Dual Action Antibodies for Treatment of Progressive Multiple Sclerosis

Linzey M#., DeCristofano L*., Phillips R*., Gilli F#., and **Tran H*.**

*Abzyme Therapeutics, 321 Jones Boulevard, Suite 300, Royersford, PA 19468

Department of Neurology, Dartmouth Hitchcock Medical Center and Geisel School of Medicine at Dartmouth, Lebanon, NH

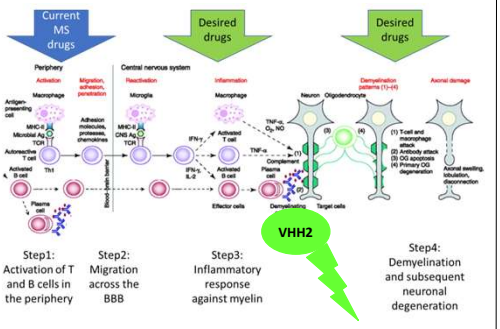
Corresponding author: tran@abzymetx.com

Abstract

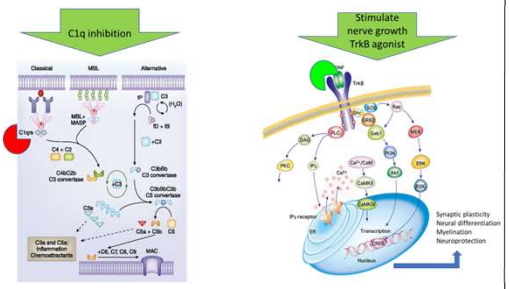
Central Nervous System (CNS) physical injuries, including bacterial or viral infection, can induce chronic neuroinflammation that is believed to persist for an individual's lifetime. Among the other inflammatory events, it is recognized that both acute and chronic activation of the complement pathway plays a role in the development of secondary brain injuries by inducing neuronal cell loss and synaptic pruning. Complement over-activation is also firmly implicated in the pathology that underlies the irreversible progression of multiple sclerosis (MS), a common inflammatory and neurodegenerative disease of the CNS. We hypothesize that therapeutic inhibition of the complement system and concurrent stimulation of nerve growth may prevent CNS tissue damage and slow or even block the progression of MS.

We have successfully produced TrkB agonistic and C1q antagonistic nanobodies validated in *in-vitro* cellular functional assays. Therapeutic efficacy was further endorsed *in-vivo* in a well-characterized murine model of progressive MS, Theiler's murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD). Treatment with either the anti-C1q or the anti-TrkB nanobodies significantly improved the progressive neurological impairment in TMEV-IDD mice. Both the anti-TrkB and anti-C1q nanobodies were successfully humanized to VHH antibodies retaining their target binding activities.

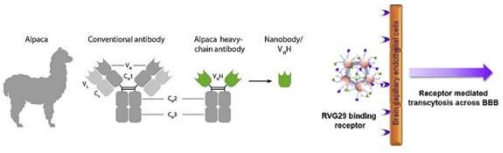
Background and Rationale



Prevent tissue destruction and stimulate nerve growth



Nanobodies to overcome BBB

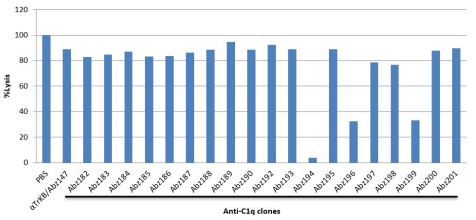


RESULTS

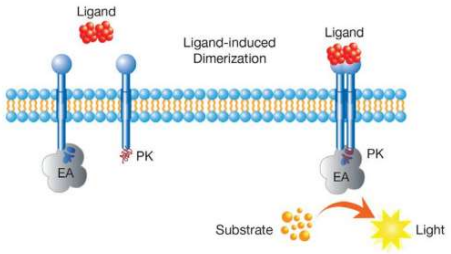
Interspecies reactive VHH TrkB and C1q antibodies to streamline pre-clinical studies in animal models

	Process	# of clones (C1q)	# of clones (TrkB)
1	Start	1 X 10^9	1 X 10^9
2	Biopanning	4 X 10^7	1 X 10^7
3	FACS I	138,000	56,000
4	FACS II – FACS III	85,500	13,500
5	Single clone analysis	142 out of 200	117 out of 204
6	Rabbit cross-reactive (for C1q) Rabbit and rat cross-reactive (for TrkB)	118 out of 142 (53-families)	50 out of 117 (8-families)
7	Functional Assay	3 antagonists	8 agonists

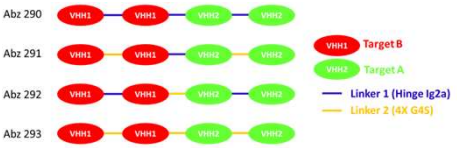
Screening Assay for C1q Inhibitory Antibodies



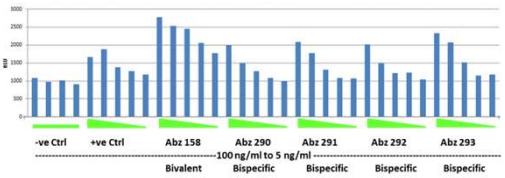
Screening Assay for TrkB Agonistic Antibodies



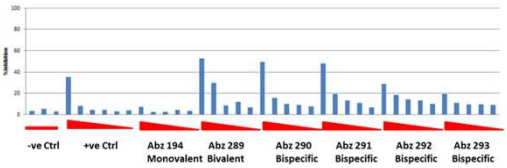
Bi-specifics for TrkB and C1q antibodies



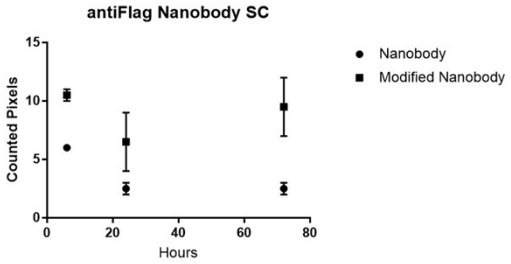
TrkB: Functional Assay Abz 290-293 Bispecifics



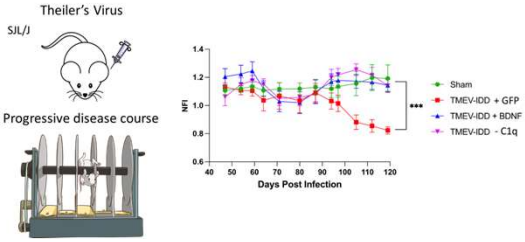
C1q: Functional Assay Abz 290-293 Bispecifics



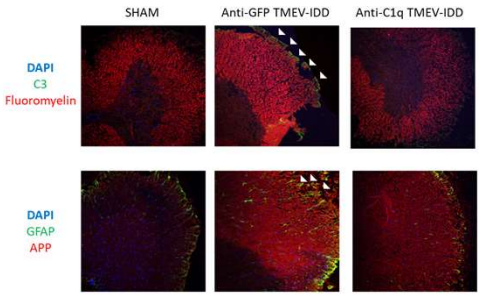
RVG29-modified VHHs were able to penetrate into CNS

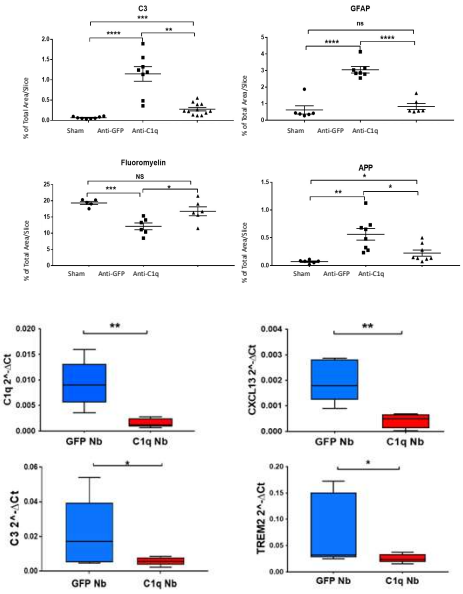


RVG29-modified C1q and TrkB VHHs improve disabilities in PMS-affected mice



RVG29-modified C1q VHHs prevent CNS inflammation





Summary and future research

We have successfully developed TrkB agonistic and C1q antagonistic nanobodies and validated in in vitro cellular functional assay. Treatment with either the anti-C1q or the anti-TrkB nanobodies significantly improved the progressive neurological impairment in TMEV-IDD mice. RVG29-modified antibodies were able to penetrate into CNS preventing TMEV-IDD induced inflammation monitored by a set of biomarkers. We continue to validate the therapeutic efficacy of humanized antibodies in various PMS murine models.

The research has been funded by NIH SBIR Phase I grant R43 NS120624-01