MHSRS-23-10468 ALPHA-1062 Reduces Phospho-Tau Deposition, is Neuroprotective and Enhances Restoration of Sensory, Motor and Cognitive Performance in a Preclinical Model of Moderate Traumatic Brain Injury

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Introduction:

Traumatic brain injury (TBI) is an especially important health problem for US military service members & Veterans. For Veterans of Operation Iraqi Freedom/Operation Enduring Freedom/Operation New Dawn (OIF/OED/OND), TBI accounts for the majority of combat casualties, with more than 85% diagnosed as mild-TBIs (mTBIs). Although most mTBI resolves over time, the percentage cases that progress to a chronic phase, termed 'persistent post-concussion symptoms' or 'post-concussion syndrome', is estimated to be between 10 & 25%. Importantly, a history of TBI is associated with increased risk of developing Alzheimer's disease. A recent assessment of 350,000 US veterans found that mTBI was significantly associated with more than a 2-fold increase in the risk of dementia diagnosis. Thus, mTBI can result in significant long-term morbidity.

Following mTBI there are significant pathological changes in CNS metabolic & cellular functions that include mitochondrial dysfunction, blood-brain-barrier (BBB) disruption which can be widespread, impairment of neurogenesis, chronic inflammation, oxidative stress, excitotoxicity, & apoptosis. The complexity & diversity of these secondary injury mechanisms may require neuroprotective approaches to target multiple delayed injury factors rather than targeting any single factor.

Rationale for the use of ALPHA-1062 to treat TBI:

ALPHA-1062 is a lipophilic, essentially pharmacologically inert, pro-drug of the Alzheimer's disease cognitive enhancer galantamine (Razadyne/Reminyl). Following intranasal administration ALPHA-1062 rapidly crosses the nasal mucosa, gains access to the blood compartment & thence the CNS where it is activated by de-esterification to galantamine. This route of administration bypasses first pass metabolism & reduces systemic [gastrointestinal] side effects.

ALPHA-1062 has been demonstrated both during pre-clinical development, & in Phase 1a & 1b single ascending dose & multiple ascending dose studies, to deliver galantamine to the brain with apparent greater tolerability than oral galantamine. In the Phase 1 studies ALPHA-1062 administration, at pharmacologically effective doses, was found to be safe & well tolerated, & exhibited evidence of cognitive benefit with improved focus/attention documented in both healthy young & elderly subjects.

ALPHA-1062's active metabolite galantamine, exhibits both acetylcholinesterase inhibition & nicotinic receptor sensitization, & has previously been demonstrated in pre-clinical models to decrease damage to the BBB following TBI, reduce neuroinflammation, decrease glutamate toxicity, activate cell survival signaling pathways, & enhance neurogenesis.

By exerting multiple effects that are beneficial to the injured brain, we believe that ALPHA-1062 will have a better opportunity to be successful in treating TBI than canidates that only address one aspect of the pathophysiology resulting from TBI.

Here we report the results of a study designed to assess the efficacy of intranasally administered ALPHA-1062 in a controlled cortical impact (CCI) model of moderate TBI in rats.

Methods:

TBI Model & Treatment Rationale: Young adult male Wistar rats (2-3 months) received an CCI injury that consisted of a single impact at 4.0 m/sec, 2.5 mm deformation to the left parietal cortex. This model of TBI consistently leads to deficits on motor, sensory, & cognitive functional tests. Following injury, animals were then administered ALPHA-1062 at 4.5 mg/kg or vehicle (purified water as treatment control) intranasally (IN), with treatment initiated 2 hr after injury & continued twice (6 h interval) daily for 35 days. A third cohort was surgically prepared for TBI but not subjected to injury (sham controls). **Treatment was initiated relatively soon following injury as we believe that the earliest practical intervention, followed by sustained treatment with an adequate level of ALPHA-1062 will have the greatest impact in preventing or blunting the development of TBI pathophysiology and promoting functional recovery**. Although aggressive, this time window for intervention is similar to that employed in the treatment of soft tissue trauma, as well as stroke.

Functional Recovery Assessment: Following TBI, functional recovery was assessed by a series of sensorimotor & cognition tests. Sensorimotor assessment began one day following CCI & was repeated weekly throughout the 35-day study. These tests consisted of a foot fault test (locomotor skill assessed), an adhesive removal test (both sensory & motor function assessed), & a modified neurological severity score (motor, sensory, balance & reflex functions assessed). During the last week of the study animals were assessed for spatial learning & memory function, using a modified Morris water maze (MWM), & recognition memory by a novel object recognition test. Both tests are sensitive measures of hippocampal function.

Neuropathology & Neurogenesis: Evidence of neuroprotection & increased neurogenesis was assessed histologically & by immunohistochemistry (IHC). All rats were sacrificed after the last MWM test 35 days after TBI, & brain tissue was subjected to the following histological analyses, determination of: lesion volume (H&E), neuronal cell loss in injured cortex & hippocampus (NeuN IHC), total Tau & pTau burden (Tau/p-Tau IHC), neuroblast number (doublecortin IHC) &, newborn neurons (NeuN/BrdU double label IHC). To label proliferating cells bromrodeoxyuridine (BrdU), had been administered to all animals for seven days starting at one day post CCI). Detailed methodology for all methods described here can be found in: Zhang Y, et al., J. Neurotrauma. 38:1535–1550 (2021).

Statistical analysis: All data are presented as the mean ± standard deviation, with one-way or two-way analysis of variance (treatment x time). Differences between means were considered statistically significant when p was < 0.05.

Results:

In comparison to the vehicle (purified water) treatment control, ALPHA-1062 treatment initiated 2 hr post injury & continued twice daily for 35 days significantly:

1) improved sensorimotor functional recovery assessed by foot-fault (Figure 1), adhesive removal (Figure 2) & modified neurological severity score(Figure 3) ;

2) improved cognitive functional recovery measured by the novel object recognition (Figure 4) & modified MWM tests (Figures 5,6) at 5 weeks after injury.

ALPHA-1062 treatment additionally provided clear evidence of neuroprotection, resulting in;

3) preserved hippocampal structure (Figure 7), and decreased gliosis in thalamus (Figure 7) suggesting preservation of cortico-thalamic tracts.

4) reduced lesion size (decrease of 28%) (Figure 8);

5) reduced neuronal cell loss in the cortex & hippocampus of the injured side (Figure 9) ;

5) enhanced neurogenesis including augmentation of doublecortin positive neuroblasts (Figure 10) as well as BrdU/NeuN positive new neurons in the Dentate Gyrus of the hippocampus (Figure 11) ;

6) reduced of p-Tau burden in the cortex & hippocampus of the injured side (Figure 12) [without changes in total-Tau (Figure 13)].

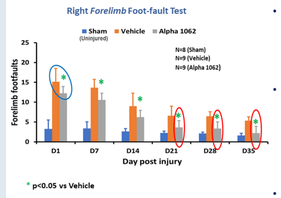
8) ALPHA-1062 did not significantly alter the body weight (data not shown) over the 35-day study indicating a lack of toxicity. This was consistent with other nonclinical toxicology studies with this dose range of intranasally administered ALPHA-1062.

Notably:

- the extent of the functional recovery was such that in, both cognition tests, & in the foot fault & adhesive removal tests, the performance of the ALPHA-1062 treated animals was statistically equivalent to that of the sham (uninjured) animals.
- in the quantitation of cell loss following TBI ALPHA-1062 administration provided sufficient neuroprotection that the cell numbers in the cortex & hippocampus of the injured side of the brain were statistically indistinguishable from those determined for sham animals.

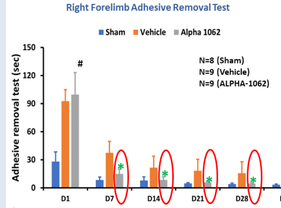
ALPHA-1062 Preserves Function and Improves Recovery Following Moderate Traumatic Brain Injury (TBI)

Figure 1: **ALPHA-1062 Administration Acutely Preserves & Persistently Improves Recovery of Locomotor Skill**



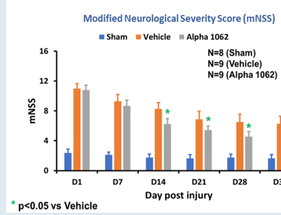
- ALPHA-1062 treatment resulted in **significant *preservation*** **of locomotor skill on the first day of treatment and *persistently improved recovery*** throughout the study, compared to the untreated/Vehicle animals.
- By Day 21 the performance of the ALPHA-1062 treated animals was **statistically *indistinguishable (p<0.05)* from that of the uninjured [sham] animals.** The acute preservation of locomotor skill suggests that the early administration of ALPHA-1062 post TBI, likely reduced the extent of the brain injury.
- Vehicle treated animals depict normal levels of functional recovery.

Figure 2: **ALPHA-1062 Administration Improves Motor Skill and Performs as well as the Uninjured Animals Beginning at One Week of Recovery**



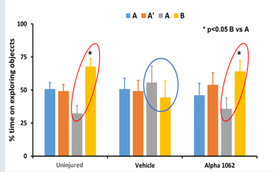
- Starting on day 7 of recovery, and throughout the remainder of the study, **ALPHA-1062 treated animals performed this motor skill as well as the uninjured [sham] animals (p<0.05).**

Figure 3: **ALPHA-1062 Administration Improved Outcomes in Motor, Sensory, Balance and Reflex Function Assessed by the mNSS**



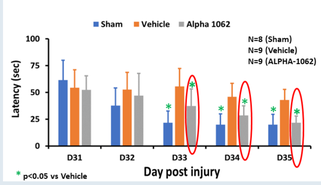
- The mNSS is a composite score assessing: motor, sensory, balance, and reflex functions.
- ALPHA-1062 treated animals performed **statistically significantly better than vehicle treated animals beginning at Week 2 of recovery and persisting throughout the duration of the study** vs Vehicle.

Figure 4: **ALPHA-1062 Administration Enables Cognitive Functional Recovery Following Moderate TBI: Novel Object Recognition**



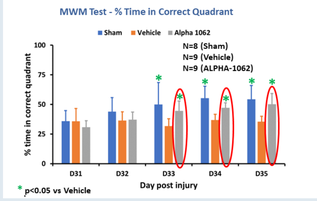
- Both ALPHA-1062 treated animals and the uninjured animals demonstrated a **significant preference** for the novel object.
- In contrast, the injured animals that received only vehicle, failed to demonstrate any preference for the novel object.

Figure 5: **ALPHA-1062 Administration Improves Spatial Learning & Memory: Performance Equivalent to Sham Animals in MWM in *Latency to Find Platform***



- On days 3,4 & 5 of testing the ALPHA-1062 treated animal performance was **statistically indistinguishable** from uninjured [sham] animals.
- Both groups performed significantly better than the vehicle treated animals.

Figure 6: **ALPHA-1062 Administration Improves Spatial Learning & Memory: Performance Equivalent to Sham Animals in *% Time in Correct Quadrant***



- On days 3,4 & 5 of testing the ALPHA-1062 treated animal performance was **statistically indistinguishable** from uninjured [sham] animals.
- Both groups performed significantly better than the vehicle treated animals.

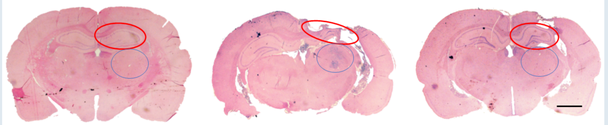
Functional Outcomes Summary:

ALPHA-1062 Acutely *Preserves* Function & Consistently *Improves Recovery* after moderate TBI

		RESULTS	
		VEHICLE	SHAM
Cognitive Functional Tests	Morris Water Maze (MWM)	SUPERIOR	EQUIVALENT TO
	Novel Object Recognition	SUPERIOR	EQUIVALENT TO
Motor and Sensory Functional Recovery	Modified Neurological Severity Score (mNSS)	SUPERIOR	–
	Footfault	SUPERIOR	EQUIVALENT TO
	Adhesive Removal	SUPERIOR	EQUIVALENT TO

ALPHA-1062 Mitigates Neuropathology Following Moderate TBI

Figure7: **ALPHA-1062 is *Neuroprotective* - Maintains Hippocampal Structure & reduces Glial Scarring in Thalamus Following TBI**



- The hippocampus plays critical role in *learning, memory formation, and spatial coding,* whilst the thalamus functions as relay center for incoming motor and sensory information routing this information to appropriate cortical region for processing.
- The hippocampus and thalamus are selectively vulnerable to damage following TBI

Scale bar = 2 mm

Figure 8: **ALPHA-1062 Significantly Reduces Injury Lesion Volume Compared to Vehicle**

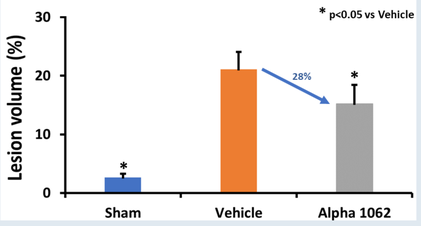
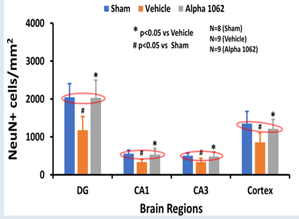
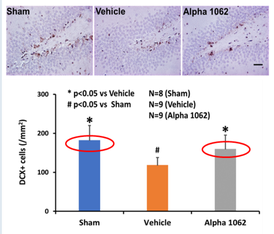


Figure 9: **ALPHA-1062 Is *Neuroprotective* Significantly Reducing Neuronal Cell Loss**



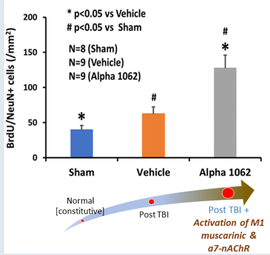
- Following ALPHA-1062 administration, the numbers of surviving neurons in the *cortex & hippocampus* of the injured side of the brain were statistically *indistinguishable* from those observed in *uninjured* animals.

Figure 10: **ALPHA-1062 Administration Significantly Enhances Production &/or Survival of Nascent Cells In The Dentate Gyrus of the Hippocampus**



- Neurons in the granule cell layer of the hippocampus are ***constantly turning over***, with newly born neurons replacing older neurons that die.
- ALPHA-1062 administration results in a level of new immature neurons that is ***indistinguishable*** from that observed in ***uninjured*** animals.
- The input region of the hippocampus, the ***dentate gyrus***, plays a ***critical role*** in the processes of learning, memory, and spatial coding.

Figure 11: **ALPHA-1062 Enhances Neurogenesis in the Dentate Gyrus of the Hippocampus – Novel Neuron Cell Counts**



ALPHA-1062S ***significantly enhances neurogenesis*** increasing the numbers of new neurons in the dentate gyrus.

Figure 12: **ALPHA-1062 Significantly Reduces pTAU Accumulation following TBI**

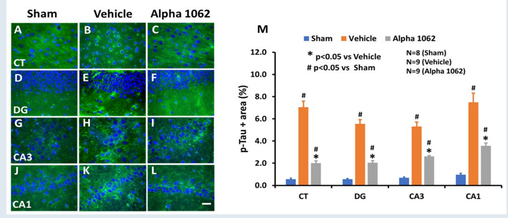
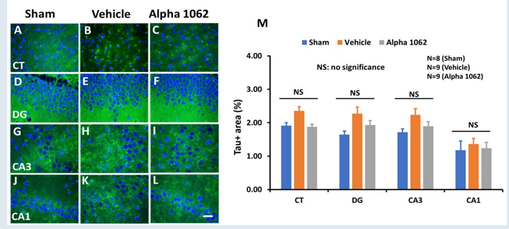


Figure 13: **ALPHA-1062 Does Not Alter Total Tau Accumulation following TBI**



Summary of Neuropathology & Neurogenesis Outcomes

Compared to the vehicle treated control animals, ALPHA-1062 treatment maintained hippocampal structure and reduced glial scarring in thalamus, while significantly:

- reducing lesion size measured at 35 days after injury
- reducing the neuronal cell loss in the cortex and hippocampus at the site of injury
- enhanceing neurogenesis including augmentation of DCX+ neuroblasts and BrdU/NeuN+ novel neurons in the Dentate Gyrus
- reduceing p-Tau accumulation in the injured cortex and hippocampus at 35 days after injury.

Conclusions

ALPHA-1062 *Effectively Treated* Moderate TBI When Administered *Acutely* Following Injury

Initiating ALPHA-1062 administration two hours following a moderate TBI, followed by 35 days of twice daily dosing:

- provided significant ***neuroprotection*** and significantly ***stimulated neurogenesis***
- significantly improved ***sensory motor*** **and** ***cognitive functional recovery*** in a rat contusion model of moderate TBI, when compared to vehicle treatment alone.
- decreased ***p-Tau burden*** suggesting the potential for acute ALPHA-1062 treatment following TBI, to reduce one the pathological drivers of the elevated risk of dementia in TBI patients.

Next steps: **Alpha Cognition is currently engaged in a DoD sponsored dose ranging & validation study of ALPHA-1062 in a military relevant Repetitive Blast mild-TBI model with our collaborators at the Seattle Institute for Biomedical and Clinical Research.**

Alpha Cognition

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