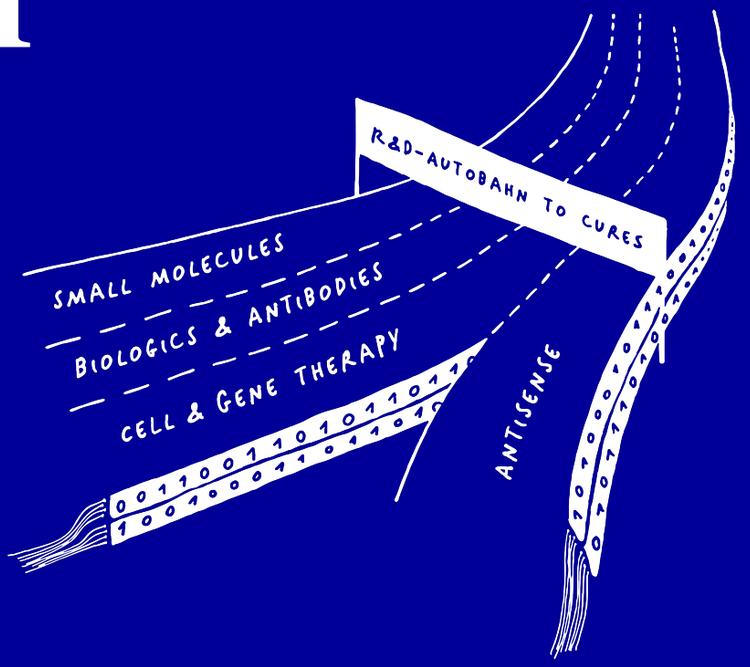
evotec

#RESEARCHNEVERSTOPS

High-sensitivity immuno-assays for biomarkers of Huntington's disease



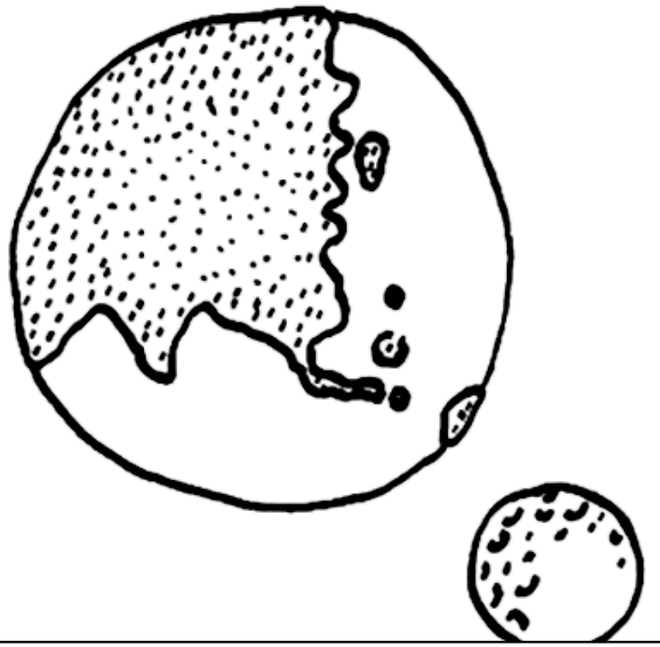
Evotec SE, Elena Vicentini, 15th EBF OS, November 2022

Agenda

Biomarkers of Huntington’s disease

Mutant Huntingtin Assay: Erenna® vs SMCxPRO™

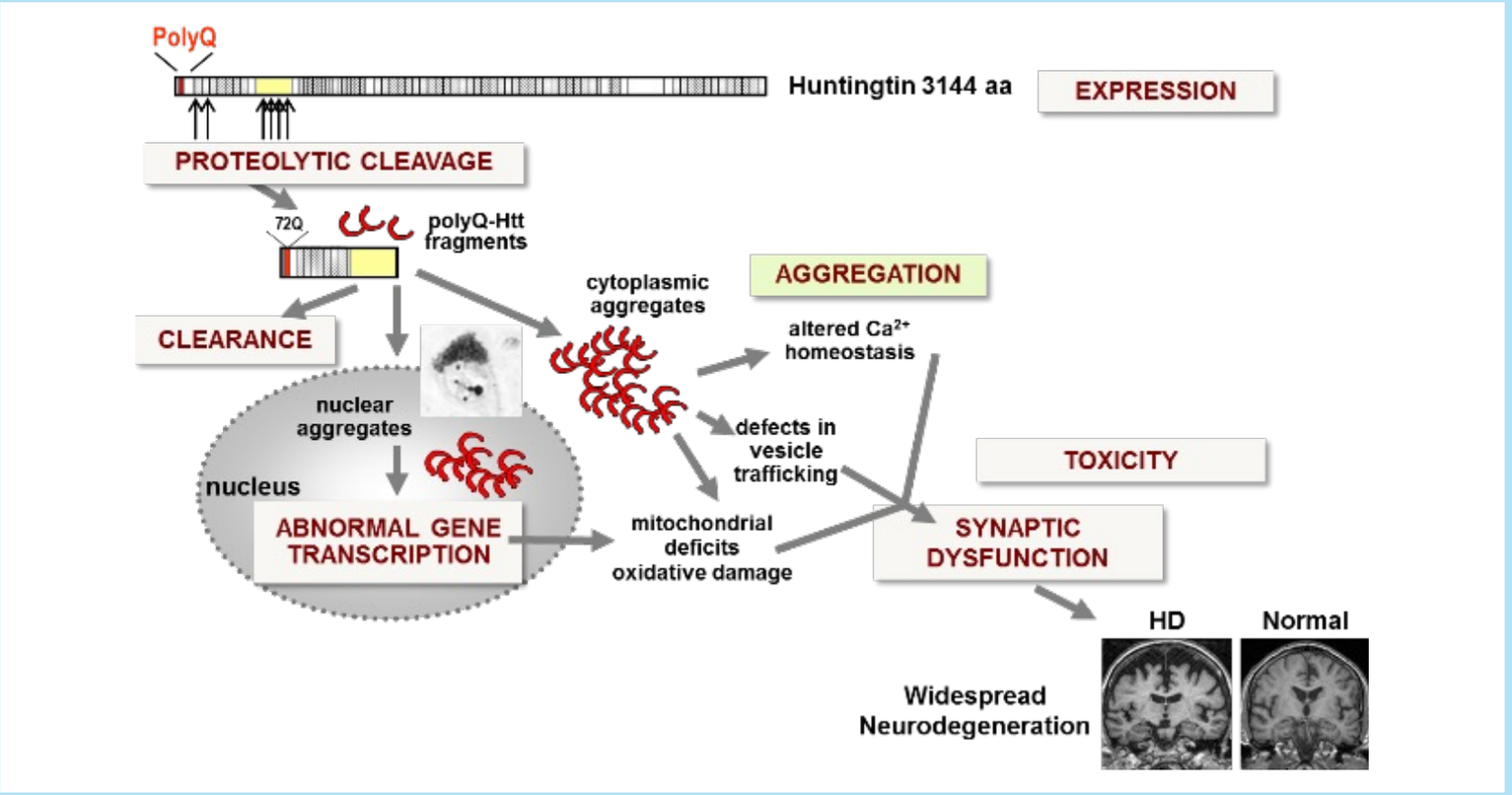
Mutant Huntingtin Assay Validation



Huntington’s Disease is caused by mutant huntingtin protein

A clear, causal pathogenic entity to measure: mutant huntingtin protein

- Huntington’s disease is a rare disease, caused by an inherited defect in a single gene (expansion of poly glutamine repeats in the huntingtin gene)
- One single copy of the defective gene is sufficient to cause the progressive degeneration of nerve cells in the brain
- Huntington’s disease results in a broad range of movement, thinking (cognitive) and psychiatric disorders
- Huntingtin protein comes in many «flavours» and needs to be quantified in different type of biofluids

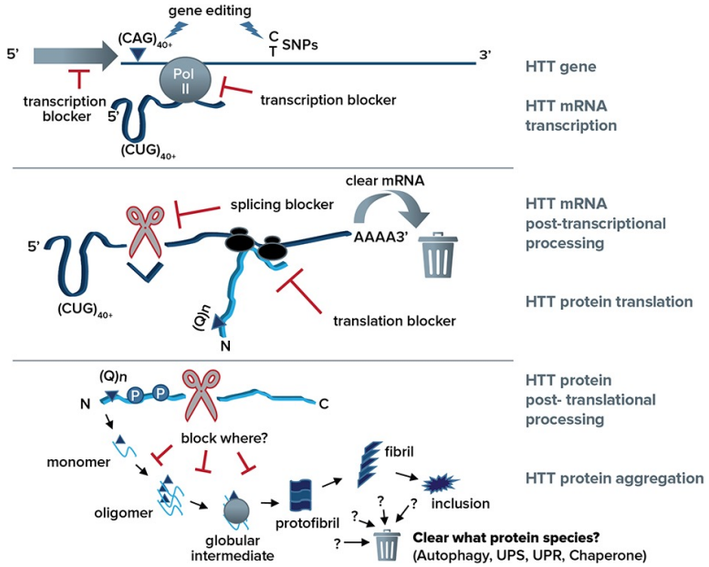


Adapted from Mayo Clinic https://www.mayoclinic.org/diseases-conditions/huntingtons-disease/symptoms-causes/syc-20356117

Huntingtin Lowering Approaches

mHTT – the most proximal therapeutic target

HTT production and life cycle lowering opportunities



Currently publicly-announced huntingtin-lowering studies

Company (drug)	Modality (delivery)
Novartis (Branaplam)	Small molecule (oral)
PTC Therapeutics (PTC518)	Small molecule (oral)
Roche (Tominersen)	ASO (intrathecal)
Takeda (Sangamo) (TAK-686)	AAV-X-(ZFP) (intracranial)
uniQure (AMT-130)	AAV5 (miRNA) (intracranial)
Voyager (VY-HTT101)	AAV1 (miRNA) (intracranial)
Wave Therapeutics (WVE-120101/2, WVE-003)	ASO (intrathecal)

- First line of intervention aimed at lowering huntingtin protein in CNS particularly
- Mutant huntingtin protein (mHTT) was the ideal candidate as a translational HTT-lowering PD biomarker

→ Need of a high sensitivity immunoassay to quantify mHTT reliably in human CSF

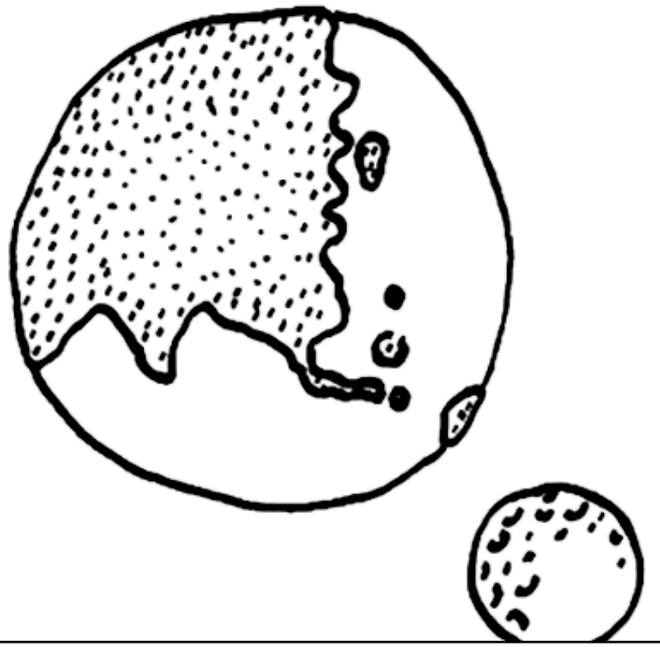
Adapted from CHDI https://chdifoundation.org/htt-lowering-mfa/

Agenda

Biomarkers of Huntington’s disease

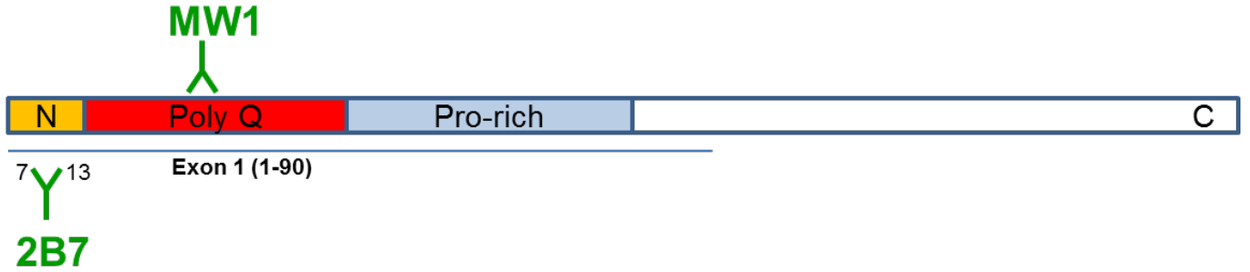
Mutant Huntingtin Assay: Erenna® vs SMCxPRO™

Mutant Huntingtin Assay Validation



Mutant Huntingtin key assay components

- **Mutant Huntingtin standard**
  - HTT-Q46, GST, 1-548
- **Antibodies**
  - Anti HTT, **clone 2B7**, IgG1, mouse monoclonal directed against the N-terminus (1-17) of huntingtin and epitope mapped to residues 7-13. **HTT-specific.**
  - Anti HTT, **clone MW1**[1)], IgG2b, mouse monoclonal antibody to **polyQ repeat** domain present in Huntingtin Epitope mapped to polyQ-stretch with minimum of 7Qs (full complementarity determining region groove binding likely requires 11Qs)
- **Matrix:** Used for standard, quality controls
  - artificial CSF

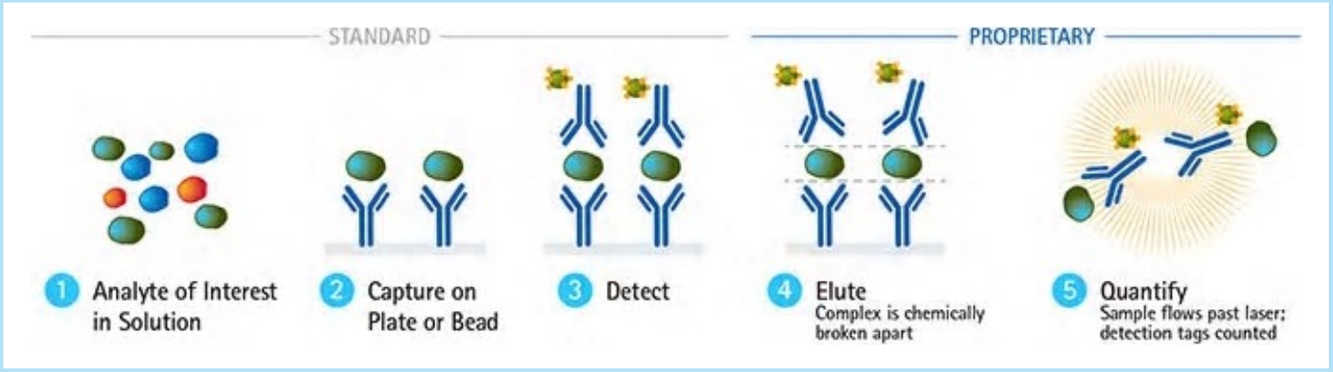


[1)] P. Patterson, Brain Res. Bull. 56(3-4), 319-329.; Proc. Natl. Acad. Sci. USA 99(2), 1002-1007.; J. Cell Sci. 122, 3262-3271.

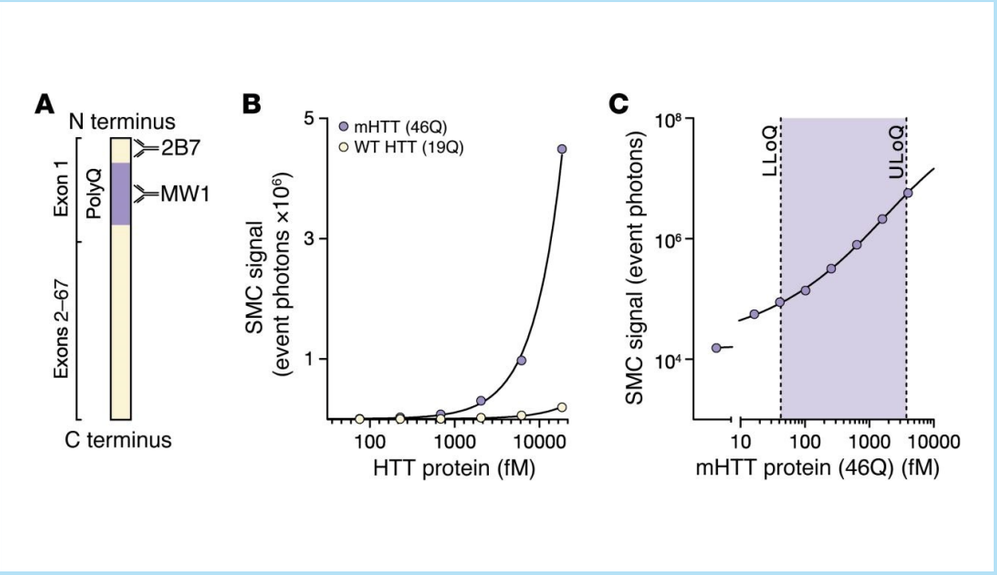
Development of mHTT assay on the Erenna® platform

SMC™ Assay Principle

- Capture and detection steps on magnetic beads
- Modified elution step, fluorescent dye-labelled detection antibodies are released from the immune complexes
- The eluate is drawn into the Erenna® System capillary tube, which contains a very small interrogation space that is illuminated by a laser
- Single fluorescently labelled molecules detected
- Signals with peak intensity above the threshold of background fluorescence are counted as digital events



- mHTT singulex assay shows great specificity for mHTT over WT HTT
- Very broad dynamic range
- LLQ 25 fM – ULQ 6114 fM



Wild at al. **Quantification of mutant huntingtin protein in cerebrospinal fluid from Huntington's disease patients**. J Clin Invest. 2015

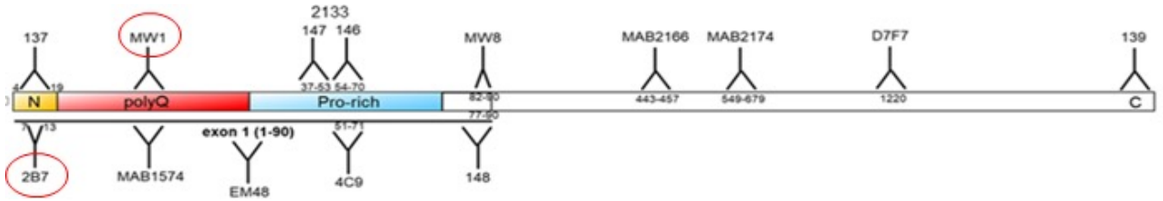
SMCxPRO™ mHTT assay

SMCxPRO™ is an innovative and ultrasensitive laser-based instrument by Merck Millipore

- It is designed to perform digital counting of low concentrated biomarkers (pM – fM range)
- It is replacing the use of Erenna® platform
- Assay principle is the same as for Erenna® platform but different plate reading (faster with no fluidic) with single Fluorescence response as read out

mHTT quantification method was transferred from Erenna® to SMCxPRO™ platform

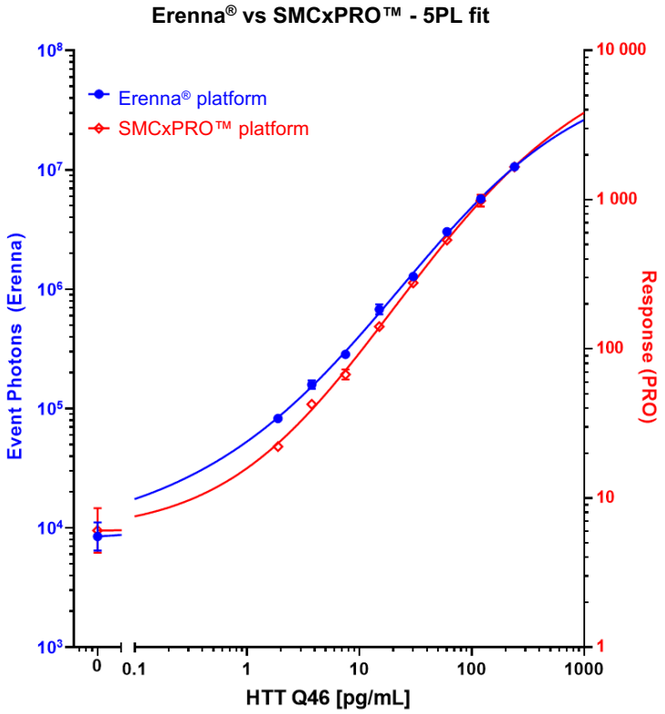
- Critical reagents and assay format are the same
- Analysis is performed in duplicate wells
- Fluorescence raw data generated by xPRO Software for sample concentration calculation using 5-PL fitting



Comparison Erenna® and SMCxPRO™

mHTT Q46 reference material

The mHTT Singulex assay performs similarly on both SMC platforms



Erenna® performance: Standards with 5PL fit

Input		Back-calculated values (pg/mL)		
(pg/mL)	(fM)	Mean	% CV	Accuracy
241	3,681	241.8	4.9	100.3%
120	1,841	118.5	7.4	98.8%
60	920	63.0	4.0	105.0%
30	460	28.3	2.6	94.4%
15	230	16.0	9.1	107.0%
7.5	115	7.1	3.8	95.0%
3.8	57.5	3.9	8.8	103.5%
1.9	28.8	1.8	7.0	96.8%

SMCxPRO™ performance: Standards with 5PL fit

Input		Back-calculated values (pg/mL)		
(pg/mL)	(fM)	Mean	% CV	Accuracy
241	3,681	239.4	1.8	99.3%
120	1,841	121.7	11.0	101.4%
60	920	60.5	4.1	100.8%
30	460	30.2	3.2	100.5%
15	230	15.2	0.1	101.3%
7.5	115	7.0	8.4	93.8%
3.8	57.5	4.2	5.6	109.5%
1.9	28.8	1.7	9.2	91.8%

Erenna® performance: Frozen QC samples

Input		Back-calculated values (pg/mL)		
(pg/mL)	(fM)	Mean	% CV	Accuracy
240.8	3,681	254.4	2.1	105.6%
180.4	2,758	202.1	13.9	112.0%
96.3	1,472	100.1	4.5	103.9%
9.6	147	10.5	10.9	109.2%
3.8	57.5	3.5	1.2	91.9%
1.9	28.8	1.7	11.8	91.9%

SMCxPRO™ performance: Frozen QC samples

Input		Back-calculated values (pg/mL)		
(pg/mL)	(fM)	Mean	% CV	Accuracy
240.8	3,681	264.5	3.8	109.8%
180.4	2,758	209.7	12.2	116.2%
96.3	1,472	95.6	15.0	99.3%
9.6	147	9.8	9.6	101.6%
3.8	57.5	3.5	15.6	94.0%
1.9	28.8	1.7	5.7	91.4%

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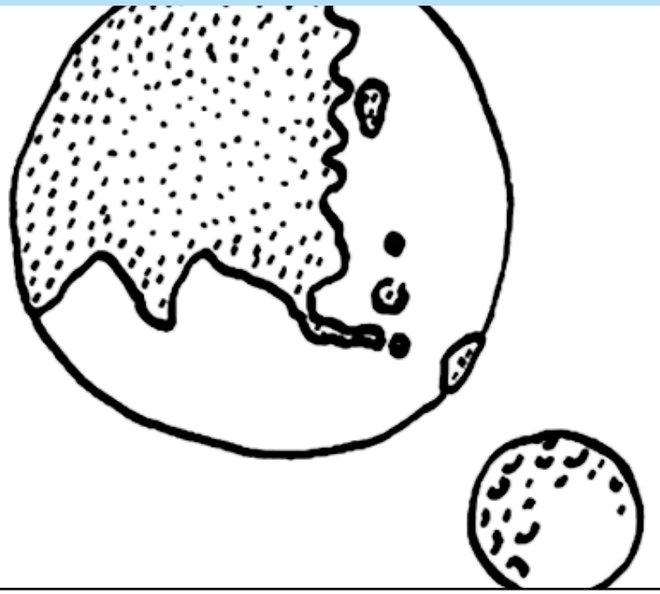
Mutant Huntingtin Assay Validation



mHTT Assay Validation in human CSF

Case study

Method developed at non-GxP site on Erenna® platform

Method transferred to GxP site and on new different platform (from Erenna® to SMCxPRO™ platform)

Non-GxP Site

mHTT hCSF method

GxP site

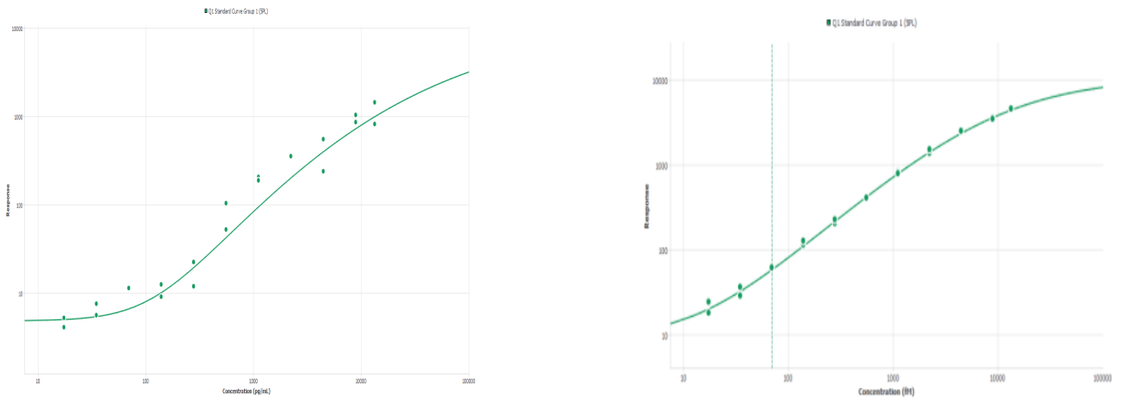
Critical Assay steps

Considerations

High sensitivity platform

- Reference Standard/Critical reagents characterization
- Need for very well trained analysts
  - Extensive training performed on the method
  - Standardized labelling procedure methods
  - Standardized scheme for CS and QC spiking
  - Well defined pipetting procedure and tips cleaning
  - Well defined preparation of capture and detection antibodies
- Plate washer protocols and cleaning procedures
- Clean and controlled environment conditions to avoid contamination and guarantee platform reading performance

CS points CV% performance affected by Capture Antibodies preparation method



Cross-laboratories assay performance assessment

Cross-Laboratories Assay Performance Assessment

Approach

CS and QC samples (4 levels) prepared at VER lab and shipped frozen to HH lab

QC samples (2 sets) tested at both sites against freshly prepared CS

Plate reading on SMCxPRO™ (VER and HH) and then on Erenna® platform (HH)

Data analysed using same fitting algorithm

% Difference calculated on the Mean value from 2 QC Sets

Comparison Verona and Hamburg QC data

Results for back-calculated QC samples

SMCxPRO™ Verona QC Set1

	Stats			
HTT Q46 (pg/mL)	Mean	SD	% CV	Accuracy
1.95	1.64	0.1	6.3	84.0%
5.86	6.17	0.4	6.9	105.3%
84.0	84.9	5.0	5.9	100.4%
175.0	194.0	7.6	3.9	110.9%

SMCxPRO™ Hamburg QC Set1

	Stats			
HTT Q46 (pg/mL)	Mean	SD	% CV	Accuracy
1.95	2.33	0.4	18.9	119.7%
5.86	6.61	0.2	2.3	112.8%
84.0	87.3	4.4	5.0	103.9%
175.0	192.0	6.3	3.3	109.6%

Erenna® Hamburg QC Set1

	Stats			
HTT Q46 (pg/mL)	Mean	SD	% CV	Accuracy
1.95	1.95	0.0	2.0	100.1%
5.86	6.73	0.6	8.7	114.9%
84.0	86.0	3.1	3.6	102.4%
175.0	179.0	3.8	2.1	102.6%

SMCxPRO™ Verona QC Set2

	Stats			
HTT Q46 (pg/mL)	Mean	SD	% CV	Accuracy
1.95	1.89	0.2	9.5	97.1%
5.86	6.41	0.1	1.0	109.4%
84.0	85.1	3.0	3.5	100.6%
175.0	184.5	10.0	5.4	105.5%

SMCxPRO™ Hamburg QC Set2

	Stats			
HTT Q46 (pg/mL)	Mean	SD	% CV	Accuracy
1.95	2.27	0.2	7.5	116.6%
5.86	6.94	0.5	6.7	118.4%
84.0	83.4	4.1	4.9	99.3%
175.0	190.0	9.1	4.8	108.4%

Erenna® Hamburg QC Set2

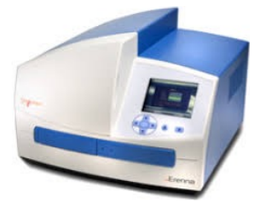
	Stats			
HTT Q46 (pg/mL)	Mean	SD	% CV	Accuracy
1.95	2.29	0.3	11.7	117.3%
5.86	7.04	0.4	5.6	120.0%
84.0	80.8	6.8	8.4	96.2%
175.0	179.0	5.2	2.9	102.3%

Inter-Lab assessment – SMCxPRO™ Verona – Hamburg

HTT Q46 (pg/mL)	Verona Mean	Hamburg Mean	% Difference
1.95	1.77	2.30	-23.4
5.86	6.29	6.78	-7.2
84.0	85.0	85.3	-0.4
175.0	189.0	191.0	-0.8

Inter-Platform assessment – SMCxPRO™ – Erenna® Hamburg

HTT Q46 (pg/mL)	SMCxPRO™ Mean	Erenna® Mean	% Difference
1.95	2.30	2.12	8.7
5.86	6.78	6.89	-1.6
84.0	85.3	83.4	2.3
175.0	191.0	179.0	6.4



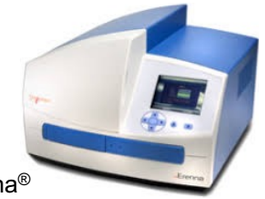
High-sensitivity immunoassays for biomarkers of Huntington's disease

Process

Development of a fit for purpose assay for the detection of expanded mutant HTT

Assay transfer
Cross lab performance assessment

mutant HTT assay validation


SMC Erenna®


SMCxPRO™

Identical assay protocol
2 platforms for readout
Erenna®: fluidics
SMCxPRO™: no fluidics


SMCxPRO™

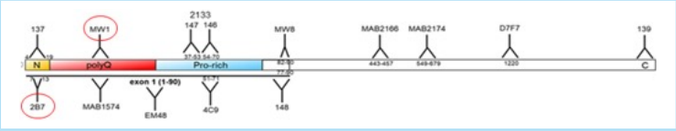
mHTT Assay Validation in human CSF

Method Performance on SMCxPRO™ Platform

Validation parameter	Results available
Calibration model	5 parameter logistic (5PL) model
Validated quantification range (in aCSF)	1.56 to 250 pg/mL (23.9 to 3823.2 fM)
Precision within run (in aCSF)	≤ 12.4%
Precision between runs (in aCSF)	≤ 11.2%
Accuracy within run (in aCSF)	88.5% ≤ Accuracy ≤ 105.8%
Accuracy between runs (in aCSF)	99.9% ≤ Accuracy ≤ 108.9%
Spike recovery (in artificial and pooled non-HD human CSF)	CV ≤ 28.7% 117.4% ≤ Recovery ≤ 123.8%
Specificity (in artificial and pooled non-HD human CSF)	No cross-reactivity between mHTT at ULQ level and wild type HTT-Q19 protein spiked up to 175 pg/mL (2676.3 fM) No cross-reactivity between mHTT at LLQ level and wild type HTT-Q19 protein spiked up to 250 pg/mL
Selectivity (10 individual non-HD human CSF matrices)	No significant matrix interference. Pass rate: 60% at LLQ, 80% at 1.95 pg/mL (29.9 fM), 100% at HVS (175 pg/mL) 100% unspiked matrices resulted BLQ
Interference with human Hemoglobin (Hb spiking in pooled non-HD human CSF)	No interference observed for mHTT at HQC level up to 4.4 µg/mL of spiked Hb Hb interference observed for mHTT at LLQ in the range 1.5 to 3.0 µg/mL of spiked Hb No interference observed for mHTT at a slightly higher level (1.65 pg/mL = 25.3 fM) up to 3.0 µg/mL of spiked Hb
LOD	0.55 pg/mL (8.43 fM)
Prozone/hook Effect	Not observed
Stability in artificial CSF	Storage for up to 4 hours at room temperature and at 2-8°C. Storage for up to 1107 days at -20°C and at -80°C
Freeze/thaw stability in artificial CSF	Tested and stable for up to 2 cycles from -20°C and from -80°C to room temperature.
Stability in human CSF	Storage for up to 2 hours at 2-8°C and up to 500 days at -20°C and up to 287 days at -80°C
Freeze/thaw stability in human CSF	Tested and stable for up to 2 cycles from -20°C to room temperature
Dilution parallelism	Demonstrated in two individual HD human CSF samples with CV < 18.4%
Stability of labelled antibodies	Storage for up to 796 days at 2-8°C for capture labelled antibody and 798 days at 2-8°C for detection labelled antibody

Assay Specifications

- 2B7 & MW1 antibodies
- HTTQ46 Reference standard
- Validated Range: 1.56-250 pg/mL



Validation

- Full Validation
- Primary Clinical Endpoint

Method Validation conducted following the Principles of GCP, in a facility that operates in compliance with the OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17, as enforced by the Italian Health Authorities (D.L. Number 50, 2 March 2007, Annexes I and II; G.U. Number 86, 13 April 2007).

mHTT Assay Validation in human CSF

Accuracy and Precision results

- **Accuracy & Precision (A&P)**
  - CS and 5 QC levels prepared in aCSF
  - One within-assay A&P run (n=6)
  - Five between-assay A&P runs (n≥3)
- **Results met Acceptance Criteria**
  - Within Assay

    CV% ≤ 12.4; 88.5% ≤ Accuracy ≤ 105.8%
  - Between Assay

    CV% ≤ 11.2; 99.9% ≤ Accuracy ≤ 108.9%
  - Total Error ≤ 15.4

	mHTT (pg/mL)				
Analyte	**LLQ**	**LQC**	**MQC**	**HQC**	**ULQ**
Nominal value	**1.56**	**5.86**	**84.60**	**175.00**	**250.00**
SAI1 (QC freshly prepared)	1.52	5.65	87.23	180.85	253.29
	1.64	4.74	86.05	163.88	236.82
	1.68	6.00	105.18	178.05	228.90
	1.59	4.64	90.57	162.72	269.71
	1.86	4.87	71.73	164.87	232.56
	1.61	5.22	95.46	186.45	279.45
Mean	**1.65**	**5.19**	**89.37**	**172.80**	**250.12**
SD	**0.1**	**0.5**	**11.1**	**10.2**	**20.9**
% CV	**7.0**	**10.5**	**12.4**	**5.9**	**8.4**
% Accuracy	**105.8**	**88.5**	**105.6**	**98.7**	**100.0**
SAI3	1.70	5.92	84.25	198.82	277.56
	1.68	5.00	89.51	173.26	282.65
	1.96	6.24	104.35	193.05	251.13
Mean	**1.78**	**5.72**	**92.70**	**188.38**	**270.44**
SD	**0.2**	**0.6**	**10.4**	**13.4**	**16.9**
% CV	**8.9**	**11.2**	**11.2**	**7.1**	**6.3**
% Accuracy	**114.1**	**97.6**	**109.6**	**107.6**	**108.2**
SAI4	1.67	6.29	95.12	182.87	282.15
	1.63	5.66	83.51	190.93	289.54
	1.98	8.13	110.57	192.39	252.65
Mean	**1.76**	**6.69**	**96.40**	**188.73**	**274.78**
SD	**0.2**	**1.3**	**13.6**	**5.1**	**19.5**
% CV	**10.7**	**19.2**	**14.1**	**2.7**	**7.1**
% Accuracy	**112.7**	**114.2**	**114.0**	**107.8**	**109.9**

	mHTT (pg/mL)				
Analyte	**LLQ**	**LQC**	**MQC**	**HQC**	**ULQ**
Nominal value	**1.56**	**5.86**	**84.60**	**175.00**	**250.00**
SAI5	1.85	5.26	80.19	185.97	253.87
	1.56	4.75	72.73	151.76	229.56
	1.85	5.94	84.43	148.20	225.06
Mean	**1.75**	**5.32**	**79.12**	**161.98**	**236.16**
SD	**0.2**	**0.6**	**5.9**	**20.9**	**15.5**
% CV	**9.6**	**11.2**	**7.5**	**12.9**	**6.6**
% Accuracy	**112.3**	**90.7**	**93.5**	**92.6**	**94.5**
SAI6	1.48	6.70	101.21	203.57	273.15
	1.54	5.62	91.30	196.14	293.79
	1.65	6.78	97.96	*184.20*	*299.27*
Mean	**1.56**	**6.37**	**96.82**	**194.63**	**288.74**
SD	**0.1**	**0.6**	**5.0**	**9.8**	**13.8**
% CV	**5.7**	**10.2**	**5.2**	**5.0**	**4.8**
% Accuracy	**99.7**	**108.6**	**114.4**	**111.2**	**115.5**
			Overall statistics		
Mean	**1.70**	**5.86**	**90.88**	**181.30**	**264.05**
SD	**0.1**	**0.7**	**7.2**	**13.5**	**20.8**
% CV	**5.6**	**11.2**	**8.0**	**7.4**	**7.9**
% Accuracy	**108.9**	**99.9**	**107.4**	**103.6**	**105.6**
% Bias	**8.9**	**-0.1**	**7.4**	**3.6**	**5.6**
TE	**14.5**	**11.3**	**15.4**	**11.0**	**13.5**

Clinical Sample Analysis

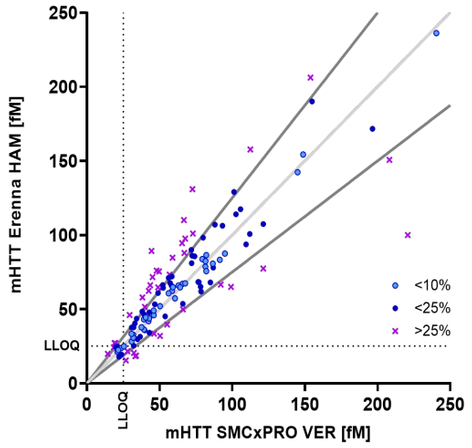
Results comparison from re-analysis testing: more than “ISR”

“Incurred Sample Re-analysis is conducted by repeating the analysis of a subset of subject or patient samples from a given study in separate runs, preferably during the study, to critically support the precision and accuracy measurements established with the QCs. The original and repeat analyses should be conducted using the same bioanalytical method procedures” **FDA BMV 2018**

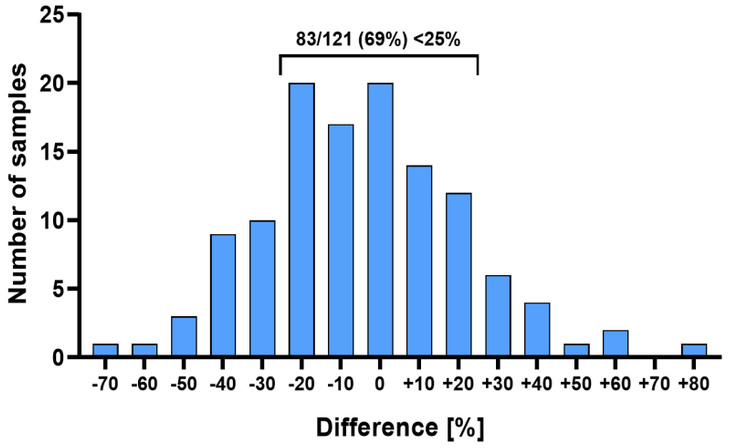
- 121 Clinical CSF Samples
- Analysis carried out in VER (SMCxPRO™) and in HH (Erenna®) on separate aliquots
- Comparison between mHTT concentration data produced
- % Difference calculation
- 69% of the results differ < ±25% in the analysis performed

LBA 67% should be ± 30% of the mean

HH Erenna® *vs* VER SMCxPRO™ % Difference



HH Erenna® *vs* VER SMCxPRO™



Conclusions

... and further development

- Assay for mHTT quantification in human CSF was successfully validated on SMCxPRO™ platform
- Clinical studies support ongoing (GCP sample analysis)
- Need for additional biomarkers

Additional HD biomarkers validated assays

Points to consider?

- Are treatments under development to lower mHTT protein, selective towards the mutant form?
- Need to monitor for the modulation of HTT form(s) (referred to total HTT)
- Correlation central *vs* peripheral quantification (CSF *vs* plasma/PBMC)
- Whole Blood: a matrix to be evaluated in clinical studies
- Need to monitor for other biomarkers, such as NfL as safety flags

Analyte	Human Matrix	Platform Ab pair	Quantification Range (pg/mL)
Total HTT	CSF	SMCxPRO 2B7-D7F7	12.4-3160
mutant HTT	Plasma	SMCxPRO 2B7-MW1	39.1-5000
mutant HTT	PBMC	SMCxPRO 2B7-MW1	781-50000
NfL	CSF	Quanterix	0.407-359 (1:4)
NfL	Plasma	Quanterix	0.407-359 (1:100)
NfL	Serum	Quanterix	0.407-359 (1:100)

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#RESEARCHNEVERSTOPS

Thank you for your attention

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