In vivo mtHTT protein reduction in the CNS and periphery by passive immunization with the monoclonal antibody C6-17

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Huntington's disease (HD) is a hereditary neurodegenerative disorder characterized by changes in personality, cognition and motor control. The cardinal neuropathological hallmark of this disease is the massive atrophy of the striatum resulting from neuronal dysfunction and loss which extends to other areas of the brain as well as peripheral organs. The genetic mutation underlying HD originates in Exon1 of the huntingtin gene gives rise to a toxic/mutated form of the huntingtin protein (mtHTT). The mtHTT protein is ubiquitously expressed but also exhibits the ability to propagate from cell-to-cell to disseminate pathology, a property which may serve as a new therapeutic focus. We have developed a monoclonal antibody C6-17 targeting a particularly exposed region close to the aa586 Caspase 6 cleavage site of the huntingtin protein and, as recently published, mAB C6-17 is able to block cell-to-cell propagation of mutated HTT *in vitro*. In order to reduce the burden of the mutant HTT protein *in vivo*, we queried whether the freely accessible and extracellular mtHTT can be targeted by an antibody. In POC experiments, using the transgenic animal model YAC128, we found that after 3 months mAB C6-17 treatment the circulating mtHTT in the peripheral as well as in the CNS tissues was reduced. Further, we could demonstrate the presence of active mAB C6-17 in PBS/heparin perfused peripheral and CNS tissues. The mAB C6-17 treated YAC128 animals showed benefits in body wight and motor behaviors and we could observe a delay in the HD disease progression. Our findings support the suitability of an antibody treatment approach in Huntington's disease and our *in vivo* data could set the ground for a new HD treatment regime based on a therapeutic antibody molecule. The obtained *in vivo* results provide the first POC data for the feasibility and efficacy of an antibody-based anti-mtHTT approach and suggest this therapeutic strategy as a potential new HD treatment possibility.

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*
- How blood can both propagate and ameliorate HD disease pathology *Rieux M et al. 2020*

Monoclonal Antibody Development

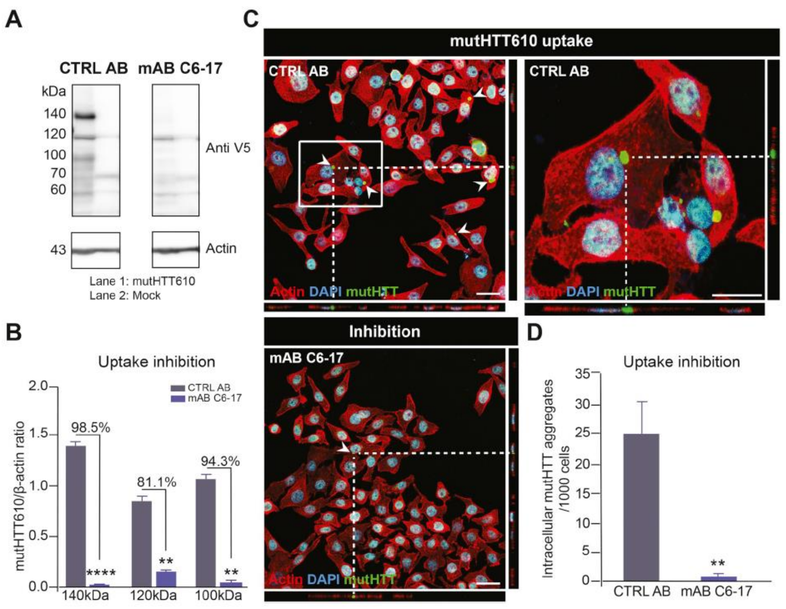
Proline Rich Region (PRR):
- Mediates stability
- prot-prot interactions
- structural epitope
- low seq. complexity
- mediocre immunogenicity for active immunization

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 C6-17 was selected for further developments**

mAB C6-17 in vitro characterization

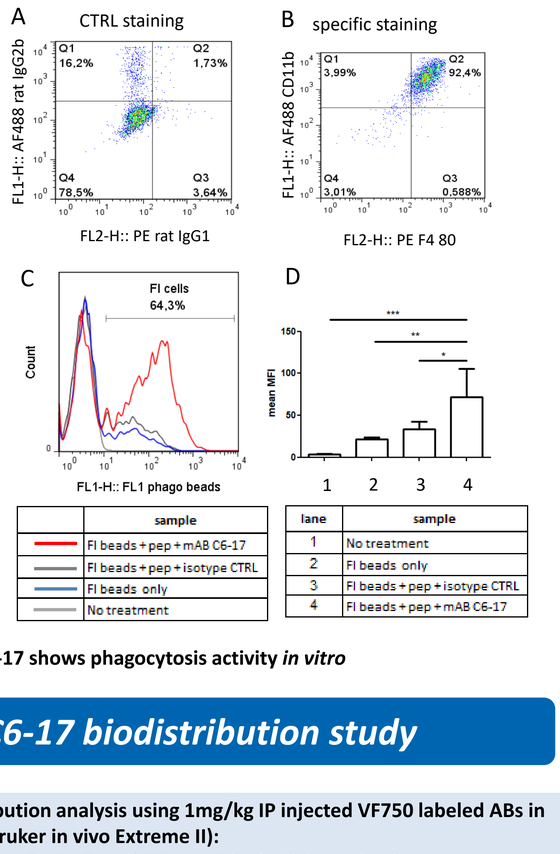
***In vitro* system to study inhibition of mtHTT uptake. The inhibitory activity of mAB C6-17 compared to a CTRL isotype antibody was confirmed by Western blot (A,B) analysis and IHC analysis (C,D).**



Results:
mAB C6-17 showed significant inhibitory activity and was able to reduce the mtHTT uptake of culture cells
(Bartl et al. 2020)

mAB C6-17 in vitro Phagocytosis activity

Monocyte differentiation:
(A) CTRL staining with isotype CTRL antibodies
(B) Specific staining with macrophage markers F4 80 and CD11b
mAB C6-17 phagocytosis assay with fluorescence peptide coated beads:
(C) FACS histogram blots of fluorescence cells, (D) mean MFI values of phagocytosis assays with CTRL and mAB C6-17



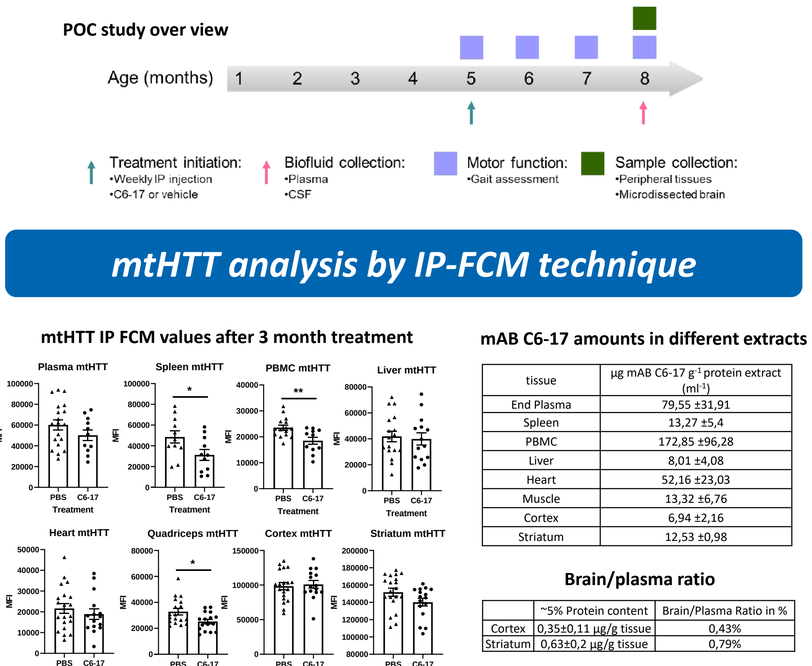
sample
FI beads + pep + mAB C6-17
FI beads + pep + isotype CTRL
FI beads only
No treatment

lane	sample
1	No treatment
2	FI beads only
3	FI beads + pep + isotype CTRL
4	FI beads + pep + mAB C6-17

Results: mAB C6-17 shows phagocytosis activity *in vitro*

mAB C6-17 biodistribution study

Time dependent biodistribution analysis using 1mg/kg IP injected VF750 labeled ABs in 5M old YAC128 animals (Bruker in vivo Extreme II):
(A) In vivo distribution of mAB C6-17 and CTRL mAB in the living animal
(B) and (C) *ex vivo* distribution analysis of CTRL and mAB C6-17 in PBS/heparin perfused brains



Results:
mAB C6-17 shows fast distribution through out the body and could be detected in PBS/Heparin perfused brains 8 days after IP injection

in vivo POC mAB C6-17 treatment study

I*n vivo* POC study in 5 months old YAC128 animals treated for 3 month with 10mg/kg mAB C6-17 (GrA) and PBS (Gr B); wt FvB PBS (Gr C)

mtHTT analysis by IP-FCM technique

mtHTT IP FCM values after 3 month treatment

mAB C6-17 amounts in different extracts

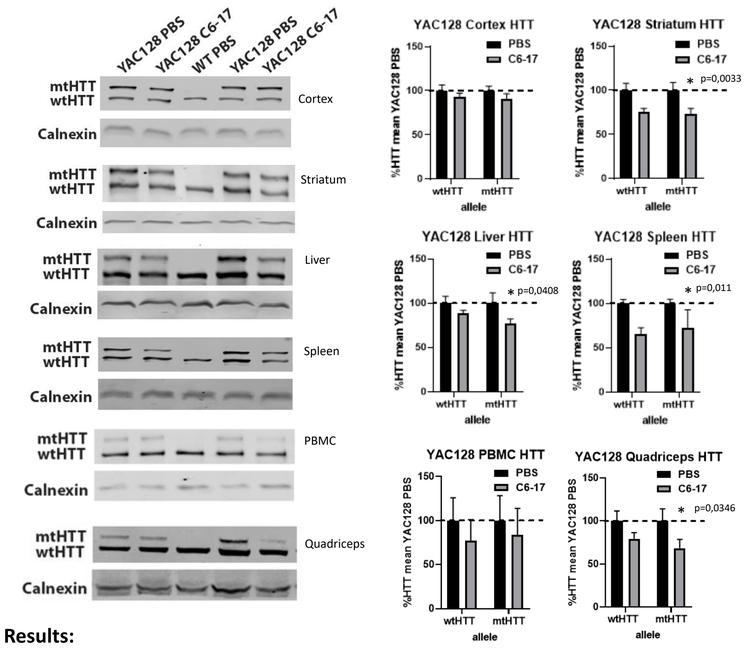
tissue	µg mAB C6-17 g^{-1} protein extract (ml^{-1})
End Plasma	79,55 ±31,91
Spleen	13,27 ±5,4
PBMC	172,85 ±96,28
Liver	8,01 ±4,08
Heart	52,16 ±23,03
Muscle	13,32 ±6,76
Cortex	6,94 ±2,16
Striatum	12,53 ±0,98

Brain/plasma ratio

	~5% Protein content	Brain/Plasma Ratio in %
Cortex	0,35±0,11 µg/g tissue	0,43%
Striatum	0,63±0,2 µg/g tissue	0,79%

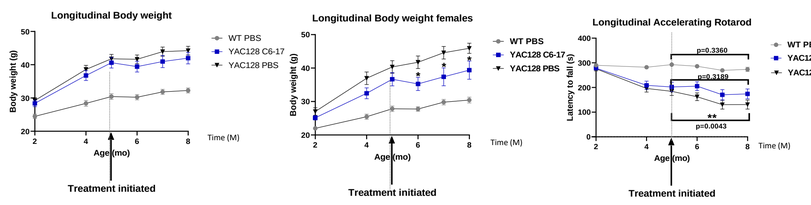
Results:
(i) mAB C6-17 could be detected in peripheral tissues and in the CNS
(ii) significant mtHTT lowering could be detected in Spleen, PBMCs and muscle, a non-significant lowering in plasma, heard and striatum

HTT and mtHTT analysis by western blot



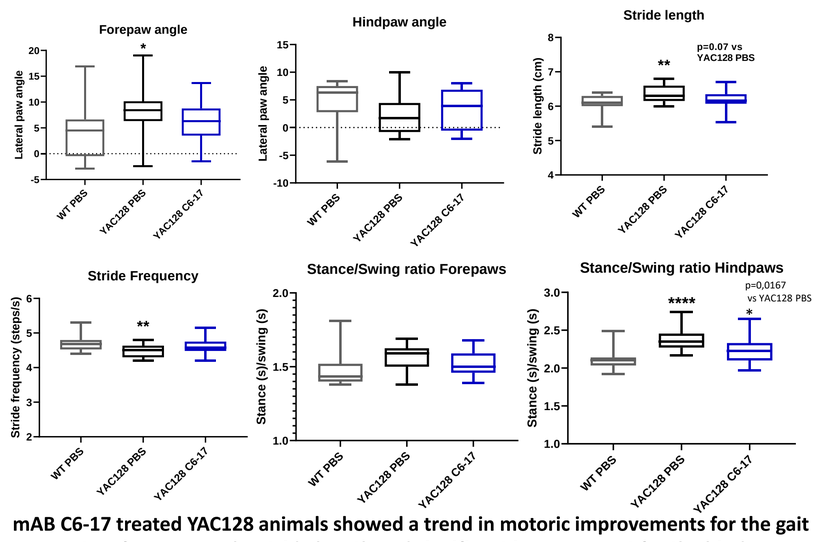
Results:
(i) A significant reduction of HTT and mtHTT in mAB C6-17 treated animals could be detected in the striatum
(ii) A non-significant reduction was detected in liver, spleen, PBMCs and muscle

Body Weight and motor performances Rotarod



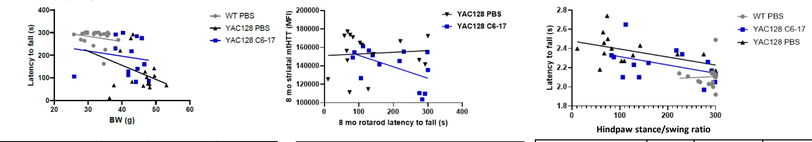
(i) mAB C6-17 treated YAC128 animals showed less body weight gain compared to the PBS group (especially female YAC128 animals)
(ii) mAB C6-17 treated YAC128 animals showed a trend toward motor improvements and a non-significant disease progression

Motor performances DigiGait



mAB C6-17 treated YAC128 animals showed a trend in motoric improvements for the gait parameters forepaw angle, stride length and significant improvement for the hindpaws stance/swing ratio (improvement of the balance)

Linear regression analysis

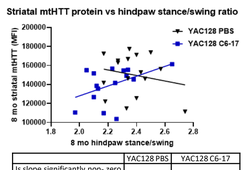


	WT PBS	YAC128 C6-17	YAC128 PBS
Is slope significantly non- zero			
F	0.9517	0.3572	2.976
DFn, DFd	1.18	1.12	1.14
P value	0.3422	0.5612	0.1065

	YAC128 PBS	YAC128 C6-17
Is slope significantly non- zero		
F	0.08404	4.998
DFn, DFd	1.14	1.12
P value	0.7761	**0.0452**

	WT PBS	YAC128 C6-17	YAC128 PBS
Is slope significantly non- zero			
F	0.03015	2.841	4.709
DFn, DFd	1.14	1.12	1.14
P value	0.8646	0.1177	**0.0477**

Linear regression analysis revealed:
(i) Body weight does not influence the motoric performances
(ii) Rotarod (RR) performances correlates significantly with mtHTT in the striatum in mAB C6-17 treated YAC128 mice
(iii) The Hindpaw stance/swing ratio values significantly correlates with RR in mAB C6-17 treated YAC mice
(iv) A strong trend towards correlation between the Hindpaw stance swing ratio and the amounts of mtHTT in the striatum in mAB C6-17 treated YAC128 mice



	YAC128 PBS	YAC128 C6-17
Is slope significantly non- zero		
F	0.5964	3.445
DFn, DFd	1.14	1.13
P value	0.4528	0.0863

Summary

- After IP application, biodistribution studies revealed fast antibody C6-17 distribution into the body and the presence of mAB C6-17 in peripheral organs and CNS
- POC studies revealed that treated YAC128 animals showed reduced mtHTT levels in peripheral organs and CNS
- mAB C6-17 treated animals revealed improved motoric performances on the classical rotarod and on the DigiGait readout
- Blocking the potentially pathological spreading mechanism by antibody intervention might slow down the HD disease progression
- In concert with other mtHTT lowering interventions focusing on mtHTT RNA/DNA level (ASO, iRNA methods) a significant benefit for HD patients could be achieved

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