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Immunomodulatory Approaches in Preclinical Gene Therapy Studies

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ABSTRACT

In-vivo gene therapy (GT) utilizing Adeno-Associated Virus (AAV) vectors or Lipid Nanoparticles (LNPs) has made considerable progress over the past several years nonetheless, activation of both the innate and adaptive immune systems remains a critical obstacle often necessitating premedication with immunomodulating or suppressive agents to mitigate adverse responses. Given the unique challenges of executing successful preclinical GT studies, we reviewed data from 30 studies conducted in the last 3 years to identify the frequency of premedication use, common single drug and combination pretreatment regimes, and incidence of clinical observations indicative of immune responses in nonhuman primates. Of the studies reviewed, ~50% utilized a pretreatment regimen prior to dosing with AAV/LNPs, sometimes in combination with other supportive medications. Clinical signs indicative of an innate immune response were not observed with premedication regimes of (A) dexamethasone at 2 mg/kg via intravenous (IV) bolus at least 1 hour prior to test article administration in 6 studies, (B) Prednisolone at 1 or 3 mg/kg over the duration of the dosing period in 3 studies, (C) Diphenhydramine at 2 mg/kg administered intramuscularly 15 to 30 minutes prior to dosing in 1 study or (D) in studies (1 each) utilizing multiple premedication drugs including 3 mg/kg Prednisolone and 5 mg/kg Diphenhydramine, 8 mg/kg Tocilizumab with and without 0.05 mg/kg Tacrolimus, or 750 mg/m^2 Rituximab with 2 mg/m^2/day Sirolimus and 4 mg/kg Diphenhydramine. In conclusion, this data review can serve as a guide to inform future GT study designs and identify suitable premedication regimes for adequate clinical translation.

INTRODUCTION

Targeted gene therapy faces significant challenges beyond precise gene editing, including immunogenicity and host reactions, which impact treatment safety and effectiveness. Immunogenicity poses a risk by potentially diminishing therapeutic benefits or triggering immune-related side effects, while host reactions, like inflammation or immune rejection, can further complicate treatment. To address these issues, researchers are exploring new immunomodulatory strategies to enhance safety and success in clinical applications.

Immunomodulatory drugs (IMDs) help by minimizing the immune reactions to therapeutic transgenes or vectors, preventing transgene immunogenicity, modulating inflammation at target sites or by improving tolerance to gene editing techniques.

Commonly used IMDs in GT to help the body accept cellular therapies and reduce inflammation include the following:

- **Corticosteroids** (e.g., Prednisone, Dexamethasone): Widely used to suppress inflammation and immune responses to GT vectors and transgene proteins.
- **Calcineurin inhibitors** (e.g., Tacrolimus, Cyclosporine): Useful for more targeted suppression of immune responses, especially in conditions requiring long-term immunomodulation.
- **mTOR inhibitors** (e.g., Rapamycin): Help modulate T-cell activation and proliferation, beneficial in reducing immune responses to both the vector and the transgene.
- **Monoclonal antibodies** (e.g., Rituximab): Used to deplete specific immune cell populations, like B-cells, which can reduce the formation of anti-drug antibodies.

OBJECTIVE

The purpose of this study was to investigate the commonly used IMDs or premedications in GT studies, that were conducted at our Testing Facility between January 2021 and January 2024.

MATERIAL AND METHODS

A total of 30 studies, conducted in the last 3 years, were screened to identify the frequency of premedication use, common single drug and combination pretreatment regimes, and incidence of clinical observations indicative of immune responses in nonhuman primates.

RESULTS

Among the studies reviewed, 50% utilized pretreatments or immunomodulatory drugs (IMDs), with Dexamethasone and Prednisolone being the most used, followed by various combinations of other treatments.

Table 1. Immunomodulatory drugs used in GT studies at Altasciences Preclinical Seattle

Pretreatment	Dose level	Dose route	Administration timing	No. of studies
Dexamethasone	2 mg/kg	IV bolus	1 hour pre-dose	6
Prednisolone	1 mg/kg	Oral	Daily during dosing period	3
Diphenhydramine	2 mg/kg	IM	15-30 minutes pre-dose	1
Prednisolone Diphenhydramine	3 mg/kg 5 mg/kg	Oral IM	Daily from Day -1, 30-minutes pre-dose	1
Tocilizumab	8 mg/kg	IV infusion	Day -1	1
Tocilizumab Tacrolimus	8 mg/kg 0.05 mg/kg	IV infusion IM	Day -3 Daily from Day -1	1
Rituximab Diphenhydramine Sirolimus	750 mg/m^2 4 mg/kg 2 mg/m^2/day	IV infusion IM Oral	Day -15 and 1 Day -15 and 1 Daily from Day -3	1
Immunosuppressant 1 or Immunosuppressant 2 and Famotidine	50 mg/kg or 0.1 or 0.5 mg/kg and 07 mg/kg	IV bolus	Days -7, -3, 1, pre-dose, and once weekly during dosing period	1

FINDINGS IN ANIMALS ADMINISTERED IMDS

Out of the 14 studies surveyed, no abnormal clinical symptoms related to IMD administration were reported, except for one study that noted exaggerated pharmacological effects of the test article.
The most common clinical observations in animals administered IMDs were consistent with those seen in control animals and typical for animals maintained under similar laboratory conditions. These observations included bruising or abnormal skin coloration, changes in feces, hair loss, abrasions, and scab or crust formation.

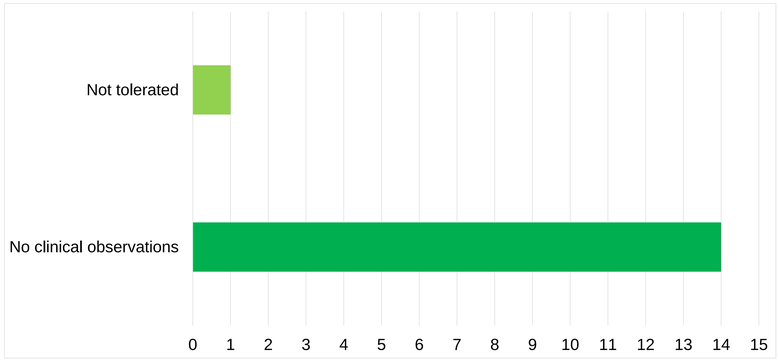

Figure 1. Clinical observations in animals administered with IMDs

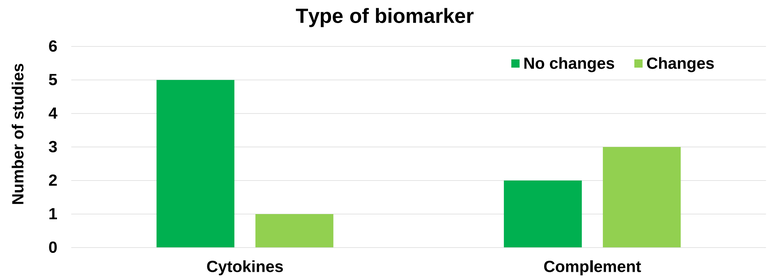

Figure 2. Biomarker findings in animals administered IMDs

Biomarker changes were observed as transient increases in MCP-1, SC5b-9 complex, and/or Bb and C3a complement fragments.

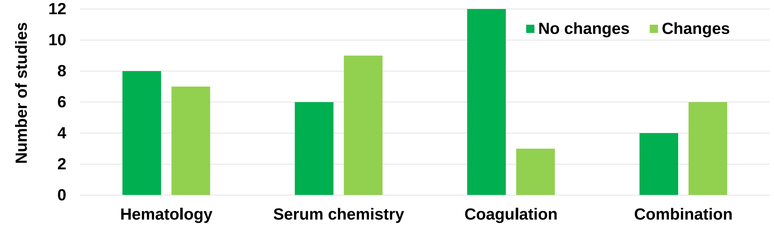

Figure 3. Clinical pathology findings in animals administered IMDs

Clinical pathology findings included varying degrees of decreased lymphocyte, monocyte, neutrophil, eosinophil, and/or platelet counts; decreased albumin and/or increased globulin levels, elevated fibrinogen, prolonged prothrombin time, and reversible increases in liver enzymes (alanine aminotransferase and aspartate aminotransferase), total bilirubin, and/or creatine kinase.

Overall, these biomarker and clinical pathology changes were generally minimal to mild, transient, or reversible. Moreover, studies that incorporated dexamethasone as a pretreatment demonstrated comparatively fewer occurrences of these findings.

CONCLUSIONS

IMDs are essential for minimizing immune reactions to therapeutic transgenes and vectors. They help reduce transgene immunogenicity, modulate inflammation at target sites, and enhance tolerance to gene-editing techniques. The data reviewed in this presentation indicate that corticosteroids, such as Dexamethasone and Prednisolone, are commonly used IMDs in gene therapy, either as standalone treatments or in combination with other premedications. Among these, Dexamethasone has shown greater suitability as a pretreatment option.

REFERENCES

Vickram *et al.,* Targeted Gene Therapy: Promises and Challenges in Disease Management. J. Bio-X Res. 2024; 7:Article0007.