Plasma LOXL2 target engagement by GB2064, a high affinity, small-molecule LOXL2 inhibitor, in a phase 1 healthy subject study

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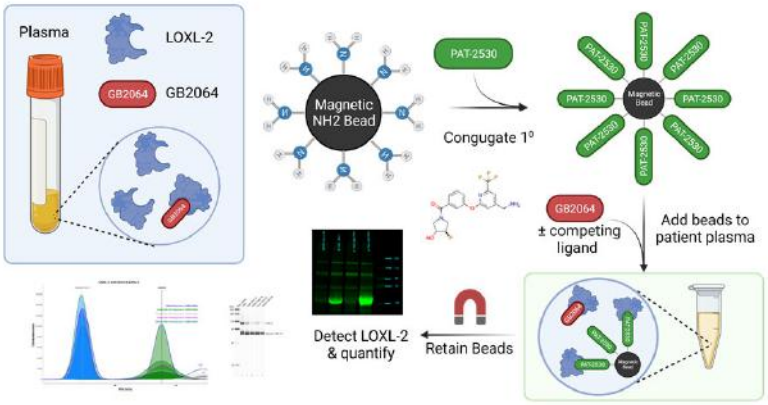
Introduction

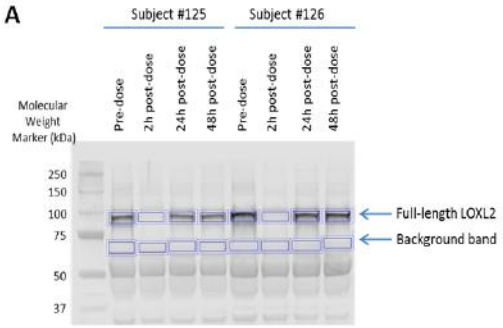
Lysyl oxidase 2 (LOXL2) is a secreted enzyme that catalyses the crosslinking of extracellular matrix collagens and elastin, which contributes to the loss of function of fibrotic organs. GB2064 (formerly PAT-1251) is a novel, high affinity, selective, pseudo-irreversible orally dosed small molecule inhibitor of LOXL-2[1] currently in development for the treatment of fibrotic disease[2]. In this study we describe the pharmacodynamic profile of GB2064 in a phase 1 study[3] together with a novel assay to measure target engagement (TE) in patient plasma samples.

Methods – Biomarker Pulldown Assay



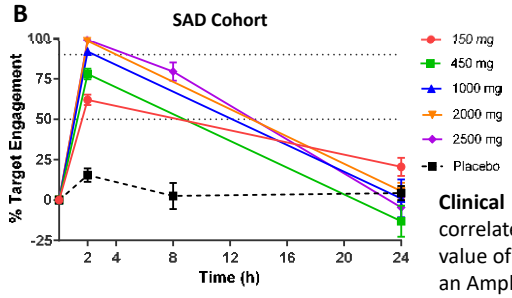
The extent to which GB2064 binds plasma LOXL2 following oral dosing was characterised in a phase 1 single ascending dose (SAD) and multiple ascending dose (MAD) study in healthy subjects. Plasma samples were taken pre-dosing (H0) and at various timepoints post dosing with GB2064. Plasma LOXL2 not bound to GB2064 was pulled down from samples using ferrite beads conjugated to PAT-2530 (a LOXL2 binding analogue of GB2064) and measured to give a readout of TE.

Results – Phase 1 SAD and MAD Studies



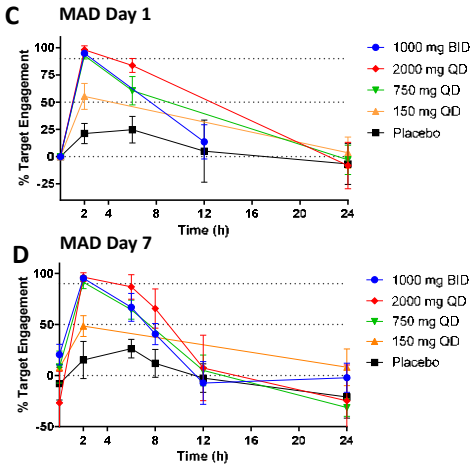
Example Blot and Quantification of LOXL2 (A): Representative western blot showing endogenous LOXL2 pulled down from the plasma of healthy subjects pre-dose (H0) and post dosing with GB2064. %TE is calculated as follows:

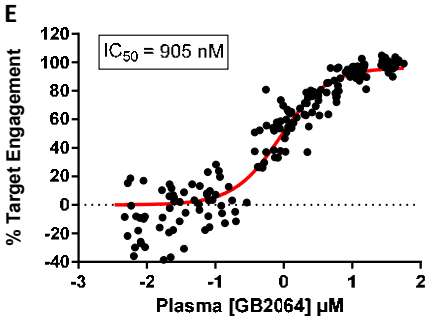
$$\%\ \text{Target Engagement} = 100 \times \frac{\text{Sample Signal} - \text{H0 Signal}}{\text{Average Background signal} - \text{H0 Signal}}$$

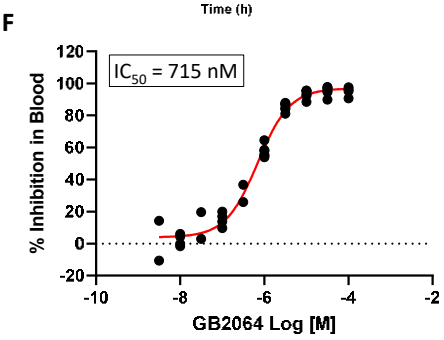


Target Engagement in SAD Cohorts (B): Plasma %TE was measured before and at 2h and 24h following single oral doses of 150, 450, 1000, 2000 and 2500mg of GB2064. At 2h post dosing, %TE was essentially complete with the top two doses, and was tending to zero for all doses at the 24h timepoint.

Target Engagement in MAD Cohorts: Plasma %TE was evaluated on Days 1 (C) and 7 (D) after oral dosing of 150, 750 or 2000mg (QD) or 1000mg (BID). On Days 1 & 7, %TE was essentially maximal at doses of 750mg and above at the 2h timepoint (93-98%TE). Similar to the kinetics observed in the SAD study, following a peak at 2h, %TE reduces over time with no appreciable TE observed at 24h.



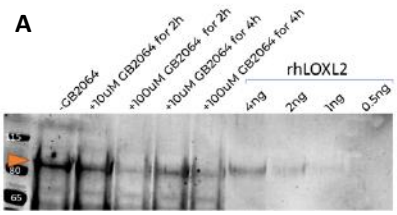


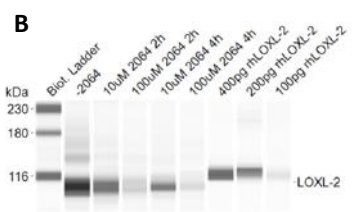


Clinical Target Engagement vs *In Vitro* Target Engagement: The %TE for all subjects in both SAD and MAD cohorts correlates very well with their respective plasma drug concentrations at the corresponding timepoints yielding an IC_{50} value of 905nM (D). When compared to *in vitro* data (E) where fresh human blood was spiked with rhLOXL2 ± GB2064 in an Amplex Red Amine Oxidase Enzymatic Assay using an artificial substrate DAP, a similar IC_{50} of 715nM was observed.

Further Work: Assay Refinement

Following the phase 1 clinical study, improvements in the detection of LOXL2 have been made that increases the sensitivity and throughput, and reduces sample requirement, and time taken to obtain results. These benefits are made through switching from traditional western blot to capillary western using the Protein Simple Jess system.





Endogenous LOXL2 was pulled down from healthy human plasma as described above. Prior to pulldown, samples were spiked with competing amounts of GB2064 and incubated for different times to simulate the patient dosing regimen. 80% of the resulting beads were run on a western blot (A) with the remaining 20% run on a Jess (B). Sensitivity limits of ~1ng are reached with western blot, whereas concentrations below 100pg are reliably detected by capillary western.

Conclusions

GB2064 was safe and well tolerated in this study. When dosed orally GB2064 binds circulating LOXL2 thereby preventing its capture by inhibitor-coupled beads. Doses of ≥750mg achieved maximal and near complete TE at 2 hours post dosing. TE decreases with decreasing plasma concentrations of GB2064, with excellent correlation in IC_{50} values determined by *in vivo* and *in vitro* assays (905nM and 715nM, respectively). TE was nearly identical on Day 1 in each treatment cohort to that of Day 7 of the MAD study and observed levels of TE in the SAD study were near identical to the same doses in the MAD study showing remarkable consistency in the data. In addition, further assay refinement has been completed using the Protein Simple Jess system to improve sensitivity and throughput, whilst reducing sample requirement, time and resource.

References

1. Rowbottom, M.W. *et al. J Med Chem* **60**, 4403–4423 (2017).
2. ClinicalTrials Identifier: NCT04679870
3. ClinicalTrials Identifier: NCT02852551

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