


GRNOPC1: Phase 1 Clinical Trial Immune Monitoring

Jane S Lebkowski, Ph.D.

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GRNOPC1 in Spinal Cord Injury: Phase 1 Clinical Trial

- **Open Label Trial**
 - **Multi-Center (7 sites)**
 - **8-10 Subjects**
 - **Subacute, Functionally Complete T3-T11 Lesions**
 - **2×10^6 Cells**
 - **Transplant 7-14 Days Post Injury**
 - **Temporary Immunosuppression**
 - **Primary Endpoint: Safety**
 - **Secondary Endpoint: Neurological Function**
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Measures of Sensory and Motor Function

- Spinal Cord Independence Measure (SCIM)
- International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI) Day 30, 60, 90, 120, 180, 270 and 365
- U of Alabama Index of Motor Recovery (UAB-IMR)
- International Spinal Cord Injury Pain Basic Data Set
- International Spinal Cord Injury Bowel and Bladder Data Set

Goals of GRNOPC1 Immune Monitoring Program

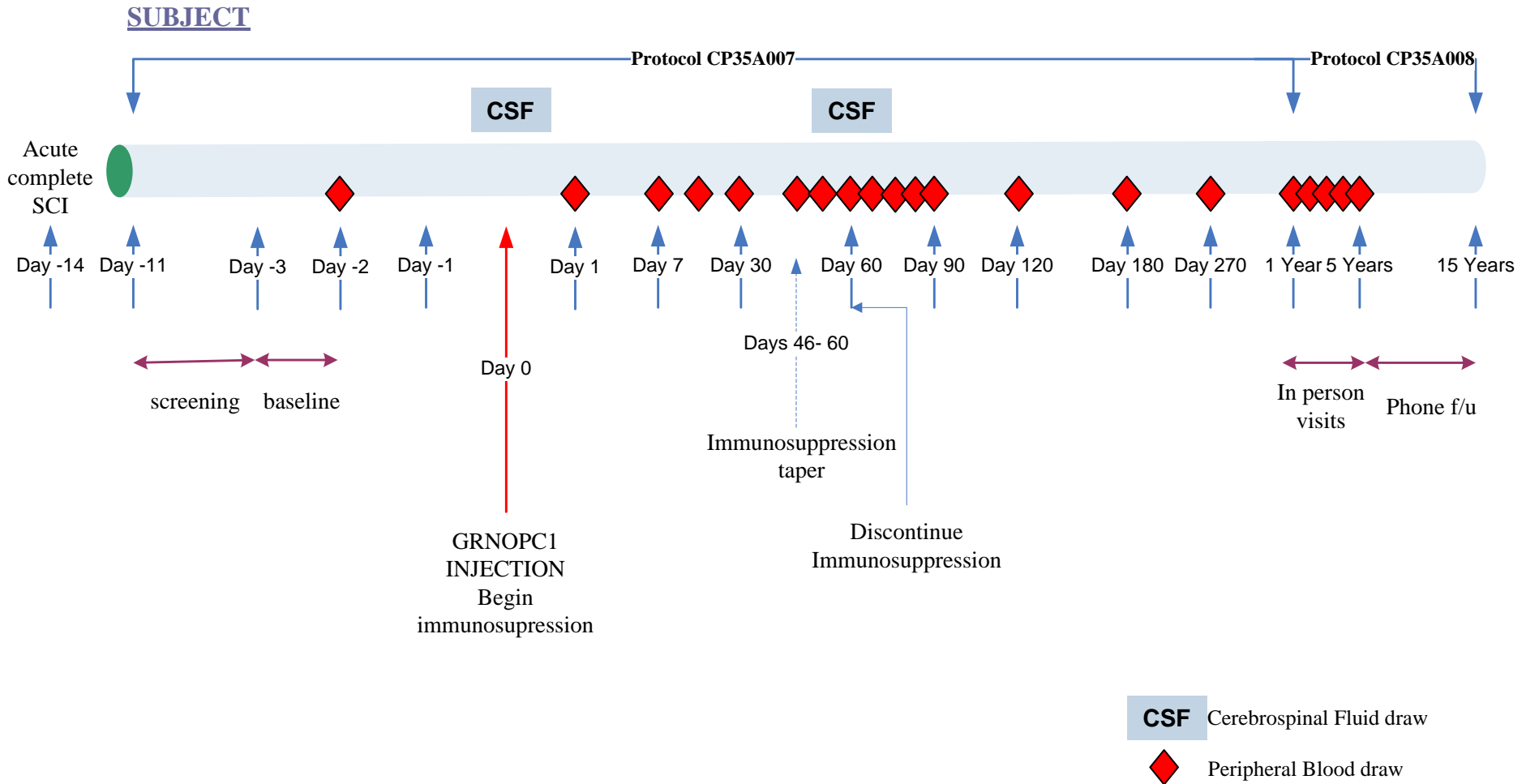
Objectives of the immune monitoring program is to:

- **Monitor immune responses against GRNOPC1**
- **Evaluate if the current immune suppression regimen prevents immune responses to GRNOPC1.**
- **Surrogates for graft survival**

Immune monitoring assays are exploratory and are not used to guide clinical decisions in the Phase I study

GRNOPC1 Phase 1 Trial - Clinical Sampling Plan

SCHEMA

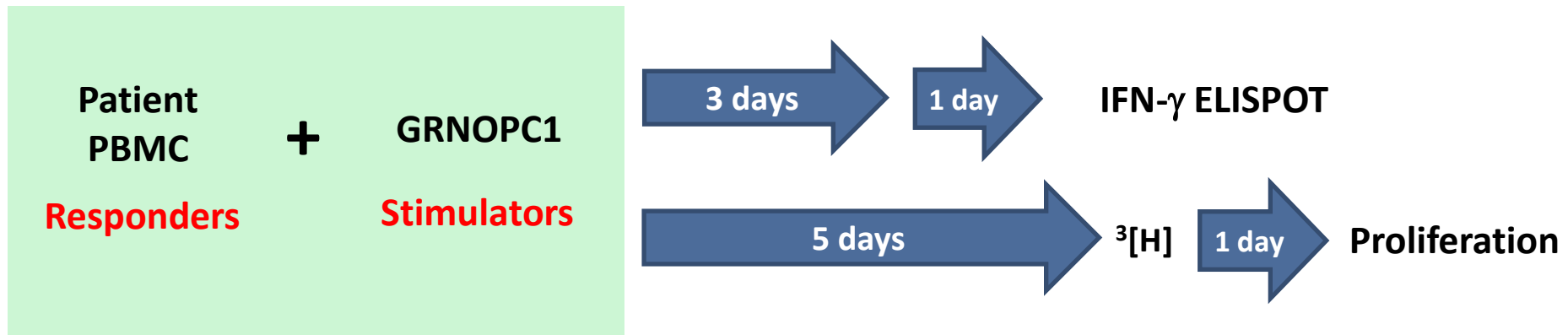


Samples Batched for Assays

Immune Monitoring Assays

Assay	Sample	Endpoint
T Cell Stimulation (Proliferation & ELISPOT)	PBMC	T cell immune responses against GRNOPC1
FlowPRA	Serum & CSF	Antibody response against GRNOPC1
Lymphocyte Phenotyping	PBMC	Non-specific assessment of immune response
mRNA Gene Expression	PBMC	Non-specific immune response assessment & biomarker discovery
Cytokines & Biomarkers	Serum & CSF	Non-specific immune response assessment & biomarker discovery

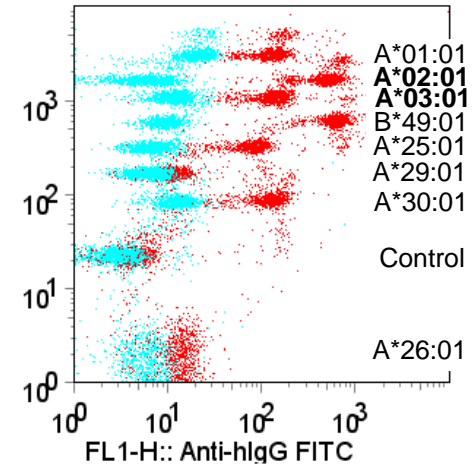
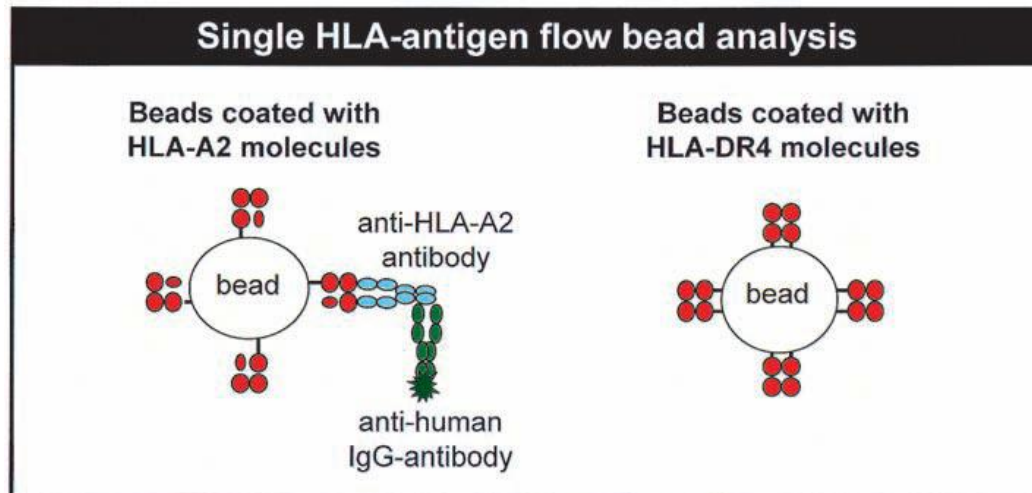
T Cell Stimulation by GRNOPC1



Positive Control Stimulators: Allogeneic PBMC & Recall Antigens

FlowPRA: Detection of GRNOPC1-Specific Anti-HLA Antibodies

- Solid phase bead-based assay to detect Donor-Specific Antibodies (DSA)
- Antibodies for specific HLA can be evaluated in patient serum and CSF samples
- Flow cytometry method (luminex method also feasible)



- Negative Control Serum
- Positive Control Serum

Antibodies to GRNOPC1 HLA antigens Not Detected in Subject Serum and CSF Samples

HLA Genotype GRNOPC1
A*02:01
A*03:01
B*08:01
B*35:01
Cw*04:01
Cw*07:01
DRB1*01:01
DRB1*03:01
DQB1*02:01
DQB1*05:01



No donor specific antibody responses against GRNOPC1-specific HLA

Immune Subset Analysis in Peripheral Blood Samples

Lymphocyte Subset	Phenotype	Role in Allogeneic Transplantation
Regulatory T cells	CD4 ⁺ CD25 ⁺ FoxP3 ⁺	Can suppress immune response; increased in tolerant vs. rejection liver allograft
T cell subsets	CD3/CD4/CD8	General indicator of immune system
NK subsets	CD3/CD16/CD56	Relative increases in NK cell populations and NK cell-related gene expression have been correlated with successful liver and kidney allograft
B cell subsets	CD19/CD27/CD69	Relative increases in B cell populations and B cell-related gene expression have been correlated with successful kidney but not liver allograft
T cell activation	CD3/CD62L/HLA-DR	T cell activation may indicate active rejection in kidney allograft
TCR $\gamma\delta$ cells	CD3 ⁺ TCR $\alpha\beta$ -TCR $\gamma\delta$ ⁺	Relative increases in TCR $\gamma\delta$ cells reported in tolerant liver allograft
Naïve/Memory T subsets	CD4/CD8/CD45RA/CD45RO/CD62L	General indicator of immune response

Additional Exploratory Assays

	Methodology	Potential Signals
Immune Cell Subsets	<ul style="list-style-type: none">• Flow Cytometry	<ul style="list-style-type: none">• Changes in Immune Cell Subset with Rejection
Cytokine & Biomarker Analysis	<ul style="list-style-type: none">• Multi- Analyte Profiling• Quantitative Immunoassays	<ul style="list-style-type: none">• Changes in Multiple Pathways• Cytokines, Chemokines, Other Biomarkers
Gene Expression Analysis	<ul style="list-style-type: none">• Microarray Analysis	<ul style="list-style-type: none">• Graft Rejection/ Acceptance Signature
Graft Detection	<ul style="list-style-type: none">• STR Genotyping• HLA TaqMan	<ul style="list-style-type: none">• Grafted Cell Identification

Wish List

Methods to Monitor :

- Immune Responses Local Site Without Biopsies
- Rejection at the Local Site Without Biopsies
- Long-term Cell Survival in Vivo