BSP 0001: A novel SOD1 antisense therapy for SOD1-linked and sporadic ALS

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Background

Limitations of current SOD1-targeted therapies:
While targeted antisense therapies to suppress superoxide dismutase (SOD1) expression have shown some therapeutic promise for SOD1 mutation positive ALS patients, the clinical benefit of recently conditionally approved Tofersen is limited. Results from the Phase 3 VALOR trial failed to demonstrate an improvement in ALSFRS-R and meet the clinical endpoint, suggesting that alternative therapies with greater SOD1 modulation potential may lead to improved outcomes. Tofersen dosing is restricted by dose limiting toxicity, a common observation for the phosphorothioate class of antisense drugs, with myelitis and hydrocephalus more frequently reported for CNS delivered phosphorothiate compounds. While Tofersen has shown promise for SOD1 patients, novel compounds with improved safety profiles could be used to overcome dose limiting toxicity, leading to greater efficacy.

A novel solution:
We report a novel antisense therapy targeting SOD1 suppression, BSP 0001, utilising the phosphorodiamidate morpholino (PMO) chemistry, not known to have any reported safety concerns otherwise associated with the phosphorothioate class.
Due to their safety, PMOs can be administered at higher doses to mediate improved and sustained target modulation, expected to result in greater clinical benefit.
Drug safety is key to pre-symptomatic patient treatment for those carrying known SOD1 mutations, as well as for expansion into the broader sporadic disease population.

BSP 0001 has potential for greater SOD1 suppression compared to other efforts, expected to result in improved clinical benefit for SOD1-mutation positive and sporadic ALS patients.

Experimental design

- BSP 0001 is our lead candidate splice-switching antisense PMO, designed to target the frame-shifting *SOD1* exon 3, leading to nonsense mediated decay of the transcript and SOD1 suppression (Figure 1).
- BSP 0001 was transfected by electroporation into SOD1-linked and sALS patient iPSC-derived motor neurons, and mature neurons analysed for SOD1 protein suppression over time, neurofilament levels, autophagy and proteasome pathways, among other readouts.
- A mimic of Tofersen, the RNase H 2´-O-Methoxyethyl (MOE) phosphorothioate ASO, was included for comparison to BSP 0001 effect on motor neuron function.
- *In vivo* evaluation of BSP 0001 was carried out in SOD1 G93A transgenic mice, with mice treated at either P30 or P60 with a single intracerebroventricular (ICV) infusion of BSP 0001 (300 nmol).
- A full battery of GLP toxicology evaluation was conducted on BSP 0001, including: Ames test, *in vitro* and *in vivo* micronucleus testing, and a 28-day rat study with 7 day recovery and up to 10-fold drug coverage.

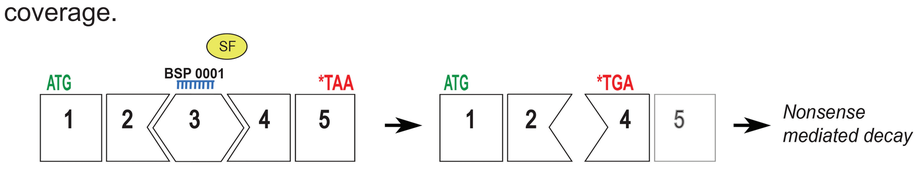


Figure 1: Schematic of the *SOD1* transcript and reading frame before and after transfection with BSP 0001. BSP 0001 sterically blocks splicing factor (SF) binding to induce targeted exon 3 removal that disrupts the reading frame, resulting in a premature termination codon and nonsense mediated decay.

In vitro efficacy of BSP 0001

1. BSP 0001 efficiently suppresses SOD1 levels and reduces protein buildup in iPSC-derived motor neurons, with long term activity over 6 weeks.

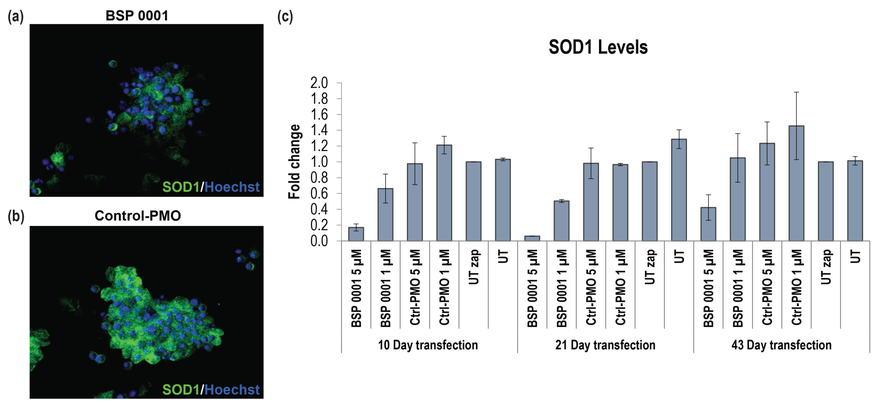


Figure 2: Representative immunofluorescent staining for SOD1 in patient iPSC-derived MNs following transfection with (a) BSP 0001 and (b) the control PMO (10 µM), and (c) graph of SOD1 levels assessed by western blot in BSP 0001 transfected MNs (5 and 1 µM), collected at 10, 21 and 43 days post transfection, compared to control (Ctrl-PMO), untreated (UT), and cells that underwent electroporation (zap).

2. BSP 0001 is effective at modifying MN function, with variable effects in SOD1 mutation positive and different sporadic ALS patient lines.

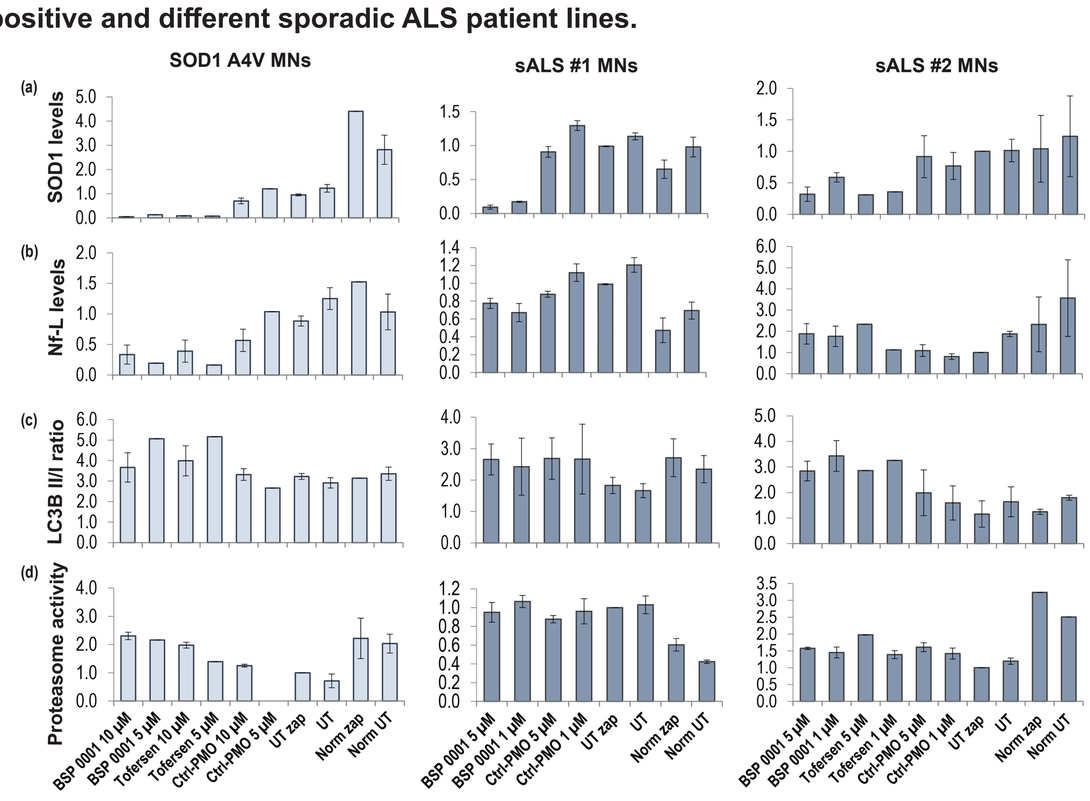


Figure 3: Graphs showing the preliminary effect of BSP 0001 and a Tofersen mimic at modulating motor neuron function following transfection into SOD1 A4V (10 and 5 µM) and two sALS (5 and 1 µM) patient iPSC-derived MNs. Functional readouts include: (a) SOD1 protein expression, (b) neurofilament light (Nf-L) expression as a biomarker and measure of axonal degeneration, (b) LC3B II/I expression as a measure of autophagic flux and (d) proteasome activity (n=2-3).

Summary of *in vitro* work

BSP 0001 is highly effective at reducing SOD1 levels and clearing SOD1 buildup in mutation positive and sALS iPSC-derived MNs, with long-term effects observed for up to 6 weeks, how this applies to a clinical scenario and the frequency of dosing to maintain long-term SOD1 suppression is still to be determined.
Preliminary evaluation of BSP 0001 effect on neuronal function is encouraging for mutation positive and sALS patients. We observed positive effects on reduction in neurofilament levels, increased autophagy (LC3B autophagic flux) and proteasome pathways in A4V patient MNs.
Preliminary functional studies in sALS MNs are interesting, and suggest that mediating SOD1 expression will have variable pathological effects on different patient lines, likely depending on the dysfunctional pathways that underpin disease pathology.
- sALS #1 demonstrated a modest decrease in Nf-L levels following BSP 0001 treatment. No change was observed in proteasome activity. Interestingly, both the BSP 0001 and control PMOs induced an shift in LC3B ratios and linked autophagic flux, but not in the untreated cells that underwent electroporation, suggesting that autophagy may be highly sensitive to the presence of any antisense compounds in these cells.
- sALS #2 had lower baseline levels of Nf-L compared to healthy controls, with Nf-L increasing following BSP 0001 and Tofersen mimic treatment. This finding was intriguing and requires follow up for interpretation. An increase in autophagic flux was also observed in these cells, induced by both SOD1 therapies. No change to proteasome activity was observed.
Further repeat experiments, as well as evaluation in additional patient lines are in progress to confirm and extend these intriguing findings.

In vivo efficacy and safety of BSP 0001

3. BSP 0001 induces 40% SOD1 suppression (p=0.01) in the spinal cord, resulting in an 11% increase in survival of SOD1 G93A transgenic mice and improved motor function from a single ICV administration (p<0.0001).

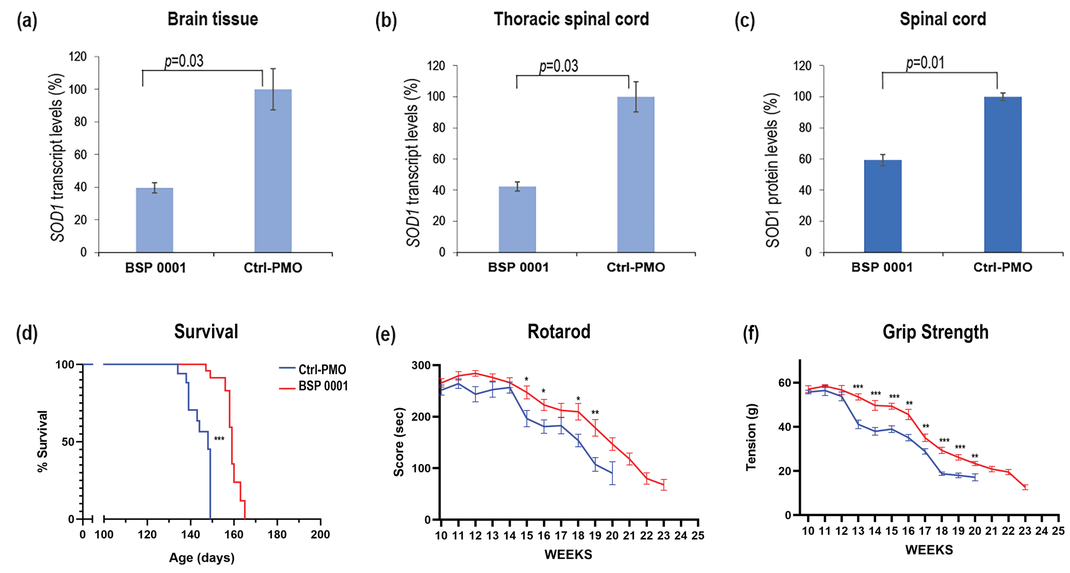


Figure 4: *In vivo* evaluation of a single ICV administration (300 nmol) of BSP 0001 or the control PMO (Ctrl-PMO), at P30 and mice sacrificed at P45 (n=3) (a-c), or a single administration on P60 with mice sacrificed upon end-stage disease onset (hind-limb paralysis, n=12) (d-e), showing *SOD1* transcript expression by qPCR in the brain (a) and spinal cord (b), SOD1 protein levels in the spinal cord, assessed by western blot and normalised to total protein (c), probability of survival (d), change in rotarod score (e), and change in grip strength (f), measured weekly. Error bars show SEM, t-test for significance (a-c), log-rank Mantel-Cox (b) and repeated measures ANOVA (e-f), *p<0.05, **p<0.001, ***p<0.0001

4. BSP 0001 has an excellent safety profile following administration to mice (ICV) and rats (intrathecal) at high doses, as assessed in GLP non-clinical toxicology studies.

- No evelated serology markers (up to 2.5 mg in mice and 10 mg in rats)
- No safety concerns reported for 28-day GLP study (up to 10 mg in rats)
- No genotoxicity findings reported in micronucleus (up to 1665 mg/kg in mice) and Ames studies

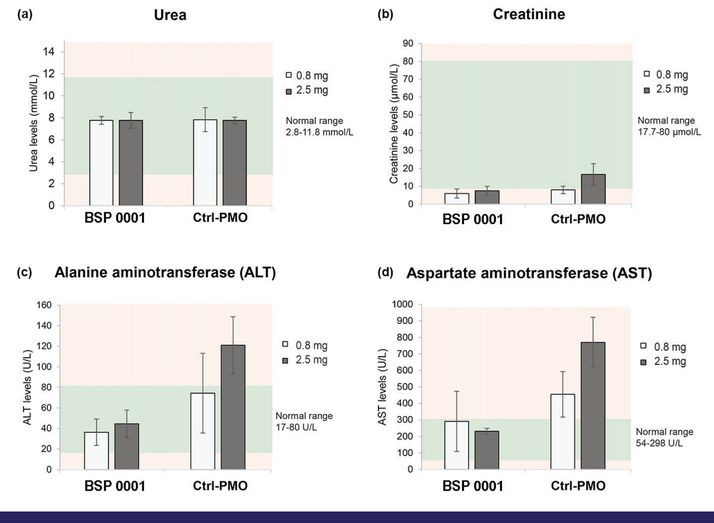


Figure 5: Graphs showing serum biochemistry from mice treated with a single ICV administration (P30) of BSP 0001 or a control PMO at 0.8 and 2.5 mg (n=3). Biochemistry readouts for common toxicity markers include, (a) urea and (b) creatinine for kidney function and (c) alanine aminotransferase (ALT) and (d) aspartate aminotransferase (AST) for liver function. The normal range is indicated in green, while elevated levels are indicated in red. Low creatinine levels are expected for a disease model characterised by muscle wasting.

Summary of *in vivo* work

BSP 0001 is highly effective at reducing SOD1 protein expression in the SOD1 G93A mouse, with up to 40% knockdown achieved with a single administration, translating to an 11% increase in survival and improved motor function. These data demonstrate the ability of BSP 0001 to ameliorate disease phenotypes in this aggressive model that will be used to inform clinical trial design.
Safety evaluations of BSP 0001 have demonstrated an excellent safety profile with high dosages and up tp 10-fold coverage, supporting its transition into clinical application.
Taken together, BSP 0001's effect at suppressing disease and its clean safety profile support high dose clinical application, suggesting that BSP 0001 has potential for greater SOD1 suppression that may lead to improved clinical outcomes.

Acknowledgements

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