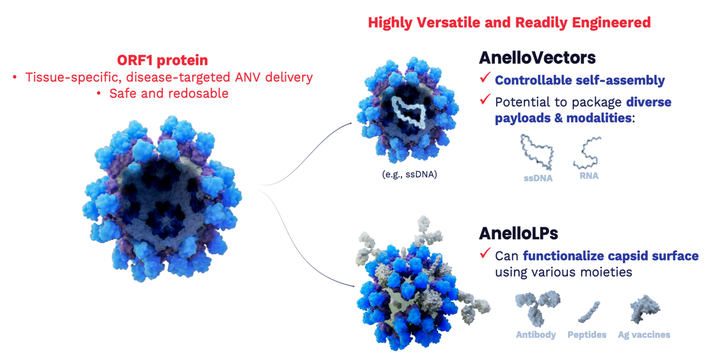
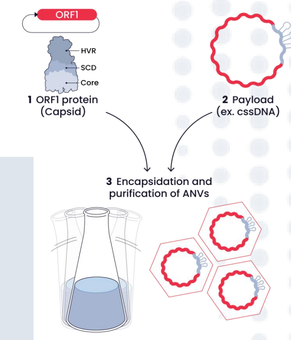


Figure 3. Modular and flexible therapeutic design.

Modular, versatile, scalable manufacturing platform

- AnelloBricks consist in the manufacture of a **single recombinant protein** (ORF1) and a **payload**. Process complexity is reduced to the level of traditional rProteins and MAbs
- This platform will be implemented for clinical material production



Advantages of the AnelloBricks Platform

Address industry-wide limitations of current Gene & Cell Therapy manufacturing systems

1. Simple production of a single capsid protein (ORF1) and payload
2. High level of robustness, purity, quality
3. Versatile payload packaging (DNA and RNA)
4. Unconstrained scalability
5. Dramatic productivity increase and CoGs reduction

Figure 4. AnelloBricks: Advanced new technology developed at Ring that eliminates the main challenges in Gene Therapy, such as low production capacity and high CoGs. AnelloBricks is based on the production of a single capsid protein (ORF1), followed by ORF1 self assembly around desired payload (encapsidation) into a functional Anellovector.

New therapeutic modalities and the growing complexity/cost of their manufacturing

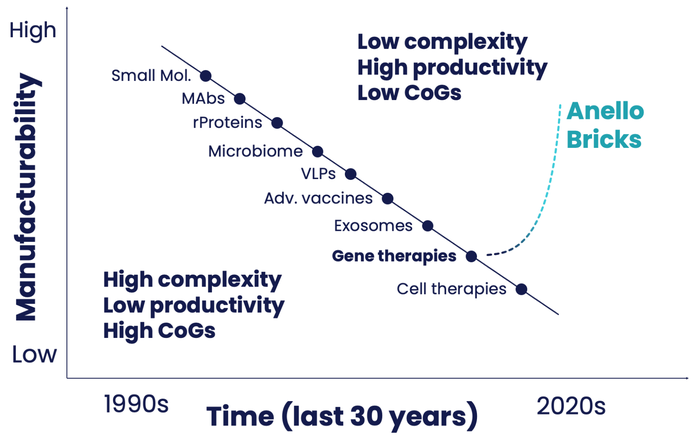
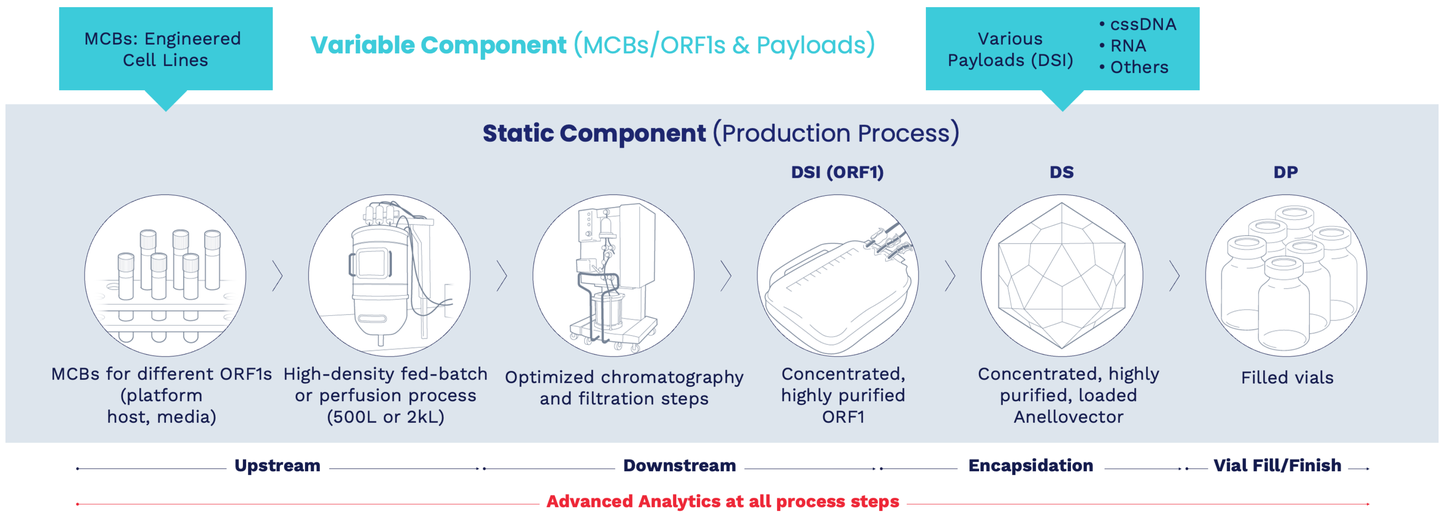


Figure 5. Path towards industrialization of Ring's Genetic Medicines manufacturing. CoGs assessment, increased production productivity/scale. The cost of goods (CoGs) reduction with AnelloBricks is estimated at two orders of magnitude.

Targeted large-scale AnelloBricks manufacturing process architecture



AnelloBricks addresses limitations of current cell-based vector production

- Uses **conventional** and **readily scalable** recombinant methods
- In vitro assembly **enables payload versatility** and **flexible design** for **broad therapeutic application**
- Highly **controllable assembly** and **manufacturing modularity** at **significantly reduced cost**

REFERENCES

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