Anellovectors: Expanding the payload capacity of anellovirus-based vectors beyond the wild-type genome capacity

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Ring Therapeutics, Cambridge, MA, USA

Oral presentation -
Thurs, May 9, 5:00-5:15 PM ET, Ballroom 4
To learn more about Anellovectors and the Anellogy platform, please attend the oral presentation by Chris Wright, MD, PhD, Chief Medical Officer.

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Abstract

We recently reported the development of the Self-Amplifying Trans-complementation of a Universal Recombinant Anellovector (SATURN) production system for producing a functional novel gene therapy class, termed Anellovectors, based on the vectorization of human commensal anelloviruses. Anelloviruses are a highly diverse family of non-enveloped negative-sense single-stranded DNA (ssDNA) viruses that are a ubiquitous component of the human virome. Despite their ubiquitous presence in human populations, anelloviruses are apathogenic and evade induction of robust humoral responses, making them attractive candidates for the next generation of genetic medicines.

Here, we report the development of an Anellovector cargo that is significantly larger than the wild-type anellovirus genome. Anelloviruses are the only truly negative-sense ssDNA viruses that infect eukaryotes. Notably, anelloviruses also lack the Rep proteins produced by other ssDNA viruses, pointing to a unique replication and packaging mechanism that may allow for the encapsidation of larger genomes. The Anellovector presented here is based on the Beta genera of human Anelloviruses which have genome sizes of ~2.8-3.0 kb. Using vector genomes of increasing size for encapsidation, we have demonstrated that Anellovectors are capable of packaging vector genomes ranging from 2.0kb - 5.0kb, representing up to 65% beyond the wild-type genome capacity. A combination of qPCR and next-generation sequencing was used to verify that the encapsidated expanded cargo DNA is intact, circular, and single-stranded. Electron microscopy was used to verify that vectors containing expanded genomes were capable of forming the expected 30nm particles. The overall potential for Anellovector cargo capacity appears to be similar to or even exceed the ~4.7 kb cargo capacity of AAV. The payload capacity of viral vectors often drives their therapeutic potential. Expanding the payload capacity of the Anellovector builds upon its intrinsic characteristics of immune favorability and tropism in efforts to reach the broadest possible patient population in need of gene therapies

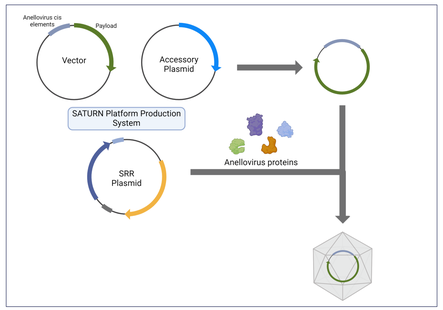

Figure 1: Representation of Anellovector production using a three-plasmid transfection system. The triple transfection comprises the vector plasmid containing Anellovirus cis elements and transgene flanked by lox sites, a Cre expression plasmid (accessory plasmid), and a self-replicating rescue plasmid (SRR plasmid) which provides Anellovirus proteins in trans.

Proposed model for ANV packaging

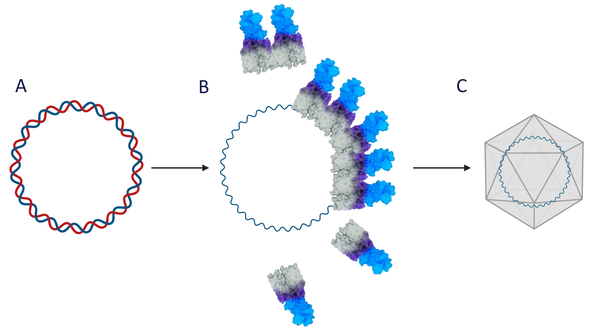

Figure 2: Diagram representing the proposed model for Anellovirus particle formation. A) The double-stranded intermediate of the Anellovirus genome expresses nonstructural and capsid proteins. B) Capsid proteins begin to spontaneously assemble around replicated or actively-replicating cssDNA. C) Mature particles are formed containing cssDNA Anellovirus genome.

ANVs can encapsidate larger payloads

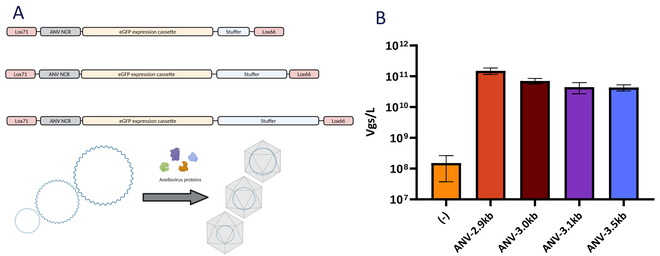

Figure 3: Beta Anellovectors can replicate and package payloads up to the size of Alpha Anelloviruses. A) Schematic representation of expanding the size of a payload in an Anellovector by altering the size of random stuffer DNA while keeping the viral non-coding region and eGFP expression transgene constant. This method replicates larger vector genomes and packages into similarly-sized capsid particles using the same viral proteins across conditions. B) Vectors were recovered with isopycnic centrifugation and physical titer was measured for DNase-protected vector DNA.

ANVs can replicate and package vectors up to 5.0kb

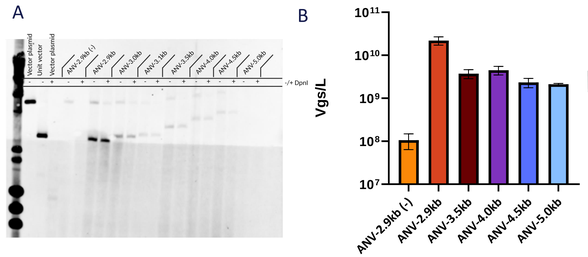

Figure 4: Anellovectors can replicate genomes larger than wild type and package them into viral-like particles. A) Southern blot probing for the eGFP gene in the vector; (+) symbol indicates the sample was digested with DpnI, an enzyme which cuts at its recognition site only where the DNA is bacterially methylated, to remove input plasmid. B) Vectors with payloads up to 5.0kb in size were recovered using isopycnic centrifugation and titer was measured for DNase-protected vector DNA.

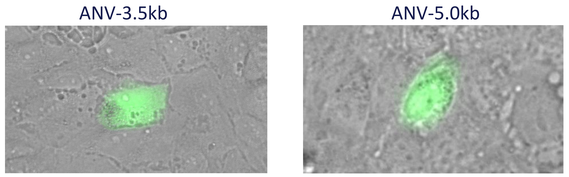

Figure 5: Transduction of RPE cells with expanded payload ANVs. ANV-3.5kb and ANV-5.0kb were transduced in RPE at MOI 2.8K vg/cell and MOI 5.1K, respectively. Cells were imaged on day 7.

Expanded payloads are intact and form uniform VLPs

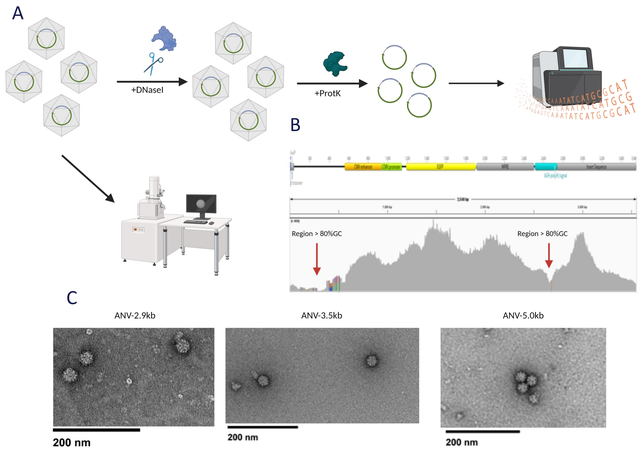

Figure 6: Verification of VLPs by next-generation sequencing and Electron Microscopy. A) Representation of VLP processing. A sample is split where one portion is imaged by electron microscopy (EM) and the other is digested sequentially – first, by DNaseI to remove residual DNA and second, by Proteinase K to break apart the capsid, releasing the vector DNA. Vector DNA is prepped and sequenced by short-read sequencing. B) Coverage plot of reads mapped to ANV-3.5kb vector DNA; red arrows indicating areas of high GC content which can interfere with NGS coverage. C) Electron micrograph of VLPs containing payloads ranging from 2.9kb to 5.0kb showing particles 30nm in size. Scale bar = 200nm.

Conclusions

- ANVs can package genomes up to 5.0kb in size, broadening therapeutic potential
- Packaged payloads are cssDNA and show complete sequence coverage across the expected vector
- Expanded payloads produce VLPs around 30nm in size, identical to WT-sized vectors

Next Steps

- Further testing is required to determine functional efficacy of expanded payloads in vitro and in vivo

REFERENCES
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