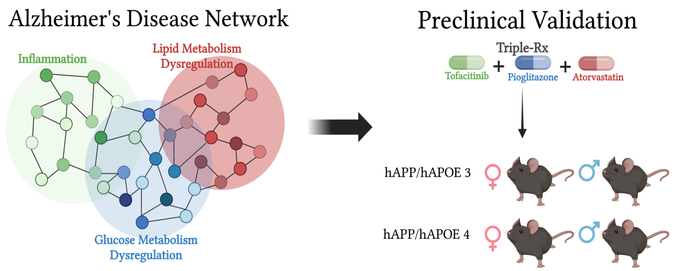
Combination Therapy Attenuates Alzheimer's Amyloid Pathology While Preserving Microglial Homeostasis in a humanized *APP/APOE* Risk Model

Sydney Ragsdale[1,2], Georgina Torrandell-Haro, Ph.D.[1,3], Hannah Van Rossum, Ph.D.[1,2], Mikaila Bantugan[1,2], Anna Parker[1], Roberta Diaz Brinton, Ph.D.[1,3,4]

[1]Center for Innovation in Brain Science, [2]Neuroscience Graduate Interdisciplinary Program, Departments of [3]Pharmacology, and [4]Neurology, University of Arizona College of Medicine, Tucson AZ

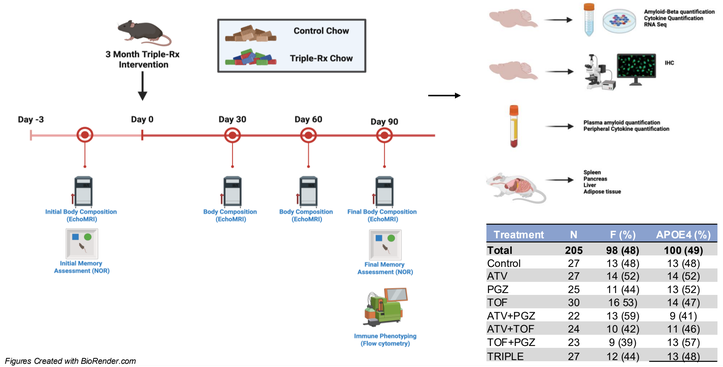
INTRODUCTION

- The prodromal phase of AD begins decades before cognitive symptoms, characterized by metabolic dysregulation, immune-metabolic vulnerability, and early amyloid accumulation[1,2]. Targeting these systems through FDA-approved therapeutics during this window could reduce AD risk[3].
- Our previous epidemiological analyses demonstrated that lipid-lowering[4], glucose-regulating[5], and anti-inflammatory[6] therapeutics were each associated with lower AD risk, with the greatest benefit when used in combination[7].
- Triple combination therapy (Triple-Rx) consisting of atorvastatin (lipid metabolism), tofacitinib (JAK inhibition), and pioglitazone (PPARγ agonism/glucose regulation), targets complementary multisystem pathways implicated in early AD pathogenesis[8,9,10].
- This study demonstrated that Triple-Rx attenuates early amyloid pathology (6E10) while preserving microglial homeostasis (Iba1), without inducing peripheral immune activation, in humanized (h) *APP*/h*APOE3* and h*APP*/h*APOE4* mice, a model recapitulating sex- and APOE-dependent AD risk vulnerabilities[11,12].



METHODS

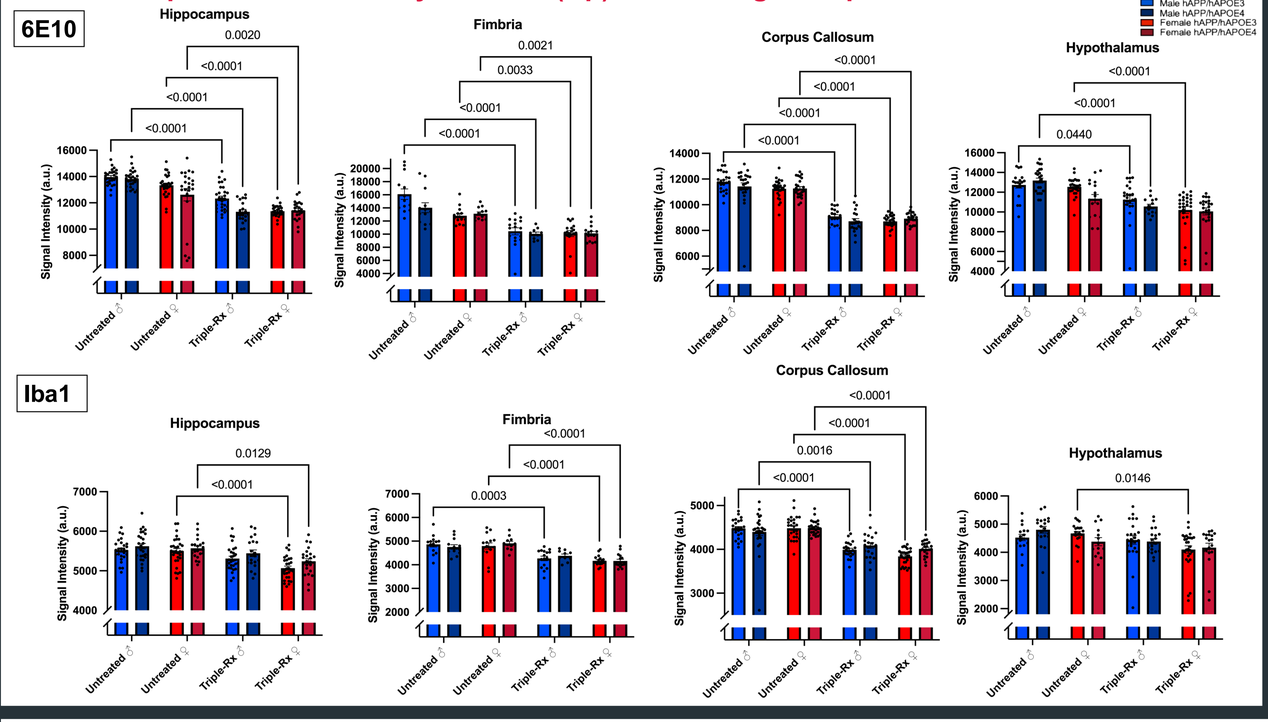
- 15-month female and male h*APP*/h*APOE3* and h*APP*/h*APOE4* mice were treated for 90 days with control chow, monotherapies (atorvastatin [ATV], tofacitinib [TOF], or pioglitazone [PGZ]), or combinatory chow regimens, including a triple-Rx: ATV+TOF+PGZ (300/250/200 mg/kg).
- Microglial-related neuroinflammatory status was assessed by Iba1 immunolabeling, while peripheral immune activation was evaluated via flow cytometry.
- Amyloid-associated pathology was assessed using 6E10 immunolabeling, and plasma Aβ42:40 ratios were measured via MSD assays to assess peripheral amyloid.
- Cognitive function was assessed via novel object recognition (NOR) and body composition was assessed via EchoMRI before and after treatment.



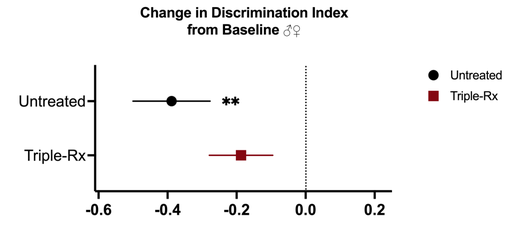
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Treatment	N	F (%)	APOE4 (%)
Total	**205**	**98 (48)**	**100 (49)**
Control	27	13 (48)	13 (48)
ATV	27	14 (52)	14 (52)
PGZ	25	11 (44)	13 (52)
TOF	30	16 53)	14 (47)
ATV+PGZ	22	13 (59)	9 (41)
ATV+TOF	24	10 (42)	11 (46)
TOF+PGZ	23	9 (39)	13 (57)
TRIPLE	27	12 (44)	13 (48)

Triple-Rx reduced amyloid-beta (Aβ) and microglial expression in the brain.

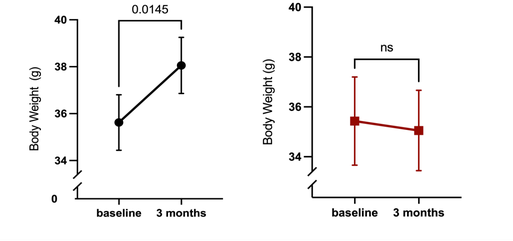


Triple-Rx prevents decline in cognition.

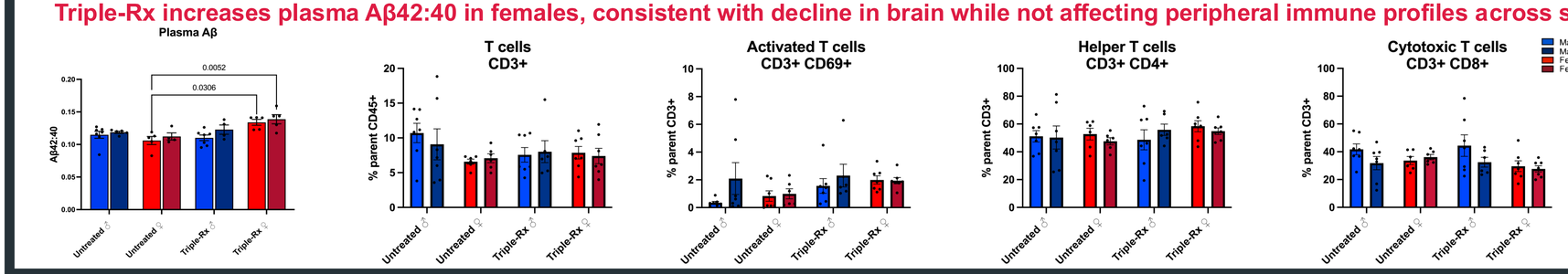


	Baseline DI	3 months DI	Paired t test	% DI Change	Unpaired t test
	Mean (SEM)	*Mean (SEM)*	*p value*	*Mean*	*p value*
Control	0.29 (0.08)	-0.08 (0.07)	***<0.01***	-38.84	*ref*
Triple	0.09 (0.07)	-0.10 (0.07)	*0.07*	-18.70	*0.17*

Triple-Rx prevents age-associated weight gain.



Triple-Rx increases plasma Aβ42:40 in females, consistent with decline in brain while not affecting peripheral immune profiles across sexes.



RESULTS & DISCUSSION

- Iba1 immunoreactivity under triple-Rx was differentially reduced across sex, genotype, and region, demonstrating preserved microglial homeostasis without triggering neuroinflammation or peripheral immune activation.
- Triple-Rx significantly reduced 6E10 across multiple brain regions in both sexes and APOE genotypes, indicating broad attenuation of early amyloid-associated pathology in a pre-symptomatic disease context.
- Elevated plasma Aβ42:40 ratios in triple-Rx animals suggest enhanced peripheral amyloid clearance, alongside preserved cognitive performance and body weight trajectories, supporting the safety and tolerability of this multi-pathway strategy.
- These findings support combination therapy as a precision prevention strategy for AD and highlight IHC-based amyloid and microglial endpoints as indicators of therapeutic efficacy during early, pre-pathological disease stages.

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