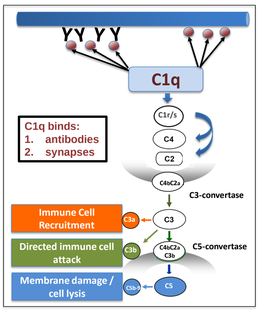
Characterization and development of ANX005, a novel function blocking anti-C1q antibody for treatment of autoimmune and neurodegenerative disease

Sethu Sankaranarayanan, Haiyan Qiu, Mario Saltarelli, Susan Kramer and Ted Yednock.
Annexon Biosciences, South San Francisco, USA.

Role of the classical complement pathway in neurological disease

- In **complement mediated neurodegeneration (CMND)**, C1q binds directly to synapses, triggering neuroinflammation, nerve damage, synapse loss and cell death
- In **autoimmune disease**, C1q binds to autoreactive antibodies bound to nerve surface antigens, initiating complement-mediated damage



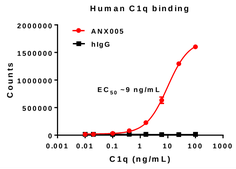
Complement mediated neurodegeneration (CMND)

- Age is a key risk factor for nearly all neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease, frontotemporal dementia and glaucoma
- C1q plays a key role in synaptic pruning in early CNS and retinal development (Stevens and Barres, Cell 2007)
- In aging mice and humans – a robust increase in C1q levels is observed at synaptic locations throughout the CNS (Stephan et al J. Neurosci., 2013)
- Early synapse loss prior to frank neuronal loss is a finding shared by many neurodegenerative diseases (Terry et al, Ann. Neurol., 1991)
- Age-dependent C1q tagging of synapses may sensitize the CNS for aberrant synapse elimination from a variety of neurodegenerative insults (Aβ, Tau, Htt, Synuclein), leading to progressive synaptic loss
- Multiple disease models show elevation of brain C1q and downstream complement activation products
  - C1q has directly been shown to play a role in synapse elimination in animal models of AD (Hong et al, Science 2016), Frontotemporal dementia (Liu et al, Cell 2016), and glaucoma (Howell et al J Clin. Inv. 2011, J. Neuroinflam. 2013)
  - Classical complement pathway has been genetically implicated in aberrant synapse loss in schizophrenia (Sekar et al, Nature 2016)

Summary of Findings

- Anti-C1q antibody ANX005 exhibits 10 pM affinity for human C1q and binds with similar potency to C1q from multiple species (**Dataset 1**)
- ANX005 inhibits antibody-mediated lysis of red blood cells in serum from multiple animal species, and also inhibits deposition of downstream complement components (**Dataset 2**)
- With peripheral administration, M1 (mouse precursor of ANX005) eliminated free levels of C1q in rat serum & CSF and inhibited ex-vivo RBC hemolysis (**Dataset 3**)
- M1 was effective in a mouse model of AMAN (**Dataset 4**) and in a model of Aβ-induced synapse elimination (**Dataset 5**)

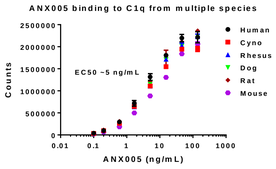
1 Robust binding of ANX005 to human C1q and serum-derived C1q from multiple species



Kinetics of binding to hC1q

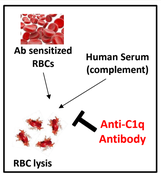
Ka (1/Ms)	kd (1/s)	K_D (pM)
9.6×10^5	6.4×10^{-6}	6.7

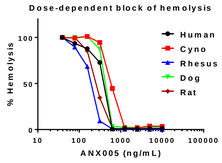
Biacore results



ANX005 shows similar binding to C1q derived from multiple species

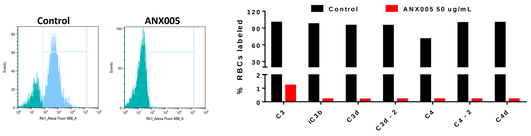
2 ANX005 blocks hemolysis & prevents deposition of activated complement onto RBCs



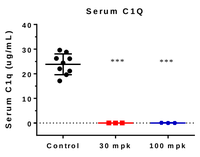


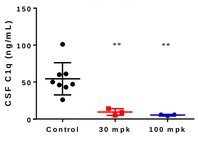
Hemolysis: Relative IC_{50} 100-400 ng/mL across all species

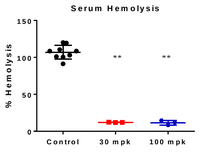
FACS analysis: C3 & C4 fragment deposition onto RBCs is blocked by ANX005



3 Reduction in Serum and CSF C1q levels & *ex vivo* RBC lysis with systemic dosing of M1 in rats



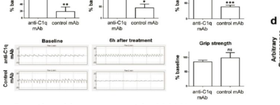




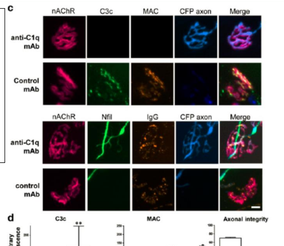
Single IP dose of M1 (murine precursor of ANX005)
30 & 100 mpk, 48 hr time point

4 M1 improves function & protects axons in a mouse model of AMAN (acute motor axonal neuropathy)

GalNAcT Tg mice lack the endogenous GalNAct gene, but overexpress GalNAcT cDNA to drive production of complex ganglioside exclusively in neurons
- Inject anti-GM1 ganglioside IgG
- 16 hr later, 50 mpk i.v. M1 or Isotype control
- Human complement (serum) i.p. 30 minutes later
- Respiratory function over 4-6 hrs
- Blood, diaphragm and soleus muscles collected for serum and IHC analysis.



Functional improvement in AMAN – Tidal volume, respiratory rate & rotorod activity improved with M1 treatment

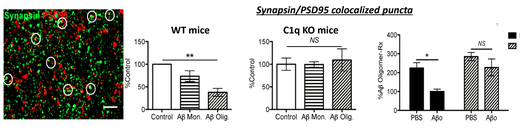


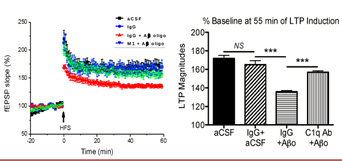
M1 reduced C3c (green) and MAC (orange) deposition, while maintaining axonal integrity (Axon – blue & Neurofilament – green)

McGonigal et al, Acta Neuropathology 2016

5 M1 blocks Aβ oligomer-mediated synapse loss and functional deficits, similar to C1q KO mice

Aβ oligomer injection induced synapse loss in WT mice
C1q KO mice or M1 administration prevented synapse loss





Aβ-oligomer induced LTP deficits prevented by M1

Hong et al, Science 2016

Acknowledgements

We thank Drs. Rhona McGonigal and Hugh Willison for their collaboration on the mouse AMAN model studies.
We thank Drs. Soyon Hong and Beth Stevens for their collaboration on the Aβ oligomer induced synaptic deficit model studies.

Conflict of interest

All authors are employees and own equity in Annexon Biosciences

Conclusions

- We have developed a novel anti-C1q antibody (ANX005) that binds with high affinity to C1q from multiple species and blocks classical complement cascade activation
  - In an *in vitro* hemolytic assay, ANX005 prevents deposition of activated complement components from human serum onto red blood cells and blocks subsequent hemolysis
- In rat, IP dosing of M1 led to significant reduction in free C1q in serum and CSF and a reduction in serum hemolytic activity in an *ex vivo* assay
- M1 improves respiratory function & protects axons in a mouse model of AMAN, an antibody-mediated autoimmune disease
- M1 blocks Aβ-oligomer-mediated synapse loss and functional deficits, similar to that observed in a C1q KO mice
- In summary, we have developed a potent anti-C1q antibody that can be used to examine complement damage and CMND in numerous animal models of autoimmune and neurodegenerative disease