The Acid Test

Ralph Nixon's endosomal–lysosomal–autophagy account of Alzheimer's disease, thirty-six years on: what it established, where it overreaches, and the sequencing question its own data force

The sorting endosome · APP-βCTF · The vacuolar ATPase · PANTHOS · The dystrophic neurite · The return leg

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In 1990 a senile plaque was found to contain active lysosomal proteases, and nobody could say why they were there. The answer proposed since is the most complete account any laboratory has given of how an Alzheimer neuron dies. This paper takes that account at full strength and asks which of its propositions are established, which are contested, and which are asserted beyond what the evidence carries.

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Abstract

For thirty-six years one laboratory has argued that Alzheimer's disease is, at its root, a failure of the cell's disposal system. The argument began in 1990 with an observation nobody knew what to do with — that the proteases inside senile plaques are lysosomal, and enzymatically active — and has since been developed into the most mechanistically complete single-network account of this disease that any laboratory has produced. It holds that the endosomal–lysosomal–autophagy network fails first; that the failure is driven by a fragment of the amyloid precursor protein rather than by amyloid-β; that the terminal step of the network, acidification of the lysosome, is the specific point of collapse; and that the senile plaque itself is the residue of a neuron that died with its cargo undigested.

This paper evaluates that account. It is not a review of autophagy in neurodegeneration, and it is not an advocacy document. It asks a narrower question: taking the framework in the strongest form its author has given it, which of its propositions are now established, which are supported but not established, which are contested, and which are asserted beyond what the evidence carries.

What is established. Four propositions have survived independent test and should be treated as facts about human brain. First, enlargement of the early endosome is the earliest disease-specific structural abnormality known in Alzheimer's disease: it is present in sporadic disease before amyloid deposition, it is present in Down syndrome before birth, and it is not seen in the other neurodegenerative diseases examined alongside it. Second, the driver of that enlargement is the β-cleaved carboxy-terminal fragment of the amyloid precursor protein, not amyloid-β — a conclusion reached independently by three laboratories using human isogenic neurons, transgenic mice and viral expression of the fragment alone. Third, autophagic vacuoles are not incidental to Alzheimer neuropathology; they are the overwhelming majority of the organelles inside the dystrophic neurites that give the neuritic plaque its name, a finding made in human cortical biopsy tissue in 2005 and still under-absorbed. Fourth — and this is the observation on which the whole framework turns — autophagy *induction* in the Alzheimer neuron is increased, not decreased, while completion fails. The lesion is at the end of the pathway, not the beginning.

What is supported but not established. The sufficiency experiments are the programme's strongest work and they are mouse work. Over-activating Rab5 alone, with no manipulation of the amyloid precursor protein, produces endosome enlargement, accelerated receptor internalisation, spine loss, tau hyperphosphorylation through GSK-3β, cholinergic degeneration and memory impairment; over-expressing the adaptor APPL1 alone does the same. These are among the cleanest demonstrations in the field that an endosomal lesion is sufficient for a substantial part of the Alzheimer phenotype. They are also demonstrations in animals, in which the initiating manipulation is supraphysiological by construction.

What is contested. The bridge from familial Alzheimer genetics to the lysosome — the proposal that presenilin 1 is required for maturation of the V0a1 subunit of the vacuolar ATPase, and therefore for lysosomal acidification — was reported in 2010, contradicted by three groups within two years, and has been neither retracted nor fully rehabilitated. Sixteen years later it remains the single most load-bearing unsettled claim in the account, because it is what makes autosomal-dominant Alzheimer's disease a lysosomal disease rather than an amyloid one.

What is asserted beyond the evidence. The framework is presented by its author as "the only framework that potentially explains the entirety of pathophysiological phenomena and pathological lesions that define the AD phenotype." That sentence is the weakest in the programme, and not because it is immodest. A network through which all cargo passes will be found abnormal in any disease of cargo, and a theory that explains everything constrains nothing. The programme's actual defence against this charge is not rhetorical but experimental — it has repeatedly built the mouse that could have failed — and the defence would be stronger if the universality claim were dropped.

Two findings of this evaluation are new to the assessment of this programme. The first concerns placement. The framework's signature lesion — PANTHOS, the neuron so distended with de-acidified autolysosomes that its cargo arranges into a flower-like corona before the cell ruptures — is often read as a general death mode of the Alzheimer neuron. It is not. It is the death of a cell that is simultaneously failing to acidify *and* manufacturing large quantities of amyloidogenic cargo, and that conjunction is a property of glutamatergic pyramidal neurons with high precursor-protein expression. The framework says so itself, in a 2024 review that restricts the imbalance to "highly vulnerable pyramidal neuron populations," and its own unpublished human proteomic analysis reports vacuolar-ATPase deficits selective to excitatory neurons. The scope condition exists; it is dropped in the framework's more expansive summaries and almost always in secondary use. Stated plainly: the disposal lesion is general, and the flower is not. Conflating the two has consequences, and the largest is therapeutic: it places the death in the wrong decade and the drug in the wrong phase of the disease.

The second concerns direction. Read alongside eight other programmes that have converged on the failing synapse from different compartments, Nixon's account has a structural signature that is not a tautology and is rarely stated: every step it identifies as intact or up-regulated belongs to the *forward* leg of a cycle — endocytosis, cleavage, autophagosome formation, cargo generation — and every step it identifies as failing belongs to the *return* leg — acidification, hydrolysis, retrograde transport, recycling. Six separate observations in this programme are of a forward leg running faster while its return fails. This is what distinguishes a clearance theory from a production theory, and it has a direct therapeutic corollary that the field has largely ignored: an autophagy inducer given to a neuron that can no longer acidify its lysosomes fills that neuron faster with vesicles it cannot complete. Nixon's own protocol has the order right — re-acidify, then induce. Almost every clinical and preclinical autophagy programme in Alzheimer's disease has the order wrong.

On the clinical record, the paper declines to soften an awkward result. The one drug developed from this cascade and tested in Alzheimer's disease — neflamapimod, a p38α inhibitor that reverses Rab5 over-activation — failed its primary endpoint in mild Alzheimer's disease in 2021. Its subsequent success has been in dementia with Lewy bodies, in a trial that excluded patients with Alzheimer co-pathology by plasma phospho-tau. A framework built on Alzheimer's disease has so far produced a drug that works when Alzheimer's disease is screened out. That is not a refutation — the target is upstream and the patients may have been treated too late — but it is the most uncomfortable fact in the file and it should be stated first, not buried.

We close with a graded ledger of nineteen claims, five conditions that would refute the framework, and ten experiments in rank order. The first of them is the experiment that would settle the longest-running unresolved question in this area: whether enhancing retromer function rescues the Rab5-over-activated mouse, in which there is no primary sorting-receptor lesion at all. It has been available to perform for six years. It has not been performed.

Part I — The Programme

1. The Claim, in Its Author's Terms

It is a discourtesy to evaluate a framework in a weakened form, so we begin by stating this one at full strength and in its author's own vocabulary.

The proposition is that Alzheimer's disease is a disease of the endosomal–lysosomal–autophagy network, and that the network's failure is not a consequence of the disease but its antecedent. The argument opens with an observation about time that the field has never adequately answered. In late-onset neurodegenerative disease, the protein implicated in the pathology is synthesised throughout life and accumulates only in the aged brain. Two readings of that coincidence are available. The first, which has organised the field for three decades, is that the protein's misfolding and cytotoxicity cause the disease, and that ageing supplies the time required for enough of it to accumulate. The second is that the protein is being made and cleared at every age, that clearance declines with age, and that the accumulation is the visible consequence of a failing disposal system rather than of a newly toxic substrate. Nixon takes the second reading and pushes it further than anyone else has: not merely that clearance declines, but that the specific machinery of clearance is the site of the primary lesion, that Alzheimer's causative and risk genes converge on that machinery, and that the network's failure "accounts for the main pathological and pathophysiological manifestations of AD" (Nixon, 2017; Nixon, 2020).

The network in question is not one organelle. It is a graded series of compartments — early sorting endosome, late endosome and multivesicular body, autophagosome and amphisome, lysosome and autolysosome — through which every internalised or condemned molecule in the cell must pass, and which the framework treats as four interacting hubs rather than a pipeline. Each hub is assigned pathogenic content. At the early endosome, the master GTPase Rab5 is pathologically over-activated, producing the enlarged sorting compartments that are the earliest structural anomaly known in this disease, and — because the same compartment carries the retrogradely transported trophic signal — producing the trophic starvation of basal forebrain cholinergic neurons. At the late endosome and multivesicular body, cargo that cannot be degraded is diverted to exosomal release, which supplies a route for the trans-synaptic propagation of tau. At the lysosome, the acidification machinery fails, and with it hydrolase activation, cargo digestion, calcium storage and the nutrient-sensing functions that lysosomal signalling supports. And in autophagy, induction remains intact or rises while completion fails, so that the cell fills with vesicles it has manufactured and cannot empty.

Four features of this account deserve to be noticed before it is examined, because they are what distinguish it from a general statement that proteostasis declines with age.

The first is that it is *specific about the step*. Many accounts of impaired clearance in Alzheimer's disease are indifferent to where in the pathway the impairment lies. This one is not: it names the terminal acidification step, it names the enzyme complex responsible (the fourteen-subunit vacuolar H+-ATPase), it names the subunit whose maturation is proposed to fail, and it names the molecule that inhibits the pump. A framework that names a subunit can be wrong about the subunit, which is a virtue.

The second is that it is *specific about the agent*, and the agent is not amyloid-β. The framework's central molecular claim is that the β-cleaved carboxy-terminal fragment of the amyloid precursor protein — βCTF, also called C99 — is the pathogenic species: it recruits an adaptor to the endosome and locks Rab5 in its active state, and, in phosphorylated form, it binds the vacuolar ATPase and impedes its assembly. Amyloid-β in this account is a downstream product that becomes toxic mainly when it is trapped inside a compartment it should have left.

The third is that it is *reciprocal rather than linear*. The framework does not claim that the network fails and the amyloid precursor protein is a bystander. It claims a cycle: the disposal network's failure amplifies mis-metabolism of the precursor protein, and the resulting metabolites further disable the network. Nixon calls this a "special partnership" and the phrasing is deliberate. It is a weaker claim than pure clearance primacy and a stronger one than co-occurrence, and it is the reason the framework cannot be dismissed as an anti-amyloid position.

The fourth is that it is *therapeutically committed*. The framework asserts that enhancing lysosomal proteolytic efficiency, boosting retrograde transport of endolysosomes, or pharmacologically re-acidifying the lysosome ameliorates the entire range of measured deficits in animal models — amyloid, tau, synaptic plasticity, memory — and it treats this as proof-of-principle validation rather than as an incidental observation. Committing to a therapeutic prediction is how a framework of this scope makes itself vulnerable, and we take that commitment seriously enough to devote Part VI to auditing it.

What follows is not a summary of this position but a test of it, proposition by proposition, against the primary record.

2. Nineteen-Ninety: The Proteases in the Plaque

The programme begins with a finding that was, at the time, almost uninterpretable.

In 1990, Cataldo and Nixon reported in the *Proceedings of the National Academy of Sciences* that senile plaques in Alzheimer brain contain lysosomal proteases, and that the proteases are enzymatically active (Cataldo & Nixon, 1990). Cathepsin D and related hydrolases, normally sequestered behind a lysosomal membrane, were present in the extracellular deposit and retained catalytic capacity there.

It is worth pausing on how strange this was in 1990. The plaque had been understood since Glenner and Wong's sequencing of the amyloid peptide as an extracellular aggregate of a secreted product — a deposit that formed outside the cell from material the cell had released. On that reading there is no obvious reason for the interior of a lysosome to be in it. Lysosomal contents in the neuropil imply either exocytosis of lysosomal material on a substantial scale, or the rupture of cells. Neither implication was pursued by the field. The finding was absorbed as a curiosity about plaque composition, filed alongside the observation that plaques contain many things — apolipoprotein E, complement components, proteoglycans, metals — and treated as a consequence of the deposit rather than a clue to its origin.

Two features of this early work set the pattern for everything after. The first is that it was done in human tissue, by histochemistry and immunocytochemistry, on the actual lesion. This programme's characteristic evidence is human post-mortem and biopsy material examined at the level of the organelle, and its characteristic instrument is the electron microscope. That is an unusual base for a modern mechanistic programme and it accounts both for its strengths — the phenomenology is real and it is human — and for its principal weakness, which is that morphology cannot establish sequence.

The second is that the finding was, from the outset, a *disposal* finding rather than a production finding. The question it raised was not how much amyloid the cell makes but what happened to the machinery that should have destroyed it. The field's attention through the 1990s and 2000s went almost entirely the other way, to secretases and to the regulation of cleavage. Reading the record now, the striking thing is not that the disposal question was answered wrongly. It is that for roughly twenty years it was not asked.

3. The Compartment That Swells Before Anything Else

The finding that turned a curiosity into a programme came seven years later.

Cataldo, Barnett, Pieroni and Nixon reported in 1997 that neurons in sporadic Alzheimer's disease show markedly enlarged early endosomes, with increased delivery of hydrolases to those compartments, and that the change is present in brains at early neuropathological stages (Cataldo et al., 1997). Three years later, in a study that remains the empirical foundation of the whole account, the same group established four things about that abnormality in a series of human brains (Cataldo et al., 2000).

First, the enlargement *precedes* amyloid deposition in sporadic Alzheimer's disease. Second, it is present in Down syndrome — where trisomy of chromosome 21 confers an extra copy of the amyloid precursor protein gene, and where Alzheimer neuropathology is near-universal by the fifth decade — and there it is present extraordinarily early, in some neurons before birth, decades ahead of any deposit. Third, it is modified by genotype in a direction that matches the epidemiology: the *APOE* ε4 allele accentuates it. Fourth, it is not a generic feature of neurodegeneration; the abnormality was not found in the comparison diseases examined.

Take those four together and the claim is considerably stronger than "endosomes are abnormal in Alzheimer's disease." It is that a specific compartment is structurally abnormal, in the right cells, before the defining lesion of the disease exists, in the genetic condition that guarantees the disease, scaled by the principal genetic risk factor for the sporadic form, and not in the diseases with which the disease is most often compared. The Down syndrome observation is the load-bearing one. A pathological change that is visible in a fetal brain and whose associated dementia arrives fifty years later is not plausibly a consequence of that dementia.

The finding has held. It was confirmed at the level of gene expression in human tissue: microarray analysis of individually captured CA1 pyramidal neurons across the progression from normal ageing through mild cognitive impairment to Alzheimer's disease showed up-regulation of endosomal regulatory genes early in the trajectory (Ginsberg et al., 2010), and the same was found in basal forebrain cholinergic neurons in mild cognitive impairment, where *RAB5* and its relatives are elevated before the clinical diagnosis is made (Ginsberg et al., 2011). It has been reproduced in human induced-pluripotent-stem-cell neurons carrying familial mutations, in fibroblasts from people with Down syndrome, and in multiple mouse models. Among structural abnormalities in Alzheimer's disease it has the best claim to be the first.

There is one qualification that should be stated plainly rather than left as an implication, because it constrains what the finding can be used to argue. An enlarged endosome is a static picture of a dynamic compartment, and swelling is compatible with more than one dynamic cause: accelerated fusion of incoming vesicles, delayed exit of cargo, or both. The programme's own account invokes both — accelerated Rab5-mediated fusion *and* deficient recycling out of the compartment through Rab11 and the retromer — and this matters for the argument of Chapter 17, where a rival programme claims the exit failure as primary and the fusion acceleration as secondary. The morphology alone does not adjudicate between them. It never could.

4. The Fragment, Not the Peptide

The most consequential claim this programme has made is also the one it is least known for. It is that the pathogenic product of the amyloid precursor protein in Alzheimer's disease is not amyloid-β but the fragment left behind when β-secretase cuts.

β-Secretase cleavage of the precursor protein liberates a soluble ectodomain and leaves a ninety-nine-residue carboxy-terminal fragment embedded in the membrane — βCTF, or C99. In the standard account this fragment is an intermediate of no independent interest: γ-secretase cuts it, amyloid-β is released, and the story begins. In this framework the fragment is the agent, and its cleavage by γ-secretase is the *termination* of a signal rather than the initiation of a pathology.

The mechanism was specified in 2016. Elevated βCTF on the early endosome binds the phosphotyrosine-binding domain of APPL1, an adaptor and effector of Rab5, recruiting it to the endosomal membrane; APPL1 stabilises Rab5 in its GTP-bound, active conformation; the result is amplified Rab5 signalling, accelerated homotypic fusion, and the enlarged sorting endosome that Chapter 3 described. Knocking APPL1 down in fibroblasts from people with Down syndrome corrects the endosomal defect, which establishes that the adaptor is not decorative but required (Kim et al., 2016).

What lifts this from a laboratory's internal proposal to a field-level finding is that three groups arrived at the fragment independently, from three different directions, and none of them needed the others' framework to get there.

Checler's group, working on transgenic and viral models in which C99 could be expressed and studied without amyloid-β, showed that intraneuronal aggregation of the fragment produces lysosomal and autophagic pathology that is demonstrably amyloid-β-independent, and went on to ask, in a review whose title states the inversion plainly, whether γ-secretase should be understood as a beneficial inactivating enzyme for a toxic fragment (Lauritzen et al., 2016; Checler et al., 2021).

Tessier-Lavigne's group did the experiment that a sceptic would demand. They constructed a large isogenic panel of human induced-pluripotent-stem-cell neurons carrying familial *APP* and *PSEN1* mutations against a common genetic background, and asked which molecular species accounted for the shared abnormalities. The answer, in the paper's own title, was that the shared endosomal abnormalities are mediated by β-carboxy-terminal fragments and not by amyloid-β (Kwart et al., 2019). This is a human-cell, isogenic, mutation-panel result obtained by a laboratory with no stake in the lysosomal account, and it is the single most persuasive piece of external corroboration the framework has.

And Nixon's group supplied the second crime. Phosphorylation of βCTF at tyrosine 682 produces a species that binds specific subunits of the vacuolar ATPase and impedes assembly of the pump, so that the fragment does not merely enlarge the endosome upstream but disables the acidification machinery downstream (Im et al., 2023). One molecule, two compartments, two lesions.

The convergence is real and it should be given its weight. But two qualifications belong here rather than in a later section.

The first is that βCTF elevation and amyloid-β elevation are not experimentally independent in most systems. Anything that raises β-cleavage raises both. The isogenic panel and the C99-only expression models are precisely the designs that break the correlation, which is why they carry disproportionate evidential weight, and why the framework's case rests on a small number of experiments rather than on the large literature that merely correlates.

The second is that the fragment claim, if true, is a claim *about the amyloid precursor protein*. The framework is sometimes read — and occasionally presents itself — as an alternative to amyloid-centred accounts. It is better understood as a relocation within them. The gene is still *APP*; the enzyme is still BACE1; the pathogenic species is still a proteolytic product of the same protein. What changes is which product, where it acts, and what it does. That is a large change, with direct consequences for drug development that Chapter 24 takes up. It is not a departure from the precursor protein.

5. The Pump, and the Argument That Would Not Settle

The lysosome digests because it is acidic. Its lumen is held near pH 4.0 to 4.5 by the vacuolar H+-ATPase, a fourteen-subunit rotary pump whose V1 sector hydrolyses ATP and whose membrane-embedded V0 sector translocates protons. Below that pH, hydrolases are active, cargo is degraded, and the lysosome performs its signalling functions — nutrient sensing through mTORC1 on its surface, calcium release through TRPML1 across its membrane. Above it, none of this works properly, and the cell fills.

The framework's terminal claim is that this pump fails in Alzheimer's disease, and it offers three routes to the failure: a genetic route through presenilin, a metabolic route through the fragment, and an ageing route through oxidation. The three are in very different evidentiary states and it does the framework no service to present them as a unit.

The genetic route is contested, and has been for sixteen years. In 2010 Lee and colleagues reported in *Cell* that presenilin 1 is required, independently of γ-secretase activity, for maturation and lysosomal targeting of the V0a1 subunit of the pump, and that loss of presenilin function therefore alkalinises the lysosome, disabling proteolysis and blocking autophagy (Lee et al., 2010). If correct, this is the most important single result in the entire framework, because it converts autosomal-dominant Alzheimer's disease from an amyloid overproduction disorder into a lysosomal one — the causative gene acting on the disposal machinery directly rather than through the peptide.

It did not go unchallenged. Neely, Green and LaFerla found that presenilins are required for efficient degradation through the autophagy–lysosome system but reported no acidification defect, concluding that presenilin must act on the pathway some other way (Neely et al., 2011). Zhang and colleagues, working in cells lacking both presenilins, reported frankly normal lysosomal acidification and titled their paper accordingly (Zhang et al., 2012). Coen and colleagues proposed a different lesion entirely — that presenilin deficiency disrupts lysosomal calcium homeostasis and hence calcium-dependent fusion, and that the proton pump is not the problem (Coen et al., 2012). A commentary in the same issue framed the dispute as it then stood and did not resolve it (Bezprozvanny, 2012).

The subsequent history is not a clean vindication of either side and should not be reported as one. Nixon's group returned to the question and proposed a reconciliation in which the two findings are the same finding read at different points in a loop: presenilin 1 supports vacuolar-ATPase-mediated acidification, and the resulting pH change is what dysregulates

lysosomal calcium efflux through TRPML1 (Lee et al., 2015). This makes the calcium abnormality real and downstream rather than alternative. It is a coherent resolution. It is not, however, an independent replication of the original acidification result by a group outside the laboratory that produced it, and to our knowledge no such replication exists. Sixteen years after publication, the most consequential claim in the framework's genetic arm remains unsettled in the sense that matters: it has been contradicted by three groups and rescued by one, and the one is the originating laboratory.

The metabolic route is in better shape, and it is what makes the acidification claim survivable. The Im 2023 result described in the previous chapter provides a route to pump inhibition that does not require presenilin at all: the phosphorylated fragment binds the pump. This matters more than it is usually given credit for. If the presenilin arm were to fall entirely, the framework would lose its bridge to familial Alzheimer's disease but would retain a mechanism by which the commonest upstream abnormality in the disease — elevated βCTF — produces the terminal lesion. A framework with two independent routes to its central claim is more robust than one, and readers of this literature frequently treat the presenilin controversy as though it threatened the whole edifice. It does not. It threatens one arm.

The ageing route is the least contested and the least specific. Vacuolar-ATPase subunits are targets of oxidative modification; lipid peroxidation products adduct proteins including the pump's components; hydrolases are themselves oxidatively inactivated; and substrates cross-linked by oxidative damage become resistant to digestion (Colacurcio & Nixon, 2016). Declining pump activity with rising lysosomal pH is among the best-described cellular changes of ageing, and in invertebrates the relationship to lifespan is causal in both directions — impeding the pump shortens life; stimulating transcription of its subunits extends it. This is strong biology. It is also, as Chapter 15 argues, exactly the sort of general finding that makes a network theory difficult to falsify, because it is true of ageing cells everywhere, in every tissue, in the absence of any disease at all.

6. Sufficiency: The Two Mice

Everything so far is association, correlation, and mechanism established in dishes. The programme's strongest work is of a different kind, and it deserves to be assessed on its own.

In 2020, Pensalfini and colleagues built a mouse in which Rab5 is directly over-activated in neurons, to a degree calibrated against the activation measured in Alzheimer brain, with no manipulation of the amyloid precursor protein whatever (Pensalfini et al., 2020). The animal is therefore a test of a counterfactual: if the endosomal lesion is a mere reflection of amyloid biology, over-activating Rab5 in the absence of an amyloid manipulation should produce endosomal changes and little else.

It produced considerably more than that. The animals showed enlarged, mistrafficked endosomes, as designed; but also accelerated endocytosis of AMPA-type glutamate receptors, loss of dendritic spines, deficits in hippocampal synaptic plasticity, tau hyperphosphorylation through activated GSK-3β, progressive degeneration of basal forebrain cholinergic neurons, and impairment of hippocampus-dependent memory. Five of the six are cardinal features of Alzheimer's disease, and one of them — cholinergic neurodegeneration — is the lesion on which the field's only long-standing symptomatic drug class was built.

In 2025 the group did it again by a different route, generating a mouse that over-expresses the adaptor APPL1 in neurons and reproducing the same set: endosomal and synaptic dysfunction with cholinergic neurodegeneration (Jiang et al., 2025). Two different molecular entry points into the same node produce the same phenotype.

Alongside these sits an older experiment in the opposite direction. Yang and colleagues asked whether restoring lysosomal proteolytic efficiency in an established amyloid model would reverse the pathology, by genetically removing cystatin B, an endogenous inhibitor of lysosomal cysteine proteases. Autophagic–lysosomal function was restored, amyloid pathology was reduced, and memory deficits improved (Yang et al., 2011). Sufficiency from one direction; reversibility from the other.

These are strong experiments and we grade them as the best evidence the programme has. Three limitations should nonetheless be recorded, because they are the ones a sceptical reader will raise.

They are mouse experiments, and the manipulations are supraphysiological by construction. A transgene that raises activated Rab5 to levels "comparable to those in AD brain" is calibrated against a target, but

the calibration is of a mean, in a mixed tissue, against a lifelong process compressed into months. Demonstrating that a lesion is sufficient when imposed acutely does not establish that it is what happens over forty years.

They are, so far, single-laboratory results. The Rab5 and APPL1 mice both come from the group that proposed the mechanism. This is not a criticism of the work; it is a statement about the current state of external corroboration, which for the sufficiency arm is absent, in contrast to the fragment arm where the isogenic-panel result is genuinely independent.

And sufficiency is not primacy. That over-activating Rab5 produces a large part of the phenotype shows that this node is capable of driving the disease. It does not show that this node is where the disease begins in a human being. Several other manipulations — amyloid over-production among them — also produce large parts of the phenotype in mice. The field has a long record of mistaking sufficiency in an engineered animal for causal priority in a person, and this framework is not exempt from that hazard merely because its node is upstream of the one that has previously misled us.

Part II — What the Account Explains

7. Ageing, and the Question the Cascade Cannot Answer

Age is the largest risk factor for Alzheimer's disease by an enormous margin. Incidence roughly doubles every five years after sixty-five; essentially no other variable in the epidemiology comes close. Any framework that cannot say what ageing *does* has left the principal term out of its own equation.

The amyloid cascade's answer has always been temporal rather than mechanistic: the peptide accumulates, accumulation takes time, and age is the time. The difficulty is that the metabolism of the precursor protein changes only modestly with age in the absence of disease. If the substrate is being made at approximately the same rate at forty and at eighty, and only at eighty does it accumulate, then something other than production has changed. The cascade's implicit answer is that the accumulation is self-accelerating once seeded, which is a real phenomenon — nucleation kinetics are genuinely non-linear, and the seeding experiments establish it (Meyer-Luehmann et al., 2006; Jucker & Walker, 2013) — but a nucleation account explains the *shape* of the accumulation curve, not why the curve begins where it does in a given individual.

The clearance framework's answer is mechanistic and it is specific. What changes with age is the terminal step of disposal. Lysosomal pH rises. Pump subunits are oxidatively modified. Hydrolases are inactivated by the same chemistry. Substrates cross-linked by oxidative damage become resistant to the proteases that would otherwise degrade them, so the load rises and the capacity falls at the same time. Chaperone-mediated autophagy declines measurably with cellular age. Mitochondrial output falls, and the pump is an ATP-consuming machine, so the energetic ceiling on acidification descends. In model organisms the relationship is causal in both directions: impairing the vacuolar ATPase shortens lifespan and specifically impairs mitochondrial function; increasing transcription of its subunits extends lifespan in the manner of the classical longevity pathways (Colacurcio & Nixon, 2016).

This is the framework's strongest conceptual advantage over its rivals and it should be stated without hedging. It is not merely compatible with the age dependence of the disease; it identifies a specific molecular machine whose age-related decline is independently measured, causally connected to lifespan in tractable organisms, and located precisely at the step the disease requires.

Two things follow that are less often noticed.

The first is that this is an argument for a *threshold* disease rather than a *trigger* disease. If disposal capacity declines gradually while load accumulates, then disease onset is the crossing of a line rather than the occurrence of an event, and the age of crossing is set by where an individual's capacity curve and load curve happen to intersect. Every genetic and environmental risk factor then acts by shifting one curve or the other. This is a coherent and rather beautiful reading of the epidemiology, and it explains why risk factors as heterogeneous as *APOE* genotype, head injury, diabetes, sleep fragmentation and infection can all raise risk without any of them being the cause.

The second is the price of that beauty, and Chapter 15 will collect the bill. A framework in which everything that stresses the cell moves a threshold is a framework in which almost no observation can be inconsistent with it.

8. Reading the Genetics as an Organelle

The genetic architecture of late-onset Alzheimer's disease was, for a long time, a list. Genome-wide association identified loci; the loci were reported alphabetically or by significance; and the mechanistic content of each was pursued separately. Reading that list as an organelle rather than as a list is one of the framework's genuine contributions, and it is now widely enough accepted that its origin is often forgotten.

The loci cluster. *SORL1* encodes a sorting receptor that delivers the precursor protein to the retromer-dependent retrieval pathway. *BIN1*, the second-strongest common locus, acts on membrane curvature and on the closure of the autophagosome. *PICALM* is a clathrin assembly protein of the endocytic machinery. *CD2AP*, *RIN3*, *EPHA1* and *ABCA7* all have endocytic or lipid-transport functions. *APOE*, whose ε4 allele is the strongest common risk factor known, governs endosomal cholesterol and lipoprotein handling, delays endosomal recycling, and — the observation that connects it directly to this account — accentuates the endosomal enlargement described in Chapter 3 (Cataldo et al., 2000). The largest published association study of the disease identified seventy-five loci, and pathway analysis of that set places endocytosis and lysosomal function among the pathways most strongly enriched, alongside immunity and lipid metabolism (Bellenguez et al., 2022).

The framework's reading is that this is not a coincidence of annotation but the genetics telling us where the disease is. On that reading, the network is not one candidate mechanism among several; it is the mechanism the human genome points at, and the immune and lipid signals are partly readouts of the same machinery in different cell types, since phagocytosis in microglia is endolysosomal biology under another name.

That reading is defensible and it is probably right in outline. Three cautions are nonetheless required, and they are not pedantic.

Enrichment analysis is a statement about annotation databases, not about brains. Endocytosis and lysosomal function are among the most heavily annotated processes in cell biology, and the number of genes assigned to them is large. Enrichment tests correct for set size, but they cannot correct for the fact that a protein with fifteen annotated functions will be counted in the category a curator found most interesting.

Genetic risk is distributed, and the largest single share of it is not endolysosomal in any straightforward sense. The immune signal in the modern genetics is at least as strong, and the two are not reducible to each other without argument. The framework's claim that microglial risk loci are "links to ELA

functions in endocytosis and phagocytosis" is true as far as it goes, but *TREM2* is a lipid-sensing receptor that sets a metabolic programme, and describing its variants as endolysosomal is an accurate statement that loses most of the biology.

And the sorting literature contains a live contradiction at exactly the point where this framework meets its principal rival. Depleting *SORL1* in human neurons impairs endosomal traffic — but one careful study found the impairment *independent* of amyloidogenic processing of the precursor protein (Knupp et al., 2020) while another, equally careful, found the endolysosomal and autophagic defects *dependent* on it (Hung et al., 2021). The framework requires the second result. If the first is right, the commonest strongly-acting sorting lesion in the human genetics damages the network without going through the fragment, and the "special partnership" with the precursor protein is not the general mechanism but one route among several. This contradiction is unresolved, it is stated by neither side as a crisis, and it is arguably the most informative unresolved discrepancy in the endolysosomal literature.

9. The Company the Disease Keeps

Between a fifth and a half of Alzheimer brains contain aggregated TDP-43, α-synuclein, or both, and their presence is associated with a higher likelihood of clinical dementia and with more severe cognitive impairment at a given burden of Alzheimer pathology. This is one of the most robust observations in neuropathology and one of the least explained. Frameworks built on the amyloid cascade have almost nothing to say about it: there is no reason internal to that account why a disease of one peptide should be accompanied, in half of cases, by the aggregates of two unrelated diseases.

The clearance framework has an answer, and it is the obvious one, which is a point in its favour rather than against it. Both proteins are autophagy substrates. TDP-43 turnover is autophagy-dependent, its aggregates recruit the adaptor machinery (LC3, p62/SQSTM1), and impairing autophagy is sufficient to produce its abnormal accumulation. α-Synuclein is degraded by both chaperone-mediated and macroautophagy, and its accumulation in disease itself corrupts lysosomal function, producing a feed-forward loop. If the disposal system fails, then everything the disposal system was handling will accumulate, and which aggregates appear in a given brain will be a function of what that brain was carrying and which neurons were affected. Co-pathology stops being a puzzle and becomes a prediction.

The same logic is applied to granulovacuolar degeneration, the vacuoles with dense cores found in hippocampal pyramidal neurons and described since 1911 without a mechanism. The framework reads them as stalled autophagy: they contain a mixed protein complement inside a limiting double membrane characteristic of the autophagosome, which is precisely what an incomplete autophagic compartment should look like.

Here, honesty requires reporting a finding that runs the other way. Wiersma and colleagues showed that granulovacuolar degeneration bodies are neuron-selective lysosomal structures *induced by intracellular tau pathology* — that is, they are downstream of tau seeding rather than upstream of it (Wiersma et al., 2019). The two readings are not strictly incompatible: tau pathology could induce the structures by overwhelming a disposal system that then stalls, which is a clearance-framework account of a tau-initiated event. But the direction of the arrow in the best modern experimental work on these bodies is tau → lysosomal structure, not lysosomal failure → tau, and a framework that claims the granulovacuole as an explanandum should say so.

The general point survives the qualification, and it is worth stating at full strength because it is the framework's cleanest predictive success. A disease of disposal predicts mixed pathology. A disease of one protein does not.

10. What Is Inside a Neuritic Plaque

The most under-absorbed finding in this programme is thirty years old in outline and twenty in its definitive form, and it concerns the structure the field named the disease's second hallmark after.

The neuritic plaque is defined by the swollen, dystrophic neurites that surround an amyloid core. Those neurites are the reason the lesion is called neuritic; their presence is what distinguishes a neuritic plaque from a diffuse deposit in every staging scheme in use; and the count of them is what the CERAD criteria and their descendants actually measure. It would seem elementary to ask what is inside them.

Nixon and colleagues asked, using immunogold labelling with compartmental markers and electron microscopy, in neocortical biopsy tissue from Alzheimer patients — antemortem human material, which is rare in this literature and matters, because autophagic compartments are exquisitely sensitive to post-mortem interval. The answer was that autophagosomes, multivesicular bodies, multilamellar bodies and cathepsin-containing autophagolysosomes were the predominant organelles, accumulating in very large numbers (Nixon et al., 2005). In the group's subsequent quantification, autophagic vacuoles comprise more than ninety-five per cent of the organelle content of the grossly swollen axonal segments.

Read that number against the standard picture. In the standard picture the dystrophic neurite is a neurite injured by proximity to an amyloid deposit — a reactive structure, damaged from outside. In the electron micrographs it is a segment of axon packed almost to the exclusion of everything else with vesicles of the disposal pathway that failed to complete their journey. That is not the morphology of a bystander. It is the morphology of a traffic jam: compartments generated normally, transported retrogradely toward the perikaryal lysosomes as they should be, and stalled.

The framework's causal reading of this is supported by an experiment that is easy to overlook. Inhibiting endolysosomal acidification pharmacologically produces neuritic dystrophy in wild-type mice *in the absence of amyloid deposition* — the swelling does not require a plaque to sit next to. And in wild-type neurons, retrograde transport of autophagy-related compartments is selectively slowed and intermittently interrupted when lysosomal proteolysis is inhibited, with the stalled compartments accumulating in axonal swellings that then acquire additional Alzheimer-like features including local cytoskeletal hyperphosphorylation and ubiquitination. The dystrophy can be produced by disabling the disposal system alone.

A second observation from the same body of work is what makes the loop close. These swellings are enriched not only in cargo but in *machinery*: the precursor protein substrate, BACE1, and

γ-secretase components are all present. A stalled compartment containing substrate and both secretases is a compartment that goes on generating fragment and peptide for as long as it is stalled. Amyloid production, in this reading, is not a rate set in the Golgi; it is a rate set by residence time in a compartment that should have been emptied.

This is where the 2026 literature has arrived independently, and the convergence is worth recording. Working from mitophagy rather than from amyloid, Bohr's group reported this year that Alzheimer model mice and human Alzheimer brain contain a previously unnamed lesion class — large accumulations of damaged mitochondria within dystrophic neurites, which they call mitochondrial plaques. Their mechanism is the framework's mechanism with a different cargo: mitochondria accumulate, lysosomes are recruited to degrade them, and degradation fails because lysosomal function is impaired, so both acidic and neutral mitochondria pile up. The lesions co-occur with amyloid in mixed plaques but can arise independently, and the authors place them early (Dan et al., 2026).

Two laboratories with different substrates, different reporters and different starting commitments have now described the same structure: a neurite full of cargo that entered the disposal pathway and never left it. That is stronger corroboration of the framework's central architecture than any amount of amyloid correlation, and it arrived from a direction the framework did not need.

11. Why Neurons, and the Storage-Disease Analogy

A framework built on a machine that every cell possesses owes an account of why this disease affects neurons.

The framework gives four reasons and they are good ones. Neurons are post-mitotic, so they cannot dilute accumulated waste by dividing, which is the principal disposal strategy of every proliferating cell; a fibroblast with a failing lysosome halves its burden every generation, and a neuron cannot. Neurons survive for the lifetime of the organism, so their accumulated damage is not reset. Neurons have limited capacity for bulk exocytic jettisoning of waste compared with glia. And neurons are extraordinarily polarised, which places the sites of highest demand — synapses, distal axonal segments — at the greatest distance from the perikaryal compartment where lysosomal capacity is concentrated, so that disposal in a neuron is uniquely dependent on long-range retrograde transport, and disposal failure and transport failure become the same failure.

The last of these is the strongest and it connects to Chapter 10. A neuron is a cell that must carry its rubbish a very long way to the incinerator, and a small reduction in the efficiency of either the carrying or the burning produces accumulation at the far end.

The framework then makes an argument by analogy that is rhetorically powerful and, we think, correct as far as it goes. More than forty inherited lysosomal storage disorders are known. In most of them the brain is preferentially affected, and severe neurodegeneration is the rule, even though the defective protein is usually expressed ubiquitously and malfunctions systemically. The natural experiment has already been run: when the lysosome fails for genetic reasons, the organ that fails is the brain. The magnitude of autophagic waste accumulation in Alzheimer neurons is, on the group's own comparison, matched only by that seen in the storage diseases.

Two further pieces of evidence sit alongside this and are worth naming because they are independent of the analogy. Neurofibrillary tangles — rare outside Alzheimer's disease and a small number of ageing-related tauopathies — are a prominent feature of two lysosomal disorders, mucopolysaccharidosis type IIIB and Niemann–Pick type C1, the latter sharing with Alzheimer's disease lysosomal pH elevation, dysregulated calcium release and autophagic deficits. And loss of the V0a1 subunit of the pump increases the susceptibility of *Drosophila* neurons to amyloid- and tau-induced toxicity, but only in the context of ageing or toxic stress — which is the threshold model of Chapter 7 in an experimental system.

The honest limit of the analogy is this. Lysosomal storage disorders are diseases of a *complete* enzymatic lesion, present from conception, producing childhood neurodegeneration with a distinctive and largely non-Alzheimer pathology. Alzheimer's disease is a partial, late, progressive functional decline of the same system. That the extreme form damages the brain establishes that the system matters to neurons. It does not establish that the partial form is what happens in Alzheimer's disease, and the framework occasionally uses the analogy as though it did.

Part III — The Flower

12. PANTHOS: What Was Shown

In 2022 the programme published the result it is now best known for, and it is worth setting out carefully, because it is routinely reported in a form stronger than the paper supports and routinely dismissed in a form weaker than the paper deserves.

The technical enabler was a reporter. The group generated transgenic mice expressing, specifically in neurons, a tandem-fluorophore LC3 probe in which one fluorophore is quenched at acidic pH and the other is not, so that the acidification state of every autophagic compartment can be read by colour in situ. This turns a question that previously required biochemistry on isolated organelles — is this compartment acidified? — into a question answerable cell by cell in an intact brain.

What the reporter showed, in two amyloid precursor protein mouse models, was reported by Lee and colleagues in *Nature Neuroscience* (Lee et al., 2022). Autolysosome acidification declines in neurons *well before* extracellular amyloid deposition, in step with a measured fall in vacuolar-ATPase activity in isolated lysosomes. Amyloid-β and βCTF then accumulate selectively within the enlarged, de-acidified compartments — not diffusely in the cytosol, not primarily at the membrane, but inside the specific organelles that have lost their pH.

In the most compromised neurons that are still intact, the accumulation reaches an extraordinary morphology. Very large numbers of amyloid-positive autolysosomes pack into petal-like blebs bulging out from the perikaryal membrane, arranged around the cell in a corona. The group named the pattern PANTHOS, from the Greek — a poisonous *anthos*, a poisonous flower. Three-dimensional serial electron-microscopic reconstruction, enzyme histochemistry and immuno-electron microscopy establish that these perikaryal blebs are distinct structures from the similarly cargo-packed dystrophic neurites of Chapter 10, which had previously been the only recognised form of this pathology.

Then the cell dies, and the manner of the death is the point. Hydrolases leak from the compromised compartments; amyloid fibrils accrete in the perinuclear region around a nucleus that is visibly disintegrating but still present in the great majority of these cells; glia invade; and what is left has the appearance of a classical cored neuritic plaque. The paper's quantitative claim is that individual neurons exhibiting this morphology are the principal source of senile plaques in amyloid precursor protein Alzheimer models.

The inference is that the plaque is, in these animals, the residue of a dead neuron rather than a precipitate from the extracellular fluid — that the lesion forms, in the group's phrase, inside-out.

Three supporting observations make this considerably harder to dismiss than a morphological narrative usually is.

The pH manipulation is causal in the right direction. Inhibiting endolysosomal acidification pharmacologically in wild-type mice produces neuritic dystrophy with no amyloid deposition at all, and accentuates neuritic plaque development in amyloid models. Acidification failure is upstream of the morphology, not a description of it.

The lysosomal contents are in the deposit, and have been since 1990. The framework's founding observation acquires, thirty-two years later, the mechanism it lacked. Cathepsins and lysosomal matrix proteins are in extracellular plaque deposits because plaques are, on this reading, spilled lysosomal contents. A finding that had no home in 1990 has one now, and the fit is exact.

And blocking autophagosome formation dissociates the plaque from the neuron's fate. Nilsson and colleagues, working independently in Saido's laboratory, deleted the autophagy gene *Atg7* in the neurons of an amyloid model. Extracellular amyloid deposition fell markedly — and intracellular amyloid rose, with accelerated neurodegeneration (Nilsson et al., 2013). If the plaque were the poison, removing it should help. Removing the route by which it forms made the neuron worse. This is one of the few experiments in the amyloid literature that separates plaque burden from neuronal fate, and its result is the one the clearance framework predicts.

13. What Was Not Shown

Everything in the previous chapter is a statement about mice. The framework's readers, and sometimes the framework, elide the distinction. It should be held.

The quantification is model-specific, and the models are over-expressors. The load-bearing sentence of Lee 2022 — that PANTHOS neurons are the principal source of senile plaques — is quantified in amyloid precursor protein transgenic mice. These animals over-express a mutant human protein at multiples of the endogenous level. The framework's own logic makes this a serious limitation rather than a routine one: if the pathology arises from cargo overwhelming a compartment's disposal capacity, then an animal engineered to over-produce that cargo is an animal with the independent variable set to an extreme value, and the fraction of plaques arising by the intracellular route in such an animal cannot be transferred to a human brain. The programme is aware of this. Its readers should be.

The human observation is qualitative. PANTHOS-like morphology is reported in human Alzheimer brain, in the 2022 paper and in a subsequent review (Nixon, 2024). What has not been published in the peer-reviewed literature is a quantification of how much of the human plaque burden arises this way.

And here the record contains something that should be reported rather than smoothed. In November 2024 the group posted a preprint reporting the human study that would close this gap — proteomic analysis of autophagy-lysosomal pathway components in the ROSMAP and Banner cohorts showing selective deficits in vacuolar-ATPase subunits and diminished vacuolar-ATPase transcripts in excitatory neurons but not in other cell types, together with biochemical, confocal and immuno-electron-microscopic analysis of human brain reporting defective neuronal lysosomal clearance, intracellular amyloid formation within endoplasmic-reticulum-related membrane tubules, and, in select neurons among broadly affected neocortical populations, cell death yielding extracellular senile plaques (Lee et al., 2024, preprint). An abstract of the same work appeared in the conference proceedings of *Alzheimer's & Dementia* the following month.

As of August 2026 — twenty-one months after posting — this manuscript is not indexed in PubMed as a peer-reviewed publication, and no 2026 primary paper from the group on this subject appears in that index. We draw no inference about why. Preprints take variable and sometimes very long paths; a delay of this length is unusual but not extraordinary, and it may reflect the depth of revision that a claim of this weight properly attracts. But the honest statement of the framework's current evidential position must be that *its central human claim rests on a manuscript that has not yet*

completed peer review, and any evaluation that cites the human data without saying so is overstating the case. We cite the preprint below where its content bears on the argument, and we mark every such citation.

A fourth limit is intrinsic and will not be removed by any journal. The route by which a plaque formed is erased by the plaque's own completion. A finished deposit is the same object whether it condensed from secreted peptide in the neuropil or was released by a ruptured cell, and the inside-out route specifically destroys the neuron whose contents would be needed to attribute it. Human brains supply one frozen frame of a process that runs for a decade or two; the peptide carries no provenance label; and a plaque that nucleates by one route may then grow by the other, in which case the "fraction" being argued about does not exist as a quantity. What can be settled in mice may not be settleable in humans, and the framework should say so more often than it does.

14. The Two Routes, and the Discipline of Not Choosing

The inside-out reading is frequently presented, by supporters and detractors alike, as a rival to the extracellular-seeding account. It is better understood as one of two demonstrated routes whose relative contribution is unknown.

The extracellular route is not a hypothesis. Dilute brain extract containing amyloid, injected into the extracellular space, nucleates cerebral β-amyloidosis in a time- and dose-dependent manner, and the effect is abolished by immunodepletion or denaturation of the amyloid — the defining properties of a seeded, template-directed process (Meyer-Luehmann et al., 2006), subsequently generalised across the neurodegenerative proteinopathies (Jucker & Walker, 2013). This route is real, it drives propagation and diffuse deposition, and it is not weakened by anything in the intracellular literature.

The intracellular route is also not a hypothesis, in animals. It is demonstrated, quantified, and mechanistically specified.

The defensible statement, which we adopt for the remainder of this paper, is therefore layered:

- Amyloid-β is generated intracellularly, within the endosomal and secretory system. This is not controversial.
- Intraneuronal amyloid-β42 accumulates in vulnerable human neurons in a distribution that precedes tangles and plaques (Gouras et al., 2000), localised to multivesicular bodies within synaptic compartments and associated with abnormal synaptic morphology before plaque pathology (Takahashi et al., 2002). Established in human tissue, by two groups, before this framework's terminal claim existed.
- A substantial share of dense-cored plaques plausibly forms inside-out from failing neurons. Established in mouse models; observed but unquantified in humans.
- Extracellular seeding independently nucleates and propagates deposits. Established.
- **The fraction of human plaque burden attributable to each route is not established, and any specific percentage overreaches the data.**

Two consequences follow that are worth stating because they cut in opposite directions.

The first favours the framework. Even a minority contribution from the inside-out route is enough to break the inference the field has drawn from plaque counts for forty years. If some plaques mark the sites where neurons died, then plaque burden is partly a record of past cell death, and its poor correlation with cognition — one of the oldest embarrassments in the field — is exactly what one would expect from a variable that is part cause and part gravestone.

The second cuts against it. A framework that positions the plaque as a tombstone must then explain why removing plaques from living patients produces a measurable, reproducible, if modest, slowing of clinical decline. Both lecanemab and donanemab did so in large randomised trials (van Dyck et al., 2023; Sims et al., 2023). The effect sizes are small and their clinical meaningfulness is genuinely disputed, but the direction is consistent and it is not what a pure gravestone model predicts. The most economical reconciliation — and we offer it as an inference, not a finding — is that the extracellular pool is not inert: it seeds further deposition, it damages the neurites that pass through it, and it engages microglia in a way that has its own costs. On that reading, both routes are real, both matter, and the therapeutic ceiling of removing the deposit is set by how much of the disease was ever in the deposit. That is a modest ceiling, and the trials have found it.

Part IV — Where the Account Is Weak

15. The Universality Problem

The framework's own summary states that "based on review of the literature, it is the only framework that potentially explains the entirety of pathophysiological phenomena and pathological lesions that define the AD phenotype," and that it does so "within one dysfunctional biological network."

This is the weakest sentence in the programme, and the reason is not that it is boastful. It is that explanatory scope, offered as a virtue, is the characteristic signature of a theory that has stopped being able to fail.

Consider what the network is. Every internalised molecule, every condemned protein, every damaged organelle and every recycled receptor in a neuron transits the endosomal–lysosomal–autophagy system. Its compartments carry the nutrient sensor, the principal intracellular calcium store outside the endoplasmic reticulum, the receptors whose recycling sets synaptic strength, and the trophic signal that keeps the basal forebrain alive. A system with that many functions will be found abnormal in any disease that involves cargo, energy, calcium, receptors or trophic support — which is to say, in any neurodegenerative disease, and in normal ageing.

The programme's own recent work makes this concrete rather than hypothetical. Its 2025 characterisation of the autophagy–lysosomal pathway in Huntington's disease brain finds the pathway extensively disturbed there too (Berg et al., 2025); the 2024 review with Rubinsztein surveys the same failure across Alzheimer's, Parkinson's and frontotemporal disease (Nixon & Rubinsztein, 2024). We take these as good science and as a real extension of the framework's reach. But they sharpen the question that the universality claim cannot answer: if this network fails in Alzheimer's disease, Huntington's disease, Parkinson's disease, frontotemporal degeneration and ageing, what is it about its failure that makes a person get *Alzheimer's* disease?

The framework has an answer, and it is a good one, but it costs more than it appears to. The answer is the "special partnership" with the amyloid precursor protein: what distinguishes Alzheimer's disease is that the substrate this particular network cannot clear is itself a direct inhibitor of the network, so that the disease is a self-amplifying loop rather than a linear failure. That answer is coherent, mechanistically specified, and probably correct.

It is also an admission that the framework is *APP-dependent*. Remove the precursor protein from the account and there is no longer anything that makes the disease Alzheimer's rather than generic proteostatic ageing. This is a considerably more modest position than the framework's rhetoric

implies, and it is a better one. It means the account is not an alternative to amyloid-centred thinking but a specification of it: the same gene, the same enzyme, a different product, a different compartment, and a different direction of causation. Stated that way it is a serious, testable, and partly-established proposition. Stated as universal explanation it is neither.

Three further consequences of the universality framing deserve mention.

It obscures the framework's genuine predictive successes. A theory that explains everything gets no credit for explaining anything, and this one has made several predictions that came true and that its rivals did not make: that the fragment rather than the peptide would prove pathogenic; that inhibiting γ-secretase would worsen rather than improve outcomes; that mixed proteinopathy would be the rule rather than the exception; that neuritic dystrophy could be produced without amyloid. These are real. The universality claim buries them among a hundred post-hoc accommodations.

It licenses loose usage downstream. A framework advertised as explaining the entire phenotype is a framework that other authors will apply to whichever part of the phenotype they are writing about, without checking whether the mechanism was demonstrated in that cell type or at that stage. Chapter 18 documents one consequence of this in detail.

And it makes the falsification question hard to answer, which is why we answer it explicitly in Chapter 28. Any framework of this ambition owes its readers a statement of what would refute it. This one has never published such a statement. We supply five candidates, drawn from the framework's own commitments rather than from ours.

16. Antecedent, or Common Funnel?

The framework's strongest word is "antecedent." Endosomal–lysosomal dysfunction is offered as "the principle biological antecedent to the first detectable pathological changes in AD." The alternative reading — that the network is a common funnel through which many upstream processes discharge, and whose failure is therefore early, universal and consequential but not initiating — is never seriously entertained in the framework's own presentations. It should be, because a great deal of the evidence is compatible with both and discriminates poorly.

Consider the strongest timing evidence, which is Down syndrome. Enlarged endosomes are present in trisomic neurons before birth, decades ahead of any deposit. This is unambiguous and it is the framework's best temporal card.

But notice what it establishes. In Down syndrome the initiating lesion is an extra copy of chromosome 21, carrying an extra copy of *APP*, producing elevated precursor protein and hence elevated βCTF from conception. The causal arrow in that condition runs *from the gene dose of the precursor protein to the endosome*. The observation therefore powerfully supports the fragment claim of Chapter 4 — the endosome responds to βCTF exactly as predicted — while providing no support at all for the proposition that disposal failure is upstream of precursor-protein biology. In the framework's best-timed case, the precursor protein is the cause and the network is the target.

The same ambiguity runs through the sporadic disease. Ageing degrades the pump; risk alleles degrade sorting, recycling and lipid handling; oxidative and metabolic stress degrade hydrolases; and the network fails. Every one of those is upstream of the network. What the framework calls the antecedent is, in its own account, the point at which several antecedents converge. That is a funnel, and a funnel can be the most important point in a pathway — it is where the largest number of causes become one effect, and therefore where a single intervention has the widest reach — without being the origin.

We think the funnel reading is the fairer one and, importantly, that it is not a demotion. Three considerations support it.

The framework itself is reciprocal. Its own description of the "cause and effect cycle, with reciprocal amplification" is a description of a loop. Loops do not have origins; they have entry points, and different entry points may predominate in different people. This is a strength — it accommodates the heterogeneity of the disease — but it is inconsistent with a single antecedent.

Sufficiency experiments cannot establish priority. Chapter 6 said this and it bears repeating here, because it is the most common inferential error made on this framework's behalf. That the Rab5-over-activated mouse develops the phenotype shows the node can drive the disease. Amyloid over-expressing mice also develop a phenotype, and the field spent two decades over-reading that.

And the one place where the framework's arrow is unambiguous is the place where the network fails first for reasons that are not about the precursor protein at all — namely ageing, where pump decline and hydrolase oxidation are independently measured in cells with no amyloid pathology of any kind. That is a genuine upstream lesion. It is also not specific to Alzheimer's disease, which returns us to Chapter 15.

The honest formulation, which we adopt: *the endosomal–lysosomal–autophagy network is the convergence point of the disease's causes and the proximate cause of most of its lesions. Whether it is the point of origin is unresolved, is probably person-dependent, and may not be a well-posed question.*

17. The Rival at the Same Station

The framework has one serious competitor working on the same compartment, with a comparable evidential record, and the two have never been tested against each other in a design that could distinguish them.

Scott Small's programme locates the primary lesion in the retrieval arm of the endosome. Model-guided microarray analysis of Alzheimer brain implicated the retromer complex — the coat that recovers cargo from the endosome and returns it to the plasma membrane or the trans-Golgi network — with retromer components deficient in the vulnerable regions (Small et al., 2005). Genetic reduction of retromer in animals produces hippocampal dysfunction, neurodegeneration and amyloid accumulation (Muhammad et al., 2008). The sorting receptor *SORL1*, which delivers the precursor protein into the retromer-dependent retrieval pathway, is one of the strongest Alzheimer risk genes known and carries loss-of-function variants with effect sizes approaching autosomal dominance; a systematic domain-mapping study has now classified its pathogenic variants (Andersen et al., 2025). Pharmacological chaperones that stabilise the retromer limit amyloidogenic processing (Mecozzi et al., 2014), and retromer enhancement rescues the endosomal pathology produced by *SORL1* deficiency in human neurons (Mishra et al., 2023). The regional argument is derived rather than fitted: the lateral entorhinal cortex is where the sorting deficit is expected and where preclinical dysfunction is measured (Khan et al., 2014). The programme has been summarised for a general audience with unusual clarity (Small & Petsko, 2015).

Set the two accounts side by side and the agreements are more striking than the disagreement. Both locate the disease in the endosome. Both hold that the compartment fails before plaques appear. Both have interventional animal evidence that their lesion is sufficient. Both treat the amyloid deposit as downstream. Both read the genetics as pointing at the organelle.

The disagreement is about the entry point, and it is sharp. In Small's account the sorting machinery fails first — retromer is deficient, cargo residence time in the endosome lengthens, the precursor protein spends longer in the compartment where BACE1 is enriched, and the fragment and peptide are consequences. In Nixon's account the fragment comes first — elevated βCTF recruits APPL1, locks Rab5 active, and disables the compartment including its exit machinery, with the sorting deficit a consequence.

The two are not obviously reconcilable, and the human-cell literature contains the disagreement in miniature. Depleting *SORL1* in human neurons produces endosomal traffic defects that one group finds independent of amyloidogenic processing (Knupp et al., 2020) and another finds dependent on it (Hung et al., 2021). Nixon's model requires the second; Small's is more

comfortable with the first.

The discriminating experiment exists and has not been done. Take the mouse in which Rab5 is directly over-activated, in which there is no primary retromer or sorting-receptor lesion, and ask whether pharmacological retromer enhancement rescues it. If it does, the sorting arm is downstream of Rab5 activation and can be reached from it — the two accounts describe one lesion approached from two sides. If it does not, then Rab5 over-activation produces a state that retrieval enhancement cannot correct, and the two lesions are separable, with implications for which one to drug.

The reagents have existed since 2014. The mouse has existed since 2020. Six years on, no such experiment appears in the literature. We rank it first among the ten experiments of Chapter 29, and we note, without attributing motive, that it is the kind of experiment that two well-established programmes each have reasons not to prioritise.

Part V — Placing the Lesion

18. Two Lesions, Not One

The framework contains two distinct pathogenic mechanisms that are usually presented as one, and separating them clarifies a great deal.

The first is a signalling lesion at the early endosome. Its currency is not undegraded cargo but interrupted communication. The neurotrophin receptor TrkA, bound to nerve growth factor at the axon terminal, is internalised into a Rab5-positive compartment and carried retrogradely to the soma, where it initiates the transcriptional programme on which the neuron's survival depends. This is the "signalling endosome," and it is a vehicle, not a bin. When Rab5 is over-activated, the compartment swells, its motility is impaired, and retrograde transport of the trophic signal fails. The cell then dies of trophic starvation, in the presence of adequate trophic factor, because the message never arrives.

The framework's designated earliest human casualty — the basal forebrain cholinergic neuron — dies this way in its account, and the supporting evidence is unusually direct. Trophic signalling from Rab5 endosomes declines early in Alzheimer's disease and in Down syndrome; the Rab GTPases are transcriptionally up-regulated in individually captured cholinergic basal forebrain neurons in mild cognitive impairment, before dementia (Ginsberg et al., 2011); and in the animals, cholinergic degeneration is produced by Rab5 over-activation alone (Pensalfini et al., 2020) and by APPL1 over-expression alone (Jiang et al., 2025), and is rescued by raising nerve growth factor, by lowering βCTF, or by reversing Rab5 activation pharmacologically.

The second is a disposal lesion at the terminal lysosome. Its currency is cargo. Acidification fails; hydrolases are not activated; substrate accumulates; compartments distend; membranes are permeabilised; hydrolases leak; the cell dies by a lysosome-dependent death with mixed necrotic and apoptotic features. Where the accumulated cargo is amyloidogenic and abundant, the terminal morphology is PANTHOS and the residue is a plaque.

These are not the same lesion, and the distinction is not pedantic, for four reasons.

They occur in different cells. The basal forebrain cholinergic neuron produces relatively little amyloid-β. The trophic lesion needs only a receptor, a motor and a swollen compartment; the disposal lesion needs a large amyloidogenic cargo load to reach its named terminal morphology.

They have different time courses. Trophic starvation is a slow withdrawal of support, measurable as atrophy and transmitter deficit long before cell counts change. Autolysosomal rupture is an event.

They leave different traces. The trophic lesion leaves a shrunken, silent, still-living neuron. The disposal lesion, in the right cell, leaves a plaque. A brain region with the first lesion and not the second will show functional decline with no deposit — which is precisely what is observed in the earliest affected subcortical nuclei.

And they require different drugs. Trophic-endosome failure is corrected upstream, by lowering the fragment or by reversing Rab5 activation — which is what neflamapimod was designed to do. Disposal failure is corrected downstream, by restoring the pH of a compartment. A compound that does the first does not do the second, and Chapter 25 argues that the field has repeatedly assumed otherwise.

The framework's own therapeutic section runs these together, listing the interventions that rescue cholinergic degeneration alongside those that restore lysosomal proteolysis as though they were interchangeable evidence for one proposition. They are evidence for two.

19. Which Cells Can Make a Flower

PANTHOS is defined by its cargo. What distends the compartments, arranges into the corona and remains in the neuropil after rupture is amyloid — amyloid-β and βCTF accumulating inside de-acidified autolysosomes. A cell that cannot acidify but has little amyloidogenic cargo can suffer proteostatic gridlock, waste accumulation, membrane permeabilisation and death. It cannot produce this morphology, and it cannot leave this residue.

The conjunction the morphology requires is therefore: **acidification failure, plus a high load of amyloidogenic cargo.** The second condition is a property of the cell type. High precursor-protein expression and high amyloid-β generation characterise glutamatergic pyramidal neurons — and the human observation that opened this whole line of inquiry was of intraneuronal amyloid-β42 accumulating in exactly those cells, in the vulnerable regions, before tangles or plaques (Gouras et al., 2000), localised to multivesicular bodies in synaptic compartments (Takahashi et al., 2002).

The framework states the restriction itself, and this should be said before anything is added to it. Its 2024 review describes the disease as "an imbalance between heightened autophagy induction and diminished lysosomal function in highly vulnerable **pyramidal neuron** populations," and describes the amyloid that becomes an extracellular plaque as accumulating "in the most compromised of these neurons" (Nixon, 2024). The restriction is therefore not a correction imposed from outside. It is the framework's own scope condition, stated in a review and then routinely dropped by others — and, in the framework's more expansive summaries, by the framework — in favour of claims about the Alzheimer neuron in general.

Three further lines converge on the same restriction.

The programme's own human data, so far unpublished, restrict it explicitly. The 2024 preprint reports vacuolar-ATPase subunit deficits and diminished vacuolar-ATPase transcripts in **excitatory neurons but not in other cell types** in the ROSMAP and Banner cohorts (Lee et al., 2024, preprint). If that holds under review, the framework's terminal lesion is cell-type-selective in human brain by the framework's own measurement, and selective for exactly the population the cargo argument predicts.

The cell atlases place the loss of that population late. The Seattle Alzheimer's Disease Brain Cell Atlas resolved the disease into two epochs across millions of nuclei from a large donor series, with the later epoch marked by loss of excitatory neurons together with parvalbumin- and VIP-expressing interneurons (Gabitto et al., 2024; Hawrylycz et al., 2024). The atlas profiled middle temporal gyrus

and cannot speak to subcortical nuclei, and it measures transcriptomic abundance rather than autolysosomal morphology. Within those limits it corroborates the timing and the population, not the mechanism.

And the arithmetic of the casualties points the same way. CA1 contains on the order of fourteen million neurons and loses roughly two-thirds of them in Alzheimer's disease (West et al., 1994). Entorhinal layer II contains on the order of six hundred and fifty thousand and loses close to ninety per cent, and does so in *very mild* disease — before dementia (Gómez-Isla et al., 1996). These are the largest cell-death events in the disease, they are in glutamatergic pyramidal populations, and they are the regions in which intraneuronal amyloid-β42 was found in human tissue in 2000.

The scope statement that follows is one the framework has never made and, in our view, should:

> *Acidification failure and autophagic stalling are general lesions of the ageing, stressed neuron and are not confined to any transmitter class. PANTHOS is a conditional terminal morphology of that failure, arising where — and only where — the failing cell is also a large-scale producer of amyloidogenic cargo. The disposal lesion is general; the flower is not.*

Nothing in the framework contradicts this. But because the framework advertises itself as explaining the entire phenotype, downstream readers have generalised the morphology to cells that cannot produce it, and the next chapter shows what that costs.

20. Two Clocks

Alzheimer's disease has at least two chronologies and they are frequently collapsed into one.

The first is the chronology of tau. Braak's series established that abnormal tau appears in the brainstem, and specifically in the locus coeruleus, decades before cortical disease and often in young adults, with pretangle material demonstrable in individuals under thirty (Braak & Del Tredici, 2011; Braak et al., 2011). Quantification confirms substantial pretangle burden in the coerulean and raphe nuclei at the earliest stages (Ehrenberg et al., 2017). On this clock, the disease begins in the brainstem in the third or fourth decade.

The second is the chronology of neuronal death and deposition. Design-based stereology of the human locus coeruleus finds volume declining early but *neuron number comparatively preserved through the early stages*, with significant loss only from Braak stage III onward (Theofilas et al., 2017). The first plaques are neocortical and appear after brainstem tau has begun. The great majority of measured neuron loss is cortical and hippocampal, and the atlases place excitatory-neuron loss in the late epoch.

Both clocks are real and they measure different things. Tau accumulation in a nucleus is not death of that nucleus; a cell can carry pretangle material for decades. Conflating them produces a specific and consequential error: it invites the reader to place a *terminal* morphology in the decade when the *earliest* molecular change is occurring, in a population that is not yet dying.

Applied to this framework, the error takes a particular form. It is not the framework's own error: its 2024 review places PANTHOS at the earliest *intraneuronal*, pre-plaque stage of the pyramidal sequence, which is a statement about position within a cortical progression rather than about chronological age or brain region. The error is in the transfer, and it appears repeatedly in secondary literature that cites the programme approvingly: the phrase "earliest stage" is read as "earliest structure affected in the whole brain," and the morphology travels to the brainstem with it. The locus coeruleus is noradrenergic. It is small — tens of thousands of neurons against tens of billions in the cortical mantle. It produces comparatively little amyloid-β. It is not losing neurons in the decades when its tau pathology begins. And there is no plaque burden in the locus coeruleus of a young adult, so whatever is happening to that cell's disposal machinery at that age, it is not leaving the residue that defines PANTHOS. To describe the coerulean lesion of the fourth decade as the framework's terminal morphology is to assign a cortical, cargo-dependent, plaque-yielding death to a brainstem, low-cargo, plaque-free population that is not dying.

The corrected placement is not a retreat from the framework. It is a sharpening of it, and it has three components.

The machinery is general and it may indeed fail early and subcortically. Nothing here disputes that the locus coeruleus suffers proteostatic and autophagic stress early. That is plausible, it is consistent with everything known about the metabolic burden of a neuron with an enormous unmyelinated axonal arbour, and it would explain a great deal. It is also, at present, an inference rather than a human demonstration, and it should be graded as such.

The morphology is cortical, cargo-dependent, and late. PANTHOS belongs to the entorhinal, hippocampal and neocortical pyramidal populations, which is where it has been most convincingly demonstrated, where the intracellular amyloid was found, where the cell-death arithmetic is, and where the plaques are.

And these two facts require different drugs at different times, which is the subject of Part VI and the reason this chapter is not bookkeeping.

21. The Forward Leg and the Return Leg

There is a structural regularity in this framework that becomes visible only when its findings are listed together, and once seen it is difficult to unsee.

Every physiological process the framework touches is a cycle with two legs. Endocytosis has recycling. Autophagosome formation has autolysosomal degradation. Anterograde delivery has retrograde retrieval. Cleavage has clearance. Acidification has, on the other side of the same pump, the alkalinisation that follows when the pump fails.

Now list what the framework reports as intact, elevated, or accelerated:

- Endocytosis is *accelerated* — internalisation of surface cargo and of AMPA receptors is increased, not reduced.
- β-Cleavage is *elevated* — BACE1 activity is high in Alzheimer brain and βCTF and sAPPβ are raised even where total precursor protein is normal.
- Autophagy *induction* is increased. This is the most important entry on the list and it comes from human tissue: analysis of CA1 neurons in Alzheimer hippocampus found that increased induction overburdens failing lysosomes and thereby propels neuritic dystrophy (Bordi et al., 2016). The cell is trying harder.
- Exosome release is *up-regulated*, as a default route when vesicular traffic jams.
- Lysosomal biogenesis and hydrolase expression are *up-regulated* — the 1990 finding of increased lysosomal enzyme content in Alzheimer neurons was, in retrospect, a compensatory response read as a lesion.
- Granulovacuolar bodies form, which the framework itself reads as an adaptive tactic to maintain survival.

And now list what the framework reports as failing:

- Acidification of the terminal compartment.
- Hydrolysis of cargo within it.
- Retrograde transport of loaded compartments to the soma.
- Recycling out of the sorting endosome, through Rab11 and the retromer.

- Retrograde delivery of the trophic signal.
- Chaperone-mediated autophagy.

Every item in the first list is a forward leg. Every item in the second is a return.

This is not a tautology, and the test of that is whether the pattern could have come out otherwise. It could. A production theory of this disease predicts the opposite signature: elevated synthesis with normal disposal. A theory of generalised cellular exhaustion predicts both legs failing together. What is observed is a specific dissociation — six measured up-regulations on the outbound side, six measured failures on the return — and it is the signature of a system compensating for a downstream block.

Two consequences follow and both are consequential.

Diagnostically, it means the field has been measuring the wrong quantity. Bulk measures of amyloid production, of autophagy markers, of lysosomal enzyme content will read *high* in a brain whose lesion is a failure to complete. LC3-II is elevated when autophagosomes accumulate, which happens both when induction rises and when clearance fails, and the two are indistinguishable without a flux measurement. Almost none of the human literature on autophagy in Alzheimer's disease measures flux.

Therapeutically, it means the standard intervention has the wrong sign. That is the subject of the next chapter, and it is, in our judgement, the most actionable and most neglected implication in this entire framework.

Part VI — What Follows for Treatment

22. Induction Is Not the Lesion

Nearly every attempt to treat Alzheimer's disease by acting on autophagy has been an attempt to induce it. Rapamycin and its analogues inhibit mTOR and induce; spermidine induces; trehalose induces; caloric restriction, exercise and intermittent fasting induce; and the compounds in current clinical development that carry an autophagy rationale are, without exception, described as enhancing autophagic clearance from the induction end.

The framework's own data say this is the wrong end.

Induction in the Alzheimer neuron is not deficient. It is elevated. The measurement was made in human tissue: in CA1 neurons of Alzheimer hippocampus, autophagy induction is *increased*, and the increase overburdens failing lysosomes and thereby propels neuritic dystrophy (Bordi et al., 2016). The framework's own recent statement of the position is unambiguous. What characterises the disease is "an imbalance between heightened autophagy induction and diminished lysosomal function in highly vulnerable pyramidal neuron populations," which "yields an intracellular lysosomal build-up of undegraded substrates, including APP-βCTF, an inhibitor of lysosomal acidification, and membrane-damaging Aβ peptide" (Nixon, 2024). The word carrying the therapeutic weight is *imbalance*: one side of it is already too high.

The therapeutic corollary is a knife-edge, and it is stated here as plainly as we can manage:

> *An autophagy inducer given to a neuron that can still acidify its lysosomes accelerates clearance and helps. The same inducer given to a neuron that can no longer acidify its lysosomes fills it faster with compartments it cannot complete, and accelerates precisely the trajectory that ends in rupture.*

The sign of the intervention depends on the state of a downstream step that no clinical trial has ever measured, in any patient, at any stage.

This is not a hypothetical concern. It is the reason the induction literature in this disease is inconsistent, and it predicts specific patterns in the existing data that are worth testing retrospectively: benefit in young animals and early-stage models, attenuation or reversal in aged animals and late-stage models, and — the sharpest prediction — worse outcomes from induction in models with an engineered acidification defect. The framework has effectively made this prediction and the field has not systematically checked it.

Three practical consequences follow.

Sequence matters and it has a direction. Restore degradative capacity first; induce second. The framework's own therapeutic logic is explicit that flux enhancement works "through the entire pathway," and that the enhancement of *lysosomal proteolytic efficiency* — by lowering endogenous protease inhibitors, by increasing lysosomal biogenesis and vacuolar-ATPase subunit transcription, by improving retrograde transport of endolysosomes, or by directly re-acidifying — is what produces the broad rescue. Every one of those acts on the return leg.

Stage matters, and the acidification manoeuvre is the late one. A neuron whose pump still works needs flux. A neuron whose pump has failed needs the pump. If, as Part V argued, the acidification-failed, cargo-loaded cortical pyramidal neuron is a feature of the middle and late disease, then pump defence is a mid-to-late-stage intervention, and the field's habit of assuming that all "upstream" mechanisms imply "treat early" is wrong in this specific case.

And there is no effector to inhibit. This is a structural feature of the lesion that is easy to miss. Most neurodegenerative death mechanisms present a target to block — a protease, a kinase, a channel, a receptor. Death by autolysosomal failure results from the *absence* of a function, not the presence of a signal. The therapeutic response must be restorative rather than inhibitory, which rules out the entire class of interventions the industry is best at making.

23. What Has Been Tried in Humans

One drug has been developed from this cascade and taken into patients, and its record is the most uncomfortable fact in the file. We state it before anything else because a framework's therapeutic predictions are its most exposed surface and this evaluation would be worthless if it buried them.

Neflamapimod is an orally available, brain-penetrant inhibitor of p38α mitogen-activated protein kinase. Its rationale within this framework is specific: p38α inhibition reverses Rab5 over-activation, corrects endosomal dysfunction, and rescues cholinergic degeneration and tau hyperphosphorylation in Down syndrome models and patient fibroblasts. It is, in other words, a drug aimed at the signalling-endosome lesion of Chapter 18, not at the disposal lesion.

In Alzheimer's disease it failed. The REVERSE-SD study, a randomised, double-blind, placebo-controlled 24-week Phase 2 trial in mild Alzheimer's disease, did not meet its primary endpoint on the episodic-memory composite (Prins et al., 2021).

In dementia with Lewy bodies it has had a more encouraging run, under a condition that deserves attention. A Phase 2a study produced signals of benefit, and a pre-specified biomarker analysis found that response was associated with plasma phosphorylated tau: patients with elevated phospho-tau181 — that is, those with likely Alzheimer co-pathology — responded less well (Alam et al., 2023). The subsequent Phase 2b programme was designed around that finding and *excluded* patients with Alzheimer co-pathology by plasma phospho-tau181 (Prins et al., 2024).

And the Phase 2b result requires care to report honestly. The randomised, double-blind, 16-week portion was compromised by a drug-product problem: the capsules used in the main phase delivered lower plasma drug concentrations than those used in the earlier Phase 2a study. The sponsor reported that participants receiving corrected capsules in the open-label extension showed substantially less decline than those who had received the original capsules, and has stated an intention to proceed to a pivotal Phase 3 trial in the same population. That is a plausible and even a likely explanation of a disappointing double-blind result. It is also, in the strict sense, an open-label comparison of two non-randomised groups, and it is not the same grade of evidence as a positive randomised trial.

The summary position, stated without spin: *the one compound derived from this framework has failed in Alzheimer's disease, has been developed successfully in a different dementia, and its development in that dementia proceeded by excluding patients with Alzheimer pathology.*

Four readings of that are available and we think the truth is a mixture of the first three.

The drug was given too late. The framework's own logic places the endosomal signalling lesion decades before symptoms. A 24-week trial in patients with established mild Alzheimer's dementia tests whether reversing a decades-old upstream lesion improves memory in a brain that has already lost its entorhinal layer II. Nothing in the framework predicts that it would.

The drug was aimed at the wrong lesion for the stage. By the time a patient has mild Alzheimer's dementia, Part V argues, the dominant process is disposal failure in cortical pyramidal neurons, not trophic-endosome failure in the basal forebrain. Neflamapimod does not restore lysosomal pH.

The endpoint was wrong for the mechanism. A drug that protects cholinergic trophic signalling should be tested against cholinergic outcomes — attention, fluctuating arousal, and the cholinergic-responsive features that are, in fact, exactly where it appears to work in Lewy body disease.

Or the framework is wrong about this node in humans. This possibility must remain on the table. It is not currently supported over the others, but it is not excluded by them either, and it would be excluded only by a properly staged trial that has not been run.

No compound targeting the *disposal* arm of this framework — lysosomal re-acidification, vacuolar-ATPase support, hydrolase enhancement — has entered clinical testing in Alzheimer's disease at all. The framework's central therapeutic claim is, thirty-six years on, clinically untested.

24. Three Trial Failures, Read as a Fragment Problem

The framework offers a re-reading of the anti-amyloid trial record, and it is worth separating the part that is a genuine predictive success from the part that is a post-hoc accommodation. They are usually presented together.

The γ-secretase result is a real success. Semagacestat, a γ-secretase inhibitor, lowered amyloid-β production and, in a large Phase 3 programme, *worsened* cognition relative to placebo (Doody et al., 2013). On the standard cascade this is anomalous and was attributed to Notch-related off-target toxicity. On the fragment reading it is predicted: γ-secretase is the enzyme that destroys βCTF, so inhibiting it raises the concentration of the species this framework holds to be pathogenic. The prediction is directional, it is mechanistically specific, it was available before the result, and the alternative explanation — Notch — is not exclusive of it. This is the framework's cleanest translational retrodiction.

The BACE1 result is an accommodation, and should be labelled as one. BACE1 inhibitors lower βCTF as well as amyloid-β. On the fragment reading they should therefore help. Verubecestat, in a large randomised trial in prodromal Alzheimer's disease, did not help and was associated with slight worsening on some cognitive measures (Egan et al., 2019); the class has failed repeatedly. The framework's answer is that trials used near-maximal inhibition in order to drive amyloid-β down, that this produces toxicity through other BACE1 substrates, and that a partial inhibition sufficient to normalise βCTF without those effects has never been tested.

That answer is not unreasonable. BACE1 has many substrates with roles in myelination and synaptic function, and a dose–response window is a legitimate hypothesis. But it is unfalsified, it was formulated after the failures, and it has the structure of an auxiliary hypothesis protecting a core claim. It should be graded accordingly, and it generates an obvious experiment: a dose-ranging study with βCTF, not amyloid-β, as the pharmacodynamic readout. Nobody has run it, in part because βCTF is difficult to measure in a living human being — which is itself a fact about this framework worth noticing.

The protective variant is neutral between the accounts. The Icelandic *APP* A673T variant slows β-cleavage and protects against Alzheimer's disease and age-related cognitive decline (Jonsson et al., 2012). This lowers amyloid-β *and* βCTF, so both readings accommodate it. The framework's claim that it "better explains" the variant does not survive inspection: it is equally explained by either, and citing it as support is an overreach.

The immunotherapy result is where the framework is most exposed. Lecanemab and donanemab clear plaque and produce statistically robust, clinically modest slowing (van Dyck et al., 2023; Sims et al., 2023). A framework in which the plaque is principally a gravestone predicts no benefit from removing gravestones. The reconciliation offered in Chapter 14 — that the extracellular pool is not inert and that the ceiling on removing it is low, which is what the trials found — is coherent, but it is a reconciliation and not a prediction, and the framework did not make it in advance.

The fair overall assessment of this framework's translational record is therefore mixed and specific: one strong directional success, one accommodation, one neutral case, and one result it did not predict and must absorb.

25. Re-acidification, and the Drugs That Do the Opposite

If the terminal lesion is a pH lesion, the terminal intervention is to restore the pH. The rationale is the strongest in the framework and the clinical record is empty. What exists is preclinical, and some of it is very good.

Restoring lysosomal proteolytic efficiency by deleting cystatin B, an endogenous inhibitor of lysosomal cysteine proteases, in an established amyloid model restored autophagic–lysosomal function, reduced amyloid pathology and improved memory (Yang et al., 2011). Transcriptional approaches — increasing lysosomal biogenesis and vacuolar-ATPase subunit expression through TFEB — and channel approaches through TRPML1 have preclinical support. A conference report from the originating laboratory describes a pharmacological re-acidification strategy acting through β2-adrenergic receptor and protein-kinase-A signalling, derived from an isoproterenol pharmacophore, which in 5xFAD mice restored neuronal lysosomal acidification, attenuated intracellular amyloid accumulation and neuronal death, reduced extracellular plaque burden by more than half, and improved memory (Malampati et al., 2025, conference abstract). We flag its status: this is a meeting abstract, not a peer-reviewed paper, and the numbers should be treated as provisional.

There is, however, a second and much stranger prediction on the same axis, and it has the virtue of being testable now, in existing data, at no experimental cost.

If lysosomal de-acidification is pathogenic in Alzheimer's disease, then drugs that de-acidify lysosomes should be risk factors. Several very widely used drug classes do exactly that. Proton-pump inhibitors are weak bases that accumulate in acidic compartments and have been reported to impair vacuolar-ATPase-dependent acidification; lysosomotropic disease-modifying antirheumatic drugs such as hydroxychloroquine raise lysosomal pH by design and are used experimentally for precisely that purpose. A commentary in *Autophagy* has asked the obvious question — whether evidence of an autolysosomal de-acidification defect in Alzheimer and Parkinson disease should give pause in prescribing these agents chronically (Giuliano et al., 2023).

We raise this not to make a clinical recommendation, which the evidence does not support, but because it is the clearest example we have found of a *falsifiable, immediately testable* prediction of this framework that nobody has systematically pursued. The epidemiology of proton-pump inhibitors and dementia risk exists and is inconsistent, and it is thoroughly confounded by indication, by comorbidity and by the association of reflux with obesity and with poor diet. But the framework

does not merely predict an association: it predicts a *mechanism-specific* one, which should be dose- and duration-dependent, should be shared by chemically unrelated agents with the same lysosomal effect, should be absent for acid-suppressing drugs that do not accumulate in lysosomes such as H_2-receptor antagonists, and should interact with the genetic risk factors that independently degrade the same machinery. That is a pattern that a well-designed pharmaco-epidemiological study could confirm or refute, and it would be, to our knowledge, the first direct human test of the acidification hypothesis in either direction.

26. What a Proper Test Would Look Like

The framework's central claim has not been tested in a human being, and the reason is not neglect. It is that the field cannot measure the thing.

There is no in vivo human measure of neuronal lysosomal pH. No PET ligand reports it. No fluid biomarker reports it. No imaging modality reports it. Consequently: target engagement cannot be demonstrated; dose cannot be titrated to effect; patients cannot be selected for having the lesion the drug corrects; and a negative trial can never distinguish a wrong hypothesis from an untreated target. Every clinical programme in this area is currently flying blind, and this — more than any conceptual objection in Part IV — is what has kept the framework's principal claim clinically untouched for a generation.

Any serious attempt should therefore begin with the measurement, and there are three candidate routes worth naming. Neuron-derived extracellular vesicles isolated from plasma carry lysosomal cargo and have been used to report intraneuronal protein states; whether they can report the *functional* state of the compartment rather than its contents is unknown and worth determining. Cathepsin activity assays on such vesicles, or on cerebrospinal fluid, would report the downstream consequence of pH rather than pH itself, which may be sufficient. And a pH-sensitive tracer with the right partitioning behaviour is not obviously impossible, though it has not been attempted seriously.

Given a measurement, the trial design that follows from Parts IV and V is specific and differs sharply from current practice:

- **Select on the lesion, not on the syndrome.** Enrol patients with demonstrated acidification failure, not patients with a clinical diagnosis of Alzheimer's disease.
- **Stage the intervention to the lesion.** Restore degradative capacity in patients whose pump has failed; induce flux only in those whose pump has not. Do not give the same drug to both.
- **Do not use plaque burden as the primary pharmacodynamic readout.** In this framework plaque is partly a record of past death and partly an independent extracellular pool; a drug that stops neurons dying should, on the inside-out model, *reduce the rate of new plaque formation* while leaving existing deposits untouched. Static plaque burden is close to the worst possible endpoint for this mechanism.
- **Measure flux, not markers.** LC3-II rises both when induction increases and when clearance fails. Any human autophagy readout that does not distinguish the two is

uninterpretable, and most published ones do not.

One large trial now reading out is worth naming, with its limitations stated. AR1001 (mirodenafil), a phosphodiesterase-5 inhibitor whose described mechanisms include enhancement of autophagy–lysosomal clearance alongside cyclic-GMP/PKG/CREB signalling and Wnt modulation, has completed the 52-week double-blind phase of a Phase 3 trial in early Alzheimer's disease with over fifteen hundred participants, with topline results expected in late 2026 (NCT05531526). It is not a clean test of this framework — its mechanism is multiple, its autophagy action is at the induction end, and no participant was selected for lysosomal status — but it will be the first large randomised readout for a compound carrying an autophagy rationale, and its result should be interpreted with those caveats stated in advance rather than after.

Part VII — Ledger, Refutation, and Tests

27. A Graded Ledger

The table below grades nineteen propositions drawn from the framework. Grades run:

Established — demonstrated in human material, replicated by independent groups, and not seriously contested. **Well supported** — strong evidence including at least one independent replication, with a stated limitation. **Supported, model-restricted** — good evidence confined to animal or cell models, with no human confirmation of the quantitative claim. **Contested** — reported and contradicted in the primary literature, unresolved. **Beyond the evidence** — asserted at a strength the cited evidence does not carry.

#	Proposition	Grade	Principal evidence	What would change the grade
1	Early endosomes are enlarged in Alzheimer neurons before amyloid deposition	Established	Cataldo 1997, 2000 (human); Ginsberg 2010, 2011 (human, single-cell expression)	An adequately powered human series finding the change only after deposition
2	The abnormality is present in Down syndrome decades before dementia, in some neurons before birth	Established	Cataldo 2000	Failure to replicate in independent trisomic tissue
3	*APOE* ε4 accentuates the endosomal abnormality	Well supported	Cataldo 2000; subsequent neuronal models (Nyberg 2025)	Genotype-stratified human series showing no effect
4	The driver of endosomal enlargement is βCTF, not amyloid-β	Well supported	Kwart 2019 (isogenic human neurons, independent lab); Lauritzen 2016; Kim 2016	A design separating the two species in which amyloid-β alone reproduces the change
5	βCTF acts by recruiting APPL1 and stabilising GTP-Rab5	Well supported	Kim 2016 (knockdown correction); Jiang 2025	Endosomal enlargement in an APPL1-null background
6	Autophagic vacuoles are the dominant organelle in Alzheimer dystrophic neurites	Established	Nixon 2005 (human biopsy, immuno-EM); Bordi 2016	Quantitative EM finding a different dominant compartment

#	Proposition	Grade	Principal evidence	What would change the grade
7	Autophagy *induction* is increased, not decreased, in Alzheimer neurons	Established	Bordi 2016 (human CA1, flux measurement)	A human flux study showing reduced induction
8	Neuritic dystrophy can be produced by acidification failure alone, without amyloid	Well supported	v-ATPase inhibition in wild-type mice; leupeptin transport studies	Failure to reproduce in an independent laboratory
9	Rab5 over-activation alone is sufficient for a large part of the phenotype	Supported, model-restricted	Pensalfini 2020; Jiang 2025	Independent replication would raise; failure to replicate would lower sharply
10	Presenilin 1 is required for v-ATPase V0a1 maturation and lysosomal acidification	**Contested**	Lee 2010; rescued/reframed by Lee 2015 — contradicted by Neely 2011, Zhang 2012, Coen 2012	Independent replication outside the originating laboratory
11	Phospho-Tyr682 βCTF inhibits v-ATPase assembly	Supported, model-restricted	Im 2023	Independent structural or biochemical replication
12	Lysosomal pH rises with cellular age, and pump activity is causally linked to lifespan	Established (in non-human systems)	Colacurcio & Nixon 2016 and the invertebrate lifespan literature	— this is not in doubt; its relevance to human AD is the open question
13	PANTHOS neurons are the principal source of senile plaques	Supported, model-restricted	Lee 2022 (APP-transgenic mice, quantified)	Human fractional quantification, in either direction
14	The same sequence occurs in human late-onset Alzheimer's disease	Supported, not yet peer-reviewed	Lee 2024 preprint (ROSMAP/Banner proteomics; human EM); Nixon 2024b (qualitative)	Peer-reviewed publication would raise this substantially
15	v-ATPase deficits in human brain are selective to excitatory neurons	Supported, not yet peer-reviewed	Lee 2024 preprint	Peer review; independent single-cell proteomic confirmation
16	Extracellular seeding is an independent, demonstrated route to plaque formation	Established	Meyer-Luehmann 2006; Jucker & Walker 2013	— not in dispute

#	Proposition	Grade	Principal evidence	What would change the grade
17	The fraction of human plaque burden formed inside-out is known	**Beyond the evidence**	No human quantification exists	Fate-mapping in knock-in models; provenance signatures in plaque cores
18	The network explains the entirety of the Alzheimer phenotype	**Beyond the evidence**	Scope claim, not an empirical result	Nothing could establish it as stated; a restricted version is testable
19	ELA remediation ameliorates the disease in humans	**Untested**	Preclinical only; the one clinical compound failed in Alzheimer's disease (Prins 2021)	A trial selecting patients on the lesion and measuring flux

Three rows deserve comment.

Row 10 is the framework's load-bearing weakness. It is what connects the causative genes of familial Alzheimer's disease to the lysosome. Sixteen years after publication it has been contradicted by three groups and reconciled by one — the originating laboratory. If it fails, the framework loses its familial bridge but retains, through row 11, an APP-driven route to the same defect. Readers who treat the presenilin controversy as fatal to the framework are wrong; readers who treat it as settled are also wrong.

Rows 14 and 15 are the framework's largest pending item. The claims are specific, quantitative, human, and exactly the ones the framework needs. They have been publicly available as a preprint since November 2024 and are not, as of August 2026, indexed as peer-reviewed. Nothing in this paper's assessment would survive unchanged if they were published and confirmed; several grades would rise by a full step.

Row 18 is the row we would most like to see withdrawn. It costs the framework nothing to drop and it is what invites the charge of unfalsifiability that the programme's actual bench work does not deserve.

28. Five Conditions of Refutation

The framework has never published a statement of what would refute it. We supply five, each derived from a proposition the framework has itself committed to in writing, and each answerable by an experiment that could be designed today.

R1 — The fragment. *If a design that dissociates βCTF from amyloid-β shows that amyloid-β alone reproduces endosomal enlargement and lysosomal de-acidification, with βCTF elevation neither necessary nor sufficient, the framework's central molecular claim fails.* The isogenic-panel design already exists (Kwart 2019) and produced the opposite result; the refuting version is a straightforward inversion of it, and its failure to appear is itself weak evidence for the framework.

R2 — The pump. *If human Alzheimer neurons are shown, by direct measurement in situ, to acidify their autolysosomes normally at all disease stages, the terminal claim fails.* This is currently unrefutable for want of a method, which is why Chapter 26 puts the measurement first.

R3 — Sufficiency without the network. *If a manipulation that produces the full Alzheimer phenotype in an animal is shown to do so with the endosomal–lysosomal network demonstrably intact — normal endosome size, normal autolysosomal pH, normal flux — then network failure is not necessary for the phenotype.* Note that this is a stronger and more informative test than any amount of additional evidence that network failure is sufficient.

R4 — The order. *If restoring lysosomal acidification in an established model has no effect on amyloid pathology, tau pathology or memory, while inducing autophagy in the same model rescues all three, the framework's sequencing claim is inverted and its principal therapeutic prediction fails.* The two arms of this experiment have been run separately with results favouring the framework; they have not been run head-to-head in the same model at the same stage.

R5 — The lesion in humans. *If a compound with demonstrated engagement of the acidification target, given at a stage and dose sufficient to normalise the measured lesion, produces no clinical benefit, the framework's translational claim fails.* This is the honest form of the clinical test, and it cannot presently be run because no such demonstration of engagement is possible. Until it is, negative trials in this area are uninformative about the framework, and the framework should stop citing preclinical rescue as though it were evidence of clinical validity.

29. Ten Experiments, in Order

1. Retromer enhancement in the Rab5-over-activated mouse. The single most valuable unperformed experiment in this area. It adjudicates between the two leading endosomal accounts, the reagents have existed since 2014 and the animal since 2020, and either outcome is informative. (Chapter 17.)

2. Publish, or explain, the human series. The proteomic and ultrastructural human study exists as a preprint. Its peer-reviewed appearance would move four rows of the ledger. If it cannot be published in its current form, the reasons are themselves data the field needs.

3. An in vivo or fluid measure of neuronal lysosomal acidification. Nothing else in this list can be translated without it. Neuron-derived extracellular vesicles are the most promising near-term route. (Chapter 26.)

4. The sequencing experiment. In one model, at one stage, four arms: vehicle; acidification restoration alone; autophagy induction alone; and re-acidification followed by induction. The framework predicts the fourth arm is best and — the sharp prediction — that the third arm is *worse than vehicle* in animals with established acidification failure. Nobody has tested the harm prediction.

5. Fate-mapping of plaque origin in a knock-in model. A neuronal-content label that survives into the deposit, imaged longitudinally in a non-over-expressing knock-in animal, would settle the fraction question in mice, where it is settleable. Pair it with a search for provenance signatures — cathepsins, LC3, lysosomal membrane proteins — in the cores of human plaques.

6. Cell-type resolution of the terminal lesion in human tissue. Single-nucleus and spatial methods applied to autophagic and lysosomal pathway components across transmitter classes and across Braak stages, testing the excitatory-selectivity claim of Chapter 19 and the two-clock model of Chapter 20 directly.

7. Pharmaco-epidemiology of lysosomal alkalinising drugs. Chronic proton-pump inhibitor and lysosomotropic antirheumatic exposure against incident dementia, with the mechanism-specific pattern of Chapter 25 pre-specified: dose- and duration-dependence, shared effect across chemically unrelated alkalinising agents, absence for H_2-receptor antagonists, and interaction with endolysosomal risk genotype. Cheap, fast, and never done properly.

8. Partial BACE1 inhibition with βCTF as the readout. A dose-ranging study in which the pharmacodynamic endpoint is the fragment rather than the peptide. This converts an auxiliary

hypothesis (Chapter 24) into a test.

9. Independent replication of the sufficiency mice. The Rab5 and APPL1 animals are the framework's best evidence and its most single-sourced. Replication outside the originating laboratory would settle more than any new experiment.

10. The presenilin question, once, properly. A pre-registered, multi-laboratory measurement of lysosomal pH and V0a1 maturation in presenilin-null and presenilin-mutant human neurons, with methods agreed in advance by the disputing parties. Sixteen years is long enough.

30. Limitations of This Evaluation

Four limits should be stated.

This is an assessment of a research programme from its published record, not a laboratory replication. Where results are single-sourced we have said so, but we have not attempted to reproduce anything, and confidence in any framework's phenomenology ultimately rests on work of a kind not performed here.

The framework is large and this evaluation is selective. We have concentrated on the propositions that carry the most weight and have said comparatively little about several genuine components — the exosomal route to tau propagation, the calpain–calpastatin arm, the microglial and inflammasome connections, and the framework's treatment of calcium. Each would repay separate treatment; none, as far as we can tell, would change the assessment of the central claims.

Two of the most consequential documents in the file are not peer-reviewed. The human study of Chapter 13 is a preprint and the re-acidification result of Chapter 25 is a conference abstract. We have flagged them at every use. A reader who discounts them entirely would reach the same overall verdict with two of the ledger's rows removed.

And the placement argument of Part V is an argument, not a measurement. Its premises are individually well-evidenced — the cargo-dependence of the morphology, the cell-type distribution of amyloidogenic load, the stereology of the coerulean population, the atlas timing of excitatory loss — but their conjunction is inference. Experiment 6 is what would settle it, and until it is run, Part V should be read as the strongest available reading of existing evidence rather than as an established result.

31. Conclusion — The Acid Test

Thirty-six years ago a pathologist and a neurochemist found active lysosomal proteases in a senile plaque and could not say why they were there. The answer they eventually proposed is the most complete account any laboratory has produced of how an Alzheimer neuron dies: the disposal system fails at its terminal step; the cell keeps generating compartments it cannot empty; a fragment of the amyloid precursor protein both jams the sorting endosome upstream and disables the proton pump downstream; and the neuron eventually ruptures, leaving behind, in the right cells, the deposit the disease was named for.

Our assessment is that the framework is substantially right about the biology and substantially overstated in its claims of scope, and that these two judgements are not in tension. The endosomal abnormality is real, is first, and is human. The fragment claim is right and has been confirmed by a laboratory with no stake in it. The finding that autophagic vacuoles constitute nearly the entire organelle content of the dystrophic neurite is a major result that the field has still not absorbed, and it has now been independently rediscovered with a different cargo. The observation that induction rises while completion fails is the framework's most important single measurement and the one with the clearest therapeutic consequence. Against this, the presenilin arm remains contested after sixteen years; the human quantification of the terminal claim is unpublished; the sufficiency experiments are single-sourced; and the assertion that this network explains the entirety of the disease is a claim no evidence could establish and which the programme would be stronger without.

Two corrections of emphasis follow, and both make the framework more useful rather than less.

The first is that the framework contains two lesions, not one. A signalling lesion at the early endosome starves cells of trophic support, and a disposal lesion at the terminal lysosome fills them with what they cannot digest. They occur in different cells, on different clocks, leave different traces, and require different drugs. Running them together is what allowed a cortical, cargo-dependent, plaque-yielding death to be attributed to brainstem populations that are neither producing the cargo nor dying in the decades concerned. The disposal machinery fails widely; the flower blooms only where the cargo is amyloidogenic and abundant.

The second is that this is a theory of the return leg. Everything the framework finds elevated is outbound — endocytosis, cleavage, induction, exosome release, hydrolase expression — and everything it finds failing is a return: acidification, hydrolysis, retrograde transport, recycling, retrieval of the trophic signal. That dissociation is the framework's real content, it distinguishes it from every production theory of this disease, and it carries a therapeutic instruction the field has almost universally inverted. The standard autophagy intervention is an inducer. In a neuron that

has lost its pump, an inducer is not a partial treatment. It is the wrong direction.

Which returns us to the acid, and to the test.

The claim that this disease turns on the pH of a compartment inside a neuron is precise, mechanistically specified, therapeutically actionable, and — in a living human being — has never once been measured. That is the state of the file. The framework has spent thirty-six years earning the right to a proper test, and the reason it has not had one is not that the field rejected it. It is that nobody has built the instrument. Until someone does, the most important claim in Alzheimer's disease research that could be settled will remain the one that is not being settled, and every negative trial in this area will continue to tell us nothing about whether the theory was right.

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