What's Next for Cell & Gene?: Innovation & Insights – A Q&A with Heikki Turunen, AAV Gene Therapy Expert

With this Q&A, What's Next for Cell & Gene? delves into the transformative impact of AI/ML, the intricate challenges of immunogenicity, and the evolving landscape of gene delivery platforms.

The AI/ML Frontier in Capsid Design

Q: AI/ML approaches to capsid design and engineering, and drug discovery as a whole, have been intriguing to the field. Could you speak to the major areas of utility for this approach, i.e., does AI-guided design allow for selection of capsids more suited for specific indications, or are we focused more on AI-guided design to accelerate discovery of lead candidates?

A: The promise and hype surrounding AI/ML in drug discovery are immense. A core strength of these methods is their ability to improve models with increasing data, and data generation has accelerated rapidly. This creates a positive feedback loop, leading to exponentially improving ML models and superior capsids.

Historically, capsid engineering relied on generating large, complex gene libraries with random mutations. While billions of variants could be screened, the practically infinite sequence combinations of a 730 amino acid capsid protein meant random mutations were not a realistic pathway for truly transformative AAVs. Past experiments also showed how easily single amino acid substitutions could break the AAV capsid. However, recent studies demonstrate that AI/ML-guided designs can result in viable capsid particles even with dozens of amino acid changes from parental sequences.

I view the engineering challenge as three-fold:

1. Identify capsid protein areas responsible for specific properties.
2. Identify amino acid sequence modifications needed to improve those properties.
3. Graft those modifications into the capsid without interfering with other critical properties.

This can be achieved by combining strategies. For instance, *in vitro* screening identifies AAV receptors, then AI models design modifications to increase affinity. However, the true power of AI/ML emerges when these modifications are incorporated without breaking the capsid. Any change intended for one property can impact others. Therefore, all capsid engineering aims to improve or maintain multiple properties simultaneously, a task far better suited for AI models than random chance or limited human intuition.

In theory, AI/ML applies to all gene therapies. A variant engineered for increased tropism to a specific cell type could serve many indications affecting that cell type. Other times, unique, custom variants for a specific indication may be necessary. As most engineered variants are proprietary, different companies may develop their own capsids for the same indications.

It's still early days for AI/ML's full potential in AAV engineering. While claims of ML-designed best-in-class capsids are surfacing, broader access and independent validation are still needed. Nevertheless, I am optimistic that truly transformative capsids will emerge soon, leading to safer, more efficient, and cheaper gene therapies for many devastating diseases.

Addressing AAV Immunogenicity

Q: AAV immunogenicity is one of the key areas of focus of gene therapy, with the development of safe and efficacious AAV gene therapies being, in part, dependent on the circumvention of pre-existing immunity to AAVs. How far has capsid engineering brought the field in addressing AAV immunogenicity, and how do we see the dependency between pre-existing immunity and capsid engineering addressing the broader questions of patient inclusion in trials, and dosage?

A: Recent advances in high-resolution cryo-EM imaging and structural characterization of antibody-capsid interactions have helped identify putative target epitopes for antibodies on several serotype capsids. These regions can now theoretically be targeted for modifications to disrupt them and prevent antibody binding. The challenge, as mentioned, is making these modifications without interfering with other properties, like transducing target cells. Proof-of-concept studies suggest AI/ML approaches have the potential to solve this.

While early reports are promising, with many institutions generating antibody-evading capsid variants, it's crucial to acknowledge the limitations in assays used to evaluate AAV neutralization by antibodies. There are currently no standardized assays to measure neutralizing antibody levels. Neutralizing antibody titers are highly dependent on assay conditions, making them somewhat arbitrary and meaningless. Due to these inaccuracies, titer limits for patient exclusion have been difficult to set. This results in wide variations in literature regarding pre-existing immunity: population seropositivity estimates range from 20% to 90%, and individual exclusion titers range from 1:5 to successful outcomes even at 1:1,000. Finally, most published literature considers titer limits independently of the intended dose, even though lower antibody levels would neutralize low AAV doses but have no noticeable impact on significantly higher doses.

Crucially, the field urgently requires a unified definition for pre-existing immunity and its impact on AAV gene therapies. As 'titer' is arbitrary, it's imperative that more quantifiable measures are adopted, such as the number of capsids neutralized by a volume of sample. This would enable better predictions for patient exclusion and allow for dose adjustments based on neutralizing antibody levels to maximize efficacy.

In conclusion, as engineered AAVs become more efficient and doses hopefully decrease, I expect the impact of pre-existing immunity to be exacerbated. While capsid engineering has successfully generated AAV variants less neutralized than their parents, standardized methodologies are needed to accurately measure improvements and predict clinical outcomes given specific antibody levels and AAV doses. As for AI/ML strategies, I'm confident they will result in variants able to evade the majority of neutralizing antibodies.

Gene Editing, Viral, and Non-Viral Delivery — A Shifting Landscape

Q: Lastly, very broadly, how do you see the different approaches of gene editing, viral gene delivery, and non-viral gene delivery contribute to advancement and approval of gene therapeutics? Do we expect one to replace the other as the standard of care (e.g., non-viral gene delivery to become a new SoC for ocular disease down the road), or do we see an overlap/combination of viral and non-viral therapeutics serving different segments of the patient population for a specific indication?

A: Throughout their existence, AAV gene therapies have faced persistent challenges related to safety, efficacy, and high treatment costs. Recently, some companies have even discontinued AAV development programs, leading some to declare the era of AAVs over. However, despite their shortcomings, AAVs remain the vector of choice for many gene therapy applications.

Developing AAV gene therapies is time-consuming and expensive. For a product to be commercially successful, sales profits must cover years of development. This has been a challenge for the AAV field, as most applications target rare diseases with small patient populations. Sales of Luxturna, the first FDA-approved gene therapy, were significantly lower than projected due to overestimation of patient population size. Similarly, Pfizer recently discontinued commercialization of their FDA-approved hemophilia program, citing a lack of doctor and patient interest, likely due to existing alternative treatments. Thus, in these examples, companies are losing interest in AAVs because they're not profitable, not because they don't work. Of course, some clinical trials have also failed to meet goals, indicating that treatments are not always sufficiently efficient.

It may be tempting to view all gene therapies as similar enough for a universal, one-size-fits-all vector. However, the needs for different gene therapy modalities vary drastically: they may require transient or permanent expression, systemic or local administration, or target dividing or non-dividing cells. Different vector systems—viral or nonviral—have distinct pros and cons in each category.

It would be unwise to abandon AAV gene therapy development simply because it doesn't work well enough for all indications or all business cases aren't viable. It is undeniable that in some cases, AAVs do work, save lives, and can be highly profitable products. The numerous proof-of-concept successes offer promise that existing shortcomings can be improved, and early developmental results provide confidence that goals are achievable. However, the same arguments can be made for other delivery platforms. I do not foresee a world where multiple gene therapy delivery approaches are mutually exclusive. Work on all avenues should continue by those best positioned to do so. Ultimately, it's not the best product that wins; it's the patients.