Anelloviruses are highly diverse, ubiquitous commensal denizens of the human virome, and show promising properties as novel gene delivery vectors

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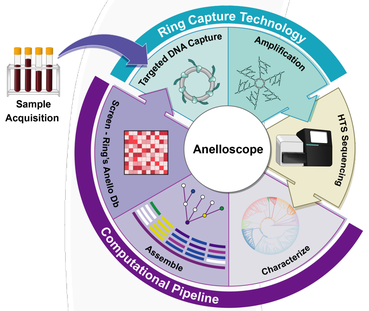
Anelloviruses are **prevalent** in humans

The human virome is an untapped resource in medicine; it houses commensal viruses that have co-evolved with us over millennia and do not elicit an immune response. The most common commensal viruses are **anelloviruses.**

Using our AnelloScope technology, we characterized anellovirus diversity and persistence within humans in a blood transfusion cohort. Utilizing anellovirus genomes, we can harness their immune stealth to develop a new class of vectors in gene therapy.



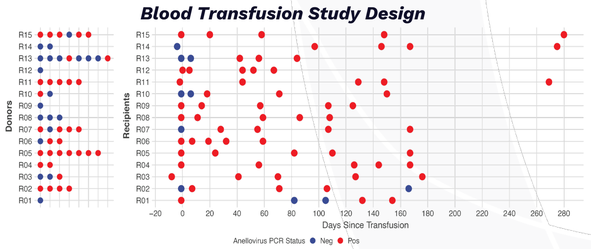


Figure 1: Study Overview. Fifteen recipients paired with one or more donors received a blood transfusion following surgery. Donor pools were unique to each of the recipients receiving a blood transfusion.

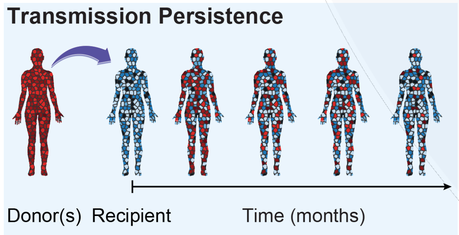


Figure 2: Transmission Persistence. The persistence of transmitted anellovirus donor lineages in a recipient over time was investigated. Through mapping of sequence data to donor anelloviruses, the relative proportion of each donor lineage at each recipient timepoint was calculated to approximate the duration of observable infection over the course of the study.

Anelloviruses are **diverse**

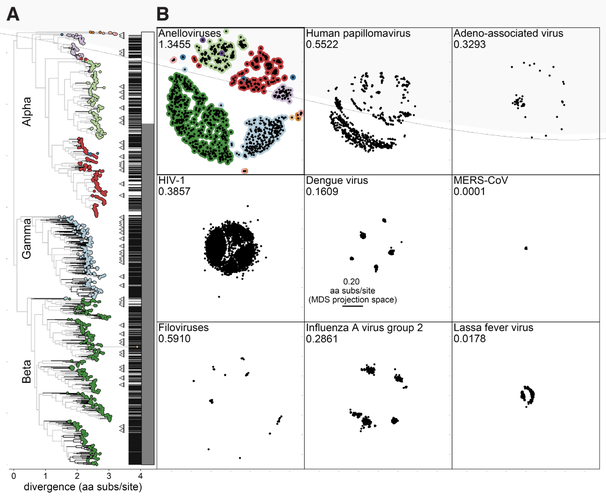


Figure 3: Anellovirus diversity. (A) Maximum-likelihood phylogeny of anellovirus ORF1 amino acid sequences (n=2,101). (B) Multidimensional Scaling analysis of 1,575 anellovirus ORF1 amino acid sequences (points are colored as in A) compared to eight other viral surface proteins.

Anelloviruses are **persistent**

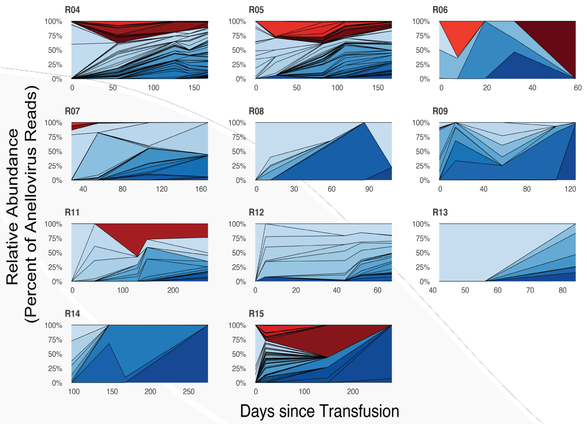


Figure 4: Anellovirus persistence after transfusion. Tracking of anellovirus relative abundance over the course of the longitudinal study following blood transfusion. Lineages colored in shades of red denote transmitted lineages from the donor(s) while shades of blue indicate resident lineages.

Anelloviruses may be **re-dosable**

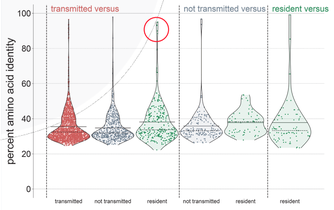


Figure 5: Anellovirus similarity between donors and recipients. Horizontal solid and dashed lines indicate the mean and median amino acid identity between each group, respectively. Similarity of anelloviruses between donor and recipient prior to transfusion does not predict transmissibility. Sequences circled in red have high sequence similarity to resident anelloviruses and are indicative of those that may be re-dosable.

Anelloviruses can be **synthesized**

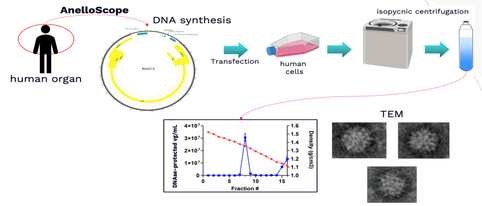


Figure 6: Rescue of recombinant human anelloviruses. Using AnelloScope, a human anellovirus genome was synthesized by standard methods and transfected into a human cell line. Following incubation, lysis of the cells, isopycnic centrifugation, and qPCR quantitation of the resulting fractions, we identified a peak fraction at a density consistent with viral particles. Transmission electron microscopy (TEM) was used to characterize purified fractions. The particles are ~30 nm in diameter, typical of anelloviruses. This is the first time a human anellovirus has been produced *in vitro*.

Anelloviruses can be **vectorized**

The Making of Anellovectors

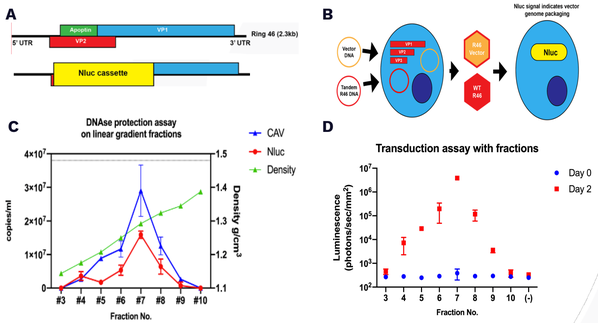


Figure 7: Vectorization of an anellovirus, Ring 46, as a prototype for human Anellovectors. The avian anellovirus CAV (chicken anemia virus) was the first Anellovector prototype due to its simplicity and ease of manipulation. (A) Linear representation of the CAV genome and the Ring 46 (R46) Anellovector construct in which a nanoluciferase (Nluc) cassette has been introduced. (B) Schematic illustration of Anellovector production by co-transfection of wildtype (WT) CAV and Anellovector DNA resulting in production of R46 and WT particles which can be added to fresh cells for detection of Nluc expression. (C) Purification of R46 by linear CsCl density gradient and detection by qPCR at the expected density. (D) R46 Anellovector fractions shown in (C) resulted in readily detectable transduction of cells *in vitro*.

Low pre-existing immunity to anelloviruses in humans

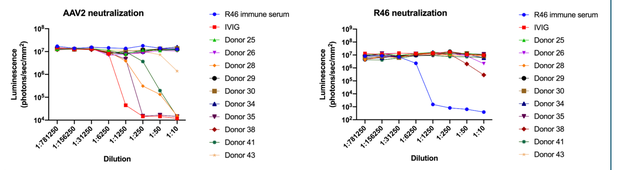


Figure 8: Very low pre-existing immunity in humans to R46 Anellovector. A luminescence assay was used to detect neutralization of R46 Anellovector and AAV2 after incubating each vector, AAV2-Nluc or R46-Nluc, with donor sera or human intravenous immunoglobulin (IVIG). While 3 of 10 donors show clear neutralizing antibodies to AAV2, in general agreement with expected seroprevalence, only the positive control serum neutralized R46 robustly, indicating that pre-existing immunity against this vector in humans is extremely low. Human IVIG is a high-concentration antibody solution prepared by pooling thousands of donor sera. The observed pattern of robust AAV2 neutralization and undetectable R46 neutralization is consistent with observations using individual sera.

Conclusions

- Anelloviruses are an extremely diverse family of human commensal viruses. This diversity suggests a promising vast pool of targets for vectorization with low human immunogenicity.
- Anelloviruses are both highly infective and durable. They are persistent in blood transfusion recipients with donor anelloviruses detected in recipients more than 200 days post transfusion (last data point of collection).
- Transfusion studies also suggest anelloviruses are redosable due to significant similarity between strains found in donors and recipients, co-existing without causing pathologic immune responses.
- Anelloviruses can be synthesized using Ring's proprietary Anellogy platform which facilitated creation of the first *in vitro* produced human anellovirus.
- Synthesized anelloviruses can be vectorized.
- Like native anelloviruses, Anellovector Ring 46 has low pre-existing immunity in humans.

Reference

Arze CA, Springer S, Dudas G, Patel S, Bhattacharyya A, Swaminathan H, Brugnara C, Delagrave S, Ong T, Kahvejian A, Echelard Y, Weinstein EG, Hajjar RJ, Andersen KG, Yozwiak NL. Global genome analysis reveals a vast and dynamic anellovirus landscape within the human virome. *Cell Host Microbe*. 2021 Aug 11;29(8):1305-1315.e6. doi: 10.1016/j.chom.2021.07.001. Epub 2021 Jul 27. PMID: 34320399.