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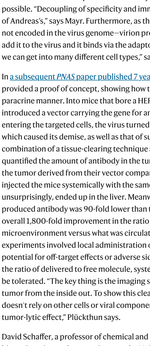
Vector BioPharma: resurrecting adenoviral gene delivery

Two veteran protein engineers team up to create a new gene-delivery vehicle.

Laura DeFrancesco

Adenoviruses as gene-delivery vectors took a hit 20 years ago, with the tragic death of a young man who was being treated for ornithine transcarbamylase deficiency using an adenovirus-delivered gene therapy. Since then, adeno-associated viruses (AAVs) and lentiviruses have been the mainstay gene deliverers. With their 2022 start-up Vector Biopharma, Andreas Plückthun of the University of Zurich and serial pharmaceutical executive Lorenz Mayr aim to bring adenoviruses back. In this, his fourth start-up, Plückthun has turned his protein engineering acumen to the task of solving several challenges that plague delivery vehicle designers: inactivation by the immune system, nonspecific uptake by the liver and restrictions on payload size. The platform, described in a 2013 *Proceedings of the National Academy of Sciences* paper and called SHREAD (for SHielded REtargeted Adenovirus), takes on all three issues. It's essentially an empty adenovirus shell, which, being devoid of any genetic material, can harbor as much as 37 kilobases of nucleic acid. It is cloaked with a human protein that makes it invisible to the immune system, and it is adorned at each vertex, with triplets of high-affinity targeting moieties. "If someone can shield [a therapy] from the immune system, if they could make it highly targeted, you can open up the door, which was pretty much blocked from the last decades," says Mayr, who, before stepping up to lead Vector as CEO, was a principal in or served on the board of several major pharma companies.



Andreas Plückthun, co-founder of Vector BioPharma Credit: Barbara Müller

The platform didn't come about overnight; it required decades of engineering to get all the pieces in place. As Plückthun describes it, "It brought together a lot of things we had done before [with engineering proteins]. My background has been to generate recombinant binding proteins, DARPins (designed ankyrin-repeat proteins), which allowed us to engineer these virions and make them [into] virus-like particles," or VLPs. The key element of the SHREAD platform is its bifunctional adaptor, consisting of two DARPINs: one that attaches to the knobs of adenovirus serotype 5 capsid, essentially blocking the adenovirus's surface protein and altering its inherent tropism for the liver, and a second that attaches to a targeting moiety, which can be anything, from an antibody to a peptide or even a non-protein. The specificity for the target is on the outside, whereas the inner face of the adaptor—which consists of a trimerized scFv antibody fragment directed against the virion coat protein—binds at each vortex of the icosahedral surface. This makes the particle invisible by covering it to the greatest degree possible. "Decoupling of specificity and immunogenicity was an extremely clever idea of Andreas's," says Mayr. Furthermore, as the adaptor protein is added afterward—it is not encoded in the virus genome—virion production is simplified. "We just have to add it to the virus and it binds via the adaptors. This gives us enormous flexibility and we can get into many different cell types," says Plückthun.

In a subsequent *PNAS* paper published 7 years later, Plückthun and colleagues provided a proof of concept, showing how their system can deliver a therapy in a paracrine manner. Into mice that bore a HER-2-sensitive tumor xenograft, they introduced a vector carrying the gene for an approved HER-2-directed antibody. Upon entering the targeted cells, the virus turned the tumor cell into an antibody factory, which caused its demise, as well as that of surrounding tumor cells. Using a combination of a tissue-clearing technique and three-dimensional imaging, they quantified the amount of antibody in the tumor, and found 21 times more antibody in the tumor derived from their vector compared to what they measured when they injected the mice systemically with the same antibody—most of which, unsurprisingly, ended up in the liver. Meanwhile, the plasma ratio of the in vivo–produced antibody was 90-fold lower than that of the injected version, resulting in an overall 1,800-fold improvement in the ratio of antibody in the tumor microenvironment versus what was circulating in the animal. Although these experiments involved local administration of their therapeutic, which reduces the potential for off-target effects or adverse side effects, the group estimates that given the ratio of delivered to free molecule, systemically delivered toxic therapeutics could be tolerated. "The key thing is the imaging showed that we were literally treating a tumor from the inside out. To show this cleanly, we wanted to use a system that doesn't rely on other cells or viral components. The antibody does all the work for the tumor-lytic effect," Plückthun says.

David Schaffer, a professor of chemical and biomolecular engineering, bioengineering and neuroscience at the University of California, Berkeley, works with several viral delivery platforms, and points out that several groups, including themselves, has tried to engineer adenovirus. "[Viruses never got evolutionarily rewarded for infecting tumor cells inside the body, so we are trying to use them for something different, which means we need to be reengineering them," he says. But adenovirus is a particularly challenge to engineer, he adds, since it is a 'very large, complicated device with intricate functions.'

Manufacturing also presents a challenge, as a virus devoid of any genetic material requires a helper virus for replication, which then must be disposed of. Schaffer says the production of helper-dependent adenovirus can be difficult to scale. To address this, Vector is looking to lower the dose required to get a therapeutic effect by improving the efficiency, for example through trimerizing the adaptors. "And by optimizing upstream processes, downstream purification as well as numerous quantification assays, we are confident that we can provide the quantities necessary for human use as soon as the actual preclinical experiments are completed," Plückthun says.

Another unknown is how well the platform will perform in humans. "There have been many situations where things have been targeted to something in a mouse, but when tried in humans, it didn't work. It's more of a limitation of the cancer models, using xenografts or syngeneic tumor, ' Schaffer says.

Several derivatives of the SHREAD platform are in the works at Vector's labs. One, a virion with three targeting molecules (CD3, CD28 and IL-2R), transduces T cells in vivo and can produce CAR-T cells of any specificity, according to Plückthun. The simultaneous engagement of the three molecules activates T cells, which improves the uptake of the VLP by the cells, he says. They demonstrated this in a mouse model with reconstituted human T cells in a recent *Molecular Therapy* paper. This, Plückthun believes, may provide a pathway toward making CAR-T cells in vivo.

In a second SHREAD variation, the therapeutic is targeted to tumor-associated fibroblasts, rather than tumor cells, so that the producer cell doesn't destroy itself. This also enables a single VLP to treat different tumors, as the surface marker FAP (fibroblast-activating protein) is always the same in different tumors.

In late 2022, Versant Ventures launched Vector out of its Basel, Switzerland–based Ridgeline Discovery Engine, with a $30 million A round of financing. In addition, the company is in discussions with several pharma companies with expertise in different areas—immuno-oncology, in vivo CAR-Ts, enzyme replacement and epigenome editing, Mayr says, "We want to bring [our platform] forward as quickly as possible with partners who understand the disease area and can bring it to commercial value today. We see broad applications, essentially any which requires delivery of large cargo of DNA to specific sites in the body, with low immunogenicity," And in the end, Schaffer says, "I wish them well. The field needs it."

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