IDENTIFICATION OF PRECLINICAL SERUM BIOMARKERS ASSOCIATED WITH rGDF11-MEDIATED RECOVERY POST ISCHEMIC STROKE

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LBP25

International Stroke Conference
American Heart Association

LBP25

Introduction

Growth differentiation factor 11 (GDF11), a member of the TGFβ superfamily, has shown significant potential as a therapeutic candidate for stroke recovery. Its mechanisms of action include promoting tissue regeneration, angiogenesis, neurogenesis, and reducing inflammation. [1, 2, 3]

This study explores the effects of ALE-001, recombinant GDF11 (rGDF11), on serum biomarkers in a rat model of ischemic stroke (pMCAO). The primary goal is to identify biomarkers that could facilitate the clinical translation of GDF11-based therapies.

Serum samples were collected longitudinally during a preclinical efficacy study and analyzed using SomaScan, aptamer-based proteomics analysis, and ELISA to identify a panel of candidate pharmacodynamic and mechanistic biomarkers to support clinical translation.

Objectives

1. To identify a panel of serum biomarkers that could be extended and validated in clinical studies for successful development of ALE-001(rGDF11) for stroke recovery.
2. To further the knowledge of potential mechanism of actions of ALE-001 (rGDF11) in stroke recovery.

Methods and Material

- Focal cerebral infarcts were made in Sprague-Dawley male 8-10 weeks rats, by permanent occlusion of the proximal right middle cerebral artery by microbipolar coagulation, with a modification of a published method. [4]
- Single or multiple doses of ALE-001 (rGDF11) or vehicle was administered via intravenous injections at 1 mg/kg per dose, initiating 24 hours following pMCAO-injury.
- Sensorimotor assessments (body swing, forelimb, and hindlimb placement) were determined on days -1, 1, 3, 5, 7, and 14 post-injury (Table 1).
- Serum samples were collected longitudinally post-injury for subjecting to ELISA and SomaScan aptamer-based proteomics analysis (SomaLogic, Inc.)

Table 1. Study Design

	Timeline	Day -1	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 7	Day 14
pMCAO + Tx: Day 1	Vehicle		Stroke	0 mg/kg						
	rGDF11			1 mg/kg						
pMCAO + Tx: Day 1,3,5	Vehicle		Stroke	0 mg/kg		0 mg/kg		0 mg/kg		
	rGDF11			1 mg/kg		1 mg/kg		1 mg/kg		
pMCAO + Tx: Day 1-5	Vehicle		Stroke	0 mg/kg	0 mg/kg	0 mg/kg	0 mg/kg	0 mg/kg		
	rGDF11			1 mg/kg	1 mg/kg	1 mg/kg	1 mg/kg	1 mg/kg		
Sham + Tx: Day 1,3,5	Vehicle		Sham	0 mg/kg		0 mg/kg		0 mg/kg		
	rGDF11			1 mg/kg		1 mg/kg		1 mg/kg		
Monitoring →		Blood & Motor Function		Motor Function	Blood	Blood & Motor Function		Blood & Motor Function	Blood & Motor Function	Blood & Motor Function

Table 1. Table describing the IV Dose Optimization Efficacy Study. Serum collected for SomaScan analysis at 16 time points are shown by red borders (n=5 per group).

Summary of Results

rGDF11 Enhances Motor Function Recovery

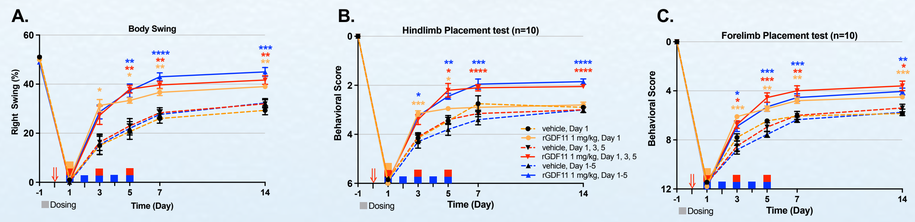

Figure 1. A-C. ALE-001 (rGDF11) administration initiated 24-hours post-stroke and treated with either a single dose (orange line), three intermittent doses (red line), or five daily doses (blue line) over a five-day period improved sensorimotor function recovery in the rat pMCAO model. Data Plotted as Mean +/- SEM and analyzed using 2-way ANOVA repeated measures with multiple comparison, comparing vehicle vs treatment for each dosing regimen, and Sidak post hoc correction. For all graphs, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

Biomarker analysis

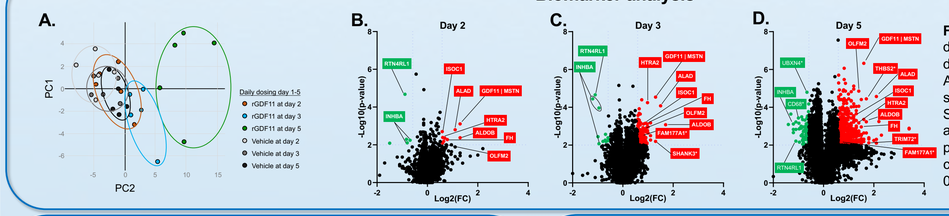

Figure 2. A. PCA analysis of biomarkers discovered using SomaScan analysis from rat serum on day 2, 3, and 5 after stroke, following once daily dosing of ALE-001 (rGDF11) for up to 5 days. **B-D**. Volcano plot showing the distribution of biomarkers identified by SomaScan analysis on day 2 (**B**), day 3 (**C**) or day 5 (**D**) after stroke. Biomarkers with a fold change >1.5 and p<0.01 are highlighted in "green" (decreased in circulation) or red (increased in circulation), when ALE-001 (rGDF11) cohorts are compared to vehicle control.

Canonical Pathways

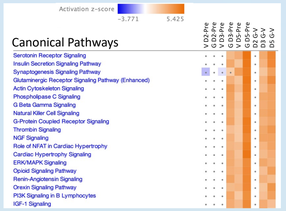

Figure 3. Canonical pathways identified from the SomaScan analysis of ALE-001 (rGDF11) or vehicle control rats comparing pre to post-pMCAO. ALE-001 (rGDF11) treated cohorts were also compared to vehicle control pMCAO rats on days 2, 3, and 5.

rGDF11 Decreases Serum CRP Concentrations Post-pMCAO

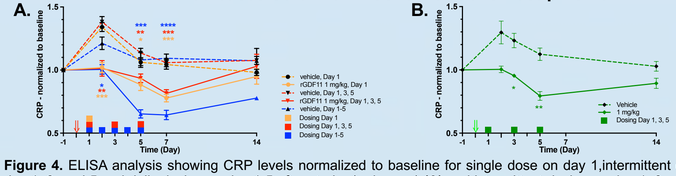

Figure 4. ELISA analysis showing CRP levels normalized to baseline for single dose on day 1,intermittent dosing on day 1, 3, and 5 and daily dosing on day 1-5 after stroke (red arrow) **(A)** and intermittent dosing on day 1, 3, and 5 after sham (green arrow) **(B)**. Colored squares show the dosing regimens for each graph. For all graphs Data Plotted as Mean +/- SEM and analyzed using Sidak multiple comparison. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

Candidate Stroke Recovery-Related Biomarkers

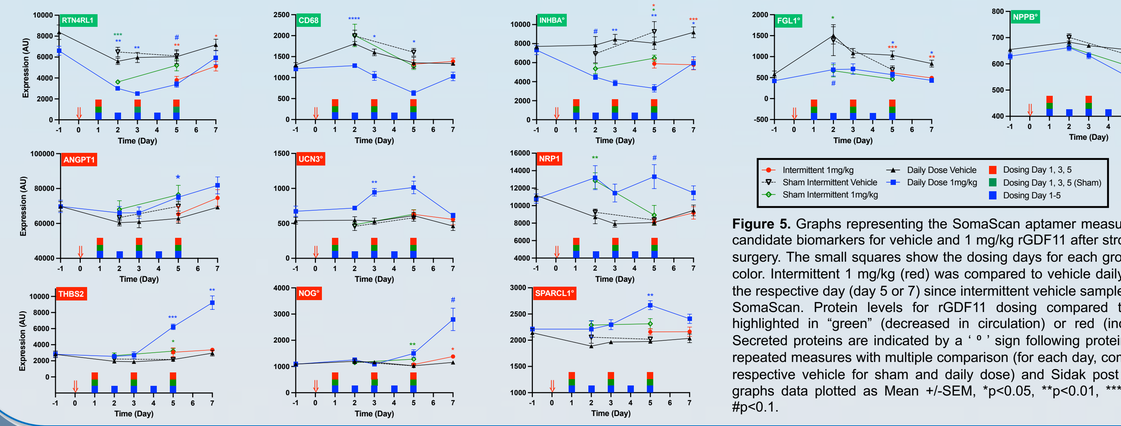

Figure 5. Graphs representing the SomaScan aptamer measured circulating levels of candidate biomarkers for vehicle and 1 mg/kg rGDF11 after stroke (red arrow) or sham surgery. The small squares show the dosing days for each group with their respective color. Intermittent 1 mg/kg (red) was compared to vehicle daily dosing (solid black) of the respective day (day 5 or 7) since intermittent vehicle samples were not analyzed by SomaScan. Protein levels for rGDF11 dosing compared to vehicle control, are highlighted in "green" (decreased in circulation) or red (increased in circulation). Secreted proteins are indicated by a ' º ' sign following protein name. 2-way ANOVA repeated measures with multiple comparison (for each day, compare treatment vs their respective vehicle for sham and daily dose) and Sidak post hoc correction. For all graphs data plotted as Mean +/-SEM, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, #p<0.1.

Conclusions & Discussion

1. We have developed and optimized an efficacious dosing regimen including dosing initiation, frequency, duration, and dose range, and demonstrated a broad therapeutic window for ALE-001 (rGDF11), in a preclinical model that informs the design of clinical studies for development of rGDF11 as a therapeutic for stroke recovery.[5]
2. rGDF11 functions through multiple mechanisms of action for stroke recovery by promoting neurogenesis and vascularization and reducing inflammation
3. Signature panels of serum biomarkers that correlate with mechanistic functions of ALE-001 (rGDF11) as demonstrated in this preclinical stroke efficacy study have been identified.
   a) Neurogenesis and/or synaptogenesis promoting: RTN4RL1↑[6], NOG↑[7], SPARCL1↑[8], NRP1↑ [9], THBS2↑[10]
   b) Angiogenesis promoting: ANGPT1↑[11] NRP1↑[9],
   c) Anti-inflammatory effects: CRP↓[12], UCN3↑[13]
4. IPA analysis identified differentially activated signaling pathways, many of which are associated with the hypothesized mechanism of stroke repair, including synaptogenesis, NGF signaling, ERK/MAPK signaling, and IGF signaling.[14]
5. Further proteomics characterization, analyses, and data validation will enable translatable biomarker identification to support proof of mechanism and predict potential for efficacy in clinical studies.

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