Positive changes to neuroinflammatory biomarkers consistent with improvement in cUHDRS in patients treated with ANX005, an inhibitor of C1q and the classical complement pathway

Ann Mongan, PhD[1]; Chenying Guo, PhD[1]; Vladislav Volman, PhD[1]; Logan Kuhn, BS[1]; Ellen Cahir-Mcfarland, PhD[1]; Benjamin Hoehn, MD, PhD[1]; Henk-Andre Kroon, MD, MBA[1]; Rajeev Kumar, MD[2]; Lauren M. Byrne, PhD[3], Edward Wild, PhD[3], Ted Yednock, PhD[1]

[1]Annexon Biosciences, Brisbane, CA, USA; [2]Rocky Mountain Movement Disorders Center, Englewood, CO, USA; [3]University College of London, UK

BACKGROUND

- Dysregulation of the classical complement pathway, which is activated by the complement component C1q, has been implicated in synapse elimination, neuroinflammation, and progressive neuronal loss in neurodegenerative disorders including Huntington's disease (HD)[1,2]
  - Levels of C4a, an activated complement protein marker within the classical pathway downstream of C1q, are elevated in cerebrospinal fluid (CSF) from patients with HD and correlate with HD disease stage[3]
  - In addition, correlation of C4a with the composite Unified Huntington Disease Rating Scale (cUHDRS) further supports a role for the classical pathway in the neurodegenerative process[3]
- In an open-label phase 2 study, patients with or at risk of manifest HD were treated with ANX005, a humanized monoclonal antibody targeting C1q, blocking initiation of the classical complement pathway
  - ANX005 treatment was associated with sustained clinical improvement (cUHDRS) over the 9-month study period in patients with high vs low baseline CSF levels of C4a (measured as the C4a/C4 ratio)
  - These results support the hypothesis that anti-C1q/classical complement therapy may preserve synapse function and prevent subsequent neurodegeneration,[4] particularly in patients with high baseline complement activity

OBJECTIVE

- To evaluate complement pathway and biomarker proteins in serum and CSF associated with improved cUHDRS observed in patients treated with ANX005

METHODS

- The ANX005 phase 2 study in HD patients (NCT04514367) was a multicenter, open-label study of intravenous ANX005 in patients who had or were at risk of manifest HD (**Figure 1**)
  - C1q target engagement and clinical outcomes were assessed in patients who completed treatment and follow-up periods
  - Outcomes were compared for patients with high and low complement activity (defined as a C4a/C4 ratio greater than or less than the cohort median, respectively) at study baseline
- Proteomic profiling of CSF samples from ANX005-treated patients as well as from natural history patients in the HD-CSF cohort (n=40) and control patients (n=20)[5,6] was performed using SomaScan (SomaLogic, Boulder, CO) and ELISA assays to compare:
  - Levels of complement proteins and complement activation markers
  - Changes in disease-relevant proteins

Figure 1. Overview of the Open-Label Phase 2 Study (NCT04514367)

24-week treatment period (n=28) → **12-week off-treatment follow-up**

Induction dosing of ANX005 administered by IV infusion on Days 1 and 5 or 6, followed by maintenance dosing every 2 weeks through Week 24

Follow up visit on Week 36

Study Population
- Adults with, or at risk of, manifest HD ("Early HD")
- Total CAP score >400
- UHDRS independence score ≥80%

Primary Endpoints
- Safety and tolerability of ANX005
- PK of ANX005 in serum & cerebrospinal fluid (CSF)
- PD as measured by C1q, C4a, and NfL concentrations in blood and/or CSF

Exploratory Endpoints
- Composite UHDRS and its components

Final Clinical Study Findings Include:
- Target engagement, clinical measures in patients who completed both treatment and follow-up periods (n=23)
- Clinical measures in patients with high (n=12) vs low (n=11) complement activity at baseline
- Changes in levels of biomarkers of neuroinflammation

CAP, CAG age product; CSF, cerebrospinal fluid; HD, Huntington's disease; PD, pharmacodynamics; PK, pharmacokinetics; UHDRS, Unified Huntington's Disease Rating Scale.

RESULTS

- In the phase 2 study, 23 of 28 patients (82%) completed treatment and follow-up periods (24-week completers) (**Table 1**)
  - Patients' baseline CSF levels of C4a and C4 were measured retrospectively
  - Baseline characteristics of patients with high (12/23; 52%) and low (11/23; 48%) C4a/C4 ratio were similar

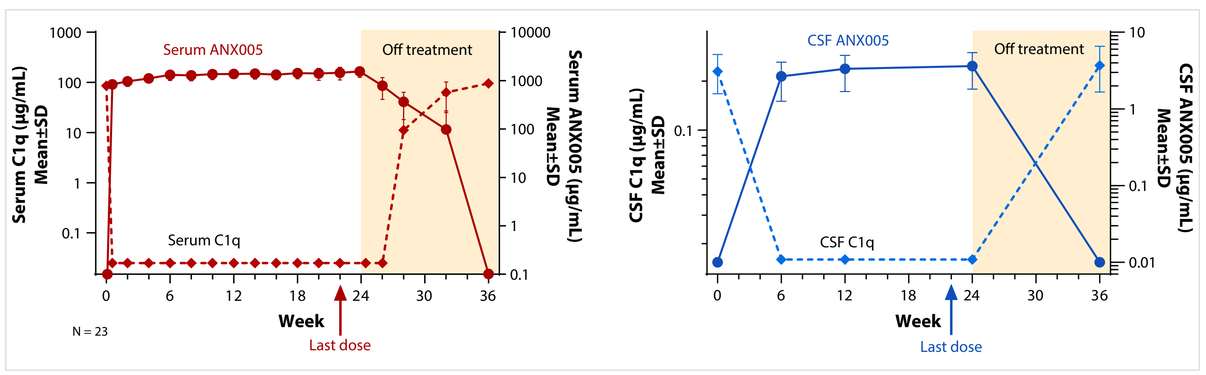
Table 1. Baseline Characteristics of Patients with High and Low Baseline C4a/C4 Ratios Were Similar to the Overall Study Population and Were Also Consistent with Prior HD Natural History Study Cohorts

Characteristic	Overall (N=28)	24-week completers: High C4a/C4 ratio (n=12)	24-week completers: Low C4a/C4 ratio (n=11)	24-week completers: Total (n=23)	TRACK-HD[a]
Age, years, mean (SD)	49.7 (12.5)	50.2 (11.2)	46.6 (15.5)	48.5 (13.3)	48.8 (9.8)
Female, %	43	42	27	35	45
CAP score, mean (SD)	505.7 (57.9)	514.97 (68.5)	509.1 (53.4)	512.2 (60.4)	NR
CAG repeat length, mean (SD)	44.6 (3.5)	44.5 (3.1)	45.7 (4.2)	45.1 (3.7)	43.7 (3.0)
Manifest HD, n (%)	25 (89)	12 (100)	9 (82)	21 (91)	123 (100)
CSF C4a, ng/mL, mean (SD)	13.9 (8.2)	15.3 (4.8)	15.1 (7.5)	15.0 (7.0)	NR
cUHDRS score, mean (SD)	10.4 (3.2)	9.8 (2.9)	10.3 (3.0)	10.1 (2.9)	11.7 (2.9)
TFC	10.6 (2.2)	10.3 (2.4)	10.5 (2.3)	10.4 (2.3)	10.9 (2.0)
TMS	21.6 (12.6)	24.7 (11.8)	19.7 (10.9)	22.3 (11.4)	23.7 (10.8)
SDMT	29.7 (11.3)	27.5 (10.5)	30.2 (11.9)	28.8 (11.0)	33.6 (10.2)
SWR	59.0 (18.7)	58.6 (19)	54.7 (14.5)	56.7 (16.7)	78.3 (19.5)

[a]Based on the TRACK-HD natural history study.[7] For illustrative purposes only—differences in patient demographics, study designs, and other factors exist, and caution should be exercised when comparing data across studies.
CAG, cytosine-adenine-guanine; CAP, CAG age product; CSF, cerebrospinal fluid; cUHDRS, composite Unified Huntington's Disease Rating Scale; HD, Huntington's disease; NR, not reported; SD, standard deviation; SDMT, Symbol Digit Modalities Test; SWR, Stroop Word Reading; TFC, Total Functional Capacity; TMS, Total Motor Score.

- Steady-state pharmacokinetics were achieved at the earliest time points tested (**Figure 2**)

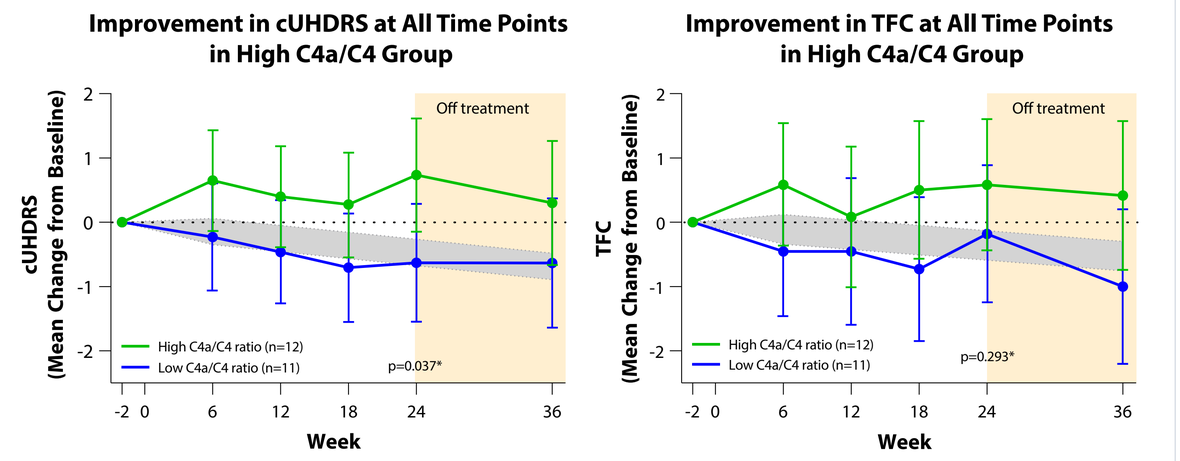
Figure 2. ANX005 Demonstrated Rapid, Complete, and Sustained Engagement of C1q in Serum and CSF



ANX005 was detectable in serum 4-10 weeks post-last dose. Drug levels in CSF were consistent with expectations (approximately 0.2% of serum levels).
CSF, cerebrospinal fluid; SD, standard deviation.

- Patients with high baseline C4a/C4 ratio exhibited improvement in cUHDRS relative to baseline over 36 weeks including 12 weeks of off-treatment follow-up, with a significant difference from patients with low baseline C4a/C4 at the end of the treatment period (Week 24; p=0.037) (**Figure 3**)
  - A similar trend was observed for Total Functional Capacity (TFC)

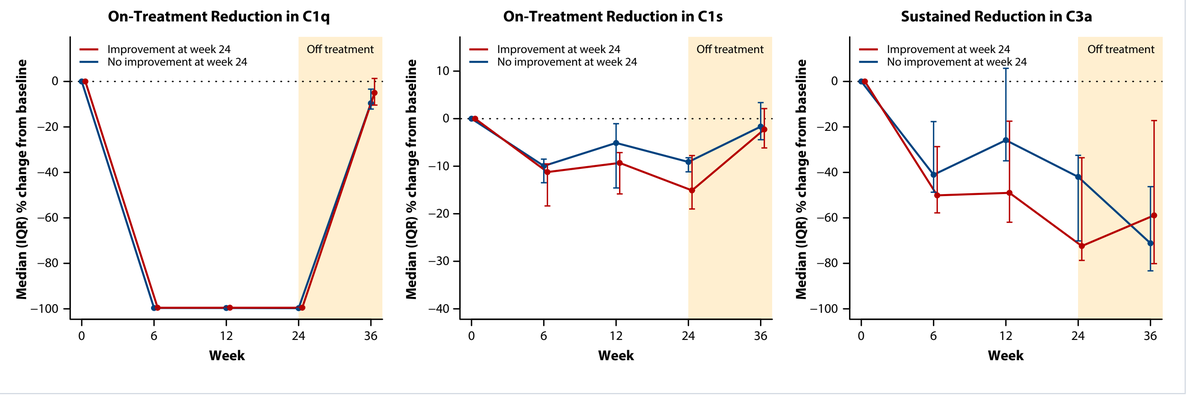
Figure 3. Rapid and Sustained Improvement in cUHDRS and TFC



LS mean change and 95% CIs based on MMRM analysis. In patients with high baseline C4a/C4, the P-value for change from baseline cUHDRS at 24 weeks = 0.10. The gray shaded areas represent expected decline based on interpolated natural history (TRACK-HD).[7] *P-value for high vs low baseline C4a/C4 at week 24.
cUHDRS, composite Unified Huntington's Disease Rating Scale; LS, least squares, MMRM, mixed model of repeated measures; TFC, Total Functional Capacity.

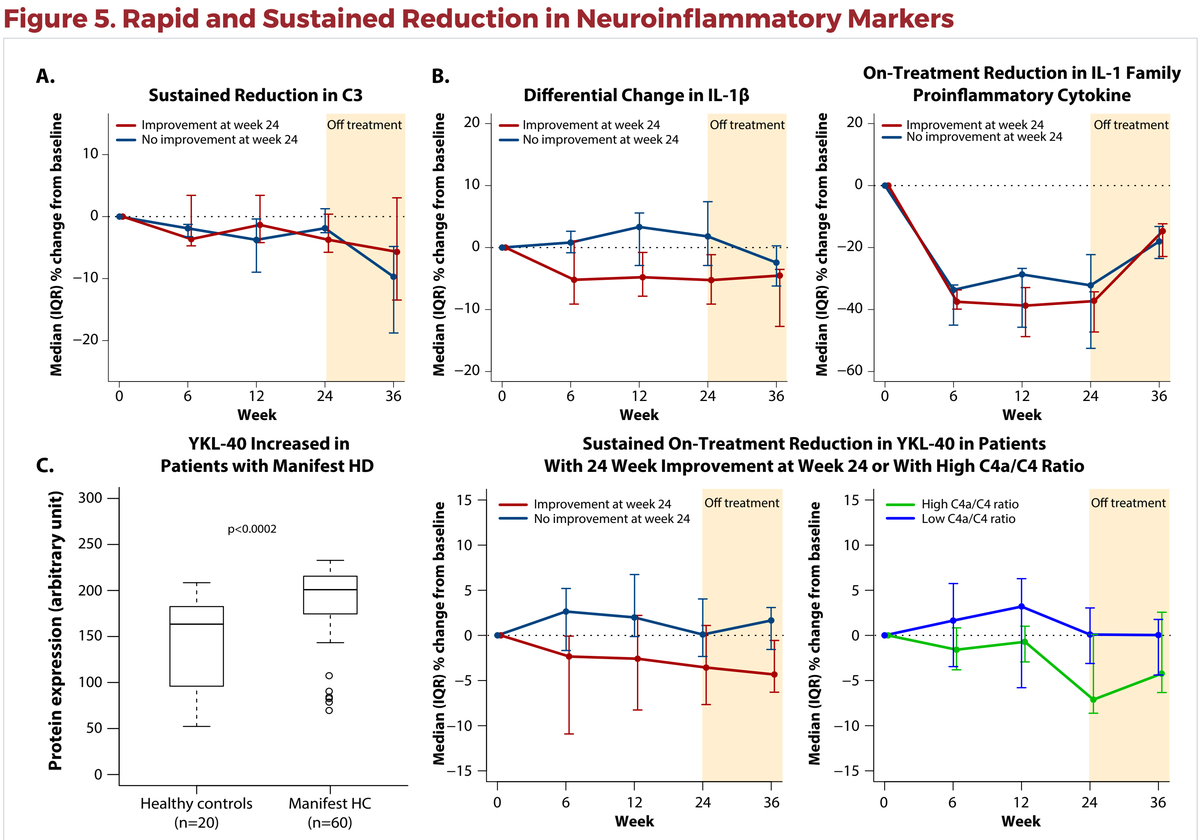
- SomaScan results confirmed the independent ELISA measures and demonstrated full target engagement of C1q in CSF during the dosing period in all patients and a return to baseline levels at the end of the study (**Figure 4**)
  - Levels of C1s, which associates with C1q, were also reduced
- Importantly, C3a, a measure of downstream complement activation, was significantly reduced in all patients ($p=2.7 \times 10^{-5}$)
- Reduced complement activation was maintained during the off-dosing period, suggesting ANX005 persistence in the CNS

Figure 4. Rapid and Sustained Reduction in Classical Complement Pathway and Activation Components in All Patients



- Proteomic profiling demonstrated reductions in four proinflammatory biomarkers (C3, two IL-1 family cytokines, and YKL-40) in patients with improved cUHDRS at week 24 (**Figure 5**)

Figure 5. Rapid and Sustained Reduction in Neuroinflammatory Markers



Panel (A). C3 is downstream of C1q and is expressed by neurotoxic astrocytes in HD patients.[8] C3 levels were significantly reduced in all patients, with reductions persisting through the off-treatment period.
Panel (B). IL-1β is expressed by microglial cells in HD.[9] IL-1β showed an apparent differential response in patients who improved with ANX005 treatment compared with those who did not. Levels of an IL-1 family proinflammatory cytokine were also decreased in all patients.
Panel (C). YKL-40 levels were increased in manifest HD patients compared with healthy controls (HD-CSF cohort). YKL-40 was selectively decreased in HD patients treated who improved with ANX005 treatment or who had high levels of baseline complement activity.

CONCLUSIONS

- CSF levels of C4a, an activated complement protein downstream of C1q, are elevated in patients with HD and are associated with disease progression
- Treatment of patients with or at risk of manifest HD produced rapid and durable suppression of C1q in the CSF
- In patients with high baseline complement activity (high C4a/C4 ratio), ANX005 treatment resulted in clinical improvement over the 9 month study period (increase from baseline cUHDRS)
- Proteomic profiling of CSF from patients in the phase 2 study confirmed inhibition of the complement pathway as well as decreased levels of the neuroinflammatory biomarkers C3, YKL-40, and IL-1 family proinflammatory cytokines
- These results support the therapeutic potential of ANX005 and classical complement inhibition for mutant Huntington carriers

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DISCLOSURES:

AM, CG, VV, LK, EC-M, BH, H-AK: employment with Annexon Biosciences; equity ownership in Annexon Biosciences. **RK:** employment with Rocky Mountain Movement Disorders Center and Research Catalyst, LLC; equity ownership in CenExel Clinical Research; consultancy/advisory role with Annexon Biosciences; research funding from Annexon paid to CenExel. **LMB:** nothing to declare. **EW:** grants from Medical Research Council UK, CHDI Foundation, and F. Hoffmann-La Roche Ltd.; personal fees from F. Hoffmann-La Roche Ltd., Triplet Therapeutics, PTC Therapeutics, Shire Therapeutics, Wave Life Sciences, Mitoconix, Takeda Pharmaceuticals, and Loqus23 (all honoraria for these consultancies were paid through the offices of UCL Consultants Ltd., a wholly owned subsidiary of University College London). **TY:** employment with Annexon Biosciences; equity ownership in Annexon Biosciences; consultancy/advisory role with Biogen and Myelin Repair Foundation.

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