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

[1]Daisy Huynh, [1]Omar Francone, [1]Teresa Wright, [1]Jenn Newman, [1]Jacinthe Gingras, [2]Pablo Morales, [2]Amber Williams, [1]Julie Jordan, [1]MiJeong Kim

[1]Homology Medicines, Inc., 1 Patriots Park, Bedford MA 01730; [2]The Mannheimer Foundation Inc., 20255 SW 360th, Homestead, FL 33034

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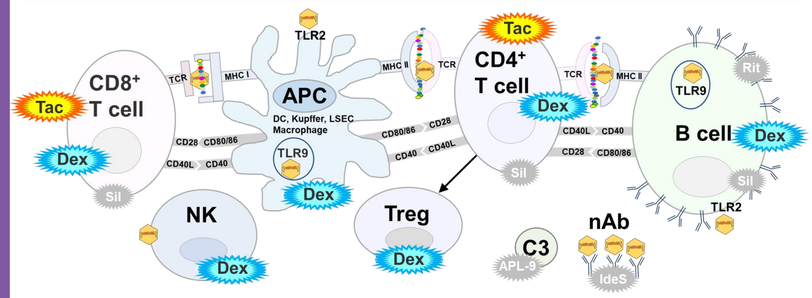
Background

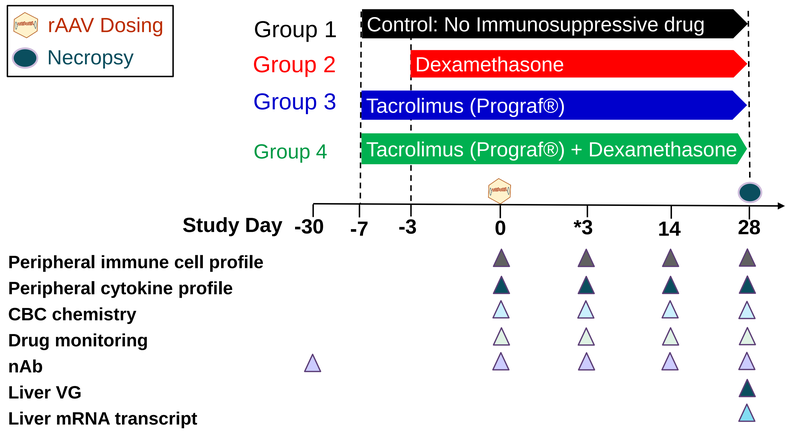
- Gene therapy using recombinant adeno-associated virus (rAAV) vectors has been successful in treating a wide range of human diseases.
- Eleven AAV hematopoietic stem cell-derived AAV (AAVHSC) belonging to Clade F have been discovered and fully characterized. Each AAVHSC has unique amino acid residues 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:
  - –Mammalian 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

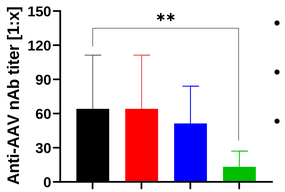


Species	*Macaca fascicularis* (commonly known as cynomolgus macaque)
Age	18-23 months
Gender	Male
Group Size	3 per group
Study Site	The Mannheimer Foundation Inc. (Homestead, FL)
Eligibility	Seronegative for anti-AAV9 nAb prior to AAV injection
rAAV Vector	AAVHSC17 with phenylalanine hydroxylase (PAH) driven by liver specific promoter (DnG: David and Goliath)
Dose	1E+14vg/kg
Dexamethasone	Daily 0.15 mg/kg s.c.
Tacrolimus	Daily 0.10 mg/kg i.m.

*CBC Chemistry for one animal at Day 3 was re-analyzed using Day 6 blood sample, due to technical challenges

Results

Dexamethasone & Tacrolimus Regimen Reduced nAb Formation

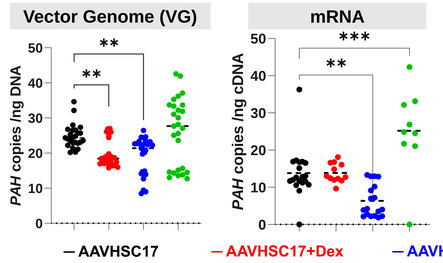


- Each NHP developed nAbs, detectable as early as Day 14 (data not shown).
- The graph on the left shows nAb titer of the terminal serum sample at Day 28.
- Dexamethasone & tacrolimus combination regimen reduced nAb formation by 4.8-fold, relative to the no- immunosuppressive regimen group.

** Statistical analysis done using ordinary one-way ANOVA

Results

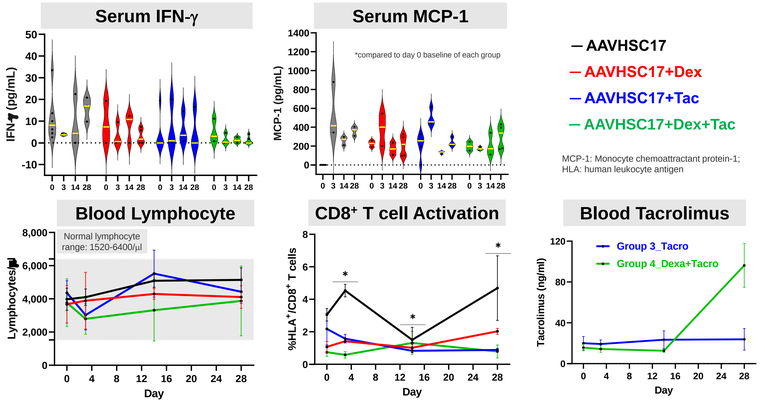
Dexamethasone & Tacrolimus Regimen Increased Gene Expression



- 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.

*Statistical analysis done using ordinary one-way ANOVA; cDNA: complementary DNA reverse transcribed from mRNA

Dexamethasone & Tacrolimus Regimen Reduced Inflammation



- Dexamethasone & tacrolimus combination regimen lowered the overall secretion of inflammatory cytokines IFN-γ and MCP-1, and the activation state of CD8+ T cells, compared to no immunosuppressive regimen group.
- The MCP-1 level at Day 0 is elevated in every group that was treated with either dexamethasone and/or tacrolimus. This is likely due to the daily handling of study animals for drug injections.
- The blood trough level of tacrolimus was above 15 ng/ml in the treated animals, which is consistent with the cytokine and immune profile.

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 HMI-203 (juMPStart) in adults with Hunter syndrome (MPS II) (NCT05238324).