Antibodies from resilient individuals: Identifying a potential novel treatment for Huntington’s disease modification

Donna Finch Session III Wed April 26th 2023
Vision: To use the power of the human immune system to discover new medicines

Discovering and developing protective, patient-originated therapeutic antibodies

- Focus on protective antibody responses
- Convergent in resilient individuals
- Target agnostic approach
- Advanced computational approaches
Humans benefit from naturally-occurring protective autoantibodies in many diseases

A human-derived antibody targets misfolded SOD1 and ameliorates motor symptoms in mouse models of amyotrophic lateral sclerosis

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ALDH4A1 is an atherosclerosis auto-antigen targeted by protective antibodies

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Protective effect of naturally occurring anti-HER2 autoantibodies on breast cancer

![Graph showing recurrence-free survival rate over time for HER2-AAbs positive and negative groups.]
Our approach finds naturally occurring protective antibodies, deconvolutes their targets and validates their function

- Repertoire analysis to select antibodies
- Proteomics to deconvolute targets
- Phenotypic assay cascade to screen for function
- Validate function in vitro & in vivo

Unbiased target and drug discovery

Resilient patient cohort with disease and healthy controls
Resilience can take many forms

Patients with years of survival with typically untreated cancer
- Pancreatic cancer survivors, alive 7+ years after diagnosis
  - Median survival: 10-12 months

Very long-lived, healthy individuals without chronic diseases
- Average >100 years, no cognitive impairment nor debilitating illness
  - ~0.004% population

Patients with susceptibility to neurodegenerative disease who do not progress
- Confirmed Beta-amyloid in CSF, APOE4 risk allele, no or very slow disease progression
We identify BCR sequences of interest through analysis of deep NGS of patient samples.

**Core Technologies**

- Repertoire analysis
- Phenotypic screening
- Target deconvolution

**Diagram:**

- **CLUSTERING**: Grouping together antibodies based on sequence relatedness
- **CONVERGENCE**: Searching for clusters containing sequences derived from multiple resilient individuals – indicates selection for similar specificities
- **VALIDATE & TRIAGE**: Validating convergent signals, and understanding features of the best antibodies

Numbers:

- 10,000,000
- 1,000,000
- 1,000
- 100
Alchemab works with leading global collaborators to secure highly curated resilient samples

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Other

- Amsterdam UMC
- Healthy ageing
  - 100+ cohort
- AD in Down’s

A total of more than 1500 patient samples from across target neurodegenerative indications
The Data Cube, containing over 350 Million BCR Sequences, generates multiple insights to support the discovery process:

- Identify which antibodies/targets most commonly occur together in resilient patients
- Identify which patients are most likely to have disease / respond to therapy based on repertoire
- Identify how our chosen antibody could be effective across diseases
- Select optimal therapeutic candidate from natural variants based on sequence insights*

*Includes: Comparison to patented antibody database for variants of known antibodies; Function based on sequence; developability; toxicity
Mining the Data Cube shows HTT antibodies may contribute to resilience in several diseases

Resilient AD cohort, 13.6% resilient FTD cohort and 8% resilient PD cohort had the sequence.

No progressors across diseases seen to have the sequence

1.9% of controls

Resilience: AD resilience defined using biomarkers or progression rate; PD resilience defined by delay in symptomatic onset; FTD resilience defined using age of onset with genetic driver

Subject distribution for individuals with ATL_005252 homologues (90% CDR3 + V + J and >75% similarity across paratope)

HTT sequences convergent across 'at risk' resilient FTD, AD and PD patients, but not in progressors

Related sequences also found in HD patients

Assessing resilience association and increasing HD cohort numbers
HTT dysfunction may play a role in FTD, AD and other dementias

HTT aggregates are seen across neurodegenerative diseases

- HTT aggregates accumulate in AD brains (Singhrao et al., 1998; Axenhus et al., 2020)
- Some FTD and ALS patients have CAG expansions of >40, which is seen x4 more often than in healthy individuals (Dewan et al., 2021)

Aggregated HTT may precede Tau, β amyloid & TDP-43 mediated changes

- Association of HTT with tau fibrils and tangles in both HD and AD (Masnata et al., 2020)
- mHTT increases the seeding properties of aggregated TDP-43 in a cellular model (Coudert et al. 2019)
Reasons to believe antibodies targeting extracellular HTT may slow spread of pathology

**Extracellular HTT Exon 1 truncated form is pathogenic**
- N-terminal fragments generated by proteolysis and alternative splicing
- Highly and directly neurotoxic in cell systems
- Clear relevant pathology in Exon 1 KI mouse models
- Greater challenge for ASOs to effectively lower truncated forms of HTT

**Extracellular mHTT can accelerate pathology by spreading and seeding**
- mHTT is present in neuronal grafts in HD patients
- CSF from HD patients contains seed competent mHTT species
- Spreading and seeding activity has been observed in cell-free, cell, and in vivo models

**CSF mHTT increases as disease progresses**
- Correlates with increases in CSF NfL (TRACK-HD)

Wild et al 2015
ATLX-1095 binds HTT with high affinity and specificity

Panel of antibody variants of convergent sequence were assessed in ELISA assays

Epitope is within Exon 1 but independent of PolyQ

Retrogenix Cell Microarray proteome platform shows no off-target binding
Can ATLX-1095 impact key biological mechanisms of interest?

- Can ATLX-1095 bind and immuno-precipitate complex multiple forms of mHTT?
- Can ATLX-1095 impact cell-free seeding of HTT?
FRASE (FRET based mHTT Aggregate seeding assay) (Ast et al, 2018)
ATLX-1095 immunoprecipitates multiple species of mHTT including high molecular weight aggregates
ATLX-1095 reduces rate of aggregation in a FRASE seeding assay

MW1 (polyQ) has no impact on seed-induced acceleration of aggregation
ATLX-1095 significantly reduces rate of seeding induced by recombinant or brain-derived seed
ATLX-1095 binds to multiple forms of muHTT

- 25Q and 48Q recombinant Ex1 HTT ELISA binding
- Immunoprecipitation with a variety of detection abs shows multiple forms of HTT are pulled down
  - mHTT over-expressing cell line lysate (LoQus23 proprietary cell line))
  - HTT R6/2 mouse brain lysate- soluble and aggregated forms

Recognizes high molecular weight aggregated and soluble forms of HTT
ATLX-1095 increases phagocytosis of mHTT by human iMicroglia

Phagocytosis of Q48Htt Exon-1 coated beads by iPSC microglia over 4hrs

- Isotype
- ATLX-1095
- HTT beads only
- Untreated

Antibody Dose response of phagocytosis at 4hrs

- Isotype control
- ATLX-1095
CNS exposure of ATLX-1095 above EC50 at achievable dose

Typical half-life of IgG1 in WT mice of 3-5 days
Typical IgG1 0.1-0.3% CNS penetrance
ATLX-1095 has an excellent early manufacturability profile

- ATLX-1095 has been engineered to remove sequence liabilities and to revert framework mutations to germline
- Good thermostability
  - No aggregation observed following incubation at 40 °C for 4 weeks (SEC-HPLC)
  - No aggregation observed following 10 freeze-thaw cycles (SEC-HPLC)
- Main species is within appropriate range for downstream processing (pH 7.5 – 9.0)

40°C 4 weeks vs -80°C 4 weeks
Control vs 10x FT cycles
Pharmacology plans in R6/1 mice - Proof of concept study

Initial proof of concept study R6/1 mouse model

- Weekly doing at 60mg/kg
- Start dosing at 5wks of age
- Endpoints at 9 wks, 13 wks and 17 weeks of age
- Free and total HTT Immunoassays
- Aggregated HTT in brain by IHC
- Behavioural endpoints (multiple)
- Neurofilament light chain

Development of inclusion bodies in R6/1 brains over time

Hansson et al, 2001
Pharmacology plans in R6/1 mice - Considering Translation to Clinic

**In vivo Pharmacokinetics, Biodistribution (PET Imaging)**
- Antibody radiolabelling
- High performance PET/CT
- Whole-body biodistribution

**Ex vivo brain penetrance – Target Engagement (Fluorescence Imaging)**
- Antibody localisation in mouse brain slices

Non-invasive monitoring of disease state using [\(^{18}\text{F-CHDI-}\)] PET radiotracers for mutant huntingtin
ATLX-1095

Discovered in Resilient Individuals ‘at risk’ of Neurodegeneration

Naturally optimised and differentiated binding profile

Targets multiple forms of mHTT

Potential for add-on to other HD lowering modalities
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