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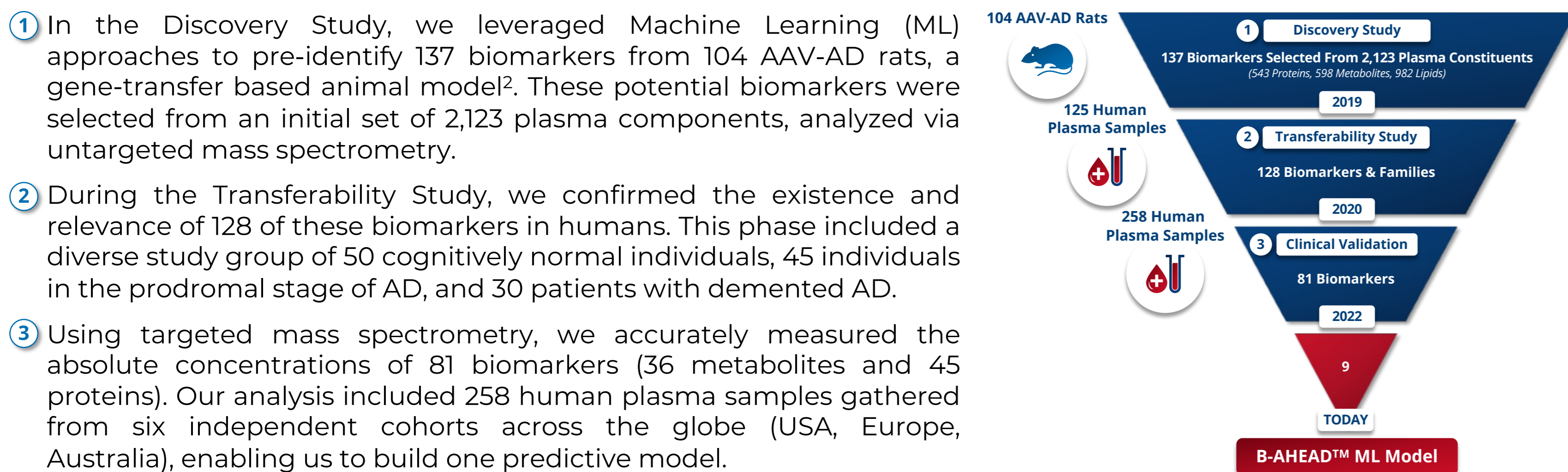
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BACKGROUND

- ▶ Amyloid biomarkers lack the specificity to predict which cognitively normal individuals will progress to Alzheimer's disease (AD) dementia. Up to 70% of amyloid-positive individuals without cognitive impairment remain non-converters over their lifetime¹.
- ▶ With the advent of disease-modifying therapies, more specific biomarkers are urgently needed to optimize secondary prevention trials, as amyloid's limited accuracy undermines both efficiency and impact.
- ▶ The onset of AD dementia symptoms likely reflects converging pathway dysfunctions, including amyloid-tau interactions, immunity and inflammation, lipid metabolism, bioenergetic failure, oxidative stress, or structure cell integrity. We hypothesize that diagnostic accuracy improves when models capture multiple pathways rather than amyloid status alone.

PRELIMINARY RESULTS: BIOMARKERS DISCOVERY

Figure 1. Identification of the Biomarkers



OBJECTIVE

- ▶ This exploratory study aimed to assess the clinical relevance and potential utility of our novel multimomics biomarkers, previously identified in a gene-transfer-based rat model, by integrating them into a machine learning model alongside APOE genotype and amyloid status. The model was evaluated retrospectively using human plasma samples from six independent cohorts (USA, Europe, Australia) to explore its ability to identify, among cognitively normal individuals, those likely to convert to Alzheimer's dementia.

METHODS

- ▶ **Mass Spectrometry:** Proprietary targeted mass spectrometry assays were developed to measure absolute concentrations of 81 biomarkers (36 metabolites and 45 proteins) from the initial set of 128 biomarkers identified in the Transferability Study.
- ▶ **Plasma Samples and Clinical Labels:** The study included 258 cognitively normal participants from seven cohorts with available APOE genotypes. Over clinical follow-up (mean 5.2 years; range [0.9-21.0] years from blood draw), 48 participants (18.6%) converted to Alzheimer's dementia (AD converters) while 210 (81.4%) remained cognitively stable (Non-converters). Participants who converted developed mild cognitive impairment after a mean of 3.6 years, and AD dementia symptoms within 6.5 years in average, whereas non-converters were followed for a mean of 4.7 years.
- ▶ **APOE Genotype:** APOE genotypes were determined locally by clinical partners per site SOPs. Genotype data were available for all 258 participants (100%). Among non-converters, 27.1% participants were APOE ε4 carriers ; among AD converters, 56.3% were APOE ε4 carriers. Among non-converters, 3.8% participants were APOE ε4/ε4 carriers ; among AD converters, 25.0% were APOE ε4/ε4 carriers.
- ▶ **Amyloid Status:** Amyloid status was established locally by clinical partners using reference tests, either amyloid PET or CSF biomarkers (p-tau181 and Aβ42). Site-specific positivity cut-offs were applied, and amyloid labels were used as provided by each site. Across the 258 participants, amyloid status was available for 82.2% of them. Among non-converters, 32.7% were amyloid-positive; among AD converters, 85.4% were amyloid-positive.

Figure 2. Retrospective Plasma Samples: Clinical Label Examples

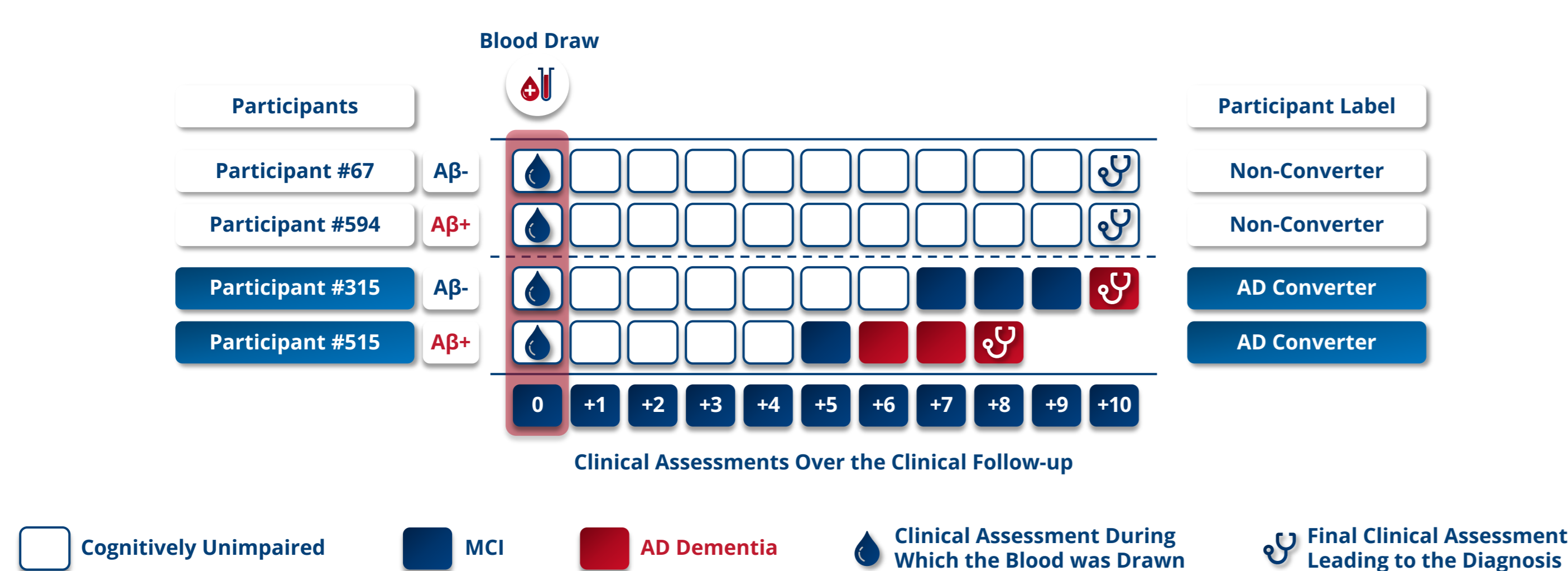
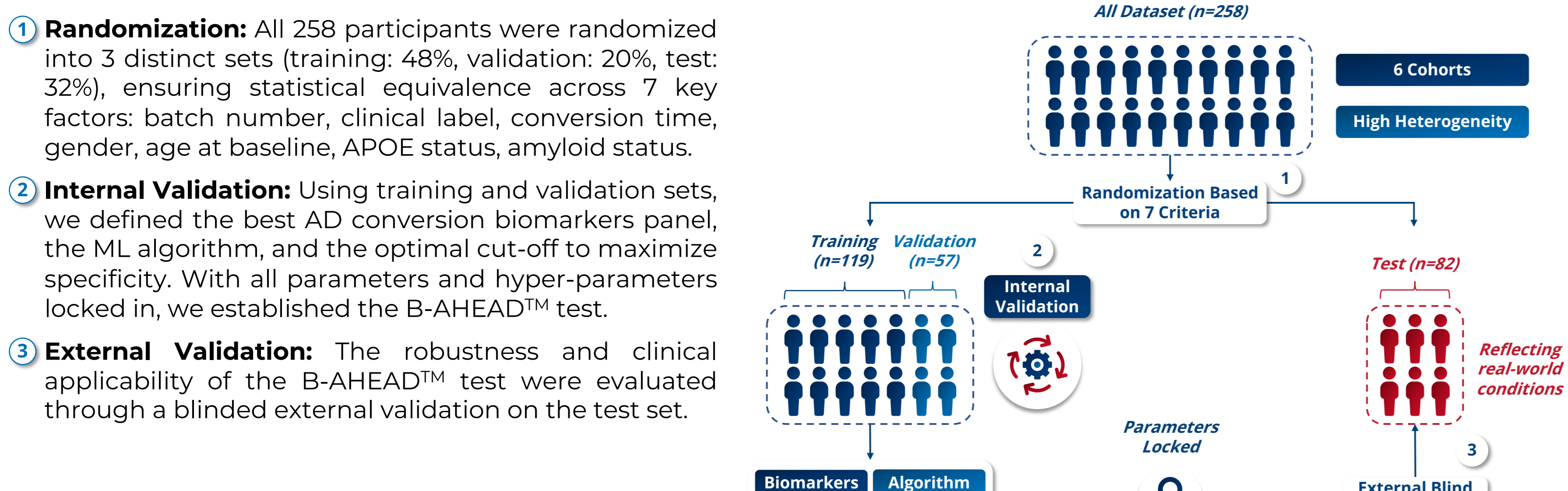


Figure 3. Machine Learning Experimental Design³



References

1. Brookmeyer, Ron and Abdalla, Nada. "Lifetime Risks of Alzheimer's Disease Dementia Using Biomarkers for Preclinical Disease." *Alzheimer's Dement.* 2018 May 22;14(8):981-988. doi: 10.1016/j.jalz.2018.03.005.
2. Audrain, Mickael et al. "βAPP Processing Drives Gradual Tau Pathology in an Age-Dependent Amyloid Rat Model of Alzheimer's Disease." *Cerebral cortex* (New York, N.Y.: 1991) vol. 28,11 (2018): 3976-3993. doi:10.1093/cercor/bbx260
3. Braudeau et al. "Optimizing Alzheimer's diagnosis and precision medicine: A narrative review unlocking the potential of multimomics markers." *NeuroMarkers*, Volume 2, Issue 3, 2025. doi: 10.1016/j.neumar.2025.100107.

RESULTS

Figure 4. Typology of the Biomarkers

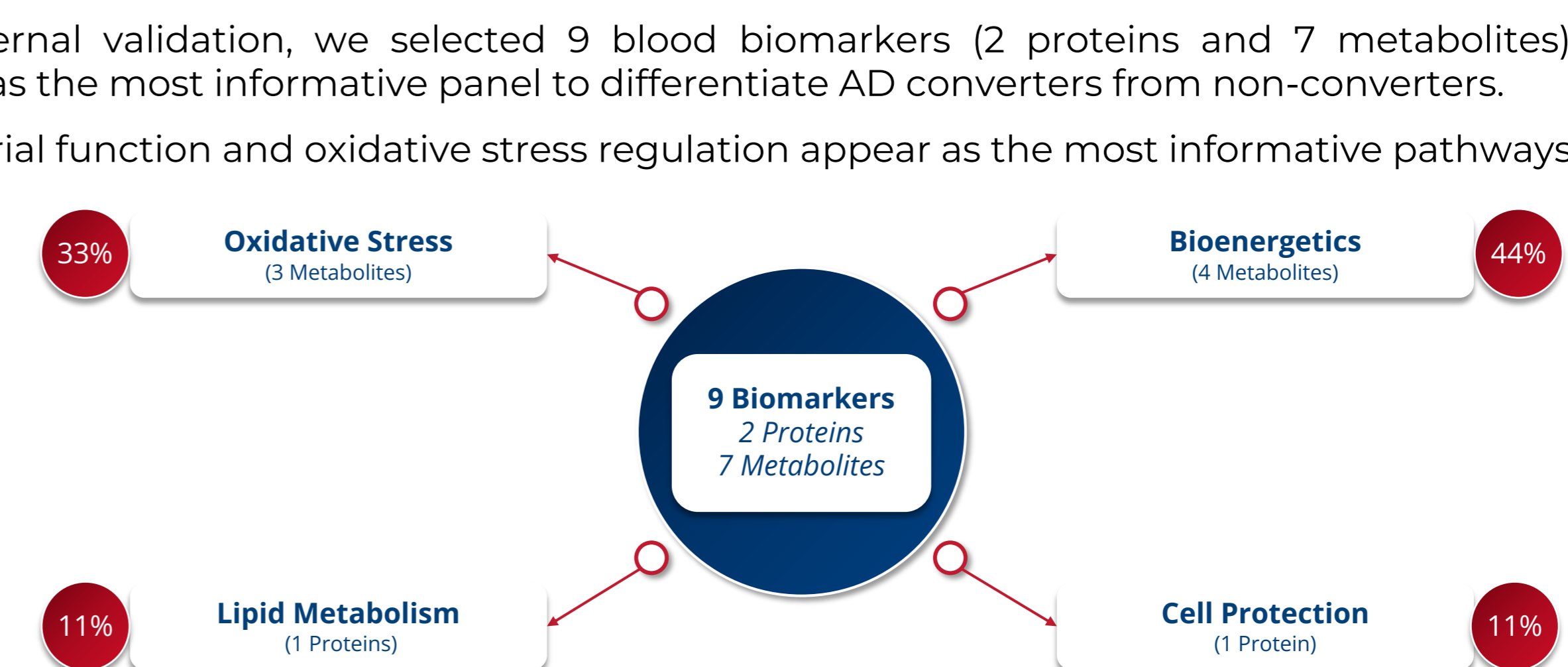
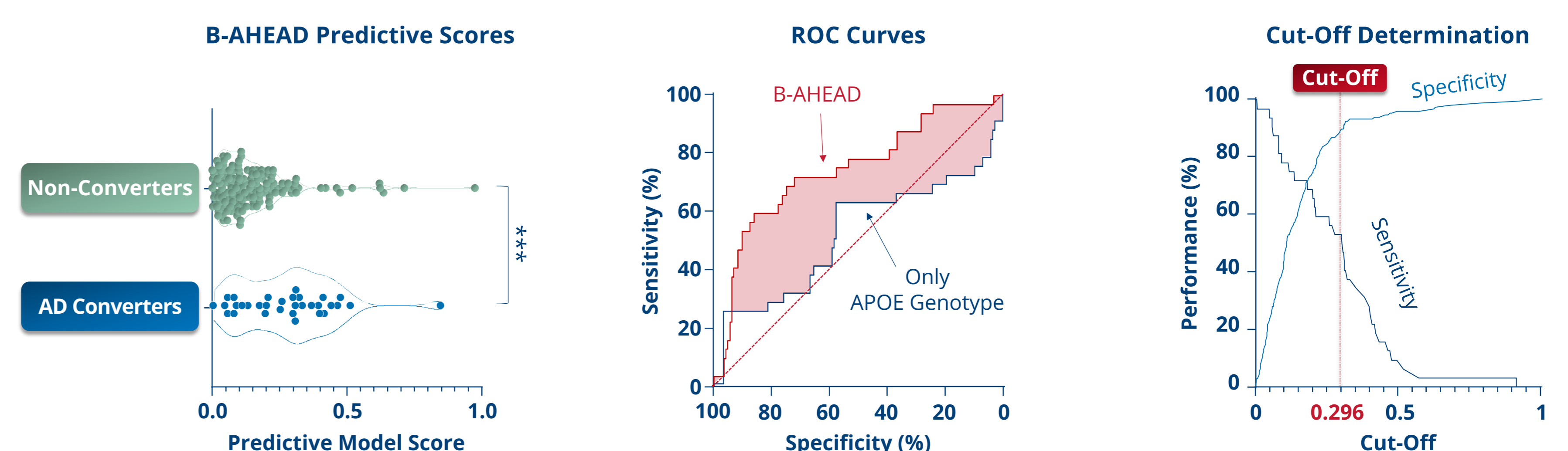


Figure 5. Internal Validation: B-AHEAD™ Predictive Performance

- ▶ **B-AHEAD Predicted AD with 89.6% Specificity:** During internal validation, the predictive ML model was calibrated to a cut-off of 0.296, resulting in 89.6% specificity in predicting AD converters from non-converters, with 53.1% sensitivity (AUROC=73.8%, p<0.0001).



Dataset	Number of Individuals			Analysis	AUROC [95% CI]	Specificity [95% CI]	Sensitivity [95% CI]
	Total	Non-Conv.	AD Conv.				
Training + Validation	176	144	32	Mean of 100x 10-fold cross-validations	73.8%*** [0.64-0.84]	89.6% [0.84-0.94]	53.1% [0.36-0.69]

Figure 6. External Validation: B-AHEAD™ Predictive Performance

- ▶ **In the external validation, B-AHEAD achieved 83.3% specificity in predicting AD converters from non-converters,** with 56.3% sensitivity (AUROC=72.4%, p=0.001). These findings strongly argue against model overfitting and affirm the robustness of the B-AHEAD test, thus suggesting its applicability in predicting AD in cognitively unimpaired populations under real-world conditions.

Dataset	Number of Individuals			Analysis	AUROC [95% CI]	Specificity [95% CI]	Sensitivity [95% CI]
	Total	Non-Conv.	AD Conv.				
Test	82	66	16	Blind prediction of the locked algorithm	72.4%** [0.57-0.88]	83.3% [0.73-0.90]	56.3% [0.33-0.77]

Figure 7. Combination of Diagnostic Criteria (Amyloid Status, ApoE Genotype, B-AHEAD)

- ▶ **In the blinded test dataset, combining B-AHEAD and amyloid test positivity markedly reduced the misdiagnosis of individuals that not progress to Alzheimer's dementia symptoms.** When both tests were applied in series, specificity reached 98.2% and sensitivity 46.2%, resulting in only 14.3% of non-converters, compared with 57.1% when using amyloid positivity alone. This combined approach could thus enable the development of secondary prevention trials enriched for truly at-risk individuals, an approach that remains currently unfeasible using amyloid biomarkers alone.

Dataset	Diagnostic Criteria	Specificity	Sensitivity	% Positive Among Test Set	Predictive Positive Value
Subpopulation of the test set with known amyloid status at blood draw time (n=68)	ApoE4/4 Genotype	96.4%	15.4%	5.9%	50.0%
	Amyloid Status (PET or CSF)	70.9%	92.3%	41.2%	42.9%
	B-AHEAD™	85.5%	46.2%	20.6%	42.9%
	ApoE4/4 Genotype + Amyloid Status	98.2%	15.4%	4.4%	66.6%
	ApoE4/4 Genotype + B-AHEAD™	98.2%	7.7%	2.9%	50.0%
	Amyloid Status + B-AHEAD™	98.2%	46.2%	10.3%	85.7%

PPV: the Predictive Positive Value (PPV) corresponds to the percentage of subjects that has been tested positive for a diagnostic criteria and that progress to AD dementia symptoms during the clinical follow-up.

CONCLUSIONS

- ▶ Peripheral multimomics plasma signatures can predict the development of AD dementia symptoms with a higher level of specificity than current amyloid tests in cognitively unimpaired population.
- ▶ The biological pathways most affected in the asymptomatic stage of AD appear to be mitochondrial function and oxidative stress regulation.
- ▶ The best combination of diagnostic criteria is a positive amyloid status and a positive B-AHEAD test. This combination of diagnostic criteria could reduce enrollment of non-converters and increases the efficiency and impact of preventive clinical trials.

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