# Targeted Approach to Immunosuppression with AAV Gene Therapy: Nonclinical Support for Clinical Approaches

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## Introduction

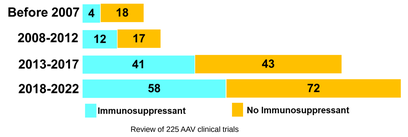
Gene therapy using recombinant adeno-associated virus (rAAV) vectors has been successful in treating a wide range of human diseases. However, patients treated with rAAV can develop unwanted immune responses to the viral capsid, including B- & T-cell activation and transaminitis, which can lead to the loss of therapeutic transgene expression and formation of neutralizing antibodies, the latter hampering the potential for re-dosing.

To combat the immune response to rAAV gene therapy and gene editing, corticosteroid usage has become more frequent over time in clinical trials, particularly with systemic AAV delivery. Given the broad mechanism of action of corticosteroids, adverse effects can be expected across a range of organ systems including hypertension, hyperglycemia, osteoporosis, and neuropsychiatric symptoms, such as insomnia and mood disturbance. Higher doses and long courses of corticosteroids have been associated with an increased likelihood of developing more severe side effects. Because of these side effects, more targeted approaches are under investigation.

## Immunosuppressant Use in AAV Clinical Trials

- Over time, the use of immunosuppressants in rAAV clinical trials has increased from 18% before 2007 to 45% in 2022, as shown in Figure 1.
- Corticosteroid treatment regimens have been widely used to manage ALT/AST levels and immune responses in rAAV-treated patients.
- Higher doses and longer courses of corticosteroids have been associated with an increased likelihood of developing more severe side effects.
- Most trials used corticosteroids, and more specific T-cell and/or B-cell inhibitors (sirolimus, tacrolimus, rituximab) were also used.
- To improve the immunosuppression regimen while reducing adverse effects in patients, drugs with more specific targeting have been introduced to AAV gene therapy. For example, mammalian target of rapamycin (mTOR) inhibitor sirolimus targets mainly B cells and T cells, and calcineurin inhibitor tacrolimus targets mainly T cells.

### Figure 1: Immunosuppression Regimen for AAV Clinical Trials




Review of 225 AAV clinical trials

Adapted from: Shen W, Liu S, Ou L. Corrigendum: rAAV immunogenicity, toxicity, and durability in 255 clinical trials: A meta-analysis. Front Immunol. 2023 Jan 18;13:1104646. doi: 10.3389/fimmu.2022.1104646. Erratum for: Front Immunol. 2022 Oct 27;13:1001263. PMID: 36741359; PMCID: PMC9891283.

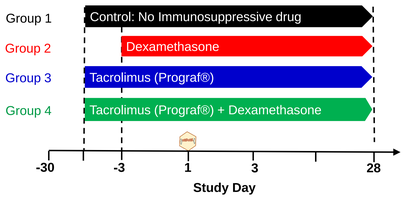
### Evolution of Immunosuppression Regimen for AAV Gene Therapy

- Immunosuppression regimens continue to evolve in the AAV field
- Shift from corticosteroids in response to elevated ALT/AST to more targeted regimens

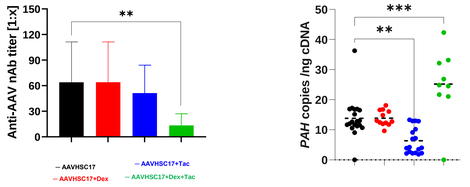
## Assessment of a Targeted Immunosuppression Regimen in Cynomolgus Monkeys

In a 28-day study in cynomolgus monkeys, we investigated the effect of a targeted immunosuppressive regimen on AAVHSC17-capsid specific neutralizing antibody (nAb) formation, immune activation, and transgene expression. The design is presented below in Figure 2. The calcineurin inhibitor, tacrolimus (Prograf®), and the corticosteroid, dexamethasone, were administered prophylactically for immunosuppression, followed by a single intravenous (I.V.) dose of rAAVHSC17 expressing human phenylalanine hydroxylase (rAAVHSC17-PAH) at study Day 1. The immunosuppressive drug(s) was administered daily to Groups 2, 3 and 4 throughout the 28-day study.

### Figure 2: Study Design: 28-Day NHP Study




### Figure 3: Dexamethasone and Tacrolimus Regimen Reduced nAb Formation and Increased Gene Expression







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

The results of the dexamethasone and tacrolimus immunosuppressive regimen used in this study are shown in Figure 3:

- Reduced nAb formation against the rAAV capsid by 8-fold, reduced overall B- and T-cell activation
- Increased transgene expression by 2-fold when compared to a control group administered rAAVHSC17-PAH without immunosuppression

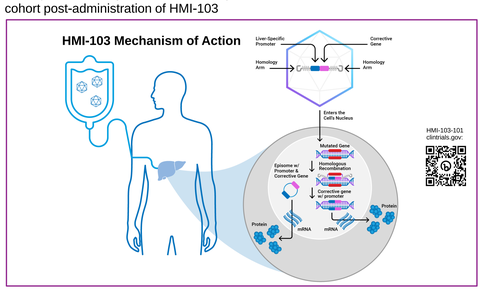
## HMI-103 and HMI-203 Clinical Trial Designs

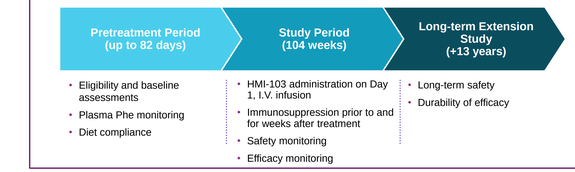
The results of this study supported the approach of a prophylactic combination of tacrolimus and dexamethasone in conjunction with dosing in clinical trials of our investigational genetic medicines. The pheEDIT and juMPStart trials are Phase 1 open-label, sequential, dose-escalation studies that will evaluate the safety and efficacy of a one-time I.V. administration of investigational gene editing and gene therapy vectors, respectively. Immune assessments in both studies include antibody and ELISPOT against capsid and transgene, immunophenotyping and cytokine assessment. Study details for pheEDIT and juMPStart are presented below in Figure 4 and Figure 5, respectively.

### Figure 4: pheEDIT Clinical Trial

The pheEDIT study will evaluate the safety and efficacy of a one-time I.V. administration of HMI-103, an investigational gene editing therapy in adult participants with phenylketonuria (PKU) (NCT05222178).

**pheEDIT primary study endpoints include:**
Incidence and severity of treatment-emergent adverse events (TEAEs) and adverse events (AEs) of special interest (hepatic dysfunction and low Phe)
Mean percent change from baseline in plasma Phe concentrations within each dose cohort post-administration of HMI-103

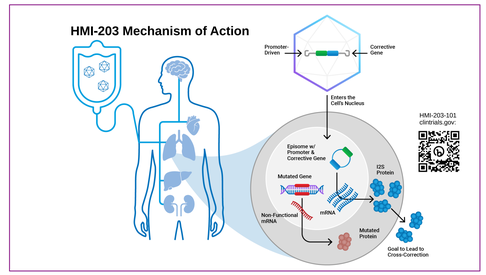


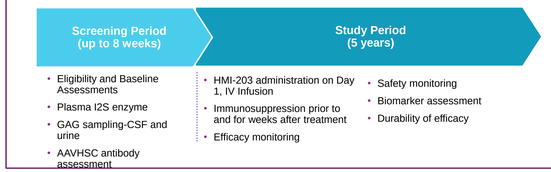




### Figure 5: juMPStart Clinical Trial

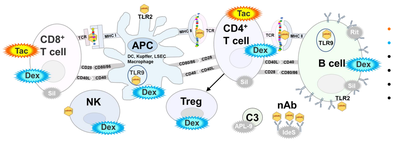
The juMPStart study will evaluate the safety and efficacy of a one-time I.V. administration of HMI-203, an investigational gene therapy, in adult participants with m\Mucopolysaccharidosis Type II, also known as Hunter syndrome (NCT05238324)

**juMPStart primary study endpoints include:**
Incidence and severity of TEAEs and AEs of special interest (hepatic dysfunction)
Mean percent change from Baseline in urine GAG levels and plasma I2S activity through Week 52 following HMI-203 administration.




### Figure 6: Corticosteroids have Broad Interactions Compared to Tacrolimus




- 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

## Conclusion

To decrease the potential for immune response to AAV gene therapy in the pheEDIT and juMPStart studies, a prophylactic immunosuppressive regimen of a corticosteroid in combination with tacrolimus are administered. Tacrolimus inhibits T-lymphocyte activation and proliferation, as well as T-helper-cell-dependent B-cell response. It is known that T-cells are important in the immune response to AAV vectors. The introduction of a T-cell inhibitor tacrolimus allows for a potential shortening of the duration of exposure to high dose corticosteroids and a more rapid taper. Corticosteroids have a more pleotropic mechanism than tacrolimus, as shown in Figure 6. Immune function endpoints, efficacy and safety are being assessed in both ongoing trials. This combination therapy is anticipated to decrease the development of nAb, optimize transgene expression and reduce the adverse events related to corticosteroid therapy.