Antibodies inhibit cell to cell transmission of mutant HTT

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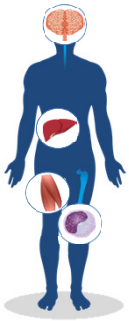
The toxic functions of the mutant Huntingtin protein (mutHTT) were studied extensively and in addition to neuronal based symptoms, also peripheral changes upon mutHTT expression were described. An important finding in Huntington's disease (HD) research from the last years is the discovery of extracellular mutHTT and evidence of cell to cell spreading of the mutant protein. This offers new opportunities for targeting mutHTT by antibodies or target-specific vaccines. Recent publications revealed that mutHTT protein was largely present in a free, non-encapsulated form in the extracellular compartment thereby making it accessible by antibodies. We previously demonstrated peripheral target engagement in actively and passively vaccinated YAC128 mice. In these experiments, mutHTT lowering was accompanied by motor improvement in rotarod assays. We sought to generate an *in vitro* model for testing the molecular mode of action of newly developed mutHTT targeting antibodies and vaccines. Lead antibody C6-17 was capable of depleting mutHTT and blocking intercellular mutHTT transmission, thereby interfering with a potentially disease amplifying mechanism. Our work sets the ground for the development of new antibody-based therapeutics targeting extracellular HD. It is expected that, besides mutHTT depletion, systemic antibody-based targeting will provide inhibition of mutHTT spreading and intercellular transmission. We understand our systemic approach as an addition to forthcoming tissue-specific mutHTT lowering approaches.

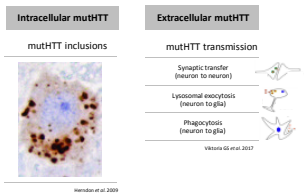
Cumulating evidence for a pathogenic role of extracellular Huntingtin (selection)

- mutHTT spreading into genetically normal and unrelated allografted neural tissue, *Cicchetti et al., 2014*
- Transneuronal propagation of mutHTT, *Pecho-Vrieseling et al., 2014*
- Transcellular spreading of Huntingtin aggregates in the Drosophila brain, *Babcok & Ganetzky 2015*
- Human to mouse prion like propagation of mutHTT, *Jeon I. et al., 2016*
- Mutant Huntingtin is secreted via a late endosomal/lsosomal unconventional secretory pathway, *Trajkovic K. et al., 2017*
- Cell-to-cell transmission of polyglutamine aggregates in C. elegans, *Kim DK et al., 2017*

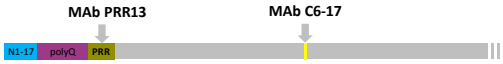
HD primarily involves the CNS but also peripheral tissues and organs



Organs (selection)	Pathology
CNS/brain	mutHTT inclusions, neuronal cell death and cerebral atrophy
Liver	Metabolic changes
Immune system	Proinflammatory cytokines
Muscle	Low ATP production, reduced strength and mutHTT inclusions



Monoclonal Antibody Development

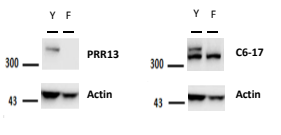


Proline Rich Region (PRR):
- Mediates stability
- prot-prot interactions
- structural epitope
- low seq. complexity
- mediocre immunogenicity for SAIT active immunization

Caspase 6 cleavage region (D586):
- Prominent, functionally characterized Caspase 6 (C6) cleavage site
- Functional role in pathogenesis (Graham 2006)
- Structurally exposed
- Neoepitopes upon cleavage

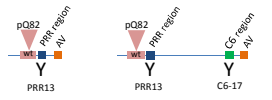
MAb Features

MAb binding to mutHTT derived from YAC128 brains (Western blot; each MAb 5ng/µl): **MAb C6-17 but not PRR13 cross reacts with denatured endogenous mouse (wt-)HTT protein.** (consistent with absence of epitope sequence conservation)



Y – YAC 128 brain extract
F – FvB brain extract
(all extracts 50µg tot. prot. conc.)

Mab binding in vitro characterizations



B	V5 mean EC50	PRR13 mean EC50	C6-17 mean EC50
HTTEx1wt	20,17	119 ± 6,61	/
HTTEx1polyQ82	17,45	174 ± 8,5	/
HTT610wt	15,85	410 ± 10,8	4,33±0,18
HTT610polyQ82	15,48	1503 ± 429	7,64 ± 0,72

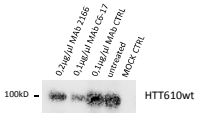
C	Peptide KD	HTTEx1polyQ KD	HTT610polyQ KD
mAB PRR13	4,6E-09	3,5E-09	n.d.
mAB C6-17	9,7E-08	/	8,66E-08

Based on the binding results Mab C6-17 was used for further developments

Selection and characterization of antibody targeting:
(A) rec. human HTT fragments used in the antibody binding analysis. All constructs contain a C-term avi tag (side directed biotinylation) and a V5 tag (AV).
(B) Epitope accessibility of MAb PRR13 and MAb C6-17 on different recombinant mutHTT fragments. Capture ELISA assays; Binding data expressed as mean EC50 values in ng/µl antibody.
(C) Biacore Analytics of MAb PRR13 and MAb C6-17,

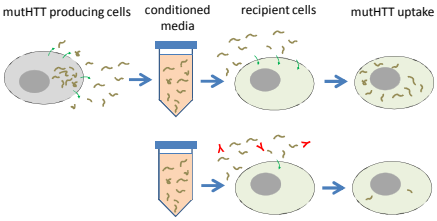
HTT depletion by monoclonal Antibodies

Depletion of extracellular HTT protein from cell culture supernatants. Conditioned media were incubated with MAbs and complexes depleted via magnetic beads. HTT detection by Western blot with anti V5 Antibody (0,2ng/µl; loading: 20µl depleted SN).

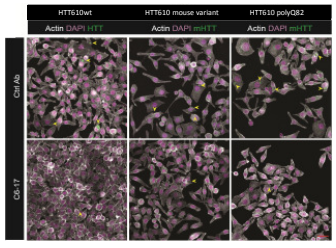


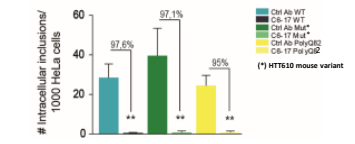
MAb C6-17 recognizes and depletes secreted HTT from cell culture supernatants of transfected cells suggesting HTT accessibility

Transmission inhibition of mutHTT in vitro

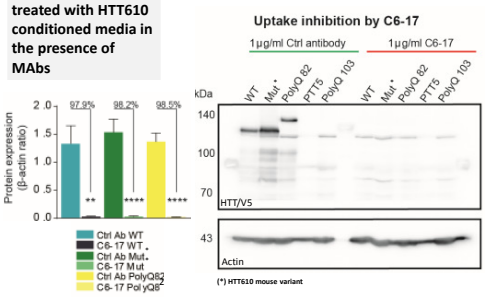


IHC analysis of Hela cells treated with HTT610 conditioned media:
Hela Cells were incubated with HTT conditioned medium for 96h in the presence of CTRL Ab and MAb C6-17. For normalization, cells were washed and identical cell number was placed on cover slips for additional 24h. HTT detection was done with anti-V5 antibody





Western blot analysis of Hela cells treated with HTT610 conditioned media in the presence of MAbs



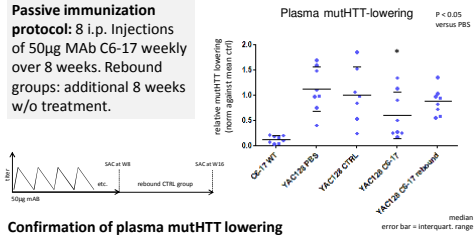
MAb C6-17 blocks intercellular mutHTT transmission

Targeting opportunities of extracellular HTT

Broad systemic activity expected
Easy delivery route
Adjuvant therapy to other treatment modalities

Short passive mutHTT lowering protocol

Passive immunization protocol: 8 i.p. Injections of 50µg MAb C6-17 weekly over 8 weeks. Rebound groups: additional 8 weeks w/o treatment.



Confirmation of plasma mutHTT lowering by MAb C6-17 upon short term Passive treatment.

anti HTT human Ab development

Humanization of the lead MAb C6-17. Two independent strategies were chosen:

Structural *in silico* 3D alignment of human antibody databases (Rees Consulting AB, Uppsala, Sweden)

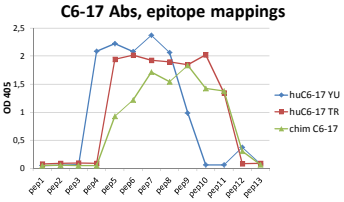
Human scFv library screening and *in vitro* selection (YUMAB GmbH, Braunschweig, Germany)

Human Ab characterizations

Ab binding characterizations via Biacore on HTT protein level. Bivalent Fitting model, subtraction of the C6-17 epitope scrambled HTT signals

KD values (M):
chim C6-17 – 2,21E-08
huC6-17 TR – 5,18E-08
huC6-17 YU – 5,64E-08

Epitope mapping ELISA Experimentes. Peptide walk using a series of C6-17 epitope specific peptides (pep 1 –pep 13)



Identical/similar epitopes between the human Abs and the chim CTRL Ab

Summary

- **Peripheral, extracellular mutHTT can be targeted by MAbs.**
- **Prototypic MAb C6-17 inhibits intercellular mutHTT spreading *in vitro* and provides mutHTT lowering in plasma of TG animals.**
- **Successful development of 2 human C6-17 prototype Abs**
- **Our work sets the ground for the development of new Ab-based therapeutic modalities targeting Ab-accessible HTT systemically**
- **Our targeting concept is proposed as a complementary, systemic approach to forthcoming CNS-directed mutHTT lowering strategies.**

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