Discovery report for Companion Target Discovery For Drug Expansion

Research Objective

Check out this new drug and its target. Come up with an another target that would pair well with it, for both efficacy and indication expansion.
https://www.roche.com/media/releases/med-cor-2025-06-16

Summary of Discoveries

Discovery 1: LRRK2 inhibition complements α-synuclein immunotherapy by aligning with Fc-receptor phagocytosis and lysosomal repair in microglia

Prasinezumab neutralizes extracellular aggregated αsynuclein but leaves predominant intracellular pathology and lysosomal stress insufficiently addressed. Convergent mechanistic and human microglial transcriptomic data support LRRK2 kinase inhibition as a complementary partner that lowers exosomal αsyn flux, restores glucocerebrosidase trafficking, and aligns with Fcreceptormediated phagocytosis without amplifying NFB inflammation.

Discovery 2: Preserving Fc-receptor phagocytosis while dampening cytokines: NLRP3 inflammasome inhibition as an alternative to XPO1 for combination with α-synuclein immunotherapy

Prasinezumab neutralizes extracellular, aggregated αsynuclein and relies on Fc receptormediated microglial uptake for clearance, but clinical efficacy has been modest, highlighting a need for complementary mechanisms that temper neuroinflammation without blunting phagocytosis. Crossdisease transcriptomic and functional data argue that exportin1 (XPO1) inhibition does not deliver this separation, whereas suppressing the NLRP3 inflammasome can reduce IL1βdriven cytokines while preserving or improving microglial clearance programs. These findings nominate NLRP3 inhibition as a pragmatic partner to prasinezumab to improve efficacy and enable indication expansion across synucleinopathies.

Discovery 3: Indication expansion landscape: LRRK2 is PD-focused, while XPO1 and EIF4E lack propagation synergy and cross-disease robustness

Prasinezumab neutralizes extracellular, Cterminal aggregated alphasynuclein and likely leaves intracellular proteostasis and inflammatory drivers insufficiently addressed. Crossdisease analyses support pairing prasinezumab with LRRK2 inhibition for Parkinsons disease (PD) using biomarkerenriched designs, whereas XPO1 and EIF4E lack direct antipropagation evidence and show inconsistent diseaselayer validation, making them weak choices for efficacy synergy or indication expansion.

LRRK2 inhibition complements α-synuclein immunotherapy by aligning with Fc-receptor phagocytosis and lysosomal repair in microglia

Summary

Prasinezumab neutralizes extracellular aggregated αsynuclein but leaves predominant intracellular pathology and lysosomal stress insufficiently addressed. Convergent mechanistic and human microglial transcriptomic data support LRRK2 kinase inhibition as a complementary partner that lowers exosomal αsyn flux, restores glucocerebrosidase trafficking, and aligns with Fcreceptormediated phagocytosis without amplifying NFB inflammation.

Background

Pathologic αsynuclein adopts seedcompetent conformers that propagate transsynaptically and accumulate within neuronal and glial endolysosomal systems, driving neurodegeneration and microgliosis. Antibody therapies can neutralize extracellular seeds and engage Fcreceptordependent microglial clearance, yet their impact is constrained by low brain exposure and the fact that most αsynuclein resides intracellularly. Parallel genetics and cell biology implicate LRRK2 kinase as a nodal regulator of Rab GTPasedependent vesicular trafficking and lysosomal homeostasis, linking it to αsynuclein release, uptake, and degradation. Aligning extracellular immunotherapy with restoration of intracellular proteostasis in microglia provides a rational path to enhance efficacy and widen the clinical footprint of disease modification in Parkinsons disease.

Results & Discussion

Prasinezumab recognizes a Cterminal epitope (aa118126) with preferential affinity for aggregated/seedcompetent αsynuclein, sequesters extracellular species to limit neurontoneuron spread, and can drive Fcdependent microglial clearance; however, clinical translation has been constrained by low central exposure (CSF $\approx$0.3%), modest or equivocal efficacy in PASADENA with at most posthoc motor signals, and the reality that intracellular proteostasis defects dominate the pathological burden [r2, xiao2025, kallunki2025, pagano2021, pagano2021a, geerts2023, nimmo2021amyloidβandαsynuclein, menon2022]. Preclinical work with the parent 9E4 antibody suggests autophagy/lysosomal engagement and reduced gliosis, but comparable restoration of lysosomal function has not been demonstrated in humans, and additional barriersincluding exosomal shielding, Cterminal truncation that weakens epitope recognition, and strain heterogeneityfurther limit target engagement in vivo [r2, menon2022, geerts2023, kallunki2025]. These features underscore the need for a mechanistically orthogonal partner that addresses intracellular αsynuclein biology and the endolysosomal axis while preserving or enhancing Fcmediated clearance.

LRRK2 kinase directly interfaces with αsynuclein secretion and glial processing. Under lysosomal stress and after exposure to preformed fibrils, LRRK2 is recruited to lysosomes, phosphorylates Rab10, and promotes exocytic/exosomal release of insoluble αsynuclein from macrophage/microglial lineages; LRRK2 also modulates Rab8/10/35 and SNARE nodes (VAMP4/7) relevant to unconventional secretion, while G2019SLRRK2 neuronal systems show increased αsynuclein release into media [r9, abe2024, zhang2024, filippini2024, outeiro2019, cresto2019]. Beyond secretion, LRRK2 loss enhances microglial uptake/clearance of extracellular αsynuclein, and kinase inhibition (e.g., MLi2) restores impaired astrocytic handling, indicating kinasedependent control of glial cargo processing [r9, cresto2019, streubelgallasch2021, ohara2020lrrk2andαsynuclein]. While these data position LRRK2 inhibition to reduce extracellular αsynuclein availability and improve downstream handling, it is important to note that direct reductions of extracellular αsynuclein after LRRK2 inhibitor treatment and formal synergy with prasinezumab have not yet been demonstrated in the cited models,

rendering the combination mechanistically plausible but experimentally unproven [r9, filippini2024]. Independently, multiple studies show that pathogenic LRRK2 activity impairs glucocerebrosidase (GCase) enzymatic function by disrupting lysosomal delivery via Rab10mediated trafficking, and that pharmacologic LRRK2 inhibition restores GCase activity, thereby repairing a core lysosomal defect that intersects αsynuclein proteostasis [r24, brooker2021, pang2022, domenicale2024].

Human microglial transcriptomics from substantia nigra provide an additional, diseaserelevant link between LRRK2 and antibodyeffector biology. In a singlenucleus RNAseq pseudobulk analysis of 30 samples (microglia identified by AIF1 or P2RY12), LRRK2 expression correlated significantly with an Fcreceptor pathway module score (Spearman $\rho = 0.577$, p = 8.37Œ10^{-4}), with a stronger effect in PD/PDD ($\rho = 0.621$, p = 2.67Œ10^{-3}) and the strongest singlegene association observed for SYK ($\rho = 0.673$, p = 4.68Œ10^{-5}) [r75]. The Fcreceptor module was defined as the arithmetic mean (per sample) of nine genes central to Fcreceptormediated phagocytosisFCGR1A, FCGR2A, FCGR3A, SYK, PIK3CA, PIK3CB, VAV1, RAC1, and CDC42capturing receptor, proximal kinase, and actinnucleation control points in a manner consistent with a consensus Fcphagocytosis gene set (FcγRs, FCER1G, SYK/Src, PI3KPLCγ, VAVRac/Cdc42, and Arp2/3WASP/WAVE) [r74, r75, norris2022, bournazos2020, li2020]. Crucially, the LRRK2Fcreceptor relationship was independent of NFBdriven inflammation: LRRK2 showed no correlation with an NFB module ($\rho \approx 0$, p = 0.971), and the partial correlation between LRRK2 and the Fcmodule controlling for NFB increased to $\rho = 0.632$ (p = 2.36Œ10^{-4}), indicating that LRRK2 aligns with a phagocytic axis rather than general inflammatory activation [r80]. These data support a model in which LRRK2 activity intersects the very microglial program required for efficient clearance of antibodyopsonized cargo, positioning LRRK2 inhibition to complement, rather than undermine, Fcdependent immunotherapy.

Together, these mechanistic and human data motivate a precision combination of

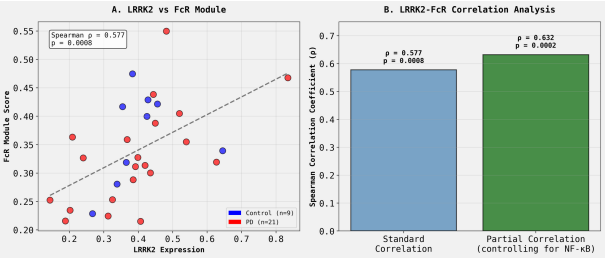

Figure 1: LRRK2 expression is positively correlated with an Fc-receptor (FcR) gene module independently of NF-B signaling. (A) Spearman correlation between LRRK2 expression and the FcR module score in control (n=9) and Parkinson's disease (PD, n=21) patient samples. (B) Bar plot comparing the standard Spearman correlation coefficient to a partial correlation coefficient after controlling for NF-B activity. These data indicate a specific relationship between LRRK2 and the FcR phagocytic machinery that is not confounded by general inflammatory signaling. (Source: [r80])

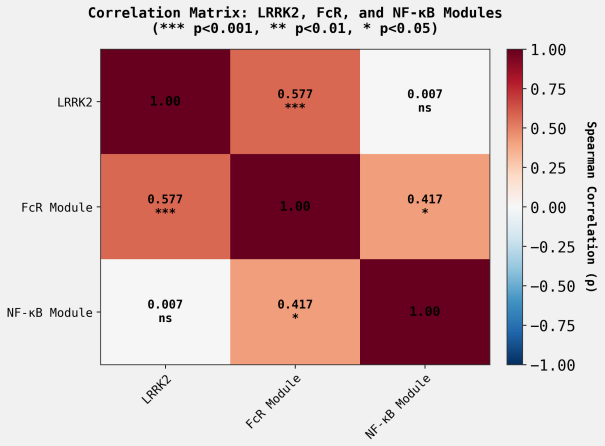

Figure 2: LRRK2 expression correlates with the Fc-receptor module but is independent of the NF-B inflammatory module. The heatmap displays the Spearman correlation matrix of transcriptomic modules for LRRK2, the Fc-receptor (FcR), and NF-B. This association pattern supports a link between LRRK2 and FcR-mediated phagocytosis that is decoupled from canonical NF-B inflammatory signaling. Significance is denoted as *p<0.05 and ***p<0.001; ns, not significant. (Source: [r80])

prasinezumab with a brainpenetrant LRRK2 inhibitor to: (i) reduce exosomal αsynuclein release via Rab10dependent lysosomal exocytosis, (ii) restore GCase trafficking and lysosomal degradative capacity, and (iii) optimize Fcreceptormediated phagocytosis of antibodyopsonized seeds while avoiding amplification of NFB inflammation [r2, r9, r24, r75, r80, abe2024, pang2022]. A biomarkeranchored trial design is feasible using established LRRK2 pharmacodynamic markerswholeblood pS935 and total LRRK2,

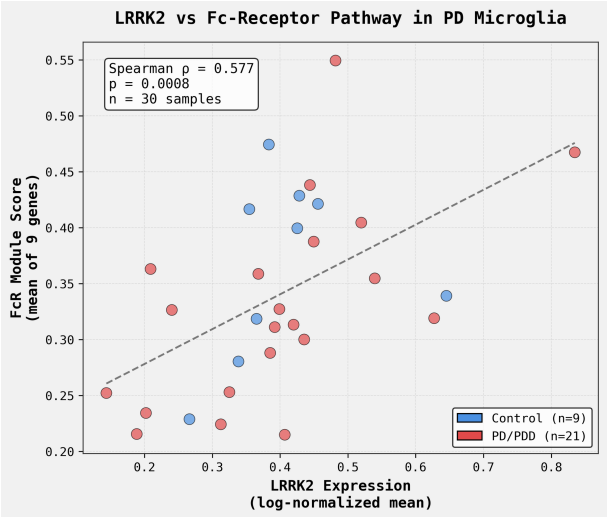

Figure 3: LRRK2 expression positively correlates with an Fc-receptor gene module score in human microglia. The scatter plot shows log-normalized mean LRRK2 expression versus the mean score of a 9-gene FcR module in samples from control (n=9) and PD/PDD (n=21) donors. This transcriptomic association suggests a functional link between LRRK2 and Fc-receptor pathways, which are critical for antibody-mediated clearance of α-synuclein. (Source: [r75])

neutrophil pRab10 (direct substrate), urinary bis(monoacylglycerol)phosphate (BMP), and urinaryexosomal pS1292 normalized to total LRRK2to select and verify target engagement in participants enriched by genotype (G2019S, R1441) or by elevated pathway activity (baseline pRab10, BMP) [r18, vissers2023, predmoreUnknownyearadvancingdevelopmentof]. Concurrent αsynuclein seedamplification assays can confirm the presence of pathogenic αsynuclein, while CSF/serum pharmacokinetics contextualize central engagement given the low CNS exposure of prasinezumab [r18, xiao2025, feldman2024]. This framework accommodates efficacy testing across idiopathic PD and PD with dementia, with prespecified subgroup analyses in LRRK2 and GBA mutation carriers to probe indication expansion through lysosomal rescue [r24, domenicale2024].

Finally, key gaps and next steps are tractable. Although convergent evidence argues that LRRK2 kinase activity elevates extracellular αsynuclein and impairs glial handling, direct demonstration that LRRK2 inhibitors lower extracellular αsynuclein in vivo and provide supraadditive benefit with prasinezumab remains to be shown; PFFseeded rodent studies quantifying interstitial and CSF exosomal αsynuclein under LRRK2 inhibition, with and without prasinezumab, should be prioritized [r9, filippini2024]. Given the observed coupling of LRRK2 to Fcphagocytic programs and its independence from NFB, ex vivo assays of human microglia should evaluate whether LRRK2 inhibition modulates FcγRSYK signaling and phagocytic indices for antibodyopsonized αsynuclein without triggering inflammatory transcription [r75, r80]. Collectively, this evidence positions LRRK2 inhibition as a mechanistically coherent and biomarkerenabled partner to prasinezumab, with the potential to augment efficacy and extend disease coverage within the αsynucleinopathy spectrum most immediately across PD and PD with dementia [r2, r9, r24, r75, r80].

Trajectory Sources

Trajectory r2: Defining prasinezumabs extracellular, Cterminalfocused engagement of aggregated alphasynuclein clarifies that core intracellular clearance defects, neuroinflammatory drivers, and mitochondrial dysfunction remain incompletely addressed and are logical targets for synergistic combination therapy (...

Trajectory r9: Preclinical evidence supports that LRRK2 kinase activity directly drives extracellular/exosomal release of pathogenic αsynuclein from macrophage/microglial lineages via Rab10, but there is no direct demonstration that LRRK2 inhibitors lower extracellular αsyn in models or that they synergize with ...

Trajectory r18: Yesthe literature contains actionable genetic, biochemical, and functional biomarkers for both XPO1 and LRRK2 that enable stratification and pharmacodynamic monitoring across PD and ALS/FTD, supporting identification of subpopulations most likely to benefit from prasinezumab plus a pathway inhibito...

Trajectory r24: The weight of evidence supports that LRRK2 kinase activity negatively regulates GCase enzymatic function, likely by impairing its lysosomal delivery via Rab10mediated trafficking disruptions (brooker2021 pages 4-6, pang2022 pages 6-8).

Trajectory r74: The literature supports compiling a consensus gene set for Fc-receptormediated phagocytosis in myeloid cells encompassing FcγRs, ITAM/Syk/Src signaling, PI3KPLCγ modules, Rho-family GTPases/GEFs, and Arp2/3WASP/WAVE actin machinery with validated roles in macrophages and related cells (norris2022...

Trajectory r75: LRRK2 expression is significantly and positively correlated with the Fc-receptor pathway module score in human PD microglia (Spearman $\rho = 0.577$, p = 8.37Œ10^{-4}, n = 30).

Trajectory r80: **ANSWER:**
The hypothesis is **STRONGLY SUPPORTED**. In PD microglia from the substantia nigra (GSE184950, n=30 samples), the positive correlation between LRRK2 and the Fc-receptor (FcR) module score is **independent** of the NF-B inflammatory signature.
Quantitative Evidence:
1. **Standard...

Preserving Fc-receptor phagocytosis while dampening cytokines: NLRP3 inflammasome inhibition as an alternative to XPO1 for combination with α-synuclein immunotherapy

Summary

Prasinezumab neutralizes extracellular, aggregated αsynuclein and relies on Fc receptormediated microglial uptake for clearance, but clinical efficacy has been modest, highlighting a need for complementary mechanisms that temper neuroinflammation without blunting phagocytosis. Crossdisease transcriptomic and functional data argue that exportin1 (XPO1) inhibition does not deliver this separation, whereas suppressing the NLRP3 inflammasome can reduce IL1βdriven cytokines while preserving or improving microglial clearance programs. These findings nominate NLRP3 inhibition as a pragmatic partner to prasinezumab to improve efficacy and enable indication expansion across synucleinopathies.

Background

Antibody therapies against αsynuclein seek to intercept extracellular seeds and limit neurontoneuron spread while engaging Fc receptordriven microglial clearance. Because microglia often coactivate inflammatory and phagocytic programs in human CNS tissue, combinations that dampen cytokines without impairing Fcdependent uptake are particularly attractive. Among candidate axes, nuclear export (XPO1) has been linked to inflammatory transcriptional programs, whereas inflammasome signaling via NLRP3 directly controls IL1β maturation largely at the posttranscriptional level. Determining which target best preserves antibodymediated phagocytosis while attenuating damaging cytokines is critical for enhancing efficacy and extending disease coverage.

Results & Discussion

Prasinezumab recognizes a Cterminal epitope on αsynuclein (aa118126) with strong preference for aggregated, seedcompetent species (subnanomolarpicomolar binding to preformed fibrils) while retaining measurable monomer affinity (KD ~620 nM), and it acts by sequestering extracellular seeds, reducing neuronal uptake, and promoting Fcdependent microglial clearance; preclinically it lowers inclusions and gliosis with incomplete rescue of dopaminergic neurons, and clinically it shows low CSF exposure (~0.3%) and modest or equivocal efficacy in PASADENA despite serum pharmacodynamic effects [r2, xiao2025, kallunki2025, geerts2023, pagano2021, pagano2021a, nimmo2021amyloidβandαsynuclein, menon2022]. These features underscore that intracellular proteostasis, neuroinflammatory drivers, and mitochondrial dysfunction remain incompletely addressed by extracellular neutralization alone and support rational combinations that preserve FcγRmediated phagocytosis while reducing inflammatory injury [r2, menon2022, geerts2023].

To identify a pairing that selectively attenuates inflammation without compromising phagocytosis, human transcriptomic analyses were reexamined using samplelevel module scores defined as the mean expression of curated gene sets for TNFα/NFB signaling (inflammation) and phagocytosis computed from pseudobulk microglial profiles and tested via Spearman correlation. In PD substantia nigra microglia, inflammatory and phagocytosis programs were strongly positively coupled (ρ = +0.751, p = 1.72 Œ 10^{-6}), arguing against a simple tradeoff between these functions; this relationship persisted after adjusting for XPO1 expression (partial r = 0.735, p = 5.53 Œ 10^{-6}) [r44]. XPO1 expression itself correlated with the NFB inflammatory module (ρ = 0.459, p = 0.0107) and was elevated in PD versus control microglia, but showed no significant association with the phagocytosis module (ρ = 0.264, p = 0.159), indicating it does not track with clearance capacity in this dataset [r41, r44]. The coupling of XPO1 to inflammatory and phagocytic modules

varied by context: in bulk spinal cord from the NYGC ALS cohort, XPO1 exhibited very strong positive correlations with both phagocytosis (ρ = 0.784, p = 2.77 Œ 10^{-33}) and NFB modules (ρ = 0.826, p = 1.22 Œ 10^{-39}) across ALS and controls, underscoring robust coactivation at tissue scale [r82]. Functionally, pharmacologic XPO1 inhibition (selinexor) reprogrammed macrophage checkpoints but did not increase phagocytic activity in the only direct assay identified, and no preclinical data were found demonstrating that XPO1 or EIF4Eaxis modulation reduces αsyn propagation, weakening the case for synergy with an antispread antibody [r16, r58, bernal2020, jimenez2020, menon2022, correddu2019, tan2020, tavassoly2021].

In contrast, convergent preclinical evidence shows that suppressing the NLRP3 inflammasome reduces cytokine output while preserving or enhancing microglial clearance. Genetic ablation of NLRP3 or ASC and smallmolecule inhibitors such as MCC950 and fenamates lower IL1β, reduce amyloid burden, improve cognition, and have been attributed to improved microglial Aβ phagocytosis, illustrating functional uncoupling of inflammatory cytokines from engulfment capacity in vivo and in vitro [r67, nizami2019]. Broader myeloid programs that bias toward proclearance while limiting inflammation (e.g., TREM2/TYROBP, TAM receptors, nuclear receptors LXR/RXR and PPARγ, and Nrf2) further validate the feasibility of reducing cytokines without blunting phagocytosis across CNS models, albeit with known context dependencies and safety considerations [r67, yu2022, chen2022, pinto2020]. Consistent with a mechanism acting posttranscriptionally, human microglial mRNA analyses showed that NLRP3 expression did not correlate with the NFB inflammatory module in PD (ρ = 0.022, p = 0.910) or AD (ρ = 0.141, p = 0.576), but did show a positive association with phagocytosis signatures (PD: ρ = 0.373, p = 0.042; AD: ρ = 0.437, p = 0.070), supporting preservation of phagocytic programs alongside potential cytokine suppression when targeting this axis [r71]. Together, these data suggest that NLRP3 inhibition can dampen inflammasomedependent cytokines while maintaining the FcγRmediated uptake that prasinezumab relies on.

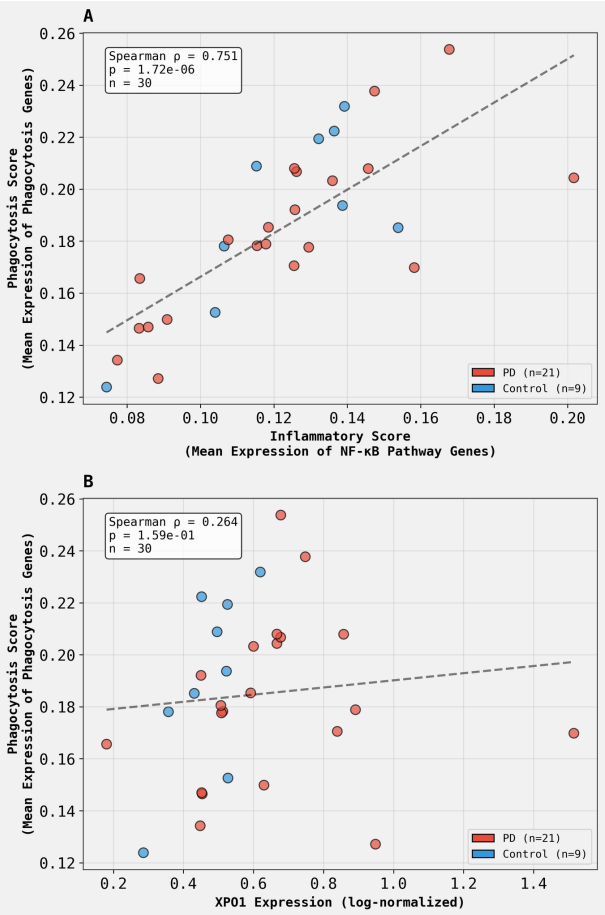

Figure 4: Phagocytosis and inflammatory gene programs are co-regulated in human brain tissue. (A) A phagocytosis gene module score shows a significant positive correlation with an NF-B pathway inflammatory score across brain samples from individuals with Parkinson's disease (PD, n=21) and controls (n=9). (B) In contrast, the correlation between the phagocytosis score and log-normalized XPO1 expression is not statistically significant in the same cohort. These findings suggest that broad suppression of inflammation may inadvertently compromise phagocytic activity due to the co-regulation of these pathways. (Source: [r44])

Practically, combining prasinezumab with a brainpenetrant NLRP3 inhibitor provides a mechanistically coherent strategy to improve efficacy by simultaneously neutralizing extracellular αsyn seeds and curbing IL1βdriven neuroinflammation without sacrificing Fc receptordependent phagocytosis. In earlystage trials, composite pharmacodynamic readouts could include CSF IL1β and related inflammasome markers, seedamplification activity for aggregated αsyn, serum free αsyn, and microglial activation imaging, alongside motor endpoints, to detect additive benefit over monotherapy [r2, pagano2021, menon2022]. Because the NLRP3phagocytosis

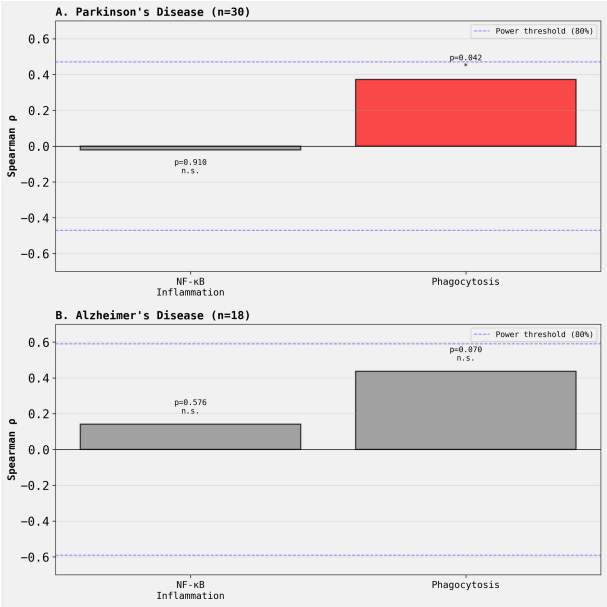

Figure 5: XPO1 expression correlates with a phagocytosis gene module in Parkinson's disease brain tissue. Bar plots show the Spearman correlation (ρ) between XPO1 transcript levels and curated module scores for NF-B inflammation and phagocytosis in post-mortem brain tissue from cohorts with (A) Parkinson's disease (n=30) and (B) Alzheimer's disease (n=18). The significant positive correlation between XPO1 and the phagocytosis module in Parkinson's disease (p=0.042) suggests that inhibiting XPO1 may compromise this clearance pathway. (Source: [r71])

relationship is observed across PD and AD microglia and cytokinephagocytosis uncoupling has been demonstrated in multiple CNS contexts, this pairing may also facilitate indication expansion to synucleinopathies beyond Parkinsons disease where extracellular seeding and microglial inflammasome activity contribute to pathology, while avoiding the contextdependent coupling and limited phagocytic support observed with XPO1directed approaches [r16, r41, r44, r58, r67, r71, r82].

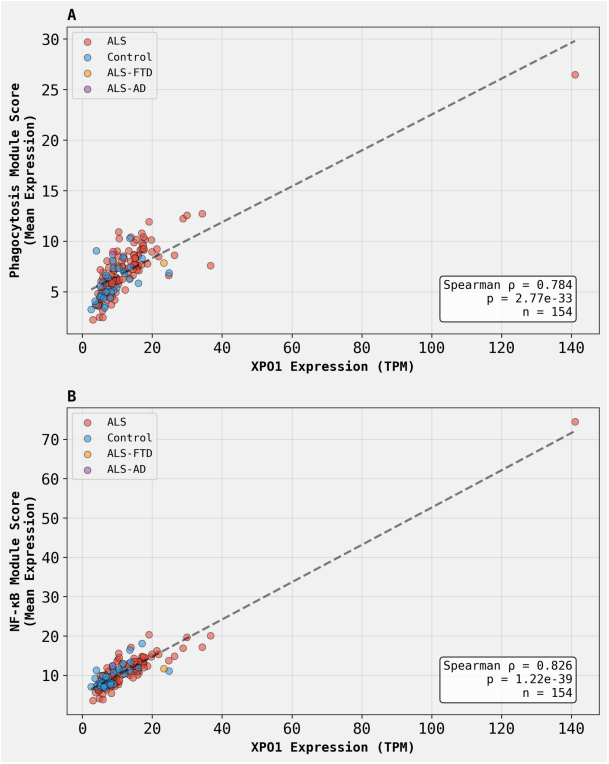

Figure 6: XPO1 expression is positively correlated with both phagocytosis and NF-B inflammatory gene module scores. Scatter plots of transcriptomic data show XPO1 expression (TPM) versus (A) a phagocytosis module score and (B) an NF-B module score in samples from control and neurodegenerative disease cohorts (n = 154). These strong correlations suggest that inhibiting XPO1 would suppress both phagocytic function and inflammation, failing to separate these two pathways. (Source: [r82])

Trajectory Sources

Trajectory r2: Defining prasinezumabs extracellular, Cterminalfocused engagement of aggregated alphasynuclein clarifies that core intracellular clearance defects, neuroinflammatory drivers, and mitochondrial dysfunction remain incompletely addressed and are logical targets for synergistic combination therapy (...

Trajectory r16: The available preclinical literature in the provided context does not demonstrate that inhibiting XPO1 (e.g., selinexor) or modulating EIF4E/cap-dependent translation (e.g., rapamycin, eIF4E/4E-BP manipulation) directly reduces cell-to-cell propagation or anatomical spread of α-synuclein, so the hyp...

Trajectory r41:
Analysis Results: XPO1 and NF-B Inflammatory Signature Correlation in PD Microglia
Primary Finding

XPO1 expression shows a **moderate, statistically significant positive correlation** with the NF-B inflammatory gene signature in microglia from substantia nigra tissue.
**Key Statistical ...

Trajectory r44:
ANALYSIS RESULTS
PRIMARY FINDING: HYPOTHESIS REJECTED
The hypothesis that pro-inflammatory state is negatively correlated with phagocytosis gene expression in PD microglia was **REJECTED**. Instead, a **strong positive correlation** was observed.
Primary Analysis Results: - **Inflamma...

Trajectory r58: The available preclinical evidence shows that pharmacologic XPO1 inhibition (selinexor) modulates myeloid phenotype but does not measurably alter phagocytic activity in macrophages, thus the hypothesis is not supported by functional assays to date (bernal2020 pages 150-157...

Trajectory r67: Published preclinical literature in neurodegeneration and stroke provides multiple examples where modulating defined myeloid pathways suppresses proinflammatory cytokine output while preserving or enhancing phagocytosis, supporting the research hypothesis. (nizami2019 pages...

Trajectory r71:
ANALYSIS RESULTS: NLRP3 Correlation with Inflammation and Phagocytosis in Neurodegenerative Diseases
Summary
NLRP3 expression does **not** exhibit the expected "uncoupling" signature (positive correlation with inflammation, no correlation with phagocytosis). Instead, across both Parkinson'...

Trajectory r82:
ANALYSIS COMPLETE: XPO1 Transcriptional Coupling in NYGC ALS Dataset
PRIMARY RESULTS
In the NYGC ALS Consortium bulk RNA-seq dataset from lumbar spinal cord (n=154: 119 ALS, 33 Control, 1 ALS-FTD, 1 ALS-AD), XPO1 expression shows **strong and highly significant positive correlations** with b...

Indication expansion landscape: LRRK2 is PD-focused, while XPO1 and EIF4E lack propagation synergy and cross-disease robustness

Summary

Prasinezumab neutralizes extracellular, Cterminal aggregated alphasynuclein and likely leaves intracellular proteostasis and inflammatory drivers insufficiently addressed. Crossdisease analyses support pairing prasinezumab with LRRK2 inhibition for Parkinsons disease (PD) using biomarkerenriched designs, whereas XPO1 and EIF4E lack direct antipropagation evidence and show inconsistent diseaselayer validation, making them weak choices for efficacy synergy or indication expansion.

Background

Alphasynucleinopathies are driven in part by prionlike spread of misfolded alphasynuclein, motivating immunotherapies that neutralize extracellular seeds to slow network propagation. Yet most toxic burden is intracellular and intertwined with lysosomal dysfunction and microglial activation, suggesting that antibodies alone may be insufficient and that rational combinations should couple extracellular seed control with restoration of intracellular clearance and immune homeostasis. Because proteinopathy biology varies across PD, multiple system atrophy (MSA), Lewy body dementia (LBD), tauopathies, and Alzheimers disease (AD), the selection of a second target should be guided by mechanistic complementarity to the antibody and by crossdisease robustness to support indication expansion.

Results & Discussion

Prasinezumab recognizes a Cterminal epitope on alphasynuclein (aa118126) with preferential affinity for aggregated/seedcompetent species (subnanomolarpicomolar to preformed fibrils) while retaining measurable monomer binding (KD ≈ 620 nM); Cterminal truncations (e.g., 1119) diminish recognition and inhibition [r2]. Proposed actions include sequestration of extracellular seeds to block neurontoneuron spread, reduced neuronal uptake from the synaptic cleft, and Fcdependent microglial clearance, with preclinical evidence that the 9E4/prasinezumab lineage can engage autophagylysosomal pathways in vitro [r2]. In vivo, prasinezumablike antibodies reduce inclusions and gliosis and preserve synaptic integrity but incompletely rescue dopaminergic neuron loss; clinically, serum free alphasynuclein decreases while central exposure is low (CSF ≈ 0.3%), PASADENA did not meet primary endpoints, and only posthoc motor signals emerged, consistent with a small extracellular target pool and limited CNS exposure [r2]. Methodofaction mapping indicates intersection with endolysosomal handling of internalized seeds and microglial activation, but no human data demonstrate restoration of lysosomal function or durable modulation of inflammatory cascades; mitochondrial rescue is unreported [r2]. Key constraints include predominantly intracellular pathology, epitope escape by Cterminal truncation, exosomal shielding, strain heterogeneity, and the lack of robust aggregatedspecies targetengagement assays, all of which argue for combinations that augment intracellular clearance and temper microglial Tolllike receptor signaling [r2].

Among candidate partners, LRRK2 stands out for PD on genetics, druggability, and orthogonal biology. LRRK2 is a gainoffunction serine/threonine kinase in monogenic and riskenriched PD, with cellular phenotypes (e.g., centrosomal alterations) rescued by kinase inhibition; multiple brainpenetrant smallmolecule inhibitors are in clinical development, with reversible pulmonary changes observed in nonhuman primates and programs that include G2019Sselective approaches [r6]. Mechanistically, LRRK2 inhibition targets intracellular trafficking, lysosomal biology, and immune cell function, complementing prasinezumabs extracellular seed neutralization; notably, many LRRK2PD cases are alphasynuclein seedingassay positive (≈67.5%), supporting synergy in synucleinopathydominant subgroups [r6]. Biomarker readiness is high: whole-

blood pS935 and total LRRK2, neutrophil pRab10 Thr73 (a direct substrate), urinary bis(monoacylglycerol)phosphate (BMP), and urinaryexosomal pS1292 normalized to total LRRK2 provide pharmacodynamic anchors; practical assay guidance favors neutrophils/whole blood and changefrombaseline analyses due to PBMC variability [r18]. A biomarkerenriched PD design should therefore combine prasinezumab with a brainpenetrant LRRK2 inhibitor, select alphasynuclein SAA-positive participants and enrich for LRRK2 mutation or elevated pathway activity (e.g., pRab10), and verify target engagement with pS935/pRab10 while tracking alphasynuclein SAA kinetics as diseaseproximal pharmacodynamics [r6, r18].

Crossdisease analyses constrain LRRK2based indication expansion. In AD microglia (snRNAseq, prefrontal cortex), LRRK2 shows no differential expression between AD and control across 4,126 nuclei (adjusted p=1.0; negligible effect size), arguing against a broad microglial transcriptional signal in AD [r26]. With tau, LRRK2 can modulate pathology in specific contextskinase inhibition (MLi2) normalized G2019Sdependent acceleration of AT8defined spread in a seedinjection modelbut evidence is mixed overall and absent for efficacy in classic tauopathy transgenics (e.g., P301S/P301L), limiting support for primary tauopathies such as PSP/CBD [r21]. In MSA, large genetic studies show no riskincreasing association and even a protective p.M2397T signal in pathologyconfirmed cohorts; mechanistic data in oligodendroglial systems are lacking [r31]. In LBD, rare pathogenic carriers exist but largescale analyses do not support a major risk effect; a locus tagging LRRK2 G2019S associates with reduced dementia within LBD, and LBDspecific mechanistic data are sparse [r54]. Together, these findings favor a PDfocused prasinezumab+LRRK2 strategy rather than nearterm expansion to AD, primary tauopathies, MSA, or LBD [r21, r26, r31, r54].

By contrast, XPO1 and EIF4E do not presently meet the bar for propagationoriented synergy or crossdisease robustness. Although AD transcriptomics reported modest XPO1 upregulation and EIF4E downregulation, an independent DIA LCMS/MS proteomics dataset (PXD034525) detected neither protein as significantly altered in hippocampus or temporal neocortex after FDR correction (FDR 0.077), with only medium, nonsignificant effect sizes in hippocampus and negligible effects elsewhere, undermining proteinlevel validity of the transcript signals [r15]. Critically, the preclinical literature surveyed here provides no demonstrations that XPO1 inhibition (e.g., SINEs) or EIF4E/4EBPdirected interventions reduce alphasynuclein propagation in PFFbased cellular or in vivo spread models; rapamycinlike autophagy induction lowers palphasyn in nonpropagation contexts but has not been shown to limit donortorecipient transfer or anatomical spread [r16]. In TDP43 systems, XPO1 inhibition does not increase nuclear TDP43 or reduce the cytoplasmic pool at tolerated doses, and redundancy of export routes (XPO1, XPO7, NXF1) further argues that blocking XPO1 alone is unlikely to constrain secretion or seeding; no studies tested TDP43 transmission endpoints under XPO1 blockade [r23]. Collectively, the lack of propagation data and discordant diseaselayer validation weaken the rationale for pairing prasinezumab with XPO1 or EIF4E for efficacy or expansion [r15, r16, r23].

Programmatically, the data prioritize a PDfo-

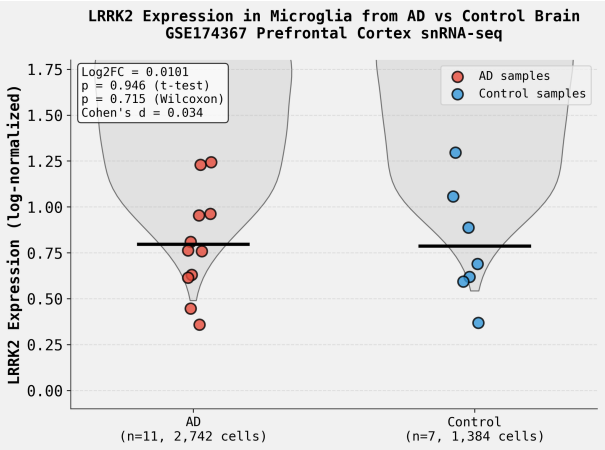

Figure 7: LRRK2 expression in microglia is not significantly altered in the Alzheimer's disease prefrontal cortex. The violin plot displays log-normalized LRRK2 expression from snRNA-seq data comparing Alzheimer's disease (AD; n=11) and control (n=7) donors, with statistical analysis showing no significant difference between groups ($p > 0.7$). This lack of differential expression in AD supports a disease-specific therapeutic rationale for LRRK2 inhibition in Parkinson's disease. (Source: [r26])

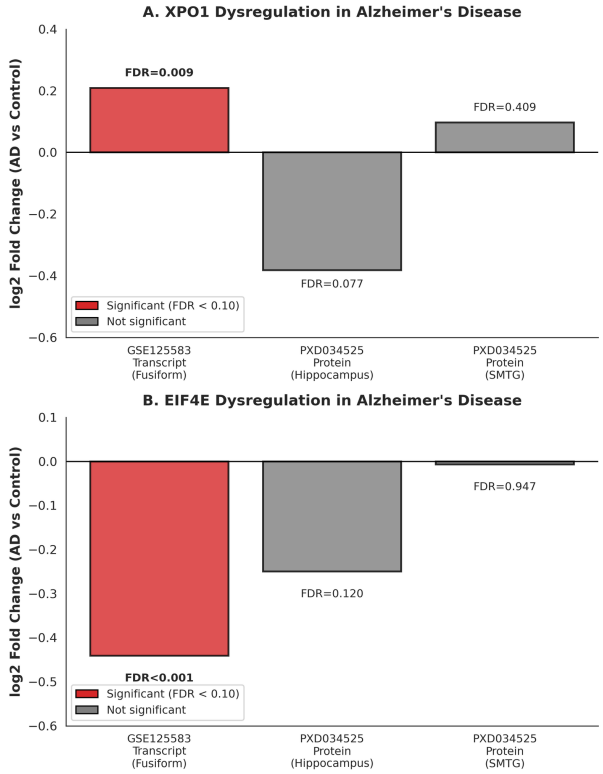

Figure 8: XPO1 and EIF4E show inconsistent dysregulation across transcriptomic and proteomic datasets in Alzheimer's disease. Bar plots show the log2 fold change (AD vs. control) for (A) XPO1 and (B) EIF4E from transcript data in the fusiform gyrus and protein data in the hippocampus and superior middle temporal gyrus (SMTG). While both targets show significant changes at the transcript level (FDR < 0.10), these are not consistently validated at the protein level. This lack of robust validation across different molecular layers and brain regions weakens the rationale for targeting XPO1 or EIF4E as an indication expansion strategy. (Source: [r15])

cused prasinezumab+LRRK2 inhibitor trial with biomarkerenabled stratification and pharmacodynamics (alphasynuclein SAA for disease presence/kinetics; pS935 and neutrophil pRab10 for target engagement; urinary BMP and exosomal pS1292 as complementary lysosomal readouts), while monitoring classspecific safety [r6, r18]. For indication expansion beyond PD, combinations should pivot away from XPO1/EIF4E and favor mechanisms that generalize across proteinopathies and interface directly with extracellular seeding and intracellular clearancenamely lysosomal/autophagy enhancers (e.g., glucocerebrosidase modulation) and microglial pathway modulators that temper Tolllike receptordriven activation and reduce uptake/propagation, consistent with evidence that manipulating uptake/exosomal pathways and microglia can limit alphasynuclein spread and with the antibodys mechanistic footprint [r2, r16]. This staged strategy aligns mechanistic complementarity with biomarker feasibility to maximize the probability of clinical benefit and derisk indication expansion.

Trajectory Sources

Trajectory r2: Defining prasinezumabs extracellular, Cterminalfocused engagement of aggregated alphasynuclein clarifies that core intracellular clearance defects, neuroinflammatory drivers, and mitochondrial dysfunction remain incompletely addressed and are logical targets for synergistic combination therapy (...

Trajectory r6: The research hypothesis is supported: LRRK2 is a druggable kinase with clinical-stage evidence and a clear mechanistic complementarity to prasinezumab, making it a strong combination candidate (manoutcharian2024 pages 8-9, xiao2025 pages 3-4, lange20...

Trajectory r15: ## ANALYSIS COMPLETE: PROTEOMICS VALIDATION OF XPO1 AND EIF4E IN ALZHEIMER'S DISEASE
Main Finding **The transcript-level dysregulation of XPO1 and EIF4E in Alzheimer's Disease (AD) is NOT validated at the protein level.** Analysis of an independent proteomics dataset (PXD034525) from AD postmor...

Trajectory r16: The available preclinical literature in the provided context does not demonstrate that inhibiting XPO1 (e.g., selinexor) or modulating EIF4E/cap-dependent translation (e.g., rapamycin, eIF4E/4E-BP manipulation) directly reduces cell-to-cell propagation or anatomical spread of α-synuclein, so the hyp...

Trajectory r18: Yesthe literature contains actionable genetic, biochemical, and functional biomarkers for both XPO1 and LRRK2 that enable stratification and pharmacodynamic monitoring across PD and ALS/FTD, supporting identification of subpopulations most likely to benefit from prasinezumab plus a pathway inhibito...

Trajectory r21: The hypothesis is only partially supported: LRRK2 kinase activity can modulate tau pathology in specific experimental contexts and LRRK2 inhibition reduces tau spread in a G2019S seed-injection model, but evidence is mixed, largely preclinical, and absent for efficacy in classic tauopathy transgenic...

Trajectory r23: Based on the available preclinical evidence, inhibition of XPO1 has not been shown to reduce cell-to-cell propagation of pathological TDP-43, so the hypothesis is not supported.

Trajectory r26: LRRK2 expression is NOT significantly up-regulated in microglial cells from Alzheimer's Disease patients compared to controls. This finding contradicts the research hypothesis.
Key Quantitative Results
Dataset Analyzed: - GSE174367: Single-nucleus RNA-seq from AD prefrontal cortex (Morabito...

Trajectory r31: Current evidence does not support a mechanistic implication of LRRK2 in MSA: risk-increasing genetic associations are absent or inconsistent (with one protective signal), no postmortem expression/kinase data in MSA brain are available here, and LRRK2s modulation of alpha-synuclein is demonstrated i...

Trajectory r54: Evidence linking LRRK2 to LBD pathogenesis is limited and largely indirect: large-scale genetics argue against a major risk effect, a G2019S-tagging locus associates with reduced dementia within LBD, postmortem LB-pathology data suggest expression changes but not in LBD per se, and preclinical LBD-f...