LymPro Assay for Alzheimer's Disease
Pilot Clinical Data - Interim Analysis

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Lymphocyte Proliferation Test: LymPro

Can we develop a blood test for Alzheimer's disease?

Measuring the Brain in Blood: AD v. HC

Lymphocytes can reflect AD

AD = Alzheimer's Disease

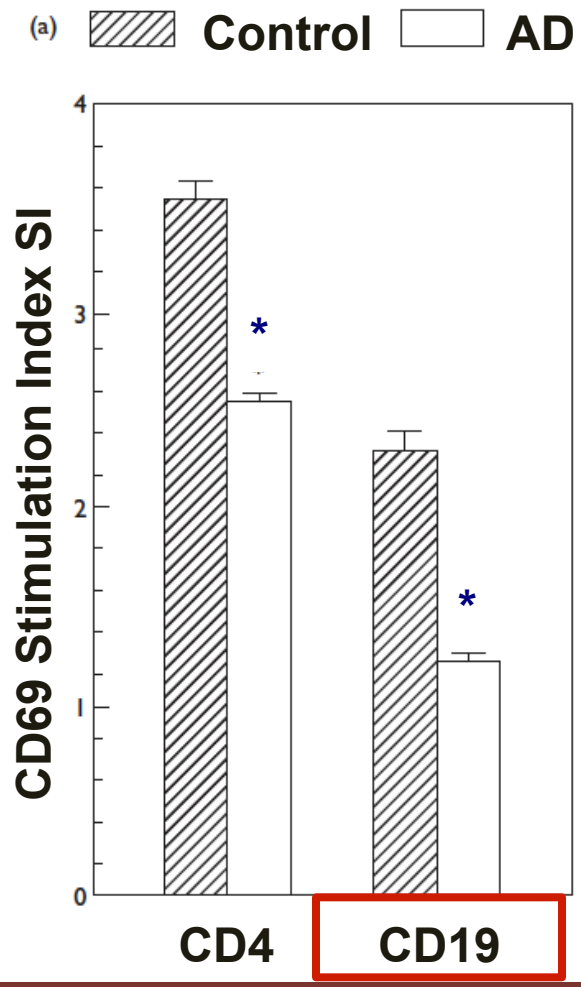
PDD = Parkinson's disease dimentia

HC = Healthy Control

CD4, CD14, CD19 = Lymphocytes

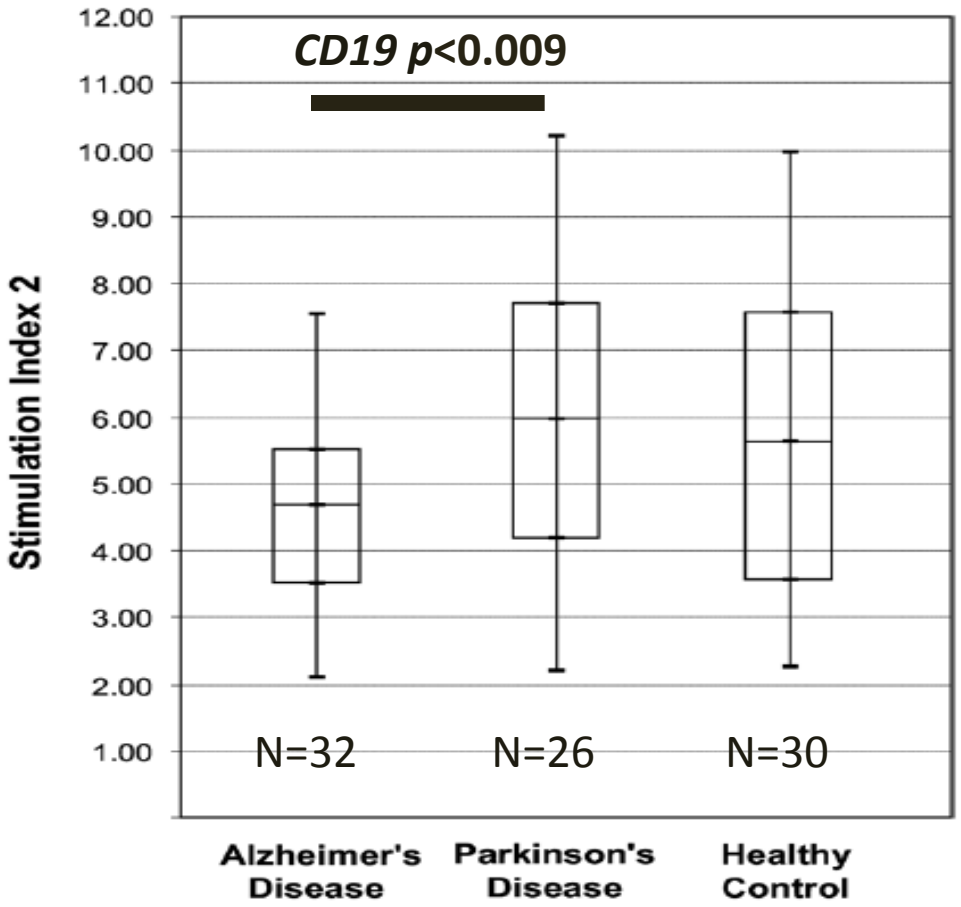
CD69 = Lymphocyte cell surface marker

SI = Stimulated / Unstimulated



Stieler, JT, and Arendt, T.; et al. Neuroreport 2001 **12(18).**

Replication of the 2001 Findings



Stieler, J et al, 2012. Neurobio Aging **33**:234-341.

LymPro Clinical Trial - LP-002 Study

Primary Study Objective:

Replicate the 2 published works that shows meaningful differences between Alzheimer's disease and Healthy Controls with LymPro

LP-002 Original Study Design

2 Cohorts → AD and Healthy Controls

Enrollment → 72 Subjects; 36 per cohort

Blinding → Assay & Clinical Operations

AD → MMSE ≤ 22 **HC → MMSE ≥ 29**

Excluded: Autoimmunity, immune meds

MMSE = Mini-mental state exam

LP-002 Assay Methodology

- 8 ml blood collection
- Purify lymphocytes and stimulate with mitogen (PHA/PWM for 4 hrs)
- Stained for:
  - Cell sub population (e.g., T-Cells, B-Cells)
  - CD69 (measure of cell division)
- Measure CD69 expression levels with flow cytometry
- Compare Alzheimer's (AD) to Healthy Controls (HC)

LP-002 Study Interim Analysis:

Objective

Evaluate the data available from the single site with reasonable patient enrollment.

Interim study: Analyzed Population

Subjects Recruited

AD = 10
HC = 34
Tot 44

Age

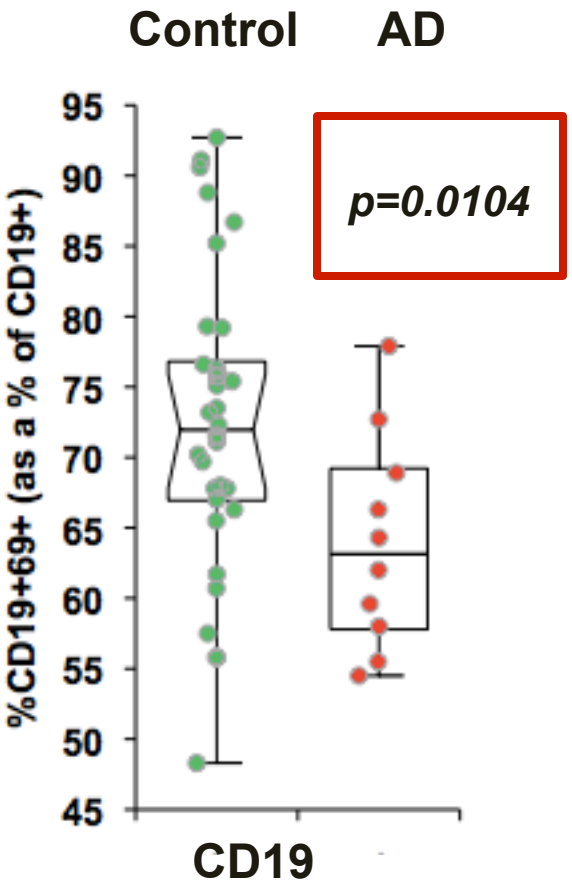
AD = 70.8
HC = 67.1

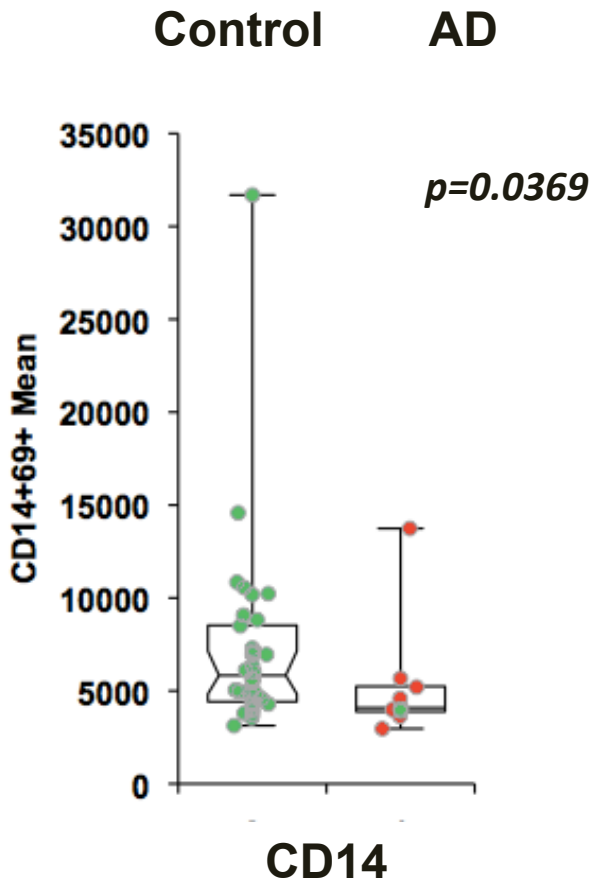
similar

Study Limitations

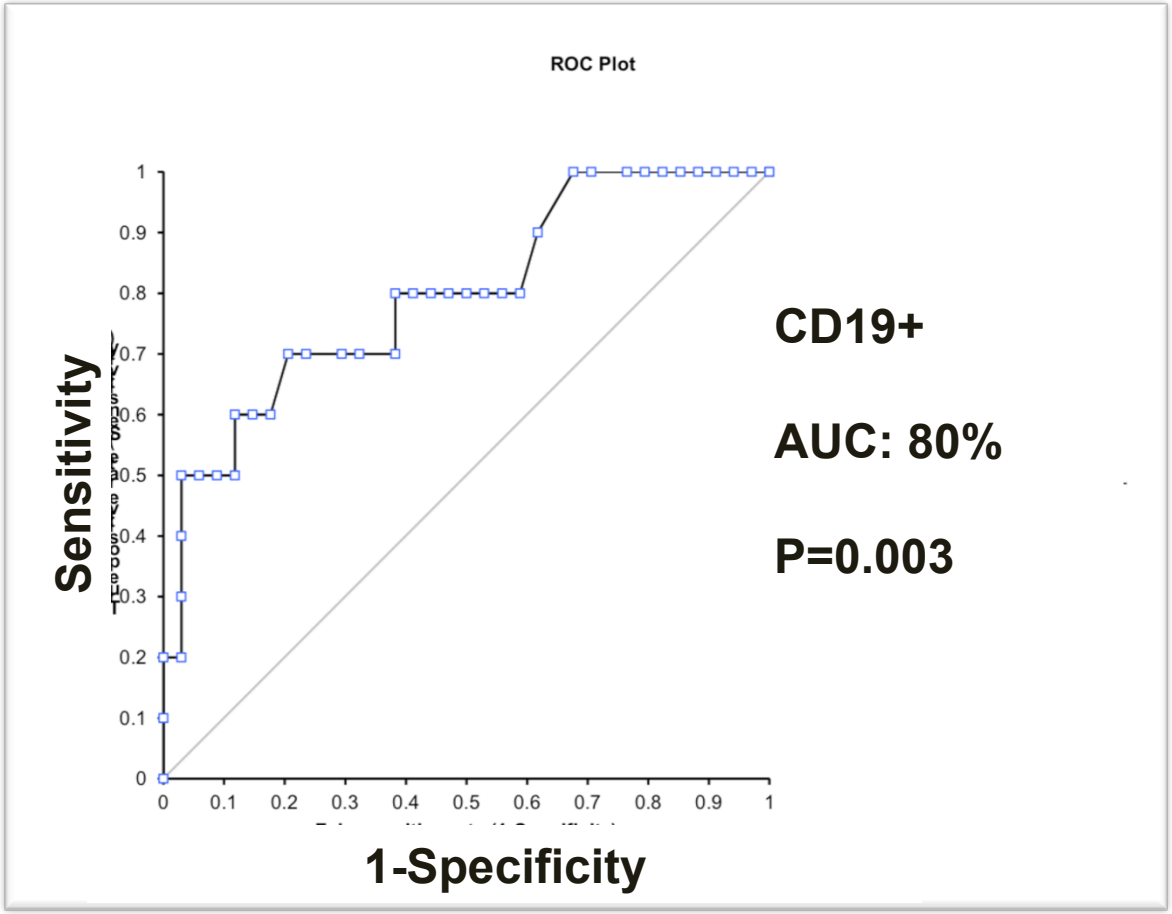
- Small, underpowered cohort size (single site)
- Interim analysis with training set only
- Assay not fully validated / characterized

Key Interim Result Findings: Replication of the previous 2 papers





Clinical Utility: CD19 Predictive Model

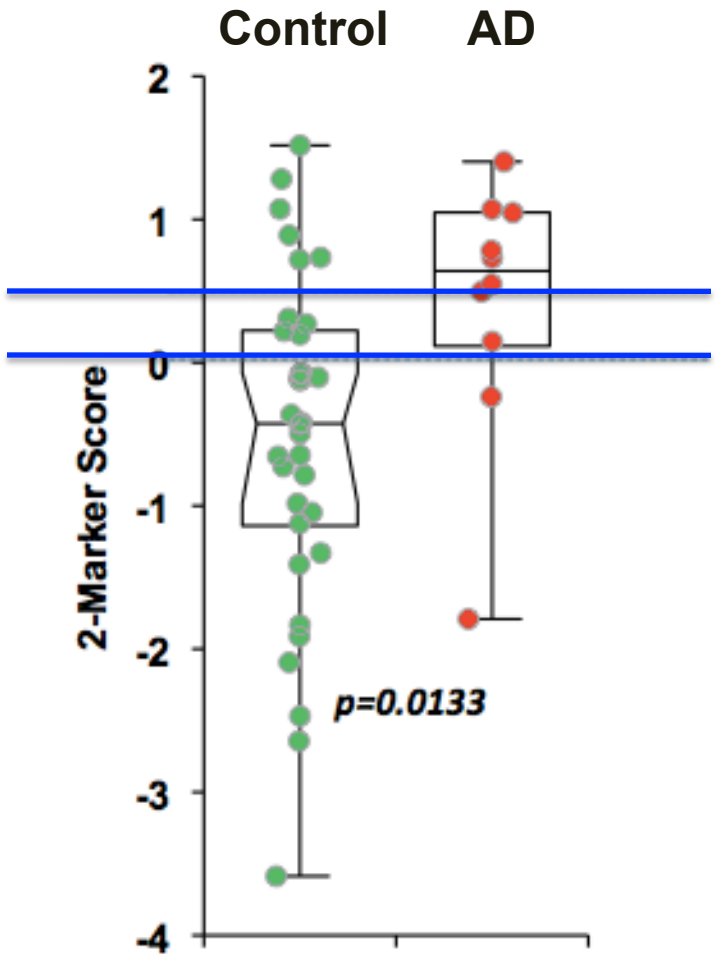


Statistical Significance of Markers

Variable (PHA4)	AUC	AUC 95% CI	Optimal Cutpoint	Sensitivity (95%CI) @optimal cutpoint	Specificity (95%CI) @optimal cutpoint	PPV (95%CI) @optimal cutpoint	NPV (95%CI) @optimal cutpoint	Odds Ratio (95% CI) @optimal cutpoint
%CD3+4+69+	0.559	0.323-0.795						
CD3+4+69+ MFI	0.553	0.351-0.755						
%CD3+8+69+	0.566	0.348-0.785						
CD3+8+69+ MFI	0.671	0.485-0.857						
%CD19+69+	***0.797***	***0.628-0.966***	***≤85.2***	***70.0 (34.8-93.3)***	***79.4 (62.1-91.3)***	***20.6 (8.7-37.9)***	***30.0 (6.7-65.2)***	***9.0 (1.8-44.0)***
CD19+69+ MFI	0.650	0.465-0.835						
%CD14+69+	0.593	0.398-0.788						
CD14+69+ MFI	0.635	0.417-0.853						

Variable (PWM4)	AUC	AUC 95% CI	Optimal Cutpoint	Sensitivity (95%CI) @optimal cutpoint	Specificity (95%CI) @optimal cutpoint	PPV (95%CI) @optimal cutpoint	NPV (95%CI) @optimal cutpoint	Odds Ratio (95% CI) @optimal cutpoint
%CD3+4+69+	0.537	0.318-0.755						
CD3+4+69+ MFI	0.568	0.372-0.764						
%CD3+8+69+	0.679	0.490-0.869						
CD3+8+69+ MFI	0.537	0.316-0.758						
%CD19+69+	***0.749***	***0.576-0.921***	***≤66.3***	***70.0 (34.8-93.3)***	***76.5 (58.8-89.3)***	***23.5 (10.7-41.2)***	***30.0 (6.7-65.2)***	***7.6 (1.6-36.4)***
CD19+69+ MFI	0.518	0.323-0.713						
%CD14+69+	0.538	0.332-0.745						
CD14+69+ MFI	***0.721***	***0.526-0.915***	***≤4146***	***60.0 (26.2-87.8)***	***82.4 (65.5-93.2)***	***17.6 (6.8-34.5)***	***40.0 (12.2-73.8)***	***7.0 (1.5-32.7)***
2-Marker Score	***0.762***	***0.588-0.935***	***>3.085***	***70.0 (34.8-93.3)***	***79.4 (62.1-91.3)***	***20.6 (8.7-37.9)***	***30.0 (6.7-65.2)***	***9.0 (1.8-44.0)***

The cutpoint can be tuned to achieve enrichment of the desired population



70% Sensitivity at 82.4% Specificity

80% Sensitivity at 70.6% Specificity

Future Work

- Evaulate the clinical performance of an alternate form of the stimulation parameters (Version 2)
- Complete and report full results from current study, better understand significant markers
- Full assay performance package at CLIA / GMP certified laboratory – negotiations ongoing
- Further studies with biomarker defined subjects in dementia, MCI and prodromal AD
- Evaluate role of LymPro in TBI/CTE

Conclusions

- The LP-002 study interim analysis corroborated findings from two previous published studies

  TREND CONFIRMED!

- LymPro markers were observed to be statistically significantly between AD and HC in Amarantus' hands
- Further studies with biomarker defined subjects may enhance our understanding of the assay
- We may be able to modify 'cut-point' to enrich trials

Thank you

Acknowledgements

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