

#### INTERVIEW

## Foamy virus: an emerging viral vector for human gene therapy



Dr Jennifer E. Adair received her Bachelor of Science degree from Youngstown State University and her Doctor of Philosophy degree from Washington State University. She is currently an Assistant Member in the Clinical Research Division at Fred Hutchinson Cancer Research Center and a Research Assistant Professor in the School of Medicine at the University of Washington in Seattle. Her laboratory develops and translates gene therapy using hematopoietic stem cells as a treatment for genetic, malignant and infectious diseases. Specifically, her lab has translated successful drug resistance gene therapy to protect blood from otherwise myelotoxic chemotherapy used to treat solid tumors, functionally corrective gene therapy for Fanconi Anemia, X-linked severe combined immunodeficiency syndrome and Wiskott-Aldrich syndrome, and anti-HIV gene therapy in combination with drug resistance gene therapy for the treatment of AIDS-lymphoma. The lessons learned from these studies provide research directives to overcome remaining barriers to mainstream clinical application of hematopoietic stem cell gene therapy.

**Q** Foamy virus is more of a recent addition to the viral vector toolbox for gene therapy. Can you tell us a little about the biology of foamy viral vectors, and how they compare with other viral vectors?

**T**he characterization of natural foamy virus vectors is largely described by the laboratories of Dr Maxine Linnial, Dr Ali Saïb and Dr Axel Rethwilm, as well as others. Foamy viral vectors are derived from the spuma retroviruses, an exogenous type of retrovirus. In nature, foamy viruses are endemic in non-human primates and other mammals. They are thought to be one of the oldest known retroviruses. They're

believed to have co-evolved with their hosts over the last 60 million years. They can infect humans and induce lifelong persistent infections, but are apathogenic. Foamy viruses are a different subfamily compared to gamma retroviruses and lentiviruses, which are ortho-retroviruses.

In general, the viral genomes of gamma-retroviruses, lentiviruses and foamy viruses have a similar principle order: long terminal repeat (LTR), *gag*, *pol*, *env*, accessory genes, LTR. All three types of retroviruses share another characteristic feature, reverse transcription and integration into the host cell genome. However, there are differences between these vectors. Probably the biggest difference is that foamy viruses actually perform a very late reverse transcription of the RNA, or the pre-genome, into double-stranded DNA before the resulting virion buds from the producing cell membrane. The resulting double-stranded DNA genome in the virion is considered to be very stable.

Additionally, the foamy virus envelope glycoproteins are able to transduce almost any cell type, meaning they have a very broad tropism. The foamy virus receptor was identified by two different groups in 2012 as the ubiquitous heparan sulfate receptor [1,2].

Foamy viruses are considered to have a larger carrying load capacity than gamma-retroviruses or lentiviruses. Their current documented carrying capacity goes up to 12 or 13 kilobase pairs (kbp). At minimum, we know they can package about 9.2 kbp, which is approximately the maximum packaging capacity of gamma-retroviruses and lentiviruses.

The current strain of foamy virus in use for gene therapy was originally isolated from infectious clones of a foamy virus isolate from a human infection, but other simian and feline foamy viruses have also been developed. They generally carry a deletion in the U3 promoter region, as well as in most of the viral genome, with the exception of cis-acting sequences in the viral *gag* and *pol* genes, which are required for packaging. They all carry Pol protein encapsidation de-

letions in the transactivator and accessory genes. Thus, both the viral promoter and transactivator are deleted, rendering these foamy virus vectors as true self-inactivating (SIN) vectors. This SIN configuration has also been developed for gamma-retroviruses and lentiviruses.

Foamy viruses can also be produced using a three- or four-plasmid transfection system. Crude vector preparations can be concentrated by centrifugation or filtration to improve the vector titers about 100-fold without an observable loss of infectivity. Head-to-head experiments were performed in earlier studies with lentivirus, gamma-retroviruses and foamy virus in transduced human and canine cells. In these studies, foamy vectors performed as efficiently or better at lower multiplicities of infection than lentiviruses or gamma-retroviruses. This work was

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conducted by the laboratories of Drs David Russell, Hans-Peter Kiem, Derek Persons and Helmut Hanenberg.

In terms of their integration profile within genomes, foamy viruses are generally considered to have a relatively more neutral integration profile, compared to lenti and gamma-retroviruses. They're less likely to integrate into regions proximal to gene promoters or within genes compared to gamma retroviruses or lentiviruses, respectively.

To date, as far as I know, transgene silencing has not been observed with foamy viral vector-delivered transgene cassettes. This suggests that foamy viruses would be good for life-long expression of therapeutic transgenes. Specifically for gene therapy, another advantage of foamy virus vectors is a documented resistance to serum inactivation, probably due to the apathogenicity in humans. Thus foamy viruses are a good candidate vector for intravenous delivery of therapeutic transgenes. In fact, this has been demonstrated in a preclinical canine model of X-linked severe combined immunodeficiency (X-SCID) by the laboratory of Dr Hans-Peter Kiem. It's not yet possible to pseudotype foamy virus particles with heterologous envelope glycoproteins, but it is possible to pseudotype other retrovirus vectors, such as lentiviruses, with foamy virus envelope glycoproteins to alter their tropism.

## Q What makes foamy a favorable vector for gene delivery into HSCs?

**I**n my opinion, the most prominent feature is the late reverse transcription of the viral genome prior to budding off the producing cell membrane during viral replication. This results in a foamy virus particle that already has a double-stranded DNA genome. Hematopoietic stem cells in particular are thought to be relatively quiescent, meaning these cells don't have a lot of free nucleotides hanging around because they're not actively trying to synthesize their DNA in cell division. Thus, a virus particle that has already completed the reverse transcription step into double-stranded DNA has an advantage. Additionally, it's known that the foamy virus pre-integration complex will hang around the centrosomes of the cellular chromosomal DNA until the cell divides. Thus, foamy virus infection in HSCs results in a stable double-stranded DNA pre-integration complex, which can wait in the nucleus until the HSC begins proliferating *in vivo*.

This work to document the late reverse transcription during viral replication and cell cycle dependence for integration was primarily conducted by Dr David Russell's, Dr Axel Rethwilm's and Dr Ali Saïb's groups. Their experiments demonstrated that foamy viruses transduced actively dividing cells at similar levels compared to gamma-retroviruses and lentiviruses, but foamy viruses transduced non-dividing cells more robustly than the other vector types.

Finally, depending on the target disease for gene therapy, the larger packaging capacity can also be very useful.

**Q** You mention X-linked SCID - what progress is being made in this particular disease?

**T**here are many groups studying gene therapy for X-SCID, a genetic disorder caused by a mutation in the common gamma chain gene, which results in defective development of immune cells such as T cells and natural killer or NK cells. The disease can be cured with a bone marrow transplant from an unaffected person, but matched donors are not available for all patients and complications from donor bone marrow transplants can be fatal. For these reasons, gene therapy to provide a functional version of the common gamma chain gene into the X-SCID patient's own blood stem cells is a beneficial alternative treatment. In both donor bone marrow transplant and gene therapy trials, it has been shown that adult and/or heavily treated X-SCID patients don't do as well as younger patients that have had less intervention. Unfortunately, unless a family has a history of X-SCID, often the diagnosis is not made until the child is symptomatic and requires intervention. In 2010, X-SCID was added to the core Recommended Uniform Screening Panel for heritable disorders in newborns in the USA. In many states, newborns are screened and diagnosed with X-SCID very early, which in turn permits early intervention.

In terms of blood cell gene therapy-based interventions, invasive procedures that involve collecting bone marrow and processing stem cells outside the body, especially in a child that could potentially be prone to more infectious complications, becomes a little bit more worrisome. This is where we formulated a multi-institutional collaboration between the Fred Hutchinson Cancer Research Center, Seattle Children's Research Institute and Washington State University to study the hypothesis that very early injection of a foamy virus vector, which is resistant to serum inactivation, encoding a functional common gamma chain gene could be a better approach. We postulated that because these children are very small at the time of treatment, smaller amounts of vector could be used successfully. To study this preclinically, we applied a canine model for XSCID originally developed at the University of Pennsylvania, which we now have here at the Fred Hutch in Seattle. Much of the disease pathology in these dogs is very similar to the human disease and breeding permits us to intervene in affected pups shortly after birth.

The ultimate goal of these studies is to hit a hematopoietic stem cell *in vivo* given that this disease doesn't only impact T cells. However, from a treatment perspective, you could do a lot of good by correcting a T-cell precursor that is very long-lived. The first study published in *Blood* in 2014, we demonstrated that intravenous injection of a common gamma chain encoding foamy virus vector was feasible and safe [3]. Current work within this collaboration aims to improve the efficacy of this approach by optimizing the promoter regulating expression of the common gamma chain gene and also improving the likelihood that hematopoietic stem cells are targeted for transduction *in vivo*.

In terms of how complex it would be to translate our observations in the dogs to a gene therapy trial for human X-SCID patients, we have to think about feasibility, safety and efficacy.

In regard to feasibility, the pups we treated were about 1kg in size and each received approximately  $4 \times 10^8$  infectious units/kg. The average baby, at least in the USA, weighs about 3.5 kg. Thus to directly translate this approach we're talking about 3.5 times the number of vector particles required for injection or we need to demonstrate some clinically acceptable way of transducing a target number of hematopoietic stem and progenitor cells safely with less vector particles.

In terms of safety, we consider short- and long-term risks. For short-term, there is obviously the immediate response to the vector administered and the potential for adverse events associated with the actual injection, such as an increased risk of immediate infection. We did not observe any adverse events associated with the foamy virus particle injections in any of the pups treated in the 2014 study, and have since followed-up with additional animals for up to 2.5 years without adverse events. Again, the apathogenicity of the foamy virus in humans is likely an advantage in this approach. For long-term risks, we consider both off-target effects and the potential for insertional mutagenesis. For example, off-target effects include tissues aside from the blood are transduced *in vivo*. In particular, we monitor for germ line transduction. We have not observed germline transduction in any dogs treated to date. For insertional mutagenesis, we are primarily concerned with the genomic locus of foamy virus integration. Monitoring these loci of insertion not only tells us whether the foamy virus has proximity to an oncogene, but it also allows us to estimate the number of transduced clones *in vivo*, which contribute to immune system reconstitution. We have established this clone tracking method in the dogs from the 2014 study and could monitor these parameters in patients enrolled in a clinical trial.

From the standpoint of efficacy, we would want to see reconstitution of a functional immune system. This includes not only development of functional T cells, but also other immune lineages that can be affected, such as NK cells. We observed partial efficacy in the original study published in 2014. Our current research is directed at improving the efficacy in the preclinical canine model.

Any disease treatment or prevention must take into account not just genetic variability between individuals, but also differences in environment and lifestyle. All of these elements together contribute to an individual's response to therapeutic intervention.

**Q** What are some of the challenges you're facing specifically around the use foamy as a vector?

**I** would say that we are always challenged to make reproducibly high-titer, concentrated foamy virus vectors. This challenge is not unique to foamy vectors, specifically when compared to lentivirus. There are several groups working on this, including our group here and the laboratory of Grant Trobridge at Washington State University. In general, we are able to produce titers in the range of  $10^6$  vector particles per milliliter. However, for *in vivo* delivery you want to infuse as many particles as possible in a very small volume, and you need to clean up the concentrated vector preparation before you would infuse it. Centrifugal concentration in our hands results in loss of vector particles. We're currently working on other methods of concentration like tangential flow filtration, which also increases our purity of intact viral particles, and removes cellular debris. Thus, concentration by this method also reduces toxicity and cleans up the preparation for infusion.

Codon optimization of the helper genes to increase expression during vector production could be another option for improving the titer. The foamy virus envelope can also be further modified. This envelope glycoprotein has already been modified to increase titer, and we think further modifications in the envelope could be possible.

Scale up of viral vector production such that one preparation could treat many patients is another issue for not just foamy viruses, but lentiviral vectors as well. Creation of a stably producing cell line to make foamy virus particles would also be advantageous. An engineered producer cell line that does not express known restriction factors for foamy virus particle production, such as Trim5 $\alpha$  or APOBEC3, could also improve the upper limit of titers and reliability of foamy virus production.

One challenge particular to our experience with foamy viral vectors is a limited stability at room temperature. To address this issue we have developed a rapid freezing protocol and optimized the cryogenic media, specifically with regard to the content of dimethylsulfoxide (DMSO), which stabilized foamy virus preparations. This was particularly important for *in vivo* administration of foamy virus vectors. Clinically, patients receive DMSO in cellular products, which are infused after thawing, thus the administration of thawed foamy virus particles formulated in DMSO at or below the concentrations received by patients infused with thawed cell products was critical for us.

**Q** Your group recently developed a prototype semi-automated closed system for point-of-care delivery of lenti-mediated HSC gene therapy. Could you tell us a little about this approach and the impact you think it could have on increasing the accessibility of gene therapies?

**F**irst, let me declare that accessibility to early phase clinical trials is something we really need to think about, and act to improve, in

the field of cell and gene therapy. Any disease treatment or prevention must take into account not just genetic variability between individuals, but also differences in environment and lifestyle. All of these elements together contribute to an individual's response to therapeutic intervention. In the USA, this is supported by the National Institute's of Health Precision Medicine Initiative (*All of Us*)<sup>®</sup>.

We started this research in improving accessibility because my work here at Fred Hutchison is translating hematopoietic stem cell gene therapies for a variety of diseases. We're fortunate to have the multimillion dollar Good Manufacturing Practices (GMP) infrastructure here in Seattle to do these kinds of studies. In contrast to our current preclinical *in vivo* work with foamy virus vectors for the treatment of X-SCID, all of our current Phase I gene therapy trials require blood or bone marrow to be collected from patients, then manipulated to parse out the stem and progenitor blood cells from the more mature blood cells, then these stem and progenitor cells have to be transduced outside of the body and finally cleaned up and prepared for infusion back into the patient. This requires clean rooms and sterile, heavily monitored equipment, reagents and materials. Thus, patients enrolled on our clinical trials have to come to Seattle for treatment.

Many private industry groups as well as academic institutions are attempting to centralize manufacturing by developing ways to ship cells back and forth from the clinic to the manufacturing site and back to the clinic for administration. However, this is expensive and introduces risks such as cell products being compromised or lost during shipment.

Personally, the issue of accessibility really resonated with me about 4 years ago, when we received a grant to translate a clinical trial of gene therapy to treat human immunodeficiency virus (HIV) infection. I was thinking about the environment and lifestyle of patients in the USA where the treatment is being developed and the prevalence of HIV worldwide. I really felt we needed a better way to distribute this sophisticated type of cell therapy to heavily HIV-infected countries, such as Africa.

For us this research was about creating a mobile gene therapy lab that could be applied to lots of different cell therapies in the local clinic where the patient is being seen or treated. Our proof-of-concept was in hematopoietic stem cells, but all the same components could be applied to T-cell gene transfer or any other cell type using that system.

I also wanted to show the field that you don't have to have GMP facility infrastructure in place to work on meaningful solutions to translating accessible cell and gene therapy. If more labs have access to genetic modification of purified cells in a simplified system, we can overcome the barriers to widespread use much more quickly and efficiently.

**Q** Could similar systems be developed for alternative viral vectors such as foamy?

**T**he way we developed the process, the user can define which vector is added to the cells in the system. We have shown it works



with the SIN lentivirus vector backbone currently in clinical use, but we could have just as easily added a foamy virus vector and shown similar results. It's amenable to any viral vector.

Therefore, I think if we can come up with a viral vector that requires a very low multiplicity of infection to get the same transduction efficiency in the target cells, that's certainly something that will be immediately applicable to the field.

### AFFILIATION

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