

CD8 T cell recognition of APP and derivative non-Aβ peptides in blood as novel biomarkers for Alzheimer’s and related MCI

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Background

We recently developed a CD8 T cell-induced mouse model that allowed development of a biomarker for Alzheimer’s disease based on quantitation of homeostatically-induced Amyloid Precursor Protein [APP] peptide-reactive CD8 T ("hiT") cells in blood (PMID: 38995966) using APP₄₇₁₋₄₇₉/Human Leukocyte Antigen(HLA)-A2 multimers (dextramers) as staining reagents in flow cytometry. Levels of hiT cells distinguished AD and related MCI from normal aging controls with high accuracyThe current version of the test requires patients to carry a common genetic marker, HLA-A2, which approximately half the population carries, and there is a need to develop assay solutions that increase the accessibility of the test.

Methods

To extend the test to patients without the HLA-A2 genetic marker, we identified an alternative non-self target peptide (ALIAPVHAV) pHLA-A2 dextramer ("Universal Dextramer") that detects hiT cells regardless of a patient’s HLA background. T cell binding to the Universal Dextramer correlated closely with binding to the original APP-based target in HLA-A2-positive patients (0.42-0.58x relative to APP/HLA-A2; $r = 0.81$; $P < 0.001$), establishing it as a reliable substitute. Using this approach, we quantified hiT Cells in frozen whole blood and frozen buffy coat samples from 137 patients total: 84 with the HLA-A2 marker (79 in established Univ. of Antwerp cohort, 5 in new validation cohort from USC ADRC) and 53 without (44 UA, 9 USC). Patients were drawn from four groups: Alzheimer’s disease (AD), mild cognitively impaired (MCI) with or without CSF biomarkers consistent with AD (MCI-AD and MCI-norm/other, respectively), and normal aging cohorts. We then assessed correspondence of dextramer+ hiT cells to AD and MCI status. As a second approach, we also tested a whole blood antigen-induced marker ("WAIM") assay developed by Beckman Coulter. This method exposes blood samples to APP protein and measures the immune response (T cell activation) it triggers. The WAIM method can detect T cell reactivity independent of a patient’s genetic background, offering another potential path to an HLA-independent assay.

Key References

AD model

Panwar et al., *Mech Ageing Dev* (2020) 191:111351
Panwar et al., *Proc Natl Acad Sci U S A* (2024) 121:e2401420121

Multi-pHLA T cells

Daniels et al. *Immunity* (2001) 15:1051-1061
Jouanneau et al., *Cancer Immunol Immunother* (2014) 63:911-924
Cordner et al., *Oncogene* (2023) 42:2088-2098

WAIM assay

Trauet et al., *Cytometry A* (2024) 105:288

P-Tau217 ROC

Palmqvist et al., *JAMA* (2020) 324:772

1 Impact of APP-Reactive T cells on Mice

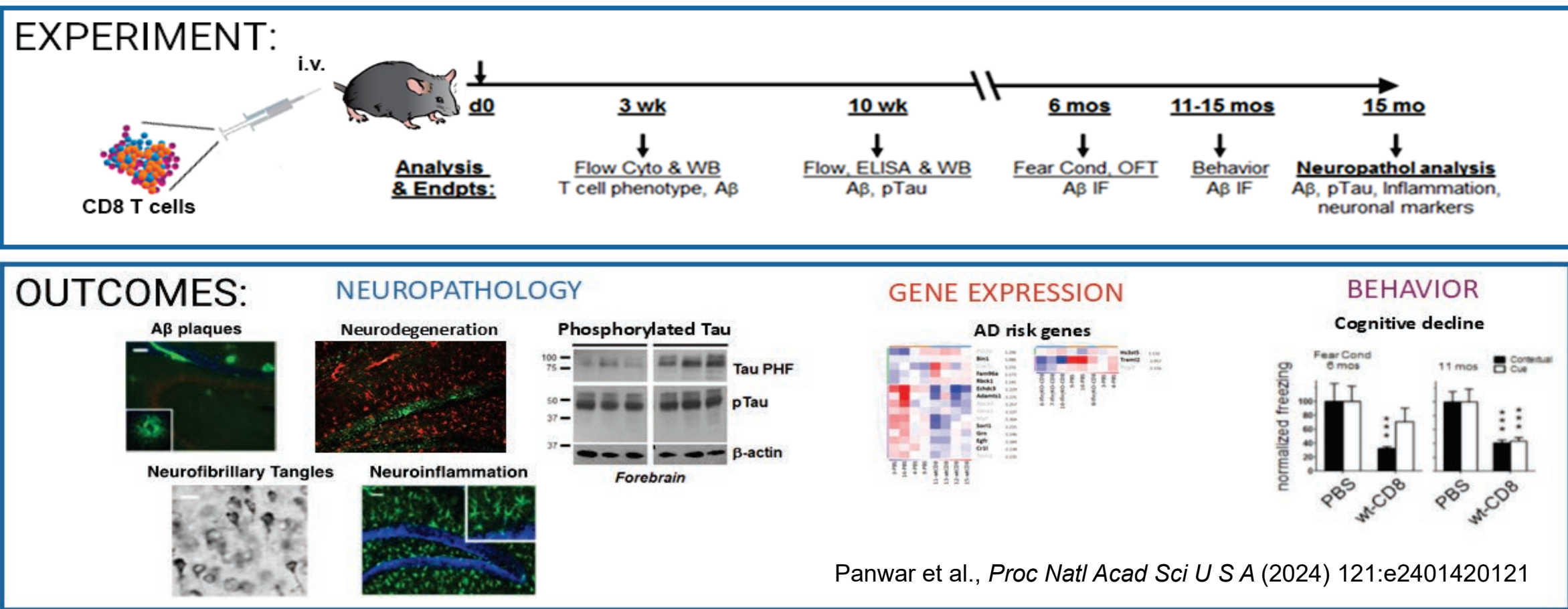


Fig. 1: Summary of published findings: Homeostatically-induced CD8 T cell ("hiT") accumulation induces AD-like pathophysiology in mice. These cells selectively accumulated in brains of these "hiT" mice. Panwar et al., 2020; Panwar et al., 2024.

2 Relevance of hiT cells to AD patients

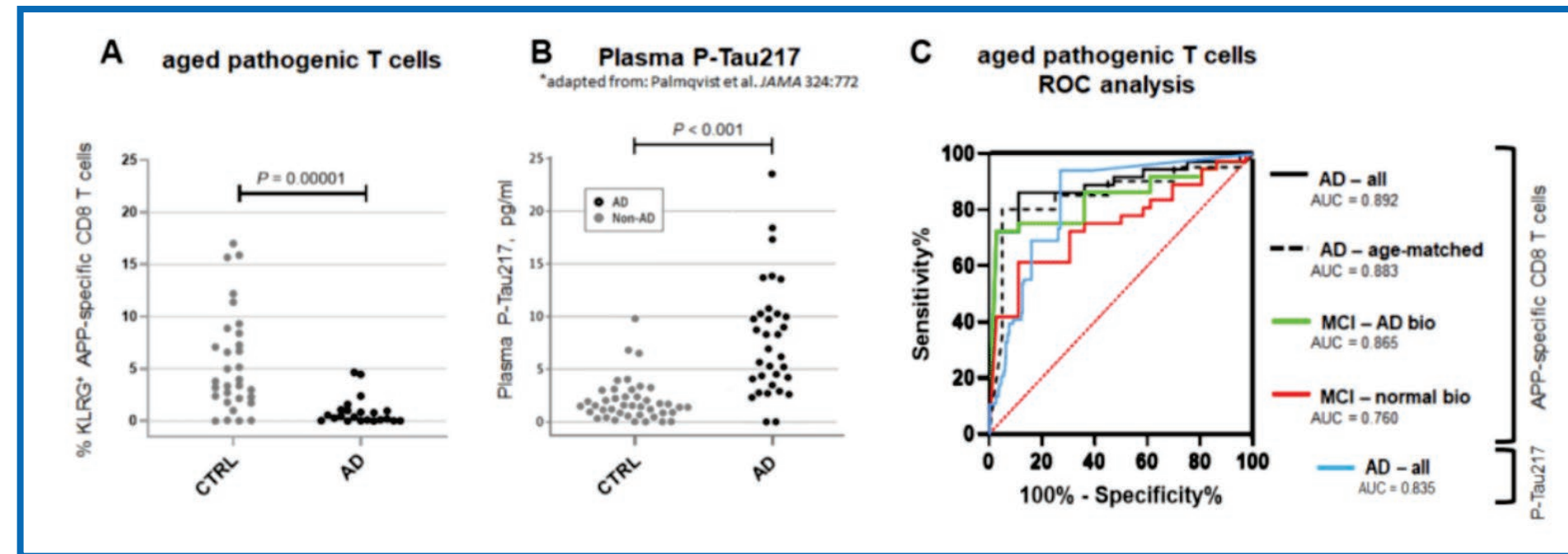


Fig. 2: Summary of published findings: Comparison with P-Tau217 and receiver operating characteristic (ROC) curves for hiT cell levels in human HLA-A2+ patients. Levels of APP/HLA-A2 dextramer-binding KLRG1+ CD8 T cells within blood lymphocytes of AD patients in our study (Panwar et al., 2024; A) relative to published plasma levels of P-Tau217 (Palmqvist et al., 2020; B). ROC plots of APP/HLA-A2 dextramer+ KLRG1+ CD8 T cells in blood of indicated patient cohorts relative to normal aging controls (C). $P \leq 0.003$ for all curves.

3 APP dex "universal" dex

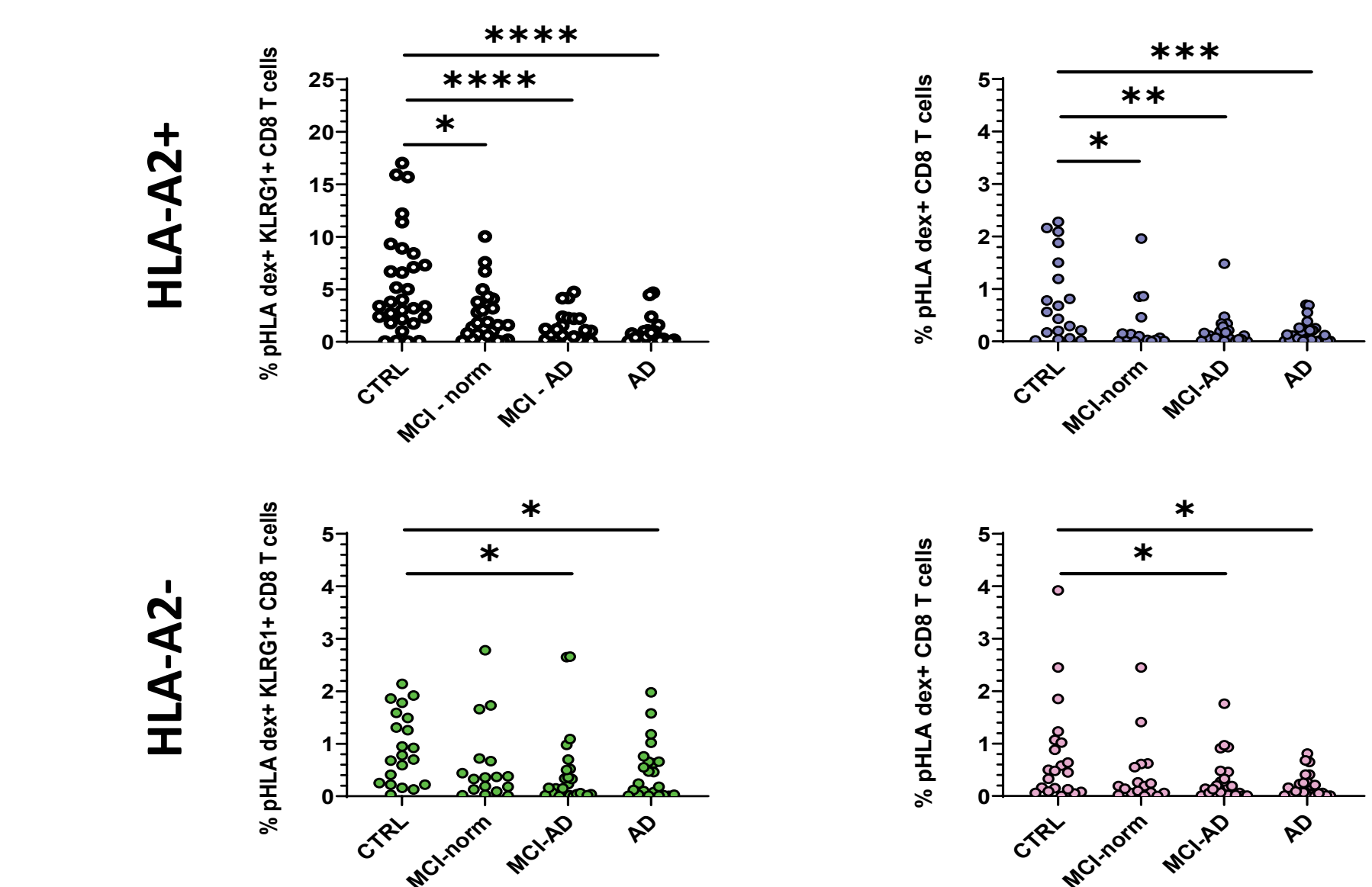


Fig. 3: New Findings: hiT cells in human Alzheimer’s. Levels of hiT cells binding APP /HLA-A2 dextramer (left column), and to non-self Universal/HLA-A0201 dextramer (right column), in blood of AD, MCI related (MCI-AD) or not (MCI-norm) to AD, and normal aging (CTRL) patients positive (top row) or negative (bottom row) for HLA-A2 from the Univ. of Antwerp cohort. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$, **** $P < 0.0005$. 2x T-test.

5 APP dex "universal" dex

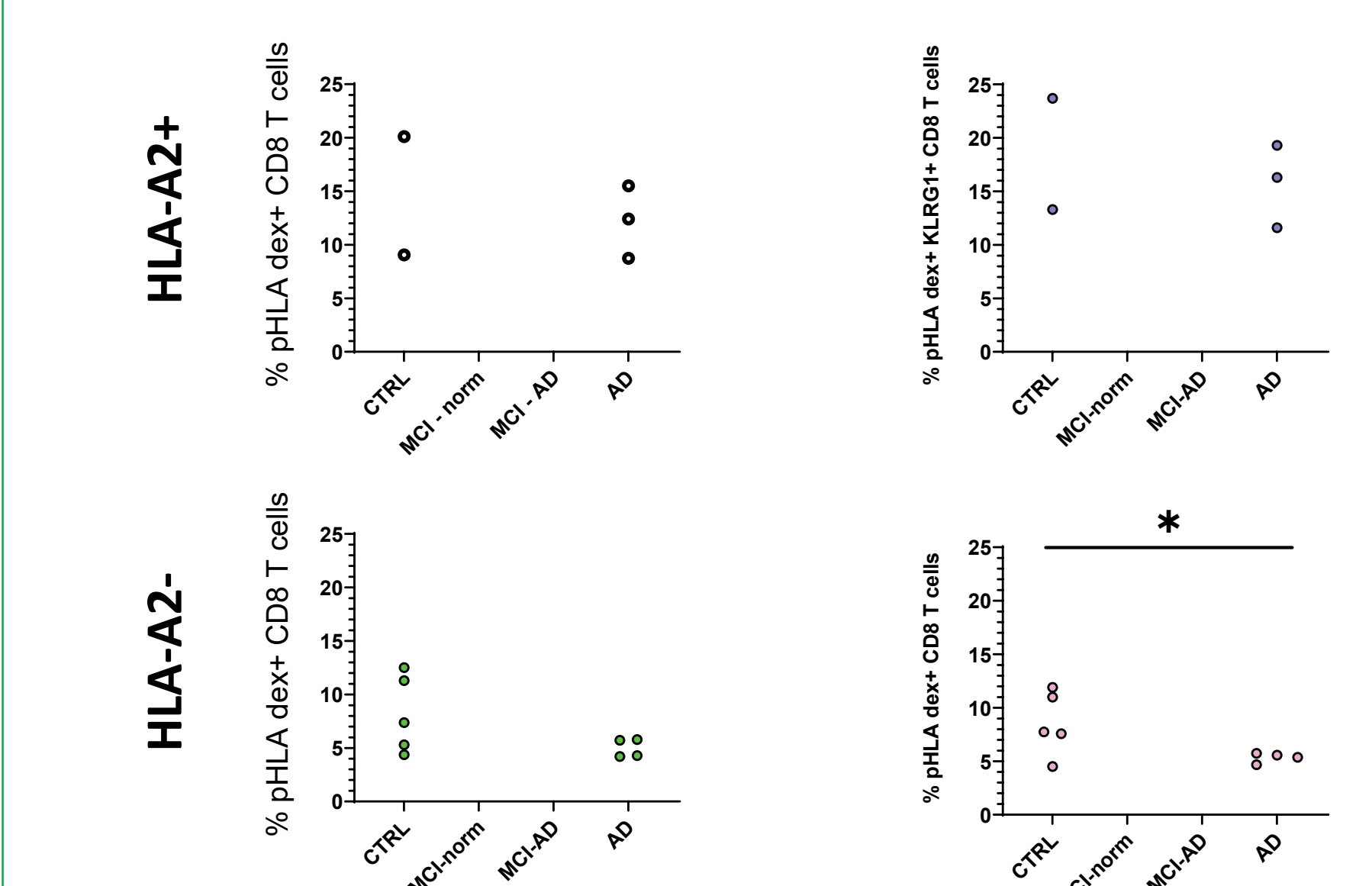


Fig. 5: Emerging Findings: Interim analysis of hiT cell metrics in USC ADRC cohort. Levels of CD8 T cells APP-specific (left column), or to "universal", HLA-A2-independent dextramer (right column), in blood of AD and normal aging (CTRL) patients positive (top row) or negative (bottom row) for HLA-A2. * $P < 0.05$, 1x T-test. Despite low numbers, the strongest trend (CTRL vs AD difference) is still observable in HLA-A2- patients, particularly with the universal dextramer.

4 A MCI-norm MCI-AD AD

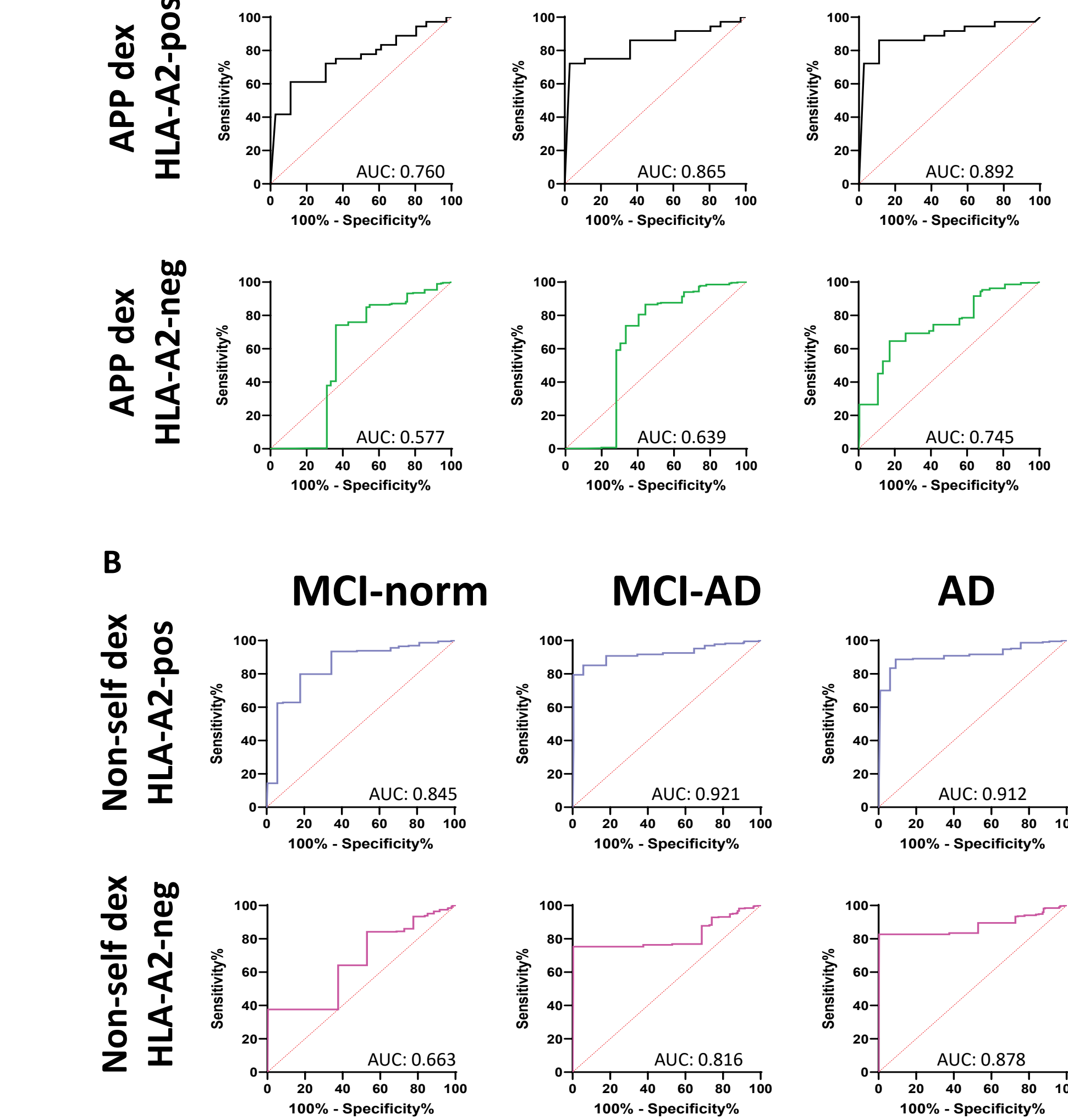


Fig. 4: New Findings: Biomarker performance for human AD and MCI. ROC plots for levels of APP/HLA-A2 (A) or "Universal"/HLA-A2 (B) – dextramer binding hiT cells in blood of MCI or AD relative to normal aging patients in the Univ. of Antwerp cohort. $P < 0.001$ for all curves.

6 CD4 CD8

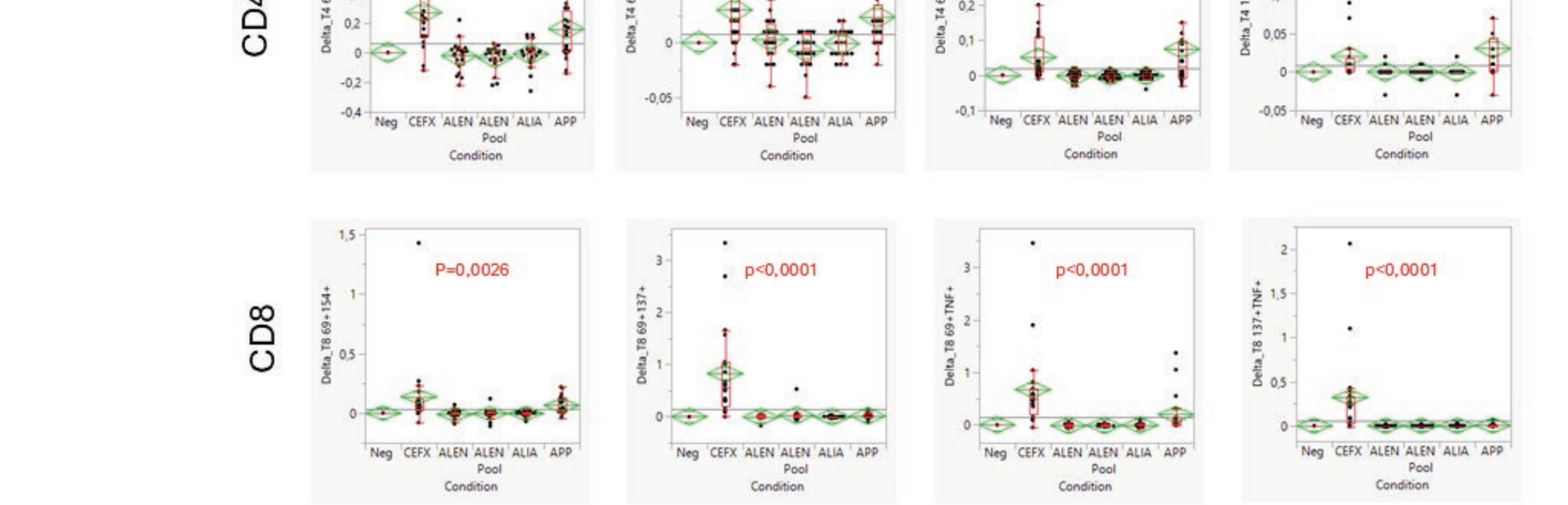


Fig.6: Emerging Findings: Whole Blood Activation-Induced Marker (WAIM) assay changes following APP stimulation. Flow cytometry screen for activation markers (CD69, CD154) on fresh blood T cells from 16 non-diseased human subjects, stimulated 16 hr. with positive control (CEFX), APP dextramer peptides, pooled APP peptides, or whole APP protein. Significance (ANOVA) vs. control is shown.

Summary of Results

As with the APP pHLA dextramer in HLA-A2+ patients, levels of hiT cells binding to the "universal" pHLA dextramer were significantly diminished in AD and MCI-AD, and independent of HLA-A2 status. In the HLA-A2- cohort, AUC for hiT cells binding to the universal dextramer distinguished AD and MCI-AD from controls (AUC 0.912 for AD; 0.921 for MCI-AD) at least as well as the APP dextramer in HLA-A2+ patients. hiT cell reduction was also seen in HLA-A2-negative AD patients from USC ADRC in interim testing, supporting further validation studies using ADRC specimens. Reactivity to APP protein by both CD4 and CD8 T cells in the WAIM analysis of normal human blood reveals additional possibilities for HLA-independent assay formats.

Conclusions

Levels of hiT cells binding and/or responding to specific antigens strongly corresponds to AD and related MCI, and existing assays can be adapted for use in both HLA-A2+ and HLA-A2- patients. Ongoing work will focus on developing additional options for assay expansion to all patients regardless of HLA subtype status.