Tacrolimus administration in combination with dexamethasone reduces neutralizing antibody formation against AAV vector and increases transgene expression in cynomolgus macaques

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Background
- Gene therapy using recombinant adeno-associated virus (rAAV) vectors has been successful in treating a wide range of human diseases.
- Eleven AAV hematopoietic stem cell-derived AAV (AAVHSC) belonging to Clade F have been discovered and fully characterized. Each AAVHSC has unique amino acid residues on the capsid proteins VP1, 2, or 3.
- Patients treated with rAAV often experience elevated ALT/AST, which is believed to be immune-mediated. Patients may also experience immune responses such as anti-AAV neutralizing antibody (nAb) formation and B & T-cell activation. These responses can lead to loss of therapeutic transgene expression over time and hamper the potential for re-dosing.
- Corticosteroid treatment regimens have been widely used to manage elevated ALT/AST levels and immune responses in rAAV-treated patients.
- However, due to the non-specific mechanism of action of corticosteroids, patients could experience adverse effects including hypertension, hyperglycemia, osteoporosis, and neuropsychiatric symptoms, such as insomnia and mood disturbance.
- The addition of drugs with more specific targeting have been introduced in AAV-based gene therapy clinical trials to improve the immunosuppressive regimen and potentially reduce adverse effects in patients. For example:
  - Mannalian target of rapamycin (mTOR) inhibitor sirolimus targets mainly B cells and T cells
  - Calcineurin inhibitor tacrolimus targets mainly T cells
  - Here, we investigated the effects of immunosuppressive regimens (different combinations of dexamethasone and/or tacrolimus) on liver and immune responses in AAVHSC17-treated cynomolgus macaques.

Mechanism of Action of Immunosuppressive Drugs

- Tacrolimus (Tac): Calcineurin inhibitor (T cell specific)
- Dexamethasone (Dex): Glucocorticoid receptor ligand
- Rituximab (Rit): CD20+ B cell inhibitor
- Sirolimus (Sil): mTOR inhibitor
- APL-9: Complement 3 inhibitor
- Imlifidase (IdeS): IgG cleaving cysteine protease

Study Design

**Results**

- **Dexamethasone & Tacrolimus Regimen Reduced nAb Formation**
  - Each NHP developed nAbs, detectable as early as Day 14 (data not shown).
  - The graph on the left shows nAb titer of the terminal serum sample at Day 28.
  - Dexamethasone & tacrolimus combination regimen reduced nAb formation by 4.8-fold, relative to the no-immunosuppressive regimen group.

Results

- **Dexamethasone & Tacrolimus Regimen Increased Gene Expression**
  - Dexamethasone & tacrolimus combination regimen increased PAH mRNA expression by 2-fold, compared to no immunosuppressive drug treatment group, greater than the slight increase observed in VG.

Summary

We demonstrated that modulating T-cell activity using tacrolimus together with dexamethasone is important in reducing B- and T-cell activity, nAb formation, and maintaining transgene expression following rAAV administration in NHPs. These results support the use of a dexamethasone & tacrolimus immunosuppressive regimen in our ongoing gene editing clinical trial with HMI-103 (pheEDIT) in adults with phenylketonuria (NCT05222178) and gene therapy trial with HM-203 (juMPStart) in adults with Hunter syndrome (MPS II) (NCT05238324).