Discovery of multiple clinical candidates for treatment of Fragile X Syndrome using AI-Enabled drug discovery

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Abstract

Fragile X syndrome (FXS) is an X-linked genetic disorder characterised by mild-to-moderate intellectual disability, anxiety and behavioural issues. It is typically caused by an expansion of the CGG triplet repeat within the FMR1 (fragile X messenger ribonucleoprotein 1) gene on the X chromosome. This results in silencing (methylation) of FMR1, which is required for the normal neuronal development and connectivity.

Healx is an AI-powered, patient-inspired biotech company using AI to develop treatments for rare diseases. Healx's tech-driven drug discovery approach centres on Healnet: an AI platform that finds novel connections between drugs and diseases. The feasibility of these predictions is then assessed by in-house experts and tested in preclinical models.

Three lead candidate drugs (HLX-0201, HLX-0205 and HLX-0206), identified through Healx's AI, were tested in two different preclinical mouse models of FXS . These compounds were able to effectively reverse several behavioural phenotypes in the FXS mouse models, including: hyperactivity, stereotypy, cognitive deficits, anxiety, impaired social recognition and aggression.

Based on these promising preclinical findings we have initiated a Phase 2a clinical trial to evaluate these drug candidates as monotherapies in male patients diagnosed with FXS.

References

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Introduction

Fragile X syndrome (FXS) is the most common inherited form of intellectual disability with an estimated frequency of 1:4,000–5,000, affecting all ethnic groups worldwide (Gross et al., 2015). FXS is a result of mutations in the FMR1 gene that block the expression of the fragile X messenger ribonucleoprotein (FMRP). FMRP is a ubiquitously expressed mRNA binding protein required for transport and translation of 4%–8% of synaptic proteins, and thus regulates a variety of synaptic functions.

Individuals with FXS are at increased risk for a range of associated behavioural issues, including: attention problems, hyperactivity, anxiety, and many features associated with autism including motor stereotypies, social avoidance, self-injurious behaviour, and aggression (Hagerman et al., 2010). Behavioural interventions and pharmacological management of discrete symptoms are offered to individuals with FXS, but there are currently no approved therapies to treat the syndrome as a whole.

Healx has identified 3 candidate drugs which have shown efficacy in reversing several behavioural phenotypes in FXS preclinical mouse models. These three lead candidate drugs have distinct mechanisms of action targeted towards different pathophysiological pathways in this complex neurodevelopmental disorder. If these drugs were combined it would provide a multi-targeted approach and could maximise the number of phenotypes treated. Three independent CROs confirmed efficacy of the 3 lead candidate drugs by using two different preclinical mouse models of FXS {Fmr1 KO1, exon 5 disruption, FVB.Sev129 (Bakker et al., 1994), and Fmr1 KO2, exon 1 disruption, C57Bl6, (Mientjes et al., 2006)}.

In silico drug discovery

We have applied the Healx AI drug discovery approach (Healnet) to FXS. Healnet integrates data from biomedical research, scientific literature, patient insights and Healx's own curated sources to form a rare disease knowledge graph. By applying state-of-the-art AI models to the graph, Healnet rapidly identifies novel disease-compound relationships with the highest chances of success. Healx pairs the platform with human drug discovery expertise to identify multiple potential ways to exploit the new disease biology identified. This includes exploring opportunities to repurpose known drugs, opportunities to combine compounds and ways to enhance efficacy of molecules.

Preclinical validation

A total of 14 drugs, with different primary modes of action, were identified for in vivo screening. Eleven of these showed activity against one or more behaviours. Following a small number of further iterative studies (Tranfaglia et al., 2019) in which different drugs and different behaviours were investigated, HLX-0201 and HLX-0205 were progressed on the basis of efficacy, commercial and regulatory reasons. HLX-0206 was a Healnet prediction which was in-licensed. These drugs were tested in two different mouse models of FXS, Fmr1 KO1 and Fmr1 KO2, and results were confirmed by 3 independent CROs. All drugs were dosed for 2 weeks prior to behaviour testing. The data presented is a composite from several experiments performed at different times.

Healx ID	Mode of Action (MoA)	Dose Tested (mg/kg)	Dose Route	Dose Frequency
HLX-0201	PPAR modulator	0.5, 1.5, 2.5, 5, 10	IP	QD
HLX-0205	Signal transduction modulator	3, 6, 12	IP	QD/BID
HLX-0206	GABA receptor modulator	0.15, 0.5, 1.5	IP	QD

Preclinical validation

Novel Object Recognition (NOR) - Recognition memory as a measure of cognition

HLX-0205 (6mg/kg) significantly improved recognition memory compared to KO Veh group but not to WT Veh levels (p=0.017). HLX-0201 (5mg/kg) significantly improved recognition memory to WT Veh levels. HLX-0205 and HLX-0201 also showed efficacy in context and cued fear conditioning (FC) . HLX-0206 showed no efficacy for NOR, NOL or FC at any doses tested (0.15mg/kg - 5 mg/kg).

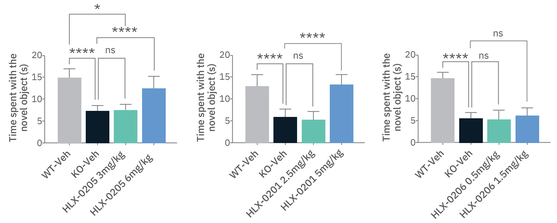

Figure 1: Novel object recognition performed in Fmr1 KO2 and WT mice. Data represents time, in seconds (s), spent investigating a novel object. One way ANOVA. *p<0.05; ****p<0.0001.

Self grooming - Stereotypy

HLX-0205 (6mg/kg) significantly reduced stereotypy compared to KO Veh group, but was unable to revert levels to that of WT Veh group (p<0.001). HLX-0201 (5mg/kg) and HLX-0206 (0.5mg/kg and 1.5mg/kg) significantly reduced stereotypy to levels comparable to WT Veh group.

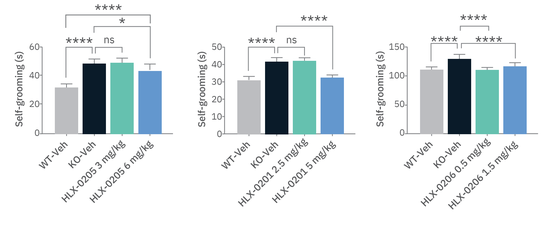

Figure 2: Self grooming as a measure of stereotypy performed in Fmr1 KO2 and WT mice. Data represents time, in seconds (s), spent self grooming. One way ANOVA. *p<0.05; ****p<0.0001.

Open Field - Hyperactivity

HLX-0205 (6mg/kg) significantly reduced hyperactivity compared to KO Veh group, but was unable to revert levels to that of WT Veh group (p<0.001). HLX-0201 (5mg/kg) and HLX-0206 (0.5mg/kg and 1.5mg/kg) significantly reduced hyperactivity to levels comparable to WT Veh group.

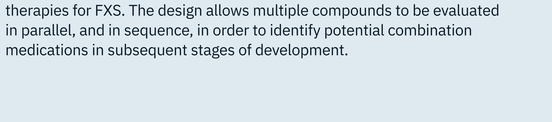

Figure 3: Activity measured in Fmr1 KO2 and WT mice over 30 minutes in a novel open field arena. Data represents distance covered, in cm, in 30 minutes. One way ANOVA. ****p<0.0001.

Partition test (Social recognition test)

HLX-0205 (6mg/kg) significantly improved social recognition memory compared to KO Veh group but was unable to reverse this behaviour to WT Veh group levels (p=0.022 vs WT Veh). HLX-0201 (5mg/kg) significantly improved social recognition to levels comparable to WT Veh group. HLX-0206 (0.5mg/kg and 1.5mg/kg) showed no efficacy.

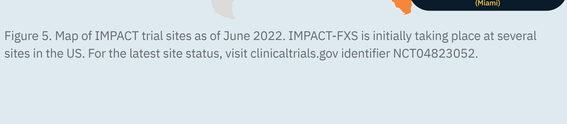

Figure 4: Social recognition test. Time mice spent investigating novel or familiar mice was recorded and exploration ratio is reported here. WT mice are able to differentiate between novel and familiar mice and as such will show a preference for interacting with the novel mouse. One way ANOVA. *p<0.05; ****p<0.0001.

Table 1: Summary of behaviours tested with HLX-0205, HLX-0201 and HLX-0206

Healx ID	Hyperactivity	Stereotypy	NOR	FC	Sociability	Anxiety	Aggression	Nesting
HLX-0205	6mg/kg	6mg/kg	6mg/kg	6mg/kg	6mg/kg	6mg/kg	6mg/kg	6mg/kg
HLX-0201	5mg/kg	5mg/kg	5mg/kg	5mg/kg	5mg/kg	5mg/kg	5mg/kg	5mg/kg
HLX-0206	0.5mg/kg 1.5mg/kg	0.5mg/kg 1.5mg/kg	0.5mg/kg 1.5mg/kg	0.5mg/kg 1.5mg/kg	0.5mg/kg 1.5mg/kg	0.5mg/kg 1.5mg/kg	0.5mg/kg 1.5mg/kg	NT

Key: Efficacy vs KO Veh, behaviour NOT returned to WT Veh level; Efficacy vs KO Veh, behaviour returned to WT Veh level; No efficacy; NT-not tested

Conclusions and next steps

We have so far identified 3 compounds with separate and distinct mechanisms of action which show efficacy in two preclinical mouse models of FXS. Targeting multiple MoAs would increase the likelihood of success in the clinic. Preclinical drug combination testing has shown some synergy between certain drugs, testing is ongoing.

Healx is conducting a Phase 2 clinical trial

IMPACT-FXS

Investigating **M**ultiple **P**athways **A**nd **C**ombined **T**reatments for **F**ragile **X** **S**yndrome

Healx is conducting a Phase 2 clinical trial to explore the safety, tolerability and efficacy of HLX-0201 and HLX-0206 in males (age 13-40 years old) with FXS at several sites across the US (Figure 5).

IMPACT-FXS study is the world's first umbrella trial for the investigation of therapies for FXS. The design allows multiple compounds to be evaluated in parallel, and in sequence, in order to identify potential combination medications in subsequent stages of development.

Healx plans to investigate additional candidate(s) such as HLX-0205 with the ultimate aim of bringing at least one novel and effective combination therapy to people with FXS in the coming years.

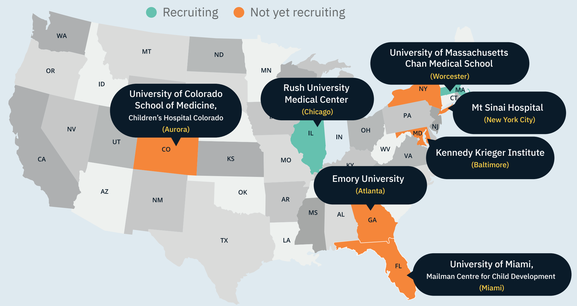

Figure 5. Map of IMPACT trial sites as of June 2022. IMPACT-FXS is initially taking place at several sites in the US. For the latest site status, visit clinicaltrials.gov identifier NCT04823052.