Outline:

- UMN DNA Repair Disorder Gene Therapy Program
- Gene Therapy 101
- Pre-clinical gene therapy development in a mouse model of XPG deficiency
- Future directions
UMN Gene Therapy Program

Clinical Studies (Dr. Kang’s team)
- Natural history studies (XP, CS, TTD, and others)
- Clinical trial planning

Pre-Clinical Studies (Pacak Lab)
- Model development
- Gene therapy optimization

Manufacturing (Aslanidi Lab)
- Capsid development
- Scaling up, product quality control

![Sweater Diagram]

Iris Gonzales
Gene Therapy

- **Gene delivery** – delivery of a healthy version of cDNA into cells with a missing or defective version
- **Genome editing** – delivery of genetic components needed to edit the genome directly (many different versions exist). The approach taken depends upon the genetic variant

**Similar Challenges Exist**

- Either approach requires the ability to deliver genetic material

Gene Therapy – Gene Delivery

- **Gene delivery** – delivery of a healthy version of cDNA into cells with a missing or defective version
Gene Therapy – Genome Editing

- **Genome editing** – delivery of genetic components needed to edit the genome directly (many different versions exist). The approach taken depends upon the genetic variant.

Gene Therapy (Gene Delivery) - Our goals:

- Long-term expression
- Focus on neurological tissues but would be wonderful to treat skin or other targets as well – avoid the liver
- Ameliorate the disease phenotype and improve quality of life
- Minimal effective dose
- **Patient safety**
Gene Delivery Vehicles

Many exist (plasmid delivery systems, retroviruses, lentiviruses, adenoviruses, adeno-associated viruses, extracellular vesicles) and all have strengths and limitations

Adeno-Associated Virus (AAV)

- Discovered as a contaminant in adenovirus preparations – requires help for replication
- Recombinant AAV (rAAV) used for gene therapy lacks elements necessary for replication

Wild-Type AAV (wtAAV)

Recombinant AAV (rAAV)

Humoral Immunity to AAV Vectors in Gene Therapy: Challenges and Potential Solutions
June 27, 2013. Elisa Masat, Giulia Pavani, Federico Mingozzi
General AAV Attributes

• rAAV persists as an episome within the nucleus – stable for many years *in vivo* in non-dividing cells and eventually diluted out in dividing cells
• Integration into the genome is extremely rare
• This has implications for treating a target like skin

Control of Expression – Many Layers of Optimization

• Capsid serotype
• Vector sequence design
• Dose
• Delivery route
Control of Expression - Capsid Serotype Selection

- Many natural and engineered serotype options – different AAV capsids have different tissue tropisms
- Capsid choice influences many things: AAV packaging, receptor binding preferences, efficiencies can be destroyed or enhanced, tendency for degradation pathways depending upon receptor, trigger host immune response

Control of Expression – Vector Sequence Design

- Promoter choice: Can be general or tissue restrictive
- Codon optimization (*human coERCC5*)
Control of Expression – Dose

• Dosage studies guide how much is needed to achieve a therapeutic effect (start low and go higher) (MED)

Control of Expression – Delivery Route

• Delivery route:
  • Intravenous (IV) (systemic)
  • Intrathecal (IT) (spinal cord)
  • Intracerebral Ventricle (ICV) (direct brain administration)
Gene Therapy Risks

- Risk tolerance of patient and family depends upon many things – gene therapy cannot be undone.
- Clinical trials determine how safe and effective a therapy is at relieving symptoms or treating aspects of a condition.
- Informed consent is essential
  - May or may not provide benefit
  - May or may not be exposed to unknown risks
- Even approved gene therapies can carry risk due to immune responses

AAV Gene Therapy Successes

- Luxturna – FDA approved therapy for inherited retinal disease (Biallelic RPE65 mutation-associated retinal dystrophy)
- Zolgensma - FDA approved therapy for spinal muscular atrophy (SMA).
  (Watching these patients to understand therapeutic durability)
Pre-Clinical Gene Therapy Studies
(*XP-CS, XP-ND, CS, TTD, etc.*)

- Model development: mice, induced pluripotent stem cells (iPSCs) differentiated into neurological cells and organoids

- Gene therapy optimization (general for the program):
  - AAV capsids
  - Promoters
  - Delivery routes
  - Doses

- Gene therapy for XPG deficiency (*ERCC5*) – good mouse model of XP-CS

### XPG/ERCC5

- Wide range of clinical phenotypes
- XPG mutations that lead exclusively to XP symptoms are deficient in NER
- XPG likely has important role/s outside of NER
- May play an important function in RNA polymerase II transcription (the XPG homologue Rad2 in *S. cerevisiae* does) through TFIIH

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2721477/
Gene Therapy for XP-CS: Experiment 1 - IV administrations

• Xpg^-/- mouse model IV delivery of AAV-ERCC5

XPG Experiment 1 (IV) Conclusions:

• Gene therapy can have a beneficial effect in XP-CS (delayed onset of neurological phenotypes and improved survival)
• However - Doses are too high
Gene Therapy for XP-CS: Experiment 1 - IT administrations

- Xpg⁻/⁻ mouse model IT delivery of AAV-ERCC5

XPG Experiment 2 (IT) Conclusions

- Delayed onset (prevention) of some aspects of neurological phenotypes
- Some improvement in survival

Will use this information to guide further optimizations (delivery route, promoter, capsids)
Ongoing Work!

Therapies and clinical trials

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A good gene therapy resource...

• American Society of Gene and Cell Therapy website (ASGCT.org – patient education)
Thank you!

Thanks to those participating in research studies!

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