Gene Therapies and Why We’re Not All Using them

What is a Gene Therapy?

- The genetic modification of cells in vivo to achieve a therapeutic effect.
- This can take many forms.
- None of these forms are at present optimal.
- Very generally, many copies of some form of DNA sequence or RNA molecules are inserted into a cell. The cell processes the DNA or RNA as though it were native, producing proteins to change cell behavior.
- In principle, gene therapy can be much more precise than any small molecule approach to therapy, as off-target and poorly understood effects on cell behavior are as minimal as they can be.

What is a Gene?

- A sequence of DNA in the genome that can be processed by genetic machinery to produce RNA and, for some, a protein.
- Genes consist of an array of intron sequences (excluded) and exon sequences (included). In some genes these can be spliced together in different ways to produce different RNAs and proteins.
- Genes are interspersed with sequences that act as promoters (activate expression), enhancers (greater expression), and silencers (stop expression) of specific genes via their interaction with genetic machinery.
- It is usually possible to come up with a better DNA sequence than an evolved gene and its regulators for any given application, given time and effort, and most patents in the industry involve this sort of work.

A Refresher on Gene Expression

- A gene is only expressed under some circumstances. This is determined by the interaction of DNA transcription machinery with promoter, enhancer, and silencer sequences that are usually adjacent to the gene.

- DNA transcription machinery reads an active gene to produce RNA molecules. Many gene sequences can be transcribed in multiple ways, based on inclusion and exclusion of components called exons and introns.

- RNA molecules locate to a ribosome for translation into protein molecules and subsequent protein folding. One RNA molecule is the blueprint for many protein molecules.

- At every step there are feedback loops between RNAs, proteins, and expression machinery. Transcription factor proteins alter transcription, while many RNAs are never translated and only exist to adjust translation of genes.

Tools of the Gene Therapy Trade: Types of Tool

- Access to the cell interior.
- Access to the cell nucleus.
- DNA and RNA cargo types to engineer expression or other effects.
- Insertion of (a) sequences into the nuclear genome, versus (b) plasmids into the nucleus, versus (c) mRNA into the cytosol.
- More targeted nuclear genome insertion via CRISPR, TALENs, etc.
- (Out of scope: engineering the mitochondrial genome – a whole topic unto itself).
- Restriction of expression to specific locations.

Tools of the Gene Therapy Trade: Cell and Nucleus Entry

- Engineered, non-replicating viruses. Lots of different ones with different characteristics: adenovirus, adeno-associated virus (AAV), lentivirus, etc. A single all-in-one package for getting into the cell and the nucleus and expressing the cargo.

- Lipid nanoparticles, for putting DNA and RNA into the cell cytosol. There are many varieties. Getting access to the nucleus remains a challenge unless target cells are replicating aggressively.
  - Nuclear targeting sequences for plasmids are a whole topic of cutting edge and underutilized research and development.

- Electroporation opens cell membranes and the nuclear envelope enough to let DNA enter, but not so much as to kill the cell.

- Cell penetrating peptides, capable of gaining entry to a cell cytosol.

Tools of the Gene Therapy Trade: DNA Cargo

- Plasmids, circular DNA that is processed in the same way as the nuclear genome, if it can only get into the cell nucleus. Many different proprietary plasmid backbones (nanoplasmids, minicircles, etc.). Some can be made to replicate.

- Promoters, enhancers, and repressors, to constrain when and where an inserted sequence is active. Design of these sequences is a dark art, poorly understood, requires a lot of experimentation. There are useful consensus “express everywhere” sequences such as CMV and Ef1a.

- The contents of promoters can include all sorts of tools for DNA insertion and expression: TALENs, CRISPR, crude DNA logic machinery such as the Oisin Biotechnologies approach to conditional expression, or can be as simple as a promoter and a gene.

- Most of what can go into a plasmid can go into a viral vector, if it fits.

Tools of the Gene Therapy Trade: RNA Cargo

- RNA interference DNA sequences, producing RNAs that block transcription (RNA to protein) of specific genes. Or just deliver the RNA molecules for a more limited, controlled effect.

- mRNA, delivering a transcript that does not have to localize to the cell nucleus, and that will then be translated into (probably) hundreds of protein molecules per mRNA molecule.

Insertion of DNA Sequences into the Genome

- DNA sequences can be inserted into the genome via many different methods with different characteristics.

- In the case of most tools, this is haphazard and breaks whatever existing sequence it is inserted into the middle of. Fortunately most of the genome is inactive in any given cell and most of the tools favor inactive regions.

- Commonly used genome insertion tools:
  - CRISPR sequences, a novel approach to more precise insertion into the genome, presently largely used to disable specific genes.
  - TALENs, a pre-CRISPR approach to more targeted insertion.
  - Transposon sequences (e.g. piggybac) that insert many copies into the genome, somewhat blindly, but usually in inactive locations.
  - Lentivirus, fewer insertions, more random than transposons.

Non-Integrating Gene Therapy Vectors and Cargo

- Having a gene therapy that doesn’t insert into the genome is great for some uses. Maybe you only need a temporary outcome, or you don’t want the FDA to give you the third degree due to a newly proposed permanent treatment.

- AAV produces plasmids that don’t integrate, and are diluted with each cell division. Eventually cell turnover removes them.

- Other plasmids operate in much the same way as AAV once in the nucleus, with durations from days to months depending on the tissue.

- mRNA operates outside the nucleus, and only lasts for a few days. Duration is more a matter of mRNA and protein stability than other factors.

The Problems (Oh, the Problems)

- Size limits
- The challenges of targeted delivery
- Dose-limiting toxicity resulting from trying to get enough vector to the destination
- The immune system hates gene therapies
- Inability to repeat treatments with many types of vector
- Extreme regulatory conservatism
- Cost: many vectors are outrageously expensive to manufacture under GMP.
- Goldilocks issues with duration of expression

Size Limits in Gene Therapy

- All approaches have size limits on the sequence that can be delivered. Some are much bigger than others.
- Size limits are quite abrupt in terms of fall-off of efficiency. Anything lower is fine, and suddenly your vector drops to 10% efficiency if you add a few hundred base pairs.
- The well established viral vectors, i.e. AAV and lentivirus, have small capacities. Some single promoter and gene sequences will not fit, and that’s before you add a tracer like a fluorescent protein.
- Plasmids have large capacities, but even these have inconvenient limits. You can’t deliver half a dozen genes in one go, and there are certainly situations in which one would want to given the ability.

Immunogenicity

- Even the best vectors, those least visible to the immune system, can be loaded up to the point of causing death via immune reaction. That places an upper limit on systemic delivery.

- Deaths and severe adverse effects due to gene therapy have been the result of immune reactions to the vector. But mRNA can be very immunogenic! It just isn’t yet used widely in large enough amounts for this to start to be a serious problem.

- But in the case of people born with a dysfunctional gene, it is possible for the immune system to react to a functional protein as though it were foreign. Similarly, there are many synthetic proteins that might be interesting to use, but the challenge lies in ensuring lack of immune reaction to a foreign sequence.

- Genetic engineering that touches on immune cells is particularly likely to produce interesting problems.

Immunogenicity Limits Repeated Treatments

- Many types of vector can only be given to a patient once, such as all viral vectors.
- The second time, it will simply be cleared by a prepared adaptive immune system that now has a memory response to the vector, possibly accompanied by an undesirable immune reaction.
- Some people are already able to clear some viral vectors naturally, limiting the patient population.
- This is largely not the case for lipid nanoparticles, which have other more general issues with innate and adaptive immune response based only on dosage.
- The degree to which the immune system can be aggravated into a response by vector payloads is unclear, very dependent on the precise details of each case.

Immunogenicity – Rolling the Dice on Clinical Translation

- It is always a question mark as to whether a given species will react badly to a given vector (common) or to cargo (particularly mRNA).
- There are cases of vectors doing fine in all species up to human, and then causing serious immune reactions in humans.
- And many more cases of felines, non-human primates, dogs, etc, reacting poorly to a vector that was harmless at a given dose in mice.
- The immune system is a black box. It cannot be predicted well.

The Challenges of Targeted Delivery

- There is always an upper limit to how much vector you can delivery systemically before causing toxicity, usually via immune reaction.
- Systemically administered vectors go everywhere, but mostly lungs and liver.
- If your target is some other tissue, how do you get enough vector to that target without delivering too much overall?
- The answer is more or less this: you inject directly, or if that isn’t possible then gene therapy is not a feasible approach to the problem at hand.
- There is as yet no vector you can soak the body in safely, allowing some form of promoter based restriction of expression to deliver sizable degrees of transfection to a small tissue in some corner of the body.

Targeted Delivery – Why the Liver is Popular

- Why did so many of the initially developed gene therapies involve the liver as a target?
- Because any systemic injection will typically deliver most of the cargo to the liver.
- Therefore much less work was needed to get the vector to where it was needed.
- Targeted delivery was – and still is – such a huge problem that it pushed the whole field in the direction of a focus on liver targeted therapies.

Gene Therapy: $$$$

- GMP manufacture of AAV is incredibly expensive. There is a treatment out there for very young children that costs more than $2 million per dose. Manufacture of that single dose requires a dedicated 100,000 sqft facility 40 days to produce, doing nothing else concurrently.

- Non-GMP, normal, sanely safe manufacture is 10x cheaper, and for AAV has basically the same outcome, but it is forbidden for medical use in most of the world.

- This is a big reason as to why new approaches to lipid nanoparticles are becoming more popular in development circles, as the GMP cost will allow prices of closer to $50,000 rather than $500,000 per dose.

Regulatory Conservatism

- Why would anyone even think about using AAV? Because it has been taken to the clinic, and thus there are established GMP manufacturers.

- There is significant risk in working with a new vector or associated technology, such as cell penetrating peptides, where “new” means “few FDA approvals or manufacturers with proven GMP track records.”

- Whether or not a path already exists is a strong influence on investors and executives, given that the FDA ensures that all attempts at gaining their approval cost so much and are so very uncertain for any new technology without an established GMP protocol.

Expression Duration

- Roughly: mRNA if you want expression for days, plasmids if you want expression for weeks to months, AAV for months to years, and lentivirus or similar for permanent changes.
- It can be economically and logistically non-viable to use mRNA if months of expression are needed for a therapeutic effect.
- It can be a regulatory show-stopper if expression lasts for a long time when only a few months would suffice.
- There is no practical months-long expression option in many cases. There is no established approach to reliably getting plasmids into the cell nucleus in vivo that has been demonstrated to be viable in the clinic, or even robustly in animal models.

Approaches to the Challenges

- Coercing the immune system into ignoring vectors
- Engineered tropism for specific tissues
- Direct injection to the target of interest
- Promoter-based limiting of expression
- Sacrifice efficacy in favor of using vectors that are already proven
- Use a cell therapy instead! Engineer cells outside the body, not inside the body.
- Give up and try a small molecule approach, because everything other than patient outcomes will be easier and better.

Make the Immune System Blind to the Vector or Cargo

- Lipid nanoparticle approaches that minimize immune reactions are emerging, but this process of development is more haphazard discovery than goal driven.

- Approaches to make the immune system tolerant to a specific viral vector have been under development for a while, but only now are practical ones emerging, e.g. Selecta Biosciences.

- mRNA is very immunogenic, but it is altered in ways to limit this. A suite of known general manipulations exist, and teams try hard to find others specific to their sequence. This is a blind exploration when conducted on a sequence by sequence basis however.

Engineered Vector Tropism

- Produce viral vector variants that are more capable of entering certain cells. An entire large industry is devoted to the interative blind exploration of this approach.

- Use molecular patterns that bind to specific cells more readily: cell targeting peptides, receptor ligands, etc. Conjugate them to your vector, whether LNP or virus. Another large industry works on interative blind exploration of these approaches.

- Tropism is largely limited in scope. If you can get a 2x effect size in terms of restricting or encouraging the percentage of your vector that ends up in a given place, you are looking good. More than that is unlikely, though in the case of viral vectors the established serotypes do see larger effect sizes than this.

- A 2x effect means that you can use 2x less vector, which can be a big deal given costs and immunogenicity.

Direct Injection – Simple Approaches Largely Work

- If you inject a therapeutic, most of it goes to cells surrounding the location in which you injected it.
- Liz Parrish underwent a hundred or so direct injections into muscle tissue for her gene therapy.
- This cannot be used in practice for deep internal organs in old people other than in cases of serious disease. Fine needle aspiration data suggests a 0.1% to 0.2% mortality rate.

Promoter Based Expression

- Find a gene expressed only in the cells of interest. This is not always possible. Most genes are active in different contexts in different tissues. Evolution loves reuse.
- Find the promoter for that gene. This is a matter of time and effort if no-one has established the promoter sequence already.
- Use this promoter rather than a general promoter like CMV in your vector cargo.
- This only works for viral vectors and plasmids. mRNA cannot be promoter driven.
- This doesn’t solve the question of how to get enough of the vector to the tissue of interest without delivering toxic levels of the vector elsewhere.

Summary: Why is there no Gene Therapy for X?

- Because it costs immense amounts of money and is very risky to put anything new into the FDA approval process, and there is no broad alternative path to reaching the clinic.
- Because it is essentially impossible to deliver therapeutic levels of the desired vector to arbitrary organs, particularly small organs, without direct injection of the tissue.
- Because there is no known promoter-based approach to limit expression of the gene therapy payload to the cells or tissues of interest.
- Because most approaches other than AAV are largely unproven or only used in a very limited way in humans, and AAV is not economically viable.
- Because your needed payload is too big for established vectors to deliver.

Summary: Why is there no Gene Therapy for X?

- Because no vector/cargo combination satisfies the ability to produce expression for a suitable length of time.

- Because developers made the rational choice to produce an objectively worse small molecule drug that they can get past the FDA at a lower cost and risk. Or a similar calculation was made for a cell therapy with ex vivo gene engineering.