INTERCEPT-AD: DEVELOPMENT AND QUALIFICATION OF A HIGHLY SENSITIVE IMMUNOASSAY DETECTING TOTAL SABIRNETUG (ACU193) IN HUMAN CSF

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Introduction

Figure 1. The proposed mechanism of action of sabirnetug (ACU193), a monoclonal antibody selectively targeting amyloid beta oligomers (AβOs) for treatment of early Alzheimer's disease (AD)

Sabirnetug is an anti-AβO monoclonal antibody (mAb) that selectively targets and inhibits toxic soluble AβOs, preventing them from binding to synapses, and thereby preserving neuronal function.[1]

An electrochemiluminescence (ECL) method was previously developed for the determination of sabirnetug in human serum. However, exposure of sabirnetug in cerebrospinal fluid (CSF) is predicted to be much lower than in serum because of the blood–brain barrier, which would make sabirnetug undetectable using a conventional serum immunoassay. An ultrasensitive assay was developed to measure sabirnetug pharmacokinetics (PK) in CSF of individuals with early AD in the Phase 1 study INTERCEPT-AD.

Methods

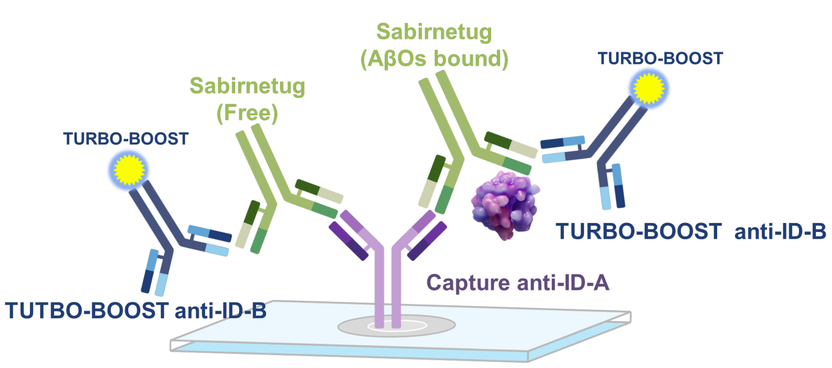

Figure 2. Schematic of PK immunoassay of sabirnetug in CSF

Sabirnetug in CSF is measured with ultrasensitive MSD S-PLEX® assay kits employing a sandwich immunoassay format using mAbsand MSD's TURBO-TAG® and TURBO-BOOST® technology. The biotinylated anti-idiotype antibody A (anti-ID-A) is coated on the MSD GOLD™ S-PLEX SECTOR Plate and incubated with diluted calibrators, quality controls (QCs), and CSF samples. After washing, TURBO-BOOST anti-ID-B detection antibody is added to the plate. TURBO-TAG is added to the washed plate to enhance electrogenerated chemiluminescence. The resulting plate is read on the MSD® plate reader following addition of read buffer.

The immunoassay was developed on the Meso Scale Discovery® (MSD) S-PLEX platform with improved sensitivity through TURBO-BOOST® and TURBO-TAG® technology. Twenty-four anti-sabirnetug antibody pairs were screened. Assay selectivity, target interference, dilutional linearity, and stability were evaluated. Assay accuracy and precision were tested, and the lower limit of quantitation (LLOQ) was determined. Target interference was tested by spiking various concentrations of monomeric Aβ and AβOs into the sabirnetug calibrators prepared in CSF. Sabirnetug bound and unbound to AβOs (total drug) in CSF can be measured using the immunoassay with the selected anti-ID antibody pair.

Results

Antibody pair screening

Capture		1A2			2B4			1H1		
Detection		1H1			Rabbit pAb			Rabbit pAb		
Calibrator	Conc. (pg/mL)	Signal	%CV	S/B	Signal	%CV	S/B	Signal	%CV	S/B
Cal-1	100,000	1,643,543	1.2	19,450	1,614,276	0	30	1,607,850	0	42
Cal-2	25,000	1,553,592	0.9	18,386	1,556,870	0.3	29	1,572,428	0.4	41
Cal-3	6,250	1,325,378	1	15,685	1,316,758	3.4	24	1,437,738	0.3	38
Cal-4	1,563	550,154	1.5	6,511	509,846	5.4	9.3	811,550	17.6	21
Cal-5	391	117,786	5.2	1,394	166,985	5.5	3.1	254,519	34.3	6.7
Cal-6	97.7	24,848	4.6	294	95,760	15.6	1.8	102,349	12.4	2.7
Cal-7	24.4	5,682	2.6	67	58,274	0.1	1.1	69,832	13.2	1.8
Cal-8	6.1	1,392	4.5	16	60,891	0.9	1.1	54,253	1.4	1.4
Cal-9	1.5	415	4.6	4.9	50,526	3.6	0.9	59,326	16.3	1.6
Cal-10	0.4	158	4.9	1.9	51,917	2.5	1	49,566	4.2	1.3
Cal-11	0.1	112	3.2	1.3	56,538	7.9	1	48,637	10.9	1.3
Cal-12	0	85	2.5	1	54,578	4.3	1	38,058	14.4	1

Table 1. Screening of capture and detection antibodies

Various combinations of capture and detection antibodies, including anti-ID antibodies, polyclonal anti-drug antibody, and mouse anti-human IgG antibody, were screened and the top 3 pairs (above) were selected for further evaluation. Anti-ID 1A2 and 1H1 were chosen as optimal capture and detection antibody, respectively, based on the signal to noise ratio (S/B) of the calibrators.

Target interference

Calibrator Diluent		CSF	CSF + Aβ 1-40 monomers				CSF + Aβ oligomers (ADDLs)					
			5 ng/mL	20 ng/mL	5 ng/mL	20 ng/mL	0.1 pM	1 pM	10 pM	0.1 pM	1 pM	10 pM
Calibrator	Conc. (pg/mL)	Signal			Normalized to CSF		Signal			Normalized to CSF		
Cal-1	6000	1,134,506	1,162,998	1,171,682	103%	103%	1,093,434	1,153,499	1,091,229	96%	102%	96%
Cal-2	1500	336,894	340,395	349,632	101%	104%	318,200	339,525	329,715	94%	101%	98%
Cal-3	375	74,436	69,625	70,741	94%	95%	67,229	69,182	69,103	90%	93%	93%
Cal-4	94	16,120	15,034	14,952	93%	93%	13,978	14,163	14,945	87%	88%	93%
Cal-5	23	3,497	3,670	3,498	105%	100%	3,272	3,393	3,709	94%	97%	106%
Cal-6	5.9	872	908	875	104%	100%	786	886	924	90%	102%	106%
Cal-7	1.5	289	293	310	102%	107%	274	267	284	95%	92%	98%
Cal-8	0	111	98	116	88%	105%	103	105	105	93%	95%	95%

Table 3. Target interference of Aβ monomers and AβOs at physiological concentrations on sabirnetug measurement

Prior to MRD in assay diluent, the calibrator curve was made in either pooled CSF, pooled CSF spiked with low and high concentration of monomeric Aβ 1-40, or pooled CSF spiked with low, medium, and high concentration of synthetic AβOs (Aβ-derived diffusible ligands, ADDLs)[2]. No interference was observed from monomeric Aβ 1-40 and AβOs in any tested concentrations. The interference data indicate that both Aβ bound and unbound sabirnetug can be detected with the immunoassay (total drug measurement).

Stability

Stability Sample	Nominal Conc. (pg/mL)	Signal	%CV	Conc. (pg/mL)	% Recovery
HQC 0 F/T (0hr RT)	4500	1,095,655	3.4	4725	105
HQC 1 F/T	4500	1,075,575	1.6	4617	103
HQC 3 F/T	4500	1,084,408	1.4	4664	104
HQC 5 F/T	4500	971,642	8.4	4081	91
HQC 12 hr RT	4500	960,837	2.4	4022	89
LQC 0 F/T (0hr RT)	4.4	1,300	7.5	4.94	112
LQC 1 F/T	4.4	1,257	1.0	4.77	108
LQC 3 F/T	4.4	1,293	6.0	4.91	112
LQC 5 F/T	4.4	1,261	2.9	4.78	109
LQC 12 hr RT	4.4	1,140	0.9	4.3	98

Table 5. Stability of sabirnetug in CSF

High-quality control (HQC) (4500pg/mL) and low-quality control (LQC) (4.4pg/mL) were prepared in pooled CSF. The sabirnetug in CSF was stable for at least 5 freeze/thaw (F/T) cycles at ≤70 ℃ and 12 hours at 23 ℃. Long-term stability will be tested during the validation.

Selectivity

Individual CSF	Unspiked	LLOQ Spike : 6 pg/mL			MQC Spike : 325 pg/mL			HQC Spike : 4770 pg/mL		
	Results (pg/mL)	Results (pg/mL)	%Bias	%CV	Results (pg/mL)	%Bias	%CV	Results (pg/mL)	%Bias	%CV
AD 01	<LLOQ	8	33.3	3.1	380	16.9	2.6	5660	18.7	1.2
AD 02	<LLOQ	7	16.7	2.5	443	36.3	2.1	5159	8.2	0.9
AD 03	<LLOQ	6	0.0	1.5	378	16.3	0.8	5236	9.8	2.2
AD 04	<LLOQ	7	16.7	2.0	351	8.0	2.1	5163	8.2	1.9
AD 05	<LLOQ	6	0.0	4.7	359	10.5	0.8	5295	11.0	0.7

Table 2. Selectivity of sabirnetug in diseased matrix

Five individual AD CSF samples were spiked with sabirnetug at LLOQ, MQC, and HQC levels. All neat samples (unspiked) were below LLOQ. The recovery and %CV at each QC level passed the criteria (≥ 80%). The assay demonstrated good selectivity for sabirnetug in individual AD samples. The selectivity will be re-evaluated in method validation using more samples.

Dilutional linearity

Diluted Sample	Dilution Fold	Nominal Conc. (pg/mL)	Signal	Observed Conc. (pg/mL)	Dilution Corrected Conc. (pg/mL)	% Recovery	%CV
Dil-1	1	100,000	1,649,479	10609	10609	11	0
Dil-2	4	25,000	1,569,476	9978	39912	40	0.4
Dil-3	16	6,250	951,179	5620	89912	90	1.7
Dil-4	64	1,563	244,355	1429	91436	91	0.3
Dil-5	256	390.6	56,423	357.6	91552	92	0.9
Dil-6	1024	97.7	13,135	91.5	93664	94	2.1
Dil-7	4096	24.4	3,107	23.1	94600	95	0.9
Dil-8	16384	6.10	829	5.75	94158	94	0.5
Dil-9	65536	1.53	354	1.71	112174	112	4.8
Dil-10	262144	0.38	193	0.171	44851	45	0.7

Table 4. Dilutional linearity of the sabirnetug immunoassay

One pooled CSF sample was spiked with sabirnetug at ultra high concentration of 100,000 pg/mL and then diluted in 4-fold serial dilutions from neat to 262,144-fold dilution in CSF. All dilutions, controls, and calibrator curve were diluted to MRD 4 in assay diluent. The data shows that the sample can be diluted up to 16,384-fold with acceptable accuracy and precision. Dilutions within quantitation range are highlighted with green.

Assay reproducibility

Calibrators/QCs	Nominal Conc. (pg/mL)	Measured Mean Conc. (pg/mL)	Intra-run %CV	Inter-run %CV	% Recovery
Cal-1	6,000	5,895.6	1.5	0.5	98
Cal-2	1,500	1,608.1	2.3	2.1	107
Cal-3	375	371.1	2.5	2.2	99
Cal-4	93.8	89.7	1.7	1.7	96
Cal-5	23.4	23.2	4.7	2.1	99
Cal-6	5.86	6.0	7.6	2.8	102
Cal-7	1.46	1.5	5.8	6.2	105
Cal-8	0	NA	11.1	16.6	NA
HQC	4,800	4,485.4	1.7	3.7	93
MQC	300	294.5	2.0	5.6	98
LQC	15	16.1	4.6	10.7	107
LLOQ	5.9	6.0	4.5	12.5	103

Table 6. Reproducibility of the sabirnetug immunoassay

Accuracy and precision were evaluated by 9 independent runs performed by 4 analysts across 4 days. All runs met acceptance criteria, indicating good reproducibility of the method.

REFERENCES

1. Siemers, E., Hitchcock, J., Sundell, K. et al. ACU193, a monoclonal antibody that selectively binds soluble Aβ oligomers: development rationale, phase 1 trial design, and clinical development plan. J Prev Alzheimers Dis. 2023;10(1):19-24.
2. Chromy BA, Nowak RJ, Lambert MP, et al. Self-assembly of Abeta (1-42) into globular neurotoxins. Biochemistry. 2003 Nov 11;42(44):12749-60.

RESEARCH HIGHLIGHTS

- Due to the blood–brain barrier, only 0.05-0.1% of a peripherally administrated mAb is expected to be delivered into brain. An ultrasensitive assay is needed to detect small amounts of sabirnetug in CSF.
- A sabirnetug immunoassay with a limit of detection of 5.9pg/mL in CSF was successfully developed and applied to the PK determination of CSF samples of the sabirnetug INTERCEPT-AD Phase 1 Study.
- The result of target interference assessment with monomeric Aβ 1-40 and AβOs indicates that the assay is detecting both bound and unbound sabirnetug (total drug) in CSF.
- Assay qualification demonstrated sensitivity, accuracy and precision, selectivity, specificity, dilutional linearity, and stability of the method.