RECOMBINANT GROWTH DIFFERENTIATION FACTOR 11 (rGDF11) IMPROVES FUNCTIONAL OUTCOMES IN MOUSE MODELS OF HEMORRHAGIC STROKE AND TRAUMATIC BRAIN INJURY

Ori S Cohen, Manisha Sinha, Yongting Wang, Berenice Charrez, Tyler Daman, Pi-Chun Li, Catherine Deatherage, Sam Jordan, Mark Allen, and Anthony Sandrasagra

Alevian, Inc., Lexington, MA

LBP16

LBP16

Introduction

- Intracerebral Hemorrhage (ICH) and Traumatic Brain Injury (TBI) and are significant contributors to long-term disability and mortality in the U.S. and worldwide, with both conditions representing major public health challenges.
- ICH involves the rupture of a blood vessel within the brain, leading to bleeding and subsequent neurological damage, while TBI occurs when external mechanical forces injure or damage the brain.
- Despite differences in etiology, both conditions share common pathological mechanisms, including inflammation, disruption of vascular integrity, neuronal death, and impaired recovery. These overlapping pathways highlight the need for therapies that target shared processes to promote neurological recovery.
- Alevian is developing ALE-001, a recombinant Growth Differentiation Factor 11 polypeptide (rGDF11), to address the unmet therapeutic needs of patients recovering from ICH and TBI.
- Preclinical research to date provides evidence that systemically administered rGDF11 exhibits broad therapeutic effects by:
  - Promoting vascular regeneration[1]
  - Activating progenitor/stem cells[1]
  - Antagonizing inflammation[1]
- rGDF11 has consistently demonstrated enhanced neurological recovery in rodent models of ischemic stroke, and aging.[1-6] This research extends these findings to ICH and TBI models.

Objectives

1. To evaluate the efficacy of ALE-001 (rGDF11) in improving recovery outcomes post - ICH and TBI.
2. To further elucidate the the potential mechanisms by which ALE-001 exerts its effects in stroke recovery.

Methods

- For ICH, intrastriatal collagenase injection was performed on mice as previously described by Lei et al., 2014.[7]
- For TBI, the murine closed head injury model with a pneumatic impactor was employed as described by Laskowitz et al., 2017.[8]
- ALE-001 (rGDF11) or vehicle was administered to male mice by intraperitoneal injection (1 mg/kg) starting 30 minutes after ICH or TBI and then every 24 hours for 7 days.
- For ICH, Neuroseverity Score (NSS)[9] and Rotarod Latency (RR)[9] behavioral assessments were conducted pre-ICH and on days 1, 2, 3, 4, 5, 6, 7, 14, 21, and 28 post-ICH. CatWalk (CW) assessment was performed on day 7 post-injury.[10]
- Brain section analyses of Microglia and vascular area were performed with F4/80 and Cd31 antibodies by immunofluorescence.
- For TBI, RR behavioral assessment was conducted pre-TBI and on days 1, 2, 3, 4, 5, 6, 7, 14, 21, and 28 post-TBI. NSS was conducted pre-TBI and on days 1 and 28 post-ICH. Total improvement was determined by subtracting day 1 score with day 28 score

Summary of Results (ICH)

ALE-001 Promotes Recovery Post Injury in a Mouse ICH Model

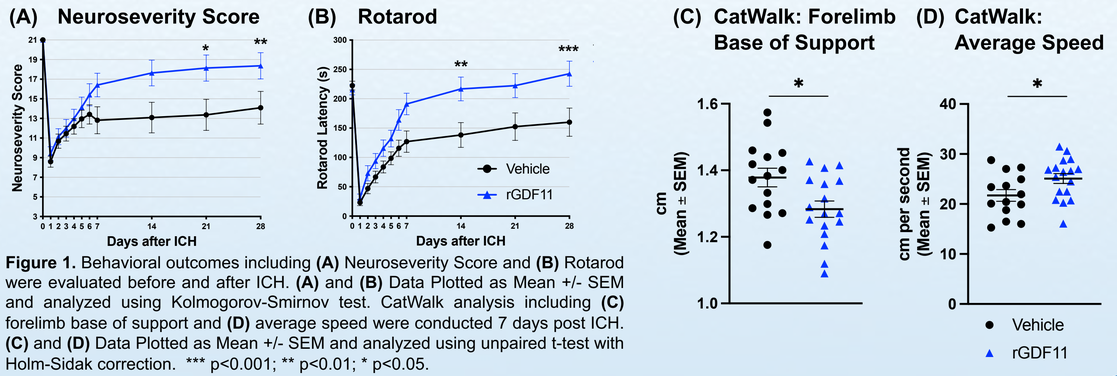

Figure 1. Behavioral outcomes including **(A)** Neuroseverity Score and **(B)** Rotarod were evaluated before and after ICH. **(A)** and **(B)** Data Plotted as Mean +/- SEM and analyzed using Kolmogorov-Smirnov test. CatWalk analysis including **(C)** forelimb base of support and **(D)** average speed were conducted 7 days post ICH. **(C)** and **(D)** Data Plotted as Mean +/- SEM and analyzed using unpaired t-test with Holm-Sidak correction. *** p<0.001; ** p<0.01; * p<0.05.

ALE-001 Reduces Microglial Density and Increases Vascular Area Post Injury in a Mouse ICH Model

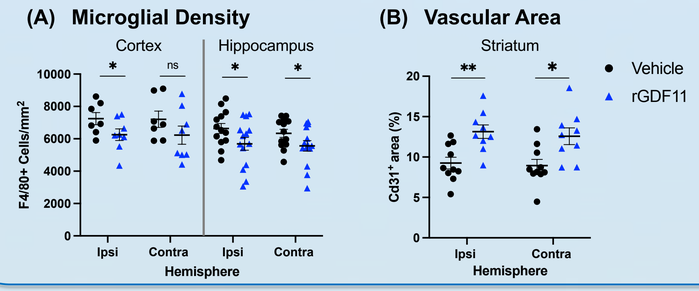

Figure 2. Unbiased quantitative stereology of brain sections 28 days post ICH using **(A)** F4/80 staining for microglia density and **(B)** Cd31 staining for vascular area. Data Plotted as Mean +/- SEM and analyzed using unpaired t-test with Holm-Sidak correction. ** p<0.01; * p<0.05; ns - not significant; Ipsi - Ipsilateral; Contra - Contralateral.

Summary of Results (TBI)

ALV-001 Promotes Recovery Post Injury in a Mouse TBI Model

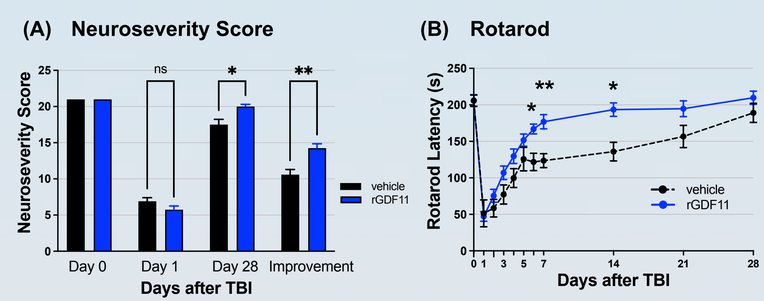

Figure 3. Behavioral outcomes including **(A)** Neuroseverity Score and **(B)** Rotarod were evaluated before and after ICH. **(A)** and **(B)** Data plotted as Mean +/- SEM. Analyzed using a 2-way ANOVA repeated measures with multiple comparison (for each day compare rGDF11 to vehicle) with Sidak post hoc correction. *** p<0.001; ** p<0.01; * p<0.05; ns - not significant.

Conclusions & Discussion

1. ALE-001 (rGDF11) improves functional recovery of neurobehavioral deficits post injury in preclinical rodent models of ICH and TBI.
2. ALE-001 functions through multiple mechanisms of action for recovery post ICH including reducing inflammation and increasing vascularization.
3. rGDF11 shows strong potential as a neurorestorative therapy for ICH and TBI. Its ability to improve motor and behavioral outcomes highlights its promise in promoting recovery through mechanisms such as neovascularization and anti-inflammatory effects, which are essential for brain repair and functional recovery.
4. Further dose regimen optimization studies, including evaluating route of administration, dosing initiation, dosing frequency, and dose levels are planned.

References

1. Cohen et al., (2025). 'Recombinant GDF11 Promotes Recovery in a Rat Permanent Ischmia Model of Subacute Stroke', *Stroke (in press)*.
2. Lu et al., (2018). 'Growth Differentiation Factor 11 Promotes Neurovascular Recovery After Stroke in Mice', *Front Cell Neurosci.*
3. Ma et al., (2018). 'Growth differentiation factor 11 improves neurobehavioral recovery and stimulates angiogenesis in rats subjected to cerebral ischemia/reperfusion', *Brain Res Bull.*
4. Hudobenko et al., (2020). 'Growth differentiation factor-11 supplementation improves survival and promotes recovery after ischemic stroke in aged mice', *Aging.*
5. Zhao et al., (2020). 'The neuroprotective and neurorestorative effects of growth differentiation factor 11 in cerebral ischemic injury', *Brain Res.*
6. Su et al., (2021). '*In situ* slow-release recombinant growth differentiation factor 11 exhibits therapeutic efficacy in ischemic stroke', *Biomedicine & Pharmatherapeutic.*
7. Lei et al., (2014). 'Intrastriatal Injection of Autologous Blood or Clostridial Collagenase as Murine Models of Intracerebral Hemorrhage', *J Vis Exp.*
8. Laskowitz et al., (2017). 'Neuroprotective pentapeptide CN-105 is associated with reduced sterile inflammation and improved functional outcomes in a traumatic brain injury murine model', *Scientific Reports.*
9. Wang et al., (2021). 'Neuroprotective Pentapeptide, CN-105, Improves Outcomes in Translational Models of Intracerebral Hemorrhage', *Neurocrit Care.*
10. Walter et al., (2020). 'The CatWalk XT is a valid tool for objective assessment of motor function in the acute phase after controlled cortical impact in mice', *Behavioural Brain Research.*

Contacts

Anthony Sandrasagra, PhD. CSO Tony@Alevian.bio
Ori Cohen, PhD. Head Preclinical & Translation Ori.Cohen@Alevian.bio