
The Assumed Switch

*cGAS in Alzheimer's Disease — What the Enzyme Does
Besides Signalling, Which DNA Activates It, Which Cell It
Acts In, and Whether It Can Be Drugged*

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September 2026

The field has been testing a pathway when it should have been characterising an enzyme.

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Abstract

Cyclic GMP-AMP synthase is treated in the Alzheimer's disease literature as a switch: DNA appears where it should not be, the switch flips, interferon follows, synapses are lost, and the therapeutic instruction is to hold the switch down. Every element of that account has experimental support. The account is nonetheless wrong in form, because cGAS is not a switch but an enzyme with several jobs, most of which do not produce a second messenger at all.

This review examines the target through four questions, each of which the switch model does not ask.

The first concerns the enzyme. Most cGAS in a cell is nuclear, tethered to chromatin, and doing something other than signalling: it inhibits homologous recombination, decelerates replication forks, binds poly-ADP-ribose, and helps maintain heterochromatin. Where this has been tested with defined mutants, these functions require nuclear entry, DNA binding and oligomerisation but not catalysis — they are not the sensing pathway operating quietly, they are a different activity of the same protein. Mice lacking the enzyme age prematurely, with derepressed repeat elements and a smoothed chromatin landscape. Not one of these functions has been examined in a neuron, a glial cell, or a brain, and the best-characterised of them requires a cycling cell, which a neuron is not.

The second concerns the ligand. No study has identified the DNA that activates cGAS in the Alzheimer's brain. Every claim in the field rests on amplicon measurement of cytosolic fractions, antibody staining, or pharmacological epistasis, none of which specifies a molecular species. This matters because the enzyme's activation is steeply length-dependent and geometry-dependent, so the difference between an intact protein-bound nucleoid and a short fragment is the difference between a ligand and a non-ligand. Each candidate has a specific problem: mitochondrial release routes have never been size-resolved in a neural cell, the canonical micronucleus route is gated by mitosis, the retroelement hypothesis is contradicted by long-read sequencing of human cortex showing lower rather than higher reverse-transcriptase activity in disease, and cell-free DNA in disease cerebrospinal fluid is reduced rather than increased.

The third concerns the cell. Expression data place the enzyme in myeloid and vascular populations, with neurons in no enrichment list. Two independent inducible microglia-restricted knockouts support a microglial mechanism. The neuronal claim rests on a single 2025 study using viral knockdown, unreplicated, and no neuron-restricted conditional deletion of the enzyme

exists in any species or disease. Yet the argument for neuronal relevance is strong on its face, because the restraints that hold the enzyme inert are mitosis-linked and a neuron never divides — an argument that is logically sound and has never been tested.

The fourth concerns the drug. No compound exists that is active against the human enzyme and demonstrated to reach brain. The only inhibitor with published brain exposure is selective for the mouse enzyme; its human-active sibling has no pharmacokinetics of any kind. No inhibitor of this enzyme or its adaptor is registered in any neurological indication anywhere. The messenger has never been measured in human cerebrospinal fluid or brain, so there is no central pharmacodynamic readout. No human loss-of-function phenotype for the enzyme has been described. And the prior for anti-inflammatory intervention in this disease is a uniform row of negative randomised trials.

A staged programme follows, ordered by the cost of being wrong. Three experiments would decide whether the target is worth the investment: whether the enzyme's nuclear functions operate in a post-mitotic neuron, whether the DNA it binds in brain can be isolated and sequenced, and whether a human-active inhibitor can be made to reach the brain at all. The argument throughout is that the field has been testing a pathway when it should have been characterising an enzyme.

1. The Switch and the Enzyme

1.1 A category error at the centre of a literature

There is a recognisable shape to the papers connecting cyclic GMP-AMP synthase to Alzheimer's disease. A source of misplaced DNA is proposed. Activation of the enzyme is inferred from downstream measurements. Deleting or inhibiting it improves a mouse. The conclusion is that the pathway is pathogenic and should be suppressed.

The work is often excellent, and the shape is not wrong so much as under-specified. It treats a protein as though its only property were to be on or off. An enzyme is not a switch. It has a location, a substrate, a set of binding partners, a catalytic rate, a physical state, and — in this case — several activities that have nothing to do with the product it is named for. A therapeutic programme aimed at a switch asks one question: how completely can it be held down? A programme aimed at an enzyme has to ask which of its activities is the harmful one, in which compartment, in which cell, at which stage, and what is lost when it is suppressed.

This review asks the second set of questions. It is organised around four of them, and the finding common to all four is that the field has largely not asked them.

1.2 The four questions

What does the enzyme do? Part I sets out the biology of cGAS that is not signalling. Most of the protein in a cell is nuclear and chromatin-bound.¹ In that location it suppresses homologous recombination, slows replication forks, binds the poly-ADP-ribose chains made at damage sites, and contributes to maintaining heterochromatin. Where the question has been asked with defined mutants, these functions require DNA binding and not catalysis, which means they are not the sensing pathway running at low level — they are a different job.^{2, 3}

What activates it? Part II asks which DNA species is the ligand in the Alzheimer's brain. The honest answer is that nobody knows, because nobody has isolated it. The enzyme is activated cooperatively and in a

length-dependent manner, and is strongly potentiated by proteins that bend DNA ⁴, ⁵, so a candidate ligand is specified only when its length, its protein associations and its compartment are specified. No candidate in this literature has been specified to that standard.

Which cell? Part III asks where the enzyme that matters resides. Nearly all disease work is microglial and two independent conditional knockouts support that. ⁶ The neuronal case rests on one unreplicated study. ⁷ The argument that neurons are a special case — because the restraints on the enzyme are tied to cell division and neurons do not divide ⁸ — is strong and untested.

Can it be drugged in a brain? Part IV assesses the target as a medicinal chemist would. The answer is that the molecule needed to test the hypothesis in a human being does not exist.

1.3 Why the synapse remains the endpoint

The quantity a mechanistic account must ultimately explain is synaptic loss, which tracks cognitive impairment more closely than plaque or tangle burden. ⁹ That constraint does not favour any particular upstream mechanism; it says only that an account which changes a pathological measurement while leaving function unchanged has not yet explained the disease. The best result in this field satisfies it: deleting the enzyme in a tauopathy model preserved synapses and memory without altering tau burden. ¹⁰ That dissociation is the reason the target is taken seriously, and this review takes it seriously too.

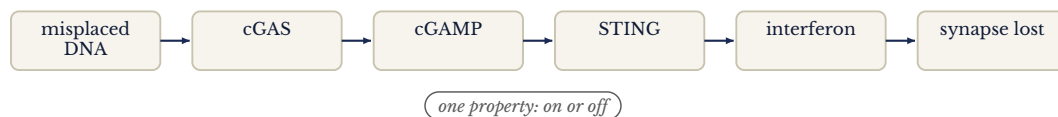
1.4 Conventions

This is a critical mechanistic review and experimental synthesis, not a systematic review and not a report of new data. It considers primary literature through 10 September 2026.

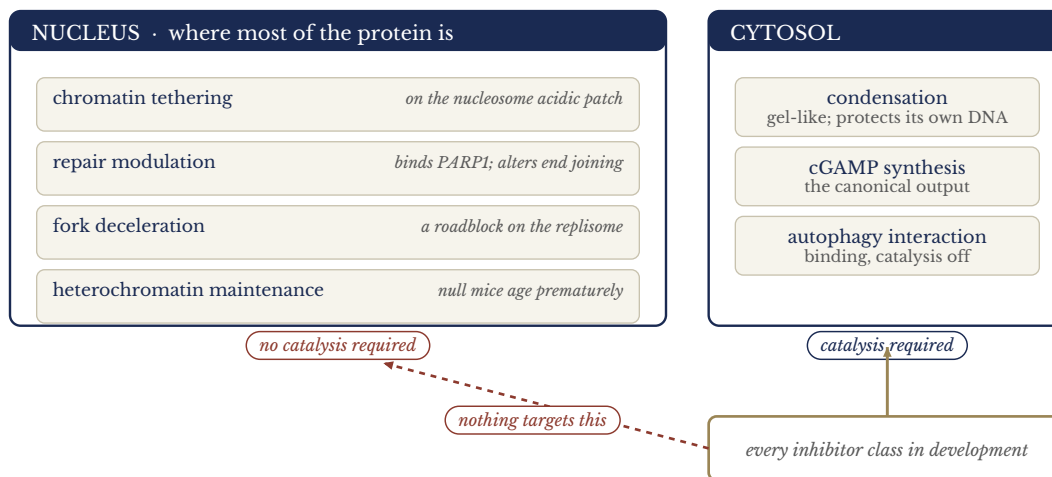
Established denotes a result reproduced by independent groups in a named system, with the system stated. Proposed denotes an integration offered without direct test. Unevidenced denotes a step the argument requires for which no study was found. Contradicted denotes a step for which the primary evidence points the other way. Corrections, retractions and failed replications are cited where the claim is made rather than deferred; three matter especially here, and are flagged in Chapters 10, 12 and 19.

Nomenclature: the human gene is *CGAS*, formerly *MB21D1* and *C6orf150*; the adaptor gene is *STING1*, formerly *TMEM173*.^{11, 12} Throughout, *the enzyme* means cGAS and *the adaptor* means STING, because a central claim of this review is that the two have been conflated and that much of what is attributed to the pathway belongs to one component or the other.

The account in use



The protein it describes



And the cell

microglia two inducible conditional knockouts	neurons one viral knockdown study, unreplicated	any brain cell no nuclear function ever examined
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— established in a named system - - - required by the account; no study found

Figure 1. The account used in the disease literature treats cGAS as a switch between misplaced DNA and interferon. The protein it describes is predominantly nuclear and chromatin-bound, where it modulates repair, decelerates replication forks and helps maintain heterochromatin. Only the cytosolic arm produces the second messenger, and only that arm is what current inhibitors are designed to stop. Sources: ^{1, 2, 3, 50, 14}.

2. What the Enzyme Is

2.1 The discovery architecture

The pathway was assembled from the middle outwards. The adaptor was identified first, by expression cloning, and was known to facilitate interferon induction before anyone knew what activated it.¹³ Five years later two companion papers supplied the upstream half: an enzyme activated by double-stranded DNA, and the cyclic dinucleotide it makes.^{14, 15} The messenger has an unusual mixed-linkage chemistry that distinguishes it from bacterial cyclic dinucleotides and gives it high affinity for the adaptor.¹⁶

Three molecular roles must be kept apart, and conflating them is the commonest error in the applied literature. Double-stranded DNA *activates* the enzyme. ATP and GTP are the *substrates*. The cyclic dinucleotide is the *product*. DNA is not consumed and is not incorporated, so a measured quantity of DNA does not correspond to a quantity of messenger, and the relationship between the two is neither linear nor fixed.

2.2 Activation is cooperative, length-dependent and geometric

Structures showed that DNA binding produces an activating conformational change, with a zinc-containing element recognising the duplex and the catalytic core becoming competent.¹⁷ An independently determined human structure placed the enzyme in the nucleotidyltransferase family alongside the RNA-responsive oligoadenylate synthetases, which is why second-messenger production is intrinsic to recognition rather than appended to it.¹⁸

Activation is not a binary occupancy event. It requires DNA-induced oligomerisation¹⁹, scales with duplex length across three orders of magnitude⁴, and is strongly potentiated by proteins that bend DNA into the geometry that permits enzyme-DNA ladders to form.⁵ Two further DNA-binding interfaces beyond the primary one contribute.^{20, 21}

The consequence is a constraint this review will apply repeatedly. A candidate ligand is not specified by naming a source. It is specified by length, by topology, by which proteins are bound to it, and by compartment. Two

DNA preparations of identical mass and identical sequence can differ by orders of magnitude in their capacity to activate this enzyme.

2.3 The human enzyme is not the mouse enzyme

One structural fact governs the interpretation of nearly every animal experiment in this literature, and it is routinely omitted. Two human-specific substitutions in the DNA-binding surface impose strict long-DNA-length specificity and sharply reduce messenger output relative to other mammalian orthologues.²² The human enzyme is the less efficient, more discriminating version.

Three consequences follow. A DNA species that activates mouse cGAS may be below the threshold for human cGAS. An inhibitor optimised against one orthologue may be inactive against the other, which Chapter 20 shows has happened repeatedly. And the difference is in the DNA-binding surface, which is precisely where the DNA-competitive inhibitors bind. Simulation work localises the divergence to differing dominance of the binding surfaces during activation.²³

2.4 Condensation: the enzyme has a physical state

DNA binding drives the enzyme into condensates, and condensation is required for efficient signalling rather than incidental to it.²⁴ The condensate concentrates enzyme and substrate, protects the bound DNA from the principal cytosolic exonuclease²⁵, and — in the most detailed reconstitution available — behaves as a gel rather than a liquid, restricting the mobility not only of DNA but of nucleotide substrates and of the reaction intermediate itself.²⁶ Whether the active state is gel-like or flowable is genuinely disputed, with one group reporting that the disordered amino terminus liquefies otherwise solid aggregates and restores catalysis.²⁷

Condensation is emphasised here because it makes the enzyme's behaviour non-linear in ligand concentration. Below a threshold there is binding without condensation and therefore without much output; above it, the complex protects its own stimulus and extends its own lifetime. An enzyme with this property does not behave like a switch, and it does not behave like a simple dose-response element either.

2.5 Convergence downstream, and what it costs the argument

The adaptor recruits a kinase and a transcription factor through a phosphorylation motif shared with two structurally unrelated adaptors serving RNA sensing and Toll-like receptor signalling.²⁸ The pathway also has an interferon-independent output through membrane trafficking and autophagy.²⁹ And the messenger moves between cells, so the cell that senses need not be the cell that responds.³⁰

Each of these is a measurement problem. Because three arms converge on one transcription factor, an interferon-stimulated gene signature in tissue does not identify the sensor that produced it, and every inference from signature to enzyme requires a genetic or pharmacological bypass. Because the pathway has a non-interferon output, suppressing the enzyme removes a function as well as a signal. And because the messenger diffuses, a tissue measurement combines the sensing and responding cells into one number. Part III returns to all three.

Part I

The Enzyme

*What cGAS does besides making a second messenger, and why
almost none of it requires catalysis*

3. The Enzyme Lives in the Nucleus

3.1 A fact that reframes the target

The name assigned to this protein locates it in the cytosol, and the disease literature follows the name. The measurement does not. Endogenous cGAS is predominantly nuclear, in every cell-cycle phase and whether or not the cell has been stimulated, and it is held there by tight association with chromatin rather than excluded from the nucleus by sequestration elsewhere.¹

This is not a minor correction of a diagram. It means the default state of the protein is bound to the very polymer it is supposed to detect, in the compartment containing nearly all of it, and that the whole problem of self-tolerance is a problem of inhibition rather than of separation. It also means that any account of what the enzyme does must explain what it is doing in the nucleus for the great majority of its residence time.

3.2 How the chromatin brake works

Five groups determined the structural basis within months of one another, which makes this one of the more securely established facts in the field. The nucleosome inhibits the enzyme by binding the acidic patch formed by the histone H2A-H2B dimer, which buries one of the enzyme's DNA-binding surfaces and blocks assembly of the active dimer.^{31, 32, 33, 34} A correction to one of these reports was subsequently published.³⁵ A further structure shows two enzyme monomers bridging two nucleosome particles rather than engaging one, a geometry that is architectural rather than catalytic.³⁶

A second restraint operates through competition: a small DNA-binding protein at the nuclear envelope outcompetes the enzyme for exposed DNA and prevents activation when the envelope is breached.³⁷

A third operates through the cell cycle, and it is the one that matters most for this review. During mitosis, when the nuclear envelope disassembles and chromatin and enzyme are in the same space with no membrane between them, two parallel restraints hold the enzyme inert: a mitotic kinase phosphorylates it, and nucleosome tethering inhibits it.⁸ This is the best

mechanistic account of self-tolerance available, and every element of it is tied to a process that a neuron never undergoes. Chapter 17 takes that up directly.

3.3 Where in the nucleus, and on what

The enzyme is not uniformly distributed on chromatin. Sequencing of enzyme-associated chromatin, together with a tagged knock-in mouse, showed preferential association with centromeric satellite DNA and, to a lesser extent, repeat elements, with the non-catalytic amino-terminal domain governing both nucleocytoplasmic partitioning and this enrichment.³⁸ The same study found that nuclear enzyme does make messenger, but roughly two hundred-fold less than after cytosolic DNA delivery. A published erratum accompanies the paper.³⁹

This remains, as Chapter 9 will note, the closest anyone has come to identifying the DNA this enzyme actually holds — and it was done in dendritic cells and cancer lines.

3.4 How it gets there, and the disagreement about that

Three distinct triggers for nuclear import have been proposed by three groups. One reports damage-induced, importin-dependent translocation released by loss of a tyrosine phosphorylation that otherwise retains the enzyme in the cytosol.⁴⁰ A second reports that the enzyme is simply nuclear at baseline and the question of import is misframed.¹ A third reports a kinase-driven serine phosphorylation that drives import in a cancer setting.⁴¹

These are not reconcilable as stated. The field does not agree on how the enzyme enters the nucleus, which is an awkward position for a literature that increasingly attributes function to it once it is there. A focused review of the nuclear literature is candid that the non-canonical function remains poorly understood.⁴²

4. Slowing the Fork, Blocking the Repair

4.1 The founding claim and the two that followed

The first report that nuclear cGAS does something other than sense described it as pathogenic: damage-induced nuclear enzyme binds PARP1 through poly-ADP-ribose, obstructs the PARP1-Timeless interaction, and suppresses homologous recombination, promoting tumorigenesis.⁴⁰ The result was independent of interferon induction, which is what made it a claim about a second job rather than about signalling.

Two subsequent studies reproduced the phenomenon and disagreed with the mechanism, and in one case with the direction.

The more complete dissection combined cells, purified proteins and mice. It found that the enzyme inhibits homologous recombination and delays break repair, that a catalytically dead enzyme does so as well as wild type, that DNA-binding and oligomerisation mutants do not, and that the adaptor is dispensable both in cells and in irradiated animals. The proposed mechanism is physical: purified enzyme compacts template DNA and blocks RAD51 filament strand invasion, with or without nucleotide substrates present. The authors state explicitly that their mechanism differs from the founding account, and that co-isolation of the enzyme with repair proteins reflects shared chromatin rather than specific complexes.²

The third study addressed replication rather than repair. Loss of the enzyme accelerates fork progression, causes premature restart after stalling, and destabilises stalled forks; the enzyme co-purifies with replisome components in a DNA-binding-dependent manner and acts as a physical roadblock without disassembling the machinery. Catalysis, phase separation and dimerisation were all dispensable; nuclear entry and DNA binding were required.³ That paper reports, contrary to the founding account, that cells lacking the enzyme are *more* sensitive to radiation and damaging agents across several untransformed and transformed lines — that is, the enzyme is genome-protective in their hands rather than tumour-promoting.

4.2 An unresolved dispute, and how to cite it

No published correction, retraction, rebuttal or Matters Arising resolves this. The disagreements exist inside the discussion sections of the papers themselves. A review of the field should therefore not present "nuclear cGAS inhibits DNA repair" as a settled mechanism with a known polarity. What is established is that nuclear cGAS binds chromatin at damage sites and changes the kinetics of repair and replication. Whether the net effect is protective or destabilising depends on the system, and the two most rigorous studies disagree.

4.3 The rest of the repair interface

Several further interactions have been described, and they do not form a single picture.

The zinc-finger domain binds poly-ADP-ribose and inhibits PARP1 activation, restraining an error-prone end-joining route and thereby favouring accurate repair — a polarity opposite to the founding account.⁴³ A complex with a core end-joining factor promotes the classical route while transcriptionally repressing the polymerase that drives the alternative one, which would make the enzyme a promoter-bound transcriptional repressor.⁴⁴ A crotonylation mark on a DNA-binding residue is removed after irradiation, lowering DNA affinity and releasing PARP1, restoring recombination — control by post-translational modification of the binding surface.⁴⁵

In the opposite direction, the MRE11-containing repair complex binds nucleosome fragments and displaces the enzyme from the acidic patch, mobilising it for activation.⁴⁶ This is the repair machinery releasing the enzyme, not the enzyme inhibiting the repair machinery; the two directions are frequently conflated in secondary literature and should not be.

Two toxicological studies reproduce the nuclear-translocation-and-repair-suppression model in non-cancer human cells with mutation-frequency and tissue endpoints.^{47, 48} A recent review synthesises the whole interface.⁴⁹

4.4 The constraint that matters for a neuron

One detail decides whether any of this can transfer to the disease under discussion. Homologous recombination operates in S and G2 phase and requires a sister chromatid as template. The recombination-suppression effect is accordingly abolished when cells are arrested before replication. A neuron is permanently post-mitotic, performs no homologous recombination, and has no sister chromatid.

The best-characterised non-canonical function of this enzyme therefore cannot operate in the cell type at the centre of this disease, at least not as published. That does not exclude a neuronal version of the phenomenon acting on a different repair route — non-homologous end joining, single-strand break repair, or transcription-coupled repair, all of which neurons use heavily. It does mean that citing the recombination literature as though it applied to neurons is an error, and Chapter 8 returns to it.

5. Binding, Not Catalysis

5.1 Why the distinction is the crux

If the enzyme's damaging activity in a tissue required catalysis, an active-site inhibitor would abolish it, and the therapeutic programme of Part IV would be coherent. If the damaging activity required only DNA binding, an active-site inhibitor would leave it untouched, and the programme would be aimed at the wrong property of the protein.

This is not a fine point. It is the difference between a target and a mistarget, and it has been answered, for several functions, in the negative.

5.2 What the mutants show

Where defined mutants have been used, the pattern is consistent. For inhibition of homologous recombination, a catalytically dead enzyme performs as well as wild type, while DNA-binding and oligomerisation mutants lose the activity, the adaptor is dispensable, and adding the messenger exogenously does nothing.² For fork deceleration, a synthesis-dead construct, a condensation-deficient construct and a dimerisation-dead construct all rescue, while nuclear-entry-dead and DNA-binding-dead constructs fail even when import is forced.³ For heterochromatin maintenance, the authors state the function is independent of both sensing and catalysis.⁵⁰ For the poly-ADP-ribose interface, the mechanism is ligand binding by the zinc-finger domain.⁴³ For the crotonylation switch, the controlled variable is DNA affinity by construction.⁴⁵

Against this, the founding paper's catalytic requirement is not established to the same standard: independence from interferon is shown, but no adaptor-knockout epistasis is reported and the mutant evidence is not presented with the rigour of the two later studies.⁴⁰

The defensible summary is narrow and worth stating precisely. *Where it has been tested with defined mutants, the DNA-repair and replication functions of cGAS require nuclear entry, DNA binding and — for recombination suppression — self-oligomerisation, but not messenger synthesis.*

5.3 Cytosolic functions that also bypass the adaptor

The same pattern appears outside the nucleus. A direct interaction with a core autophagy protein suppresses messenger synthesis while promoting autophagic clearance of cytosolic DNA — a function performed by binding, with catalysis actively switched off.⁵¹ In liver ischaemia-reperfusion, side-by-side genetics dissociate the two components cleanly: animals lacking the enzyme fare worse while animals lacking the adaptor do not.⁵² In pancreatic beta cells, both global and cell-restricted enzyme knockouts increase cell mass while adaptor knockout does nothing, with the effect running through a transcription factor.⁵³ In a cancer setting, nuclear enzyme stabilises a transcriptional co-activator by disrupting its ubiquitination, again explicitly adaptor-independent.⁴¹

And in one setting the pathway produces output without transcription at all: during mitotic arrest, slowly accumulating activated transcription factor kills cells by acting on the mitochondrial apoptotic machinery rather than on DNA, independently of its DNA-binding domain.⁵⁴

5.4 What this does to the therapeutic question

Every clinical-stage inhibitor of this enzyme, and every tool compound in the neurodegeneration literature, is an inhibitor of catalysis — competing at the nucleotide site, at the DNA-binding surface, or covalently at the active site. Chapter 20 sets out the chemistry.

If what harms the ageing brain is the enzyme's catalytic output, those compounds are appropriate. If it is chromatin occupancy, repair interference or condensate formation, they are not, and a DNA-competitive compound might even be the only class with a chance, since it targets the property the non-canonical functions require. No study in brain has distinguished these possibilities, and no compound programme has been designed around the distinction.

6. Condensation as a Physical State

6.1 The state, not the switch

Condensation deserves its own chapter because it is the clearest illustration of why a switch model fails. The enzyme and DNA form a condensed phase, and that phase is required for efficient signalling.²⁴ What the phase does has since been characterised with some precision.

The condensate protects bound DNA from the principal cytosolic exonuclease, so the complex extends the lifetime of its own stimulus — a positive feedback that has nothing to do with how much DNA arrived.²⁵ In the most detailed reconstitution available, dimerisation on duplex DNA creates a mesh that behaves as a hydrogel rather than a liquid; only duplex DNA permits the dimerisation that produces the gel, and single-stranded RNA or short duplexes dissolve it. Critically, the gel restricts the mobility of the nucleotide substrates and of the linear reaction intermediate, meaning

condensation is a kinetic microenvironment and not merely a concentrating device.²⁶

An unresolved disagreement sits here. Another group reports that the disordered amino-terminal region sets the material properties, with full-length enzyme forming flowable droplets while the catalytic domain alone forms solid aggregates of compromised activity, the disorder restoring both flow and catalysis.²⁷ Gel-active and flow-active are not the same claim, and the field has not settled it.

6.2 The threshold, and what it implies for a tissue

Both accounts agree on the consequence that matters. Output is a steeply non-linear function of ligand quantity and quality, because below a threshold there is binding without a productive phase, and above it the complex both concentrates the reaction and defends its substrate.

For a tissue argument this is decisive. A modest, chronic supply of DNA distributed across many cells may produce almost nothing, while the same total quantity concentrated at a threshold in a few cells produces a sustained response. Bulk measurement of a tissue cannot distinguish these, and every measurement in the Alzheimer's literature is a bulk or amplicon measurement.

A further refinement: single-droplet charge measurement shows that identical condensates partition into two electrostatic populations with different susceptibility to nuclease digestion, and that two pathogenic human variants abolish this control.⁵⁵ The method is new and unreplicated, but it suggests the condensate has internal states rather than one state.

6.3 Has this ever been seen in tissue?

Once, convincingly, and not in brain. Tyrosine-phosphorylated enzyme binding a myosin light chain drives condensation in lung vascular endothelium, with re-expression experiments in enzyme-deficient mice showing the condensation-dependent function operates in vivo.⁵⁶ There, condensation serves canonical interferon output, so it is evidence for the physical state in tissue rather than for a nuclear role.

The single study examining a condensate phenotype in brain is a mouse toxicology model in which lead and hypertensive exposure promote condensation in prefrontal cortex with astrocytes as the susceptible cell.⁵⁷ The

condensate disruptor used is notoriously non-specific, the readout is senescence markers rather than repair or chromatin, and no enzyme mutants or adaptor epistasis were included. It should not be over-read.

7. The Premature Ageing of the cGAS-Null Mouse

7.1 An inconvenient animal

A therapeutic proposal to inhibit an enzyme lifelong in an elderly population acquires a particular obligation when the animal lacking that enzyme ages badly.

Mice without cGAS show shortened median lifespan, increased frailty, multi-organ inflammation, derepression of repeat elements, DNA hypomethylation, a smoothed repressive chromatin landscape and increased chromatin accessibility. The authors attribute this to a role in maintaining heterochromatin organisation that is independent of both the cytoplasmic sensing function and catalysis.⁵⁰

The result is recent, from one group, and contains no brain-specific analysis. It should not be treated as settled. But it is the only whole-animal lifespan experiment on this gene, and its direction is opposite to the therapeutic premise.

7.2 Converging evidence for a structural role

It does not stand alone. In vertebrate genetic models of two genome-instability syndromes, disrupting the enzyme partially rescued germline failure, hepatic senescence and cerebellar neuroinflammation, and reversed cellular hallmarks of instability including micronucleus frequency and the repressive chromatin landscape — while loss of the enzyme on an otherwise normal background *worsened* pathology.⁵⁸ The same dual polarity, in a whole organism.

In regulatory T cells, nuclear enzyme recruits a genome organiser to specific loci through chromatin interaction, sustaining a developmental

programme, with cell-restricted knockouts and explicit demonstration that the adaptor is not involved.⁵⁹ This is the clearest single demonstration that the protein can act as a genuine chromatin and genome-architecture factor.

And the senescence literature contains a result of the same shape from the other direction: deleting the enzyme accelerates spontaneous immortalisation of primary fibroblasts, a proliferative rather than cytokine phenotype.⁶⁰ The canonical account, that cytoplasmic chromatin fragments drive the secretory phenotype through the adaptor, remains the baseline against which these are judged.⁶¹

7.3 What a therapeutic programme has to answer

Three things follow, and none of them is currently answerable.

Whether the protective chromatin function operates in a neuron — a cell whose heterochromatin organisation deteriorates with age and whose repeat elements become derepressed, as Chapter 12 describes. Whether an active-site inhibitor spares that function, which on the mutant evidence of Chapter 5 it plausibly would, since the function does not require catalysis. And whether chronic partial inhibition in an elderly human being reproduces any part of the null-mouse phenotype, which no programme has been designed to detect.

The honest position is that inhibiting this enzyme may be safe precisely because the harmful function is catalytic and the protective one is not. That would be a fortunate arrangement and it has not been demonstrated.

8. None of This Has Been Tested in a Neuron

8.1 The search, and its result

The preceding five chapters describe a substantial and partly contradictory literature on what cGAS does besides signalling: nuclear residence, chromatin tethering, repair interference, fork deceleration, poly-ADP-ribose binding, transcriptional repression, heterochromatin maintenance, condensation as a kinetic state, and several adaptor-independent cytosolic interactions.

Systematic searching across multiple query formulations located no study examining any of these functions in a neuron, in glia, in brain tissue, or in any neurodegeneration model. Every brain-related primary paper retrieved concerns the canonical route from DNA through the adaptor to interferon.

Three studies approach the boundary without crossing it. The mouse toxicology study of condensation in prefrontal cortex, with its non-specific pharmacology and senescence readouts.⁵⁷ The killifish genome-instability models, which include a cerebellar inflammatory readout but whose chromatin analyses are not brain-specific.⁵⁸ And a brain-specific negative result: in a mouse model of a genetic interferonopathy caused by defective ribonucleotide excision repair, the cerebellar phenotype is rescued by deleting p53 but not by deleting the enzyme, implicating damage signalling rather than sensing.⁶² That study tests the canonical contribution and finds it dispensable; it does not test the nuclear functions.

8.2 Why the gap is not merely an omission

Two features of the neuron make this more than an unfilled square on a grid.

The restraints are wrong for the cell. The dominant account of self-tolerance rests on mitotic phosphorylation and nucleosome tethering, and the phosphorylation arm is explicitly mitosis-linked.⁸ A cell that never divides never experiences the condition those restraints evolved to manage, and never receives the mitotic phosphorylation. Whether the tethering arm suffices alone, over eighty years, in a nucleus whose lamina erodes, is the question — and it has not been asked in a neuron. Chapter 17 develops it.

The best-characterised function is unavailable to the cell. Homologous recombination requires a sister chromatid; a neuron has none. The recombination-suppression function cannot operate there as published, so if nuclear cGAS matters in a neuron it must matter through a different route: end joining, single-strand break repair, transcription-coupled repair, or chromatin maintenance. Neurons carry a permanent, site-specific single-strand break load at enhancers⁶³ and deliberately generate double-strand breaks at promoters as a transcriptional mechanism⁶⁴, so the substrate for such a route is abundant. Nobody has looked.

8.3 The shape of Part I's conclusion

The enzyme at the centre of this disease hypothesis has a job description that the disease literature does not use. Most of the protein is nuclear. Several of its activities require binding rather than catalysis, which is the property that current drugs do not target. Losing it shortens the lifespan of a mouse. And none of this has been examined in the cell type whose loss defines the disease.

That is not an argument that the canonical pathway is unimportant. Chapter 15 will show it is well supported in microglia. It is an argument that the target has been characterised in the wrong compartment, and that the experiments needed to decide the question are straightforward and have not been done.

What defined mutants show each function requires

		catalysis	DNA binding	oligomerisation	needs STING
recombination suppression	Jiang 2019	✗	✓	✓	✗
replication fork deceleration	Chen 2020	✗	✓	✗	✗
heterochromatin maintenance	Martinez 2026	✗	✓	?	✗
poly-ADP-ribose binding	Yang 2025	✗	?	?	✗
cGAMP synthesis	canonical	✓	✓	✓	✓

what the inhibitors target
nucleotide site, DNA surface, covalent

The consequence

if the harm is catalytic the chemistry is right, and the nuclear functions are spared No experiment in brain has distinguished them.	if the harm is occupancy every compound in development addresses the wrong property
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Figure 2. Each row is a reported function; the columns record what defined mutants show it requires. Where the question has been asked, the nuclear functions need DNA binding and oligomerisation but not messenger synthesis. Every inhibitor class in development targets catalysis, which is the column the nuclear functions do not depend on. Whether that makes inhibition safe or misdirected is the most consequential open question in this review. Sources: 2, 3, 50, 43, 180.

Part II

The Ligand

*Which DNA actually activates the enzyme in the Alzheimer
brain, and why no one has ever held it*

9. The Ligand Has Never Been Identified

9.1 A missing experiment at the centre of a field

For an enzyme whose activation is the premise of a disease hypothesis, one experiment is foundational: isolate the enzyme from the diseased tissue, recover the DNA bound to it, and determine what that DNA is.

It has not been done. Systematic searching located no study that has sequenced, size-resolved or otherwise directly characterised the DNA bound to cGAS in a neuron, a microglial cell, a brain organoid, or human or mouse brain tissue. The nearest approach in any system is chromatin immunoprecipitation of nuclear enzyme in dendritic cells and cancer lines, which profiles chromatin occupancy rather than the activating cytosolic ligand.³⁸

Every ligand claim in the Alzheimer's literature is therefore inferential, and rests on one of three proxies: polymerase chain reaction of one or two amplicons in a cytosolic fraction, antibody staining for double-stranded DNA, or pharmacological and genetic epistasis. None of the three specifies a molecular species.

9.2 Why the proxies are insufficient for this particular enzyme

For most receptors, establishing that a ligand class is present would be reasonable evidence. For this enzyme it is not, because the activation function is steep in properties that the proxies do not measure.

Amplicon quantification reports that sequence from a given genome is present in a fraction. It cannot distinguish a complete sixteen-kilobase mitochondrial genome from a two-hundred-base-pair fragment, and the enzyme's response to those two differs by orders of magnitude given the length dependence.⁴ It cannot detect protein association, and DNA-bending proteins strongly potentiate activation by folding the duplex into the geometry that permits enzyme-DNA ladders.⁵ It does not establish that the material is free in the cytosol rather than inside an intact organelle carried through with the fraction, a control almost never included. And it says

nothing about whether the local concentration reaches the condensation threshold, below which there is binding without productive output.^{24, 26}

Antibody staining for duplex DNA is worse: it reports presence and approximate location, and discriminates neither length, nor protein association, nor origin among nuclear, mitochondrial and reverse-transcribed sources.

Epistasis is the strongest of the three and still does not identify a species. Showing that a phenotype requires the enzyme establishes that some ligand engages it. It does not say which.

9.3 What would count as identifying the ligand

The standard is not unreasonable and has been met in other systems. Four properties must be specified together.

Identity — the sequence class: mitochondrial, nuclear genomic, reverse-transcribed complementary DNA, or exogenous. Length distribution — because the activation function is length-dependent, and because the human enzyme is the more length-discriminating orthologue.²² Protein association — whether the DNA is naked, nucleosomal, or bound by bending proteins, since nucleosomal DNA is inhibitory³¹ while nucleoid-protein-bound DNA is activating.⁵ Compartment and concentration — free cytosolic, membrane-enclosed, or extracellular, and at what local density relative to the condensation threshold.

A candidate specified to that standard has a testable prediction: reconstituted with purified human enzyme, it should produce messenger at the observed rate. No candidate in this literature has been specified to that standard, and Chapters 10 to 13 examine each in turn against it.

9.4 The negative constraint from cerebrospinal fluid

One quantitative measurement bears on the premise that misplaced DNA is abundant in this disease, and it runs the wrong way. Cell-free mitochondrial DNA in cerebrospinal fluid is significantly *decreased* in sporadic disease, in asymptomatic at-risk subjects and in presymptomatic mutation carriers, with a validation cohort.⁶⁵ Standardised quantification places both nuclear and mitochondrial cell-free DNA in disease and control fluid below roughly one hundred genome copies per millilitre.⁶⁶

Two escapes remain open and neither has been closed. Cerebrospinal fluid is not parenchymal interstitial fluid, and no measurement of the latter exists. And a geometric, threshold-dependent mechanism is compatible with a low bulk average and a high local concentration. Both are legitimate; both are hypotheses about unmeasured quantities.

10. Mitochondrial DNA and the Species Problem

10.1 The leading candidate

Mitochondrial DNA is the most frequently invoked ligand, and the reasons are good. The organelle genome is bacterial in ancestry, present in thousands of copies, packaged by a protein that bends DNA in exactly the way that potentiates this enzyme, and released by damage of the kind this disease features. Depleting the nucleoid protein causes genome escape and primes the sensing pathway.⁶⁷ The benchmark tauopathy experiment identifies cytosolic mitochondrial DNA as the trigger.¹⁰

The problem is not whether mitochondrial DNA reaches the cytosol. It is what arrives.

10.2 Four release routes, four different products

Macropores formed by the apoptotic effectors permit inner-membrane herniation carrying matrix contents including the genome, imaged live.⁶⁸ A partly competing account emphasises sequential outer- then inner-membrane permeabilisation.⁶⁹ An oligomeric channel releases fragments rather than whole genomes.⁷⁰ And replication stress drives disposal of enlarged, still protein-bound nucleoids through endosomes that subsequently rupture.⁷¹

That last route is the most informative for the present question, because it is the only one in which the released species was characterised at all: intact nucleoids with their bending protein still attached — which is to say, the form most likely to activate this enzyme. It was demonstrated in human embryonic kidney and cervical carcinoma lines.

A fifth route is the most plausible in a long-lived cell that is not dying: sub-lethal, chronic release through partial outer-membrane permeabilisation during senescence, established in human fibroblasts and aged mice.⁷² It has not been tested in neurons.

A cytosolic topoisomerase augments the interaction as a second DNA-structuring cofactor, with release proceeding through a pore mechanism.⁷³

10.3 What has been measured in a neural cell

Very little, and never the species. Cytosolic mitochondrial DNA has been measured in primary cortical neurons and brain in a melatonin-deficiency model with sensing readouts⁷⁴; in mouse hippocampus and microglia in an anaesthetic model where pore blockade abolished both escape and pathway activation⁷⁵; and in a neuron-restricted proteasomal lesion where the readouts were whole-brain immunoblots.⁷⁶

Every one of these quantifies by amplifying one or two mitochondrial loci in a cytosolic fraction. None uses size fractionation, nuclease protection, or any control for whole-organelle carry-over. The length, topology and protein association of the released material in a neural cell are unknown.

A useful counterexample: in a microglial line, a lipid stimulus raised cytosolic *nuclear* DNA and not mitochondrial DNA, with enzyme knockdown blocking the response.⁷⁷ Microglial enzyme activation does not automatically mean organelle DNA.

10.4 Two results that complicate the account

The first is directional. Engineered double-strand breaks in the mitochondrial genome cause herniation that releases mitochondrial *RNA*, triggering the RNA-sensing arm rather than this one.⁷⁸ Damaged-organelle herniation does not necessarily produce a ligand for this enzyme, and attributing a brain interferon signature to mitochondrial DNA requires excluding the RNA route, which no study in this disease has done.

The second is a retraction. The principal *in vivo* claim that failed mitophagy drives adaptor-dependent dopaminergic neuron loss was retracted in 2025.⁷⁹ A substantial part of the narrative connecting mitochondrial quality control to nucleic-acid sensing in neurodegeneration descended from

that work, and reviews predating late 2025 that build on it require independent support.

What survives in this disease specifically is that a release-competent pore is engaged by mitochondrial amyloid in cortical neurons and that removing the pore's regulator rescues cognition — a study that predates the enzyme's discovery and measured no DNA release at all.⁸⁰ The inference is retrospective, and it is the strongest disease-specific link available.

11. Micronuclei in a Cell That Does Not Divide

11.1 The canonical route, and its gate

Micronuclei are the textbook endogenous ligand. The enzyme accumulates in them after envelope rupture, linking genome instability to innate immunity.⁸¹ A companion study established the gate: the inflammatory response to double-strand breaks requires passage through mitosis.⁸² Rupture is permissive rather than sufficient — pre-existing chromatin marks and intra-micronuclear transcription determine whether the enzyme is recruited and whether output follows.⁸³

A post-mitotic neuron does not pass through mitosis. On the canonical account, this route should be unavailable to it.

11.2 Except that neurons make micronuclei

The most surprising finding in this part of the literature is that they do. In developing mouse neocortex, migrating post-mitotic neurons generate micronuclei, release them into the extracellular space, and microglia take them up, with loss of the enzyme blunting the resulting microglial change.⁸⁴ The phenomenon extends to the aged mouse brain, where nuclear envelope fragility increases and micronuclei form in association with envelope invagination before transfer to microglia.⁸⁵ Quantitative three-dimensional imaging indicates neuronal micronucleus formation is not cell-autonomous and occurs at low frequency.⁸⁶

This is a genuinely important set of results, and it reframes the question. If neurons export micronuclei to microglia, then a neuron's genomic damage becomes a *microglial* ligand without any messenger transfer and without the neuron ever sensing anything. That is a coherent mechanism, it uses the cell type where the enzyme is actually abundant, and it is consistent with the human genetic and expression data of Part III.

11.3 What is missing

No micronuclei have been reported in human Alzheimer's disease brain tissue. The neuronal observations are in developing and aged mouse brain; the human neural evidence is in brain organoids from a genome-instability syndrome, where micronuclei accumulate and pathway inhibition suppresses senescence, but the effect is attributed to astrocytes and the organoids contain dividing progenitors from which the micronuclei may originate.⁸⁷

Nor has it been shown that neuronal micronuclei rupture, that the enzyme is recruited to them, or that their DNA is of activating length and protein composition.

11.4 The route that does not need mitosis

There is a second and mechanistically cleaner possibility for a post-mitotic cell. Chromatin fragments can bud from an intact nucleus without any mitotic event, and these cytoplasmic chromatin fragments activate the pathway and drive the senescence-associated secretory phenotype.⁸⁸ Demonstrated in fibroblasts and hepatocytes; never in neurons.

This is the route a neuron would have to use, and it has the additional merit of connecting to a documented feature of neuronal ageing: erosion of the nuclear lamina, with heterochromatin relaxation and derepression of repeat elements, observed with single-nucleus resolution in aged primate and human frontal lobe.⁸⁹ Tau drives the same lesion, uncoupling the cytoskeleton from the nucleoskeleton and misregulating lamin, with causal genetics in flies and confirmation in human tauopathy tissue.⁹⁰

A nucleus whose lamina is eroding, whose heterochromatin is relaxing and whose envelope is invaginating is a plausible source of cytoplasmic chromatin. No study has looked for cytoplasmic chromatin fragments in human Alzheimer's neurons.

12. Retroelement cDNA and the Case Against It

12.1 An attractive hypothesis

The retroelement account is the most intellectually satisfying candidate. Repeat elements are silenced by heterochromatin; heterochromatin fails with age and under pathogenic tau; derepressed elements are reverse-transcribed; the resulting complementary DNA is cytosolic by construction and is a substrate of the exonuclease whose loss causes a human brain disease.

Each link has support. Senescence derepresses one element family and cytoplasmic complementary DNA triggers interferon, with a reverse-transcriptase inhibitor reducing inflammation in aged mice.⁹¹ A companion study places the enzyme in the chain explicitly and shows nucleoside analogues improve healthspan in a progeroid model.⁹² Loss of the cytosolic exonuclease causes accumulation of retroelement-derived single-stranded DNA⁹³, and that exonuclease's loss in humans causes a severe encephalopathy.⁹⁴ Pathogenic tau depletes the small RNAs that silence these elements and drives their expression⁹⁵, confirmed across six hundred and thirty-six human brains with expression scaling with tangle burden.⁹⁶ Lamina erosion derepresses retroviral elements in aged post-mitotic neurons with an activated sensing signature.⁸⁹

12.2 The disease-specific counter-evidence

Against this stands a direct measurement in the relevant tissue, and it points the other way.

Long-read sequencing and enzymatic assays on thirty-one human cortices found that canonical full-length bicistronic transcripts of the principal element family are essentially absent, the overwhelming majority of detected transcripts being non-coding, and that endogenous reverse-transcriptase activity is *lower* in Alzheimer's disease than in aged non-demented controls.⁹⁷

If the element cannot make its own reverse transcriptase in the disease tissue, the route from derepression to cytoplasmic complementary DNA is

obstructed at its enzymatic step. The measurement is tissue-level and therefore confounded by neuronal loss, and cytoplasmic complementary DNA was not itself measured. But it is a direct assay of the required activity in human disease cortex, and it is negative.

12.3 The preprint, and how to read it

The most directly on-point study for this disease is a preprint, not peer-reviewed as of this cutoff. Using directly converted neurons from disease donors — a model that retains donor-age signatures⁹⁸ — together with spatial transcriptomics, it reports that retroelement-derived cytoplasmic DNA activates the pathway in post-mitotic neurons, with reverse-transcriptase inhibitors, antisense oligonucleotides and enzyme inhibition producing concordant effects.⁹⁹

Three qualifications. The cytoplasmic DNA is inferred to be retroelement-derived from pharmacological and antisense epistasis, not sequenced — so it does not meet the standard of Chapter 9. The authors themselves report no bulk difference in element expression between disease and control neurons, the signal residing in a long-read single-cell subset. And it is unreviewed.

12.4 Cell-type resolution, and a sensor that is not this one

Where the element is expressed in human disease brain has been resolved, and the answer implicates microglia rather than neurons in the functional follow-up of at least one study. Where retroelement nucleic acid has been introduced into brain tissue and caused degeneration, the demonstrated sensor was endosomal rather than this cytosolic enzyme, and the ligand was RNA. And a systematic review of the retroviral literature in neurodegeneration reports that findings vary substantially across element families, models and detection methods.

The position, stated carefully: retroelement derepression in this disease is well established at the transcript level; the reverse-transcription step required to produce a DNA ligand is contradicted by the one direct measurement in human disease cortex; and the connection to this enzyme in a neuron rests on an unreviewed preprint using inference rather than isolation.

13. Damage, Oxidation, and the Competing Nucleases

13.1 Damage is abundant, and that is the problem

There is no shortage of DNA damage in the Alzheimer's brain. Double-strand break markers rise focally in neurons and astrocytes from early stages¹⁰⁰, a genome-wide map reports substantially more breaks in disease frontal cortex with a distinct distribution¹⁰¹, a repair factor is depleted in disease brain and its knockdown causes breaks and memory deficits without cell death¹⁰², and oxidised bases are elevated in mitochondrial DNA in disease cortex¹⁰³ and in both genomes already at the stage of mild cognitive impairment.¹⁰⁴

The difficulty is that damage is also abundant in the healthy brain, by design. Neurons generate topoisomerase-dependent double-strand breaks at specific promoters as a normal mechanism of early-response gene induction⁶⁴, under a regulated switch that positions them preferentially at the nuclear periphery¹⁰⁵, and normal behaviour transiently raises neuronal break counts in vivo, with an amyloid model showing higher baseline and slower resolution.¹⁰⁶ Post-mitotic neurons additionally sustain continuous site-specific single-strand breakage at enhancers under permanent repair load.⁶³

So "there are breaks in disease neurons" is a weak premise. Breaks are physiological. What would matter is whether broken DNA leaves the nucleus, and no study has measured that.

A methodological correction constrains the whole literature: diffuse nuclear damage-marker staining is not associated with actual break ends, which invalidates a large body of earlier quantification that equated marker intensity with breakage.¹⁰⁰

13.2 Does oxidation make a better ligand?

This is frequently asserted and is not established. The single primary study bearing on it shows that oxidised DNA resists the principal cytosolic exonuclease and potentiates adaptor-dependent sensing.¹⁰⁷ What it demonstrates is *nuclease resistance* — a longer ligand half-life — not enhanced catalysis by this enzyme, which was not assayed, the work predating most of its biochemistry.

The defensible statement is therefore narrow: oxidation plausibly extends ligand lifetime. Whether oxidised DNA is a better or worse substrate for the enzyme itself has not been measured, in either direction.

13.3 The nucleases that compete

Any ligand's lifetime is set by the nucleases racing it, and this is where the strongest human genetics in the field sits.

Loss of the principal cytosolic exonuclease causes a severe human encephalopathy⁹⁴, with a phenotype spectrum correlating cerebrospinal fluid interferon activity with disability¹⁰⁸, and mouse epistasis proves its substrate is sensed specifically by this enzyme among candidates.¹⁰⁹ This is the strongest natural experiment that unrestrained cytosolic self-DNA is neurotoxic. It is a developmental encephalopathy, which limits extrapolation to late-life degeneration.

The lysosomal nuclease that disposes of engulfed chromosomal DNA in phagocytes causes sterile inflammation when lost in mice¹¹⁰, and biallelic human mutations define an interferonopathy — notably without a reported central nervous system phenotype, which is a real limit on placing it centrally in brain DNA clearance.¹¹¹ An extracellular nuclease digests chromatin on apoptotic-cell microparticles, establishing particle-bound extracellular chromatin as a distinct compartment with its own disposal machinery.¹¹²

Two mouse studies report that the lysosomal nuclease declines in ageing and tauopathy, with neuron-restricted knockdown driving cytoplasmic duplex DNA and tau phosphorylation through a kinase route independent of this enzyme¹¹³, and microglia-restricted knockdown activating the pathway and causing complement-dependent synapse loss.¹¹⁴ Both identify the DNA by antibody staining alone, from one laboratory. A disease risk gene encodes a further nuclease that degrades mitochondrial DNA and regulates nucleotide signalling.¹¹⁵

No study measures the expression or activity of any of these nucleases in human Alzheimer's brain with cell-type resolution.

13.4 Extracellular chromatin

One source of genuinely extracellular DNA inside the disease parenchyma is documented: neutrophils extravasate near amyloid deposits and release extracellular traps, with depletion improving memory in mouse models and traps observed in human disease brain.¹¹⁶ No study connects parenchymal extracellular chromatin to enzyme activation in any cell.

13.5 Where Part II leaves the ligand

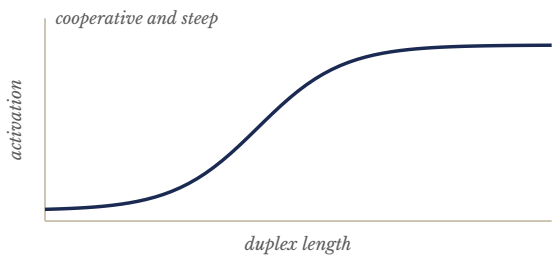
Every candidate has been examined and none is specified. Mitochondrial release routes produce different products and none has been size-resolved in a neural cell. The canonical micronucleus route is mitosis-gated, though neurons make micronuclei and export them to microglia. The retroelement route is contradicted at its enzymatic step by the one direct measurement in human disease cortex. Damage is abundant but largely physiological, and nobody has shown that broken DNA leaves the neuronal nucleus. Oxidation extends lifetime rather than improving catalysis. And the bulk cerebrospinal fluid measurement runs in the wrong direction.

The field has an enzyme, a disease, and a set of plausible substrates, and it has never held the ligand in its hand.

A ligand is specified by four properties, not by a source

	identity	length	bound proteins	compartment
mitochondrial DNA <i>four release routes, four products</i>	✓	✗	✗	✗
micronuclei <i>mitosis-gated; none seen in human tissue</i>	✓	✗	✗	✓
cytoplasmic chromatin <i>the only mitosis-free route; never sought</i>	✓	✗	✗	✗
retroelement cDNA <i>reverse transcription lower in disease</i>	✓	✗	✗	✗
extracellular chromatin <i>present in parenchyma; never linked</i>	✓	✗	✓	✓

no enzyme-bound DNA recovered from brain



why the proxies fail

- an amplicon cannot tell 16 kb from 200 bp
- an antibody cannot tell origin or length
- epistasis shows that a ligand exists

Two preparations of identical mass and sequence can differ by orders of magnitude in what they do to this enzyme.

Figure 3. A candidate ligand is specified only when its identity, length distribution, protein association and compartment are all known, because activation of this enzyme is cooperative and steeply length-dependent. No candidate in the Alzheimer's literature has been specified to that standard, and no enzyme-bound DNA has ever been recovered from brain tissue in any species. Sources: ^{4, 5, 71, 82, 97, 88}.

Part III

The Cell

*Where the enzyme is, which cell the evidence actually concerns,
and the argument that a neuron is a special case*

14. Where the Enzyme Actually Is

14.1 The question that precedes every cell-type claim

Before asking which cell's enzyme matters, it is worth asking which cells have much of it. The answer available from expression data is consistent and points away from neurons.

In the single-cell classification underlying the Human Protein Atlas, the enzyme is classified in brain as enhanced in leukocytes and central nervous system macrophages, and the adaptor as group-enriched across pericytes, choroid plexus epithelium, macrophages, leukocytes, fibroblasts and vascular cells, with microglia the highest single-cell value.¹¹⁷ Neurons appear in neither enrichment list for either gene.

Three caveats belong with that, and they cut in both directions. The classification is relative enrichment across aggregated datasets, not absolute absence. The reference human brain atlas is nuclear rather than whole-cell sequencing from three donors without disease, so low-abundance immune transcripts in neurons sit near the detection floor and failure to detect is not absence.¹¹⁸ And droplet methods are particularly poor for low expressors, which is why acutely purified populations sequenced in bulk are the more trustworthy source for this specific question, being deep enough to reveal a low but real signal and free of ambient contamination.¹¹⁹

14.2 Species and region

Two further constraints apply before any mouse result is carried across. Human and mouse glial transcriptomes diverge substantially, so cell-type assignment of an innate-immunity gene does not transfer automatically¹²⁰, and cross-species regional atlases are the appropriate check.¹²¹ Laminar and subclass denominators are available for testing whether expression is restricted to a vulnerable neuronal population.¹²² The canonical disease single-nucleus dataset is where the question of whether expression moves with pathology in neurons or microglia should be answered.¹²³

14.3 What the expression data do and do not settle

They establish that the enzyme is myeloid- and vascular-enriched in brain, and that if neurons express it they do so at levels near the limit of the dominant measurement technology.

They do not settle the functional question, for a reason worth stating explicitly. Enzyme abundance is a poor predictor of enzyme consequence when the activation function is steeply non-linear and threshold-dependent, as Chapter 6 established. A small quantity of enzyme concentrated on a locally dense ligand can produce more output than a large quantity distributed thinly. Low expression is a reason for scepticism, not a refutation.

15. The Microglial Case

15.1 What the strong version of this hypothesis looks like

The microglial account is the well-evidenced one, and setting it out plainly is the only way to see how far short the alternatives fall.

The benchmark is a tauopathy study in which genetic ablation of the enzyme, and separately its pharmacological inhibition, preserved synapses and memory without altering tau burden, with mitochondrial DNA leakage identified as the trigger and loss of a transcriptional resilience programme as the consequence.¹⁰ Necessity, a nominated ligand, a defined output, and dissociation from the proteinopathy itself.

Around it: pathway activation contributes to pathogenesis in an amyloid model¹²⁴; adaptor blockade alleviates microglial dysfunction and a broad spectrum of pathologies in knock-in models with human post-mortem expression support¹²⁵; an independent upstream route reaches the same pathway with inducible microglial depletion in vivo¹²⁶; two human risk alleles combined with sex produce enzyme-dependent microglial senescence in a tauopathy model¹²⁷; and the pathway drives ageing-related inflammation and neurodegeneration in mouse genetics and pharmacology.¹²⁸

15.2 Cell-type attribution done properly

Two studies do what most do not: delete the gene in one cell type.

An inducible microglia-restricted knockout of the enzyme crossed into an amyloid model, with deletion timed to plaque onset, restricts plaque accumulation and rescues cognition; the authors report the pathway upregulation in disease is attributable primarily to microglia.⁶ A validated inducible microglia-restricted knockout of the adaptor attenuates cortical inflammation, neuronal dysfunction and cognitive deficits after trauma.¹²⁹ In retina, global or microglia-restricted adaptor deletion rescues ganglion cell degeneration, with the dying neuron two cells downstream of the sensor.¹³⁰

A third cell type has been claimed: one study reports astrocyte-restricted deletion of the enzyme in a Parkinson's model, preventing astrocyte senescence and dopaminergic degeneration through a downstream transcriptional arm.¹³¹ Whether that deletion was achieved by a genetic driver or by viral knockdown could not be verified from the accessible record, and the distinction matters for exactly the reasons set out in Chapter 16, so the claim should be checked against the methods before being relied upon.

The inducible drivers used also label border-associated and peripheral myeloid populations transiently, which is a real but manageable caveat. This is nonetheless the standard of evidence the neuronal claim lacks entirely.

15.3 The human anchor

Two human observations support the microglial account specifically.

A protective human variant of apolipoprotein E, identified in a single carrier who resisted autosomal dominant disease despite extreme amyloid burden¹³², confers resilience against tau pathology through inhibition of this pathway in mouse, with an inhibitor of the enzyme phenocopying the variant transcriptionally.¹³³ The human anchor is one genotype and the mechanism is entirely murine, but no comparable tether exists for any other component discussed here.

And in a different neurological disease, human surgical and post-mortem brain tissue shows microglial pathway activation, with an inhibitor rescuing a mouse model.¹³⁴ This is the strongest human-tissue evidence of central pathway activation in any neurological condition, and it is microglial.

Downstream, the interferon arm is well characterised: nucleic-acid-bearing amyloid fibrils are sufficient to produce an interferon-stimulated gene signature and complement-dependent synapse elimination, with human tissue correlation¹³⁵; cell-type-restricted receptor

deletion separates microglial from neural contributions to memory impairment¹³⁶; and the human stem-cell counterpart shows interferon driving microglial dysfunction and senescence.¹³⁷

15.4 The limit of the microglial account

One human transcriptomic result constrains it. Comparing young, aged and diseased human brain across four regions, roughly forty per cent of immune probe sets changed with normal ageing while only about six per cent changed in the transition to disease.¹³⁸ The innate immune shift in human brain is predominantly an ageing phenomenon with a disease increment superimposed, consistent with the interferon signature at the choroid plexus in aged mice and aged human brain.¹³⁹

An account that presents this pathway as the disease's engine must explain why most of the signal is present in people who never develop it. The likeliest reconciliation is that the pathway sets a permissive background and that the disease-specific increment is small — which is a coherent position, and a difficult one for a therapeutic programme.

16. The Neuronal Case and the Paper It Rests On

16.1 One study

There is exactly one published claim that neuronal cGAS matters causally in Alzheimer's disease.

Viral delivery of a short hairpin against the enzyme under a neuronal promoter, into an amyloid knock-in mouse, reduced downstream pathway activation and inflammatory cytokines and rescued water-maze performance. Supporting observations include proximity-ligation signal between the enzyme and duplex DNA within neuronal nuclei in human disease hippocampus, colocalisation of the activated adaptor with a neuronal marker, and messenger and interferon-beta measured from primary hippocampal cultures treated with glycated amyloid.⁷

It is the only in vivo, causal, neuron-directed loss-of-function test of this enzyme in this disease, and it is worth taking seriously.

16.2 What it does not establish

Knockdown is not deletion: no floxed allele, no rescue arm, and no quantification of residual enzyme. The viral capsid used is broadly tropic, and although the promoter restricts expression, no sorting was performed to exclude knockdown in glia. Primary hippocampal cultures routinely contain glia and were not purified before the messenger and interferon measurements, so the cellular origin of those readouts is not established. Group sizes are small. The human evidence is proximity ligation and immunofluorescence colocalisation, both vulnerable to antibody non-specificity and — in tissue — to glial processes enveloping neuronal somata. It is a single laboratory, published in late 2025, and no independent group has reproduced it.

16.3 The experiment that does not exist

Searching across conditional-driver nomenclature located no neuron-restricted conditional knockout of this enzyme, in any brain disease model, in any species. The microglial field has two inducible cell-restricted knockouts; the neuronal field has a viral hairpin.

Nor has the messenger ever been measured in a purified or sorted neuronal population from brain tissue. The only regional measurement in aged brain is bulk cortex¹⁴⁰, and the only neuronal measurement is from unpurified cultures.⁷

16.4 Neuronal adaptor biology is better evidenced — and it argues against the neuronal enzyme

The adaptor has a substantially better neuronal literature than the enzyme, and reading it carefully undermines rather than supports the canonical neuronal claim.

In multiple sclerosis and its model, neurons induce the adaptor, and glutamate excitotoxicity detaches it from a calcium-sensor partner to drive signalling that is explicitly *non-canonical and interferon-independent*, degrading a protective enzyme and causing ferroptosis, with neuron-targeted intervention protective.¹⁴¹ In amyotrophic lateral sclerosis and frontotemporal dementia, human post-mortem tissue shows adaptor-pathway activation selectively in

the most vulnerable motor and cortical neurons.¹⁴² In peripheral sensory neurons, the best-controlled neuron-specific adaptor genetics anywhere — a nociceptor-restricted gain-of-function allele — produces a neuronal interferon-stimulated gene signature that *reduces* excitability and resolves pain.¹⁴³ Deep evolutionary evidence points the same way: a neuron-intrinsic adaptor function in insect brain that is autophagic and interferon-independent.¹⁴⁴

So the neuronal adaptor literature describes a protein that is induced in neurons, signals non-canonically without the messenger, and is sometimes protective. None of it licenses the inference that a neuron runs the canonical circuit from enzyme through messenger to adaptor to interferon in this disease.

16.5 Neurons have innate tone — but the ligand is RNA

One further result deserves prominence because it addresses the underlying intuition. Human neurons carry an intrinsically high load of immunostimulatory duplex RNA arising from long untranslated regions.¹⁴⁵ Neurons are indeed unusually loaded with an endogenous innate-immune ligand; it is simply not DNA, and it is sensed by helicases rather than by this enzyme.

Consistent with this, central neurons appear to have evolved cell-autonomous defences that bypass interferon altogether, using a neuron-specific restriction factor rather than the standard machinery.¹⁴⁶ A cell that has evolved an interferon-independent defence is a cell that may be keeping this pathway's output deliberately low.

17. Post-Mitotic Self-Tolerance

17.1 The argument

The strongest reason to care about neuronal cGAS is not an experiment. It is an argument from the structure of the restraint system, and it is sound.

The dominant account of self-tolerance rests on two parallel restraints operating when the nuclear envelope is absent and chromatin and enzyme share a compartment: mitotic phosphorylation by a cell-cycle kinase, and nucleosome tethering.⁸ The first is explicitly mitosis-linked. The second is constitutive.^{31, 1}

A neuron never divides. It never receives the mitotic phosphorylation, and it never experiences the envelope breakdown that the system evolved to manage. It therefore depends on tethering alone, continuously, for eighty years, in a nucleus whose lamina erodes with age, whose heterochromatin relaxes, and whose envelope invaginates.^{89, 85}

This is a specific, mechanistic reason to expect a post-mitotic cell to be a special case for this enzyme, and it predicts a specific failure mode: not acute activation by a burst of misplaced DNA, but slow erosion of a constitutive brake.

17.2 The experiment that has never been done

Nobody has tested whether cGAS is nuclear-tethered, chromatin-bound or nucleosome-inhibited in a neuron. The entire self-tolerance literature is in dividing cells. The question of whether the tethering arm suffices alone over a human lifespan has not been asked in any post-mitotic cell.

The reagents exist and the experiment is not difficult. Directly converted human neurons retain donor-age signatures and are the appropriate model⁹⁸; chromatin fractionation, proximity ligation and the structurally defined tethering mutants are all available. What would be measured is the fraction of enzyme that is chromatin-bound, how that fraction changes with donor age, and whether it changes in disease.

17.3 Why the prediction is testable and falsifiable

The argument makes a prediction that distinguishes it from the canonical account. On the canonical view, activation follows ligand supply: measure more cytosolic DNA, expect more output. On the tethering-erosion view, output rises because restraint falls, and ligand supply may be constant or even falling — which would reconcile the awkward observation that cell-free mitochondrial DNA is reduced rather than increased in disease cerebrospinal fluid.⁶⁵

It also predicts that the relevant measurement is not how much DNA is in the cytosol but how much enzyme is off its tether.

17.4 One result that fits, and its limits

The aged-brain result that comes closest is a mouse study in which the enzyme and its messenger both rise in aged cortex *without* downstream adaptor or transcription-factor activation, with increased duplex DNA attributed to entorhinal neurons.¹⁴⁰

Read against the canonical model this is anomalous: messenger is being made and nothing follows. Read against the tethering model it is what one would expect from a partial, subthreshold loss of restraint — enzyme freed and catalytically productive, but not to the level that engages the downstream cascade. The study is small, descriptive and uses no causal manipulation, and it is the only measurement of the messenger in aged brain by region. It should be treated as a pointer to an experiment rather than as a result.

18. The Sensing Cell Is Not the Suffering Cell

18.1 Three mechanisms of separation

A recurring error in this literature is to infer from a phenotype in one cell that the pathway ran in that cell. Three well-documented mechanisms separate the two.

The messenger diffuses and is transported. It spreads to bystander cells through gap junctions³⁰, is imported by a folate transporter¹⁴⁷ and by volume-regulated anion channels¹⁴⁸ — the latter notable because the same channel family is a major astrocytic release pathway in brain, making a glial-to-neuronal conduit mechanistically plausible and entirely untested. In neural tissue the only demonstration of messenger movement between cells is through assembled gap junctions in brain metastasis¹⁴⁹, and the only import mechanism described in neural tissue is a purinergic receptor in retinal microglia.¹⁵⁰

The ligand itself moves. Neurons export micronuclei that microglia take up, converting a neuron's genomic damage into a microglial substrate without any messenger transfer.^{84, 85}

And the sensing cell can be removed from the measurement. Pathway-driven apoptosis of brain immune cells acts as a brake on cerebral interferon, which would silently delete the sensing population from any bulk tissue assay.¹⁵¹

18.2 Studies that got the attribution right

Several papers show how to do this, and they are worth naming because their designs are the template for Chapter 27.

In a neuron-restricted transgenic model of amyotrophic lateral sclerosis, the lesion is in the neuron and the interferon response, when localised, is in astrocytes.¹⁵² In herpes infection of brain, microglia are the main source of induced interferon while the adaptor is redundant for cell-autonomous

resistance in neurons and astrocytes — yet neuronal infection worsens in whole-body adaptor-null animals, the paradigm case of a cell suffering from a pathway it does not run.¹⁵³ In axon regeneration, applied messenger activates the neuronal adaptor directly while in the peripheral arm the enzyme is upregulated in Schwann cells and infiltrating cells that supply messenger to the neuron.¹⁵⁴ A conditional mitochondrial lesion restricted to excitatory neurons causes the damage while microglia execute the death.¹⁵⁵ And in a human central interferonopathy, the most rigorous determination of which cell suffers found an astrocytic source acting on cerebral endothelium, with endothelial receptor ablation rescuing the disease — an answer nobody was looking for.¹⁵⁶

Human stem-cell co-culture work shows pathway activation in the neuronal compartment driving transfer of aggregates and damaged organelles to microglia with bystander inflammation.¹⁵⁷

18.3 Studies that did not

The clearest illustration is a neuron-restricted proteasomal lesion producing cytosolic mitochondrial DNA and pathway activation, where every sensing readout is a whole-brain immunoblot — so the pathway could be running entirely in reactive microglia.⁷⁶ The most cited application of this pathway in neurodegeneration assembles its causal chain from cell lines, patient-derived cells and mouse tissue without any cell-type-restricted deletion, leaving unsettled whether the operative enzyme is in the motor neuron or the myeloid cell.¹⁵⁸ A widely cited metabolic intervention study attributes its effect to the pathway entirely from bulk brain measurement.¹⁵⁹ A striatal disease study uses immortalised neuronal-like cells and bulk tissue with no in vivo cell-type manipulation.¹⁶⁰ An alpha-synuclein study uses whole-animal adaptor deletion and attributes the effect to glia.¹⁶¹ A traumatic injury study claims neuronal adaptor activation from culture systems and immunohistochemistry without neuron-restricted deletion.¹⁶²

A methodological template that works: microglial depletion combined with separate neuronal and microglial nuclear profiling, which separates the interferon-producing cell from the transcriptionally injured one.¹⁶³ Repair-deficient human microglia-like cells mounting a pathway-dependent chemokine response show the same discipline in vitro.¹⁶⁴

19. Four Results That Do Not Fit

19.1 Why to foreground them

A review that reports only the results supporting its subject's importance is an advertisement. Four published findings sit badly with the prevailing account, none has been retracted, and none has been adequately addressed.

19.2 The messenger rises and nothing happens

In aged mouse cortex, the enzyme and the messenger both increase without downstream adaptor or transcription-factor activation.¹⁴⁰ If messenger production does not imply interferon output, then every study inferring pathway activity from enzyme abundance or from a downstream signature has an unquantified gap in its argument. Chapter 17 offers one reading of this result; the point here is that the field has not engaged with it.

19.3 The pathway is impaired, not hyperactive

A study of human disease temporal lobe reports the opposite of the prevailing model: signalling compromised, the adaptor depleted from its normal compartment, and no interferon elevation despite abundant DNA damage.¹⁶⁵ Bulk homogenate, no cell-type resolution, small cohort, single group, unreplicated — and a direct published contradiction that the field has largely passed over.

19.4 When someone looked carefully for the neuronal trigger, it was not this enzyme

In human stem-cell-derived motor neurons from a genetic form of amyotrophic lateral sclerosis, investigators set out to test this pathway and found instead that the neuron-intrinsic interferon driver is cytosolic duplex RNA sensed by a helicase, with knockdown abolishing the response.¹⁶⁶ A negative result obtained by looking, which is the most informative kind.

19.5 The pathway may be protective

One study argues that phosphorylated tau acts as an innate immune effector and that signalling through this pathway *preserves* neurons in this disease — directionally opposite to the dominant account.¹⁶⁷ Single group, organoid causality, human data colocalisation only, cell-type attribution assumed, and not independently replicated. It is nonetheless in the literature and is not answered.

To these four should be added the three from Part I: the enzyme-null mouse ages prematurely⁵⁰, loss of the enzyme worsens pathology on a normal background in a vertebrate genome-instability model⁵⁸, and the brain-specific test in a genetic interferonopathy found the enzyme dispensable for the neuropathology.⁶²

19.6 What the discordant set implies

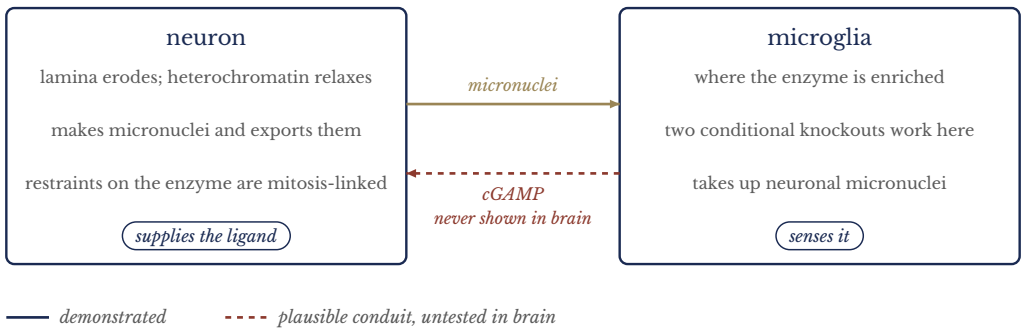
Not that the target is wrong. The microglial evidence of Chapter 15 is strong and the benchmark experiment is clean. What the set implies is that the relationship between this enzyme and this disease is conditional — on cell type, on stage, on whether catalysis or occupancy is the operative property, and possibly on direction. A field confident enough to pursue inhibitors ought to be able to say which conditions, and it cannot.

Which cell the evidence actually concerns

	microglia	neurons
cell-restricted conditional knockout	✓	✗
causal in vivo loss of function	✓	○
expression enrichment in human brain	✓	✗
messenger measured in a sorted population	✗	✗
human tissue evidence of activation	✓	○

An open circle marks a single unreplicated study.

Why the sensing cell need not be the damaged one



Read this way the neuron's role is to supply the ligand, not to sense it — which fits every expression dataset and both conditional knockouts. It also makes the single neuronal study the anomaly to replicate rather than the foundation to build on.

Figure 4. Expression places the enzyme in myeloid and vascular populations, and two independent inducible conditional knockouts support a microglial mechanism; the neuronal claim rests on one unreplicated viral-knockdown study, and no neuron-restricted deletion of the enzyme exists. The lower panel shows the two routes by which the sensing cell need not be the damaged one: the messenger diffuses and is transported, and neurons export micronuclei that microglia take up. Sources: ^{117, 6, 7, 84, 30}.

Part IV

The Drug

*The target assessed as a medicinal chemist would assess it:
chemistry, brain exposure, biomarker, safety and the prior*

20. Chemical Matter and the Species Problem

20.1 What exists

The enzyme is druggable, and there is more chemical matter than the neurodegeneration literature usually acknowledges. Five mechanistic classes have been characterised.

Active-site binders. A fragment-derived nanomolar binder with co-crystal structures — and no cellular activity, for a reason its authors state plainly: the active site is large and polar.¹⁶⁸ It never became a pharmacological probe, and that failure is informative about the site.

DNA-competitive compounds. Antimalarials block the enzyme-DNA interaction, identified by docking and validated in cells.¹⁶⁹ An acridine derivative given orally to a mouse interferonopathy model outperformed the parent compound and demonstrated target engagement by mass-spectrometric measurement of the messenger in tissue — one of very few in vivo pharmacodynamic datasets in this field.¹⁷⁰ Potency is micromolar and the compounds are highly promiscuous.

DNA-displacing compounds. A polysulfonated polyanion displaces bound DNA rather than competing at the nucleotide site, a mechanistically distinct mode, in a molecule with poor selectivity and essentially no central penetration.¹⁷¹

Optimised nucleotide-site series. The tool compounds used throughout the neurodegeneration literature descend from one chemical programme^{172, 173}, with an independent human-optimised series showing cellular activity and pathway selectivity¹⁷⁴, a covalent chemotype¹⁷⁵, natural-product chemotypes with human co-crystal structures^{176, 177}, a spiro series with oral bioavailability and efficacy in peripheral injury¹⁷⁸, and a recent large screen against the human enzyme yielding sub-micromolar leads.¹⁷⁹

The clinical series. The sponsor whose compound reached trials has published its chemistry: a metal-chelating nucleotide-site series hybridised with a tetrahydrocarboline, with leads potent against both human and mouse enzymes — dual-species potency treated as an explicit design goal.¹⁸⁰ A follow-up names neurodegenerative disease among target indications and documents that the chemotype required careful control of polarity to escape a cardiac ion-channel liability while retaining permeability.¹⁸¹ A review maps the field for this indication.^{182, 183, 184}

A sixth, endogenous mode. Acetylation at three lysines is reported to hold the enzyme inactive, and aspirin is reported to acetylate it directly, with efficacy in patient-derived cells and a mouse interferonopathy model.¹⁸⁵ The claim was received as significant¹⁸⁶, and the best pharmacological test of it since — adaptor agonists abolishing aspirin's protective effect in two vascular models — is supportive.¹⁸⁷ It should nonetheless be treated with care: no retraction, correction or rebuttal exists, and equally no independent replication of the biochemistry by a group unconnected to the original authors. Aspirin's acetyl transfer is promiscuous across hundreds of proteins, and the concentrations required for this modification are not reconciled with those achieved clinically. The point is not that the mechanism is wrong but that a widely available drug is frequently described as a cGAS inhibitor on the strength of a single laboratory's biochemistry.

20.2 Every one of them inhibits catalysis

The classes differ in where they bind — nucleotide site, DNA-binding surface, covalent active site — and agree in what they are designed to stop: production of the messenger.

Chapter 5 established that several of the enzyme's functions do not require catalysis. If what harms the ageing brain is chromatin occupancy, repair interference or condensate formation, the entire chemical armamentarium is aimed at the wrong property. The DNA-competitive class is the partial exception, since it targets the binding the non-canonical functions require — and it is the least potent and least selective class. No programme has been designed around this distinction, and no experiment in brain has determined which property matters.

20.3 The species problem, and how it is being handled

Two human-specific substitutions in the DNA-binding surface make the human enzyme less efficient and more length-discriminating than its mouse orthologue.²² Simulation localises the divergence to differing dominance of binding surfaces during activation and states the practical consequence: inhibitors designed on the mouse protein have often proved ineffective against the human one.²³ There is a counter-case, arguing the principal tool compound inhibits both orthologues in cells and human primary cells¹⁸⁸ — from the group that originated the compound, cell-based rather than by head-to-head enzymology, and in tension with the structural evidence and with practice.

Practice is the most telling evidence. The benchmark tauopathy study used *two different compounds from the same programme* — one against the mouse enzyme, one against the human — assayed under different metal conditions and incubation times.¹⁰ That is what the species problem looks like from inside a laboratory, and it means the mouse efficacy data and the human-active chemistry are not the same molecule.

No published head-to-head potency table across chemotypes and orthologues exists.

21. The Brain the Drug Cannot Reach

21.1 The single published brain-exposure dataset

For a target proposed for a brain disease, one number matters more than any potency: how much compound reaches brain.

The published literature contains one such dataset. The benchmark tauopathy study reports, for its mouse-active compound, a brain-to-plasma ratio near two with brain and plasma half-lives around twelve and ten hours after intraperitoneal dosing, measured by mass spectrometry after transcardial perfusion.¹⁰ That is genuinely good exposure.

It is for the mouse-selective compound. Its human-active sibling from the same programme has no published brain exposure, no pharmacokinetics of any kind, and no in vivo data.

21.2 The consequence, stated plainly

There is no compound that is active against the human enzyme and demonstrated to reach the brain. The molecule required to test this hypothesis in a human being does not exist in the published literature.

A later study extending the target to another neurological disease dosed the same mouse-selective compound and asserts brain penetrance by citing the earlier work rather than by new measurement.¹³⁴ The adaptor-blockade study in disease models reports no central pharmacokinetics for its compound.¹²⁵ No brain-exposure data exist for any adaptor inhibitor in the literature retrieved.

21.3 Why this is harder than it looks

The chemistry papers explain the difficulty. The active site is large and polar, which is why the first high-affinity binder had no cellular activity.¹⁶⁸ The clinical series required meticulous control of polarity to avoid a cardiac ion-channel liability while retaining permeability.¹⁸¹ Polarity that gets a molecule into a cell is not the same as polarity that gets it across the blood-brain barrier, and the optimisation pressures point in different directions.

The clinical programmes reflect this. The sponsor's central-nervous-system asset is a different compound against a different target entirely; its inhibitor of this enzyme is being developed for a systemic autoimmune indication. Conflating the two would be a serious error and is easily made.

21.4 What would have to be shown

Three things, none of which has been shown for any molecule. Potency against the human enzyme at a concentration achievable in brain. A measured brain-to-plasma ratio and free-fraction-corrected brain concentration in at least two species. And demonstrated central target engagement, which requires the biomarker that Chapter 22 shows does not exist.

22. No Central Biomarker, No Registered Trial

22.1 The messenger has never been measured in a human brain

Target engagement for this enzyme has an obvious readout: its product. The messenger has been quantified in mouse tissue by mass spectrometry¹⁷⁰, and intracellular messenger suppression has been shown in cells.¹⁸⁸ In aged mouse brain it has been measured regionally.¹⁴⁰

It has never been measured in human cerebrospinal fluid or human brain. There is therefore no established central pharmacodynamic biomarker for inhibition of this enzyme, and a trial would have to rely on a peripheral surrogate or develop a fluid assay from scratch.

22.2 The available surrogate, and what it can carry

The peripheral surrogate exists and is well characterised: a whole-blood interferon-stimulated gene score developed explicitly with diagnosis and trials in view, with a defined positivity threshold, together with historical cerebrospinal fluid interferon activity data in a central interferonopathy.¹⁸⁹ It has been shown to track clinical benefit over years in monogenic interferonopathy treated with a kinase inhibitor¹⁹⁰, and interferon-directed therapy has changed outcomes in a human central interferonopathy against a natural-history comparator.¹⁹¹

The limits are severe for the present purpose. It is a blood measure standing proxy for a brain process. It was validated where interferon tone is extremely high, not in sporadic late-life disease where — as Chapter 15 noted — the disease-specific increment over ageing is small.¹³⁸ And it measures the output of three convergent adaptors, so it cannot attribute a change to this enzyme.²⁸

22.3 The clinical landscape

Two clinical-stage inhibitors of this enzyme could be verified against trial registries. One is in a randomised phase 2a study in cutaneous lupus.¹⁹² The other is in an open-label study in monogenic interferonopathies.¹⁹³

Only one peer-reviewed publication exists on any clinical-stage inhibitor of this enzyme anywhere: single-cell sequencing of blood cells from one patient with a genetic interferonopathy and one matched control, with ex vivo exposure normalising the most elevated interferon-stimulated genes.¹⁹⁴ One participant against one control, ex vivo, no patient dosed, and two authors employed by the sponsor. The first-in-human data for both programmes exist only as press releases and conference presentations; one has no locatable registration at all, and the pharmacodynamic marker described as demonstrating target engagement is not named in any public document.

No inhibitor of this enzyme or of its adaptor is registered in any neurological or neurodegenerative indication anywhere.

22.4 What agonist trials show about the axis

The opposite direction has been tested extensively in oncology and the results bound what systemic modulation of this axis can achieve. Agonists of the adaptor produce a characteristic systemic interferon toxicity — fever, chills, injection-site reactions — with minimal efficacy and terminated programmes.^{195, 196} In the largest series, fever occurred in seventy per cent of participants with dose-limiting toxicities, and cytokine responses plateaued above a threshold dose rather than scaling.¹⁹⁷ Systemic dosing produced systemic exposure without a dose-related immune response.¹⁹⁸ The most tolerable agent confirmed pathway activation with few objective responses.¹⁹⁹

Two lessons transfer. The axis produces dose-limiting systemic effects when pushed, which is a caution for chronic modulation in either direction in an elderly population. And its pharmacodynamic response saturates, which means the relationship between exposure and pathway state is not a simple one.

23. Safety Bounds from Human Genetics

23.1 The upper bound

Human genetics establishes that too much pathway activity is severely harmful. Gain-of-function in the adaptor causes an early-onset syndrome of systemic inflammation, cutaneous vasculopathy and interstitial lung disease.²⁰⁰ Loss of the cytosolic exonuclease that restrains ligand supply causes a severe encephalopathy with a measurable interferon signature and a defined disability spectrum.^{94, 108, 201} Mouse epistasis shows that disease depends on this enzyme specifically.¹⁰⁹

That is the strongest argument for the therapeutic hypothesis: in humans, unrestrained signalling through this pathway damages the brain.

23.2 The lower bound, and what it actually covers

The corresponding question — whether losing pathway function harms people — has an unusually good answer, for the adaptor. A common allele is effectively null: homozygotes express almost no adaptor protein and their cells do not respond to cyclic dinucleotides, and they constitute a substantial fraction of some populations while being, in general, healthy.²⁰² A large number of people walk around with essentially no functional adaptor. That is a powerful argument that pharmacological suppression of the pathway is tolerable.

Two qualifications. The corresponding knock-in mouse showed a reduced response to a polysaccharide vaccine, so an infection and vaccine-response caveat is real. And — the point that matters here — this is the adaptor, not the enzyme.

23.3 The missing human genetics for the enzyme

No published human loss-of-function phenotype for this enzyme was located. There is no described null individual and no constitutional deficiency syndrome. The safety-by-genetics argument therefore rests entirely on the adaptor and must not be stated as though it were established for the enzyme itself.

This matters more than it would for most targets, because Part I established that the enzyme has functions the adaptor does not share. A person lacking the adaptor still has a fully functional nuclear enzyme maintaining heterochromatin, modulating repair, and slowing forks. A person — or a patient — lacking enzyme function does not. The tolerability of adaptor deficiency says nothing about the tolerability of enzyme deficiency, and the mouse that lacks the enzyme ages prematurely.⁵⁰

23.4 The catalytic escape

There is a plausible resolution, and it is worth naming because it would make the programme coherent. If the enzyme's protective nuclear functions require binding rather than catalysis — which is what the mutant evidence of Chapter 5 indicates — then an active-site inhibitor would suppress messenger production while leaving chromatin maintenance, fork deceleration and repair modulation intact.

On that reading, the current chemistry is targeting exactly the right property, and the null-mouse phenotype is not predictive of pharmacological inhibition. It is a fortunate arrangement, it is consistent with the available mutant data, and it has never been tested. Testing it is straightforward: dose an enzyme inhibitor chronically in an ageing animal and measure the heterochromatin landscape, repeat-element derepression and lifespan that the knockout disturbs.

24. The Negative Prior

24.1 A row of null results

Any proposal to treat this disease by suppressing an inflammatory pathway inherits a prior, and the prior is unfavourable.

The prototype antimalarial DNA-binding inhibitor of this enzyme has been tested in early disease in a randomised, double-blind, placebo-controlled trial over eighteen months. It was completely flat on function, cognition and behaviour, with high completion, so the result is not a failure of power or retention.²⁰³ Its activity against this enzyme is micromolar and unmeasured

in brain, so it is a weak test of the specific hypothesis — but it is a strong prior against repurposing a weak inhibitor of this class.

Beyond it: a selective and a non-selective anti-inflammatory produced no slowing of decline over a year in mild to moderate disease²⁰⁴; a primary prevention trial of the same class was terminated early on cardiovascular safety with hazard ratios above one in both arms, that is, no benefit and a possible harm signal from long-duration dosing in an elderly population²⁰⁵; and cytokine blockade over twenty-four weeks was tolerable and inert, with more infections on drug.²⁰⁶

24.2 Why this prior might not apply

Three arguments distinguish the present proposal from its predecessors, and they should be stated fairly.

The mechanism is upstream rather than at the effector end: these trials suppressed prostaglandins or a single cytokine, whereas this target sits at the initiation of a transcriptional programme. The genetic anchor is different in kind: a protective human variant acting through this pathway^{132, 133} is not something any previous anti-inflammatory programme possessed. And the benchmark preclinical result dissociates synaptic and cognitive rescue from the proteinopathy itself¹⁰, which the earlier classes never achieved.

24.3 Why it might apply after all

Two arguments run the other way, and they are quantitative.

The disease increment is small. Most of the innate immune transcriptional shift in human brain accompanies normal ageing, with only a small further change in the transition to disease.¹³⁸ A therapy aimed at that increment is aimed at a small signal on a large physiological background, which is a difficult place to demonstrate benefit and an easy place to cause harm.

And the timing is unfavourable. The mechanism, insofar as it is characterised, is continuously active from mid-life — which is the least favourable configuration for late intervention and the most favourable for a prevention argument that no trial design currently supports. The one prevention trial in this therapeutic area was stopped for safety.²⁰⁵

25. Reducing the Ligand Instead

25.1 A different point of attack

If the enzyme cannot yet be inhibited in a human brain, the supply of its ligand might be reduced instead. One class of agent does this: reverse-transcriptase inhibitors, which would block the production of retroelement-derived complementary DNA.

The preclinical case is reasonable. A nucleoside analogue reduced interferon and inflammation across tissues in aged mice ⁹¹ and improved healthspan in a progeroid model where the enzyme is explicitly in the causal chain. ⁹² In a tauopathy model, the same agent given in drinking water reduced tau phosphorylation, inflammation, neuronal death and atrophy with behavioural improvement. ²⁰⁷ In an amyloid model, it suppressed pathway markers, restored mitophagy and improved spatial memory, though the two effects were not separated and no genetic epistasis was performed. ²⁰⁸ In a genetic model carrying high lifetime risk of this disease, it completely rescued hippocampal recognition memory. ²⁰⁹

25.2 The human data

Two pieces of human evidence exist, and neither is a completed trial of efficacy.

Propensity-matched analysis across two large health systems found that exposure to one antiretroviral class was associated with lower disease incidence while three other classes were not. ²¹⁰ The class specificity is the inferential asset, and it is observational pharmacoepidemiology; notably, the authors attribute the effect to inflammasome inhibition rather than to reverse transcription or to this enzyme.

And one human study tests the strategy directly: a single-centre, single-arm, self-controlled study of a reverse-transcriptase inhibitor in mild cognitive impairment with biomarker-confirmed disease, measuring plasma neurocognitive markers and interferon-stimulated genes over twenty-four weeks. ²¹¹ Twenty-three participants, no placebo, biomarker endpoints only, no results posted. It is the only human study anywhere testing ligand-supply

reduction in a cognitively impaired population, and its pharmacodynamic architecture — an interferon-stimulated gene readout — is exactly what a trial of the enzyme itself would need.

25.3 The problem this strategy inherits

It depends on the ligand hypothesis of Chapter 12, which is the one contradicted at its enzymatic step by direct measurement in human disease cortex: full-length element transcripts essentially absent and reverse-transcriptase activity *lower* in disease than in aged controls.⁹⁷

If there is little endogenous reverse transcription in the disease brain, a reverse-transcriptase inhibitor has little to inhibit, and any benefit observed is proceeding through something else — which is precisely what the pharmacoepidemiological authors themselves propose. The strategy may work; if it does, the mechanism is probably not the one that motivated it.

25.4 Other points of ligand control

Two further levers exist in principle and neither has been pursued in this disease. Blocking the mitochondrial release routes of Chapter 10 — the pore whose regulator, when deleted, rescues cognition in an amyloid model⁸⁰ — targets supply at its source. And restoring nuclease capacity addresses disposal rather than production, with mouse evidence that the lysosomal nuclease declines in ageing and tauopathy and that its restoration would be protective.^{113, 114} Neither has a compound, and neither has been tested.

Part V

Assessment

*An itemised ledger of the four questions, a programme ordered
by the cost of being wrong, and what would have to be true*

26. A Ledger of Four Questions

26.1 Purpose

The chapters above are organised by question rather than by molecule, and the itemisation below follows the same order. The categories are those of Chapter 1: *established* means reproduced by independent groups in a named system, *proposed* means offered without direct test, *unevidenced* means the argument requires it and no study was found, and *contradicted* means the primary evidence points the other way.

26.2 What the enzyme does

Table 1 — What the enzyme does — status of each claim

Claim	Status	Basis and limit
Most cGAS is nuclear and chromatin-tethered	Established	Endogenous protein across cell-cycle phases ¹ ; five independent structures of the inhibitory interaction ^{31, 36}
Nuclear cGAS alters DNA repair and replication	Established	Three groups, untransformed and transformed cells, plus mice ^{40, 2, 3}
The direction of that effect	Contested	Founding account reports tumour promotion; the fork study reports the enzyme is genome-protective, with no rebuttal published either way
These functions require catalysis	Contradicted	Catalytically dead enzyme performs as wild type; binding and oligomerisation mutants do not ^{2, 3}
Condensation is a kinetic state, not just concentration	Established	Reconstitution showing substrate and intermediate mobility restricted, and nuclease protection ^{26, 25}
Losing the enzyme shortens lifespan	Proposed	One whole-animal study, single group, no brain analysis ⁵⁰
Any of this operates in a neuron or glial cell	Unevidenced	No study located across multiple search formulations
Recombination suppression could operate in a neuron	Contradicted in principle	The effect requires S/G2 and a sister chromatid; neurons have neither

26.3 What activates it

Table 2 — What activates it — the candidate ligands and their specific problems

Candidate	Status in this disease	The specific problem
Mitochondrial DNA	Proposed, best supported	Four release routes give four different products; never size-resolved or protein-resolved in any neural cell ^{68, 71}
Micronuclei	Proposed	Canonical route is mitosis-gated ⁸² ; neurons do make and export them ⁸⁴ , but none reported in human disease tissue
Cytoplasmic chromatin fragments	Proposed, mechanistically apt	The only route not requiring mitosis ⁸⁸ ; never examined in neurons
Retroelement complementary DNA	Contradicted at the enzymatic step	Full-length transcripts essentially absent and reverse-transcriptase activity lower in disease cortex ⁹⁷
Oxidised DNA as a better ligand	Unevidenced	The one study shows nuclease resistance, not enhanced catalysis ¹⁰⁷
Extracellular chromatin in parenchyma	Present, never connected	Traps documented in human disease brain ¹¹⁶ ; no link to enzyme activation tested
Abundant extracellular DNA in disease	Contradicted in cerebrospinal fluid	Cell-free mitochondrial DNA reduced; total cell-free DNA very low ^{65, 66}
The ligand has been isolated and identified	Unevidenced	No cGAS-bound DNA has ever been recovered from brain in any species

26.4 Which cell

Table 3 — Which cell — microglia and neurons compared by the experiments that exist

Claim	Microglia	Neurons
Cell-restricted conditional knockout of the enzyme	Yes, inducible ⁶	None exists in any species or disease
Causal in vivo loss of function in a disease model	Yes ^{10, 6}	One viral knockdown study, unreplicated ⁷
Expression enrichment in human brain	Yes ¹¹⁷	Not in any enrichment list
Messenger measured in a sorted population	No, but measured regionally ¹⁴⁰	No; only unpurified cultures
Human tissue evidence of activation	Yes, in another neurological disease ¹³⁴	Proximity ligation in one study ⁷
A mechanistic reason to expect a special case	—	Yes: the restraints are mitosis-linked ⁸

26.5 Whether it can be drugged

Table 4 — Whether it can be drugged — requirements and their status

Requirement	Status
Chemical matter against the enzyme	Established, five mechanistic classes ^{168, 169, 180}
Compounds that inhibit non-catalytic functions	None; every class targets catalysis
A compound active on the human enzyme with measured brain exposure	Does not exist
Brain exposure data for any inhibitor	One compound, mouse-selective ¹⁰
A central pharmacodynamic biomarker	None; the messenger has never been measured in human brain or cerebrospinal fluid
A registered trial in any neurological indication	None anywhere
Human loss-of-function safety data for the enzyme	None; the reassuring null allele is the adaptor ²⁰²
Prior for the therapeutic class in this disease	Uniformly negative ^{203, 204, 205, 206}

26.6 What the ledger shows

Three observations follow.

The strongest evidence in this field is microglial, interventional and mouse, and it is genuinely strong. The benchmark experiment dissociates synaptic rescue from proteinopathy, two independent conditional knockouts support cell-type attribution, and a protective human variant acts through the pathway. A reader who wanted only to know whether this target is worth pursuing could stop there and answer yes.

The weakest links are not at the periphery of the argument but at its centre. The ligand has never been identified in brain. The enzyme's dominant activity is nuclear and has never been examined in a neural cell. The neuronal claim rests on one unreplicated study. And the compound needed to test the hypothesis in a human being does not exist.

And the property that current drugs target — catalysis — is the one property that several of the enzyme's functions demonstrably do not require. Whether that is a fortunate coincidence that makes inhibition safe, or an indication that the drugs address the wrong activity, is undetermined and is the single most consequential open question in this review.

27. A Staged Experimental Programme

27.1 Design principle

The programme below is ordered by the cost of being wrong. Three experiments would determine whether the framing of this review is correct at all; a second stage establishes identity and order; a third addresses translation. Each is specified so that a null result is informative.

27.2 Stage one: three experiments that decide

Experiment 1 — Does the enzyme do its nuclear job in a neuron? In directly converted human neurons from young and aged donors, which retain donor-age signatures⁹⁸, determine the chromatin-bound fraction of the enzyme by fractionation and proximity ligation, and test whether it changes with donor age and with disease genotype. Use the structurally defined tethering mutants^{31, 8} to establish dependence. Measure, in the same cells, whether the enzyme influences end joining, single-strand break repair and transcription-coupled repair — the routes available to a post-mitotic cell — rather than homologous recombination, which is not.

Falsifies if: the enzyme is fully tethered irrespective of age and influences no repair route. Part I then does not transfer to this disease, and the target reduces to the microglial account of Chapter 15.

Experiment 2 — Isolate the ligand. Immunoprecipitate the enzyme from microglia and neurons sorted from mouse disease models and from human post-mortem tissue, recover bound DNA, and sequence it with a method that preserves length distribution. Report identity, length, protein association and compartment — the four properties of Chapter 9. Reconstitute the recovered material with purified human enzyme and measure messenger production.

Falsifies if: no enriched species is recoverable, or the recovered material does not activate the enzyme in reconstitution. This is the single most important missing experiment in the field, and it has no technical barrier.

Experiment 3 — Can a human-active compound reach brain? Take a compound potent against the human enzyme and measure brain-to-plasma ratio and free brain concentration in two species, with central target engagement demonstrated by messenger measurement in tissue. The dual-species chemistry now exists ¹⁸⁰, and the polarity and ion-channel constraints are documented. ¹⁸¹

Falsifies if: free brain concentration cannot reach the human potency. The therapeutic hypothesis is then untestable in humans with current chemistry, which is a decision-relevant result and should be published as one.

27.3 Stage two: identity and order

Experiment 4 — Neuron-restricted conditional deletion. Generate and cross a floxed allele of the enzyme to neuronal and astrocytic drivers in amyloid and tauopathy models, with the microglial deletion as comparator. ⁶ This is the experiment the field has assumed and never performed, and its absence is why Chapter 16 is short.

Experiment 5 — Separate catalysis from occupancy in vivo. Compare, in the same disease model, a catalytically dead knock-in allele against a DNA-binding-deficient allele against full deletion. The mutants are defined. ^{2, 3} This determines which property of the enzyme matters in brain and therefore whether the existing chemistry addresses it — the question Chapter 26 identifies as most consequential.

Experiment 6 — Where does the messenger come from and where does it go? Measure the messenger in sorted neuronal, microglial and astrocytic populations from disease tissue, and test transfer between them using the known conduits: gap junctions, the folate transporter, and volume-regulated

anion channels, the last being a plausible glial-neuronal route that has never been examined in brain.^{149, 147, 148}

Experiment 7 — Test the micronucleus-export route. Determine whether neurons in ageing and disease export micronuclei to microglia as they do in development^{84, 85}, whether the exported material engages microglial enzyme, and whether this route accounts for any part of the microglial activation attributed to mitochondrial DNA. This would reconcile the expression data with the neuronal lesion without requiring neuronal signalling at all.

Experiment 8 — Look for cytoplasmic chromatin in human disease neurons. The one ligand route available to a post-mitotic cell has never been sought in the relevant tissue.⁸⁸

27.4 Stage three: translation

Experiment 9 — Test the protective-function question directly. Dose an active-site inhibitor chronically in ageing animals and measure the endpoints the knockout disturbs: heterochromatin landscape, repeat-element derepression, frailty and lifespan.⁵⁰ If catalytic inhibition spares them, the safety argument of Chapter 23 is established rather than hoped for.

Experiment 10 — Build a central pharmacodynamic assay. Develop and validate measurement of the messenger in human cerebrospinal fluid, against the peripheral interferon score as comparator.¹⁸⁹ Without this, no trial of this target in a brain disease can demonstrate that it engaged the target.

27.5 What success would look like

The programme is designed so that its most probable outcome is a substantial narrowing. If Experiment 1 is null, the enzyme's second job is irrelevant here and Part I becomes background. If Experiment 2 recovers a defined species, the field acquires the thing it has been arguing about without possessing. If Experiment 3 fails, the honest conclusion is that this target cannot currently be tested in people, and resources should move to ligand supply or to the adaptor.

Each of those is a better position than the present one, in which a target is being advanced on the strength of a pathway diagram.

28. What Would Have to Be True

28.1 The strongest defensible version

Stripped to what the evidence supports, the hypothesis is narrower and more specific than the one usually stated, and it is worth setting out because it is testable.

Ageing brain accumulates sources of misplaced DNA continuously: organelle genomes released sub-lethally through partial membrane permeabilisation⁷², chromatin fragments budding from nuclei whose lamina is eroding^{88, 89}, and — on the most interesting reading — micronuclei exported by neurons and taken up by microglia.⁸⁴ The sensing cell is predominantly microglial, which is where the enzyme is and where the conditional knockouts work.^{117, 6} What rises with age is not necessarily ligand supply but the fraction of enzyme that is free to act, and the output is steeply non-linear because condensation is threshold-dependent.²⁶ The resulting interferon tone is chronic and low, which is what the human transcriptomic data show.¹³⁸ And the disease increment is a modest addition to a large physiological background, which is why the benchmark experiment rescues function without touching pathology.¹⁰

In this version the neuron's role is to supply the ligand, not to sense it. That is consistent with every expression dataset, with both conditional knockouts, with the micronucleus-export observation, and with the absence of a neuron-restricted deletion — and it makes the one neuronal study⁷ the anomaly to be replicated rather than the foundation to be built on.

28.2 What would have to be true for the therapeutic proposal

Four things, each currently unestablished.

That the harmful activity is catalytic, so that the existing chemistry addresses it and the enzyme's nuclear functions are spared. That a human-active compound can reach brain at a free concentration above its potency. That the disease-specific increment over ageing is large enough to move with a tolerable degree of inhibition. And that inhibition late enough to

be practical still changes the trajectory, given that the mechanism appears continuously active from mid-life.

28.3 What would remain unexplained

Selective vulnerability. The enzyme is ubiquitous, the ligands are generic, interferon tone is global, and this disease is regionally and cellularly specific. Nothing in this review addresses why the entorhinal cortex goes first. That is not a criticism of the target; it is a statement that the target, even if correct, is a permissive factor rather than a sufficient cause.

28.4 The general lesson

The four questions of this review are the questions one asks about an enzyme: what does it do, what activates it, where is it, and can it be drugged. The literature connecting this protein to this disease has largely asked a different question — whether the pathway is on — and answered it well.

The cost of the substitution is visible in the ledger. A target has advanced towards the clinic while its ligand in the relevant tissue has never been isolated, its dominant cellular activity has never been examined in the relevant cell, and the compound that would test it in a person does not exist. None of those gaps is technically hard to close. All of them have been left open because a switch does not need a job description, and an enzyme does.

29. Operational Definitions and Interpretation Guide

29.1 Definitions used throughout

Table 5 — Operational definitions used throughout

Term	Operational definition used here
The enzyme	cGAS, the product of <i>CGAS</i> ; used in preference to "the pathway" wherever the claim concerns this protein specifically

Term	Operational definition used here
The adaptor	STING, the product of <i>STING1</i> ; distinguished throughout because several claims attributed to the pathway belong to one component only
Established	Reproduced by independent groups in a named experimental system, with the system stated
Proposed	An integration offered without direct test, by cited authors or by this review
Unevidenced	A step the argument requires for which no study was located
Contradicted	A step for which the available primary evidence points the other way
Specified ligand	A DNA species characterised by identity, length distribution, protein association and compartment — all four
Target engagement	A measured change in the enzyme's product in the compartment of interest, not a change in a downstream transcriptional signature
Canonical function	DNA-activated synthesis of the cyclic dinucleotide messenger
Non-canonical function	Any activity of the same protein that does not require messenger synthesis

29.2 Interpretation rules applied in this review

A downstream signature does not identify an upstream sensor. Three unrelated adaptors converge on one transcription factor through a shared motif²⁸, so attribution requires genetic or pharmacological bypass. Applied in Chapters 15, 18 and 19.

Presence of DNA is not presence of a ligand. Activation is cooperative, length-dependent, geometry-dependent and threshold-dependent^{4, 5, 24}, so amplicon or antibody detection does not establish that a ligand exists. Applied throughout Part II.

Abundance is not consequence. A steeply non-linear activation function means a small quantity of enzyme on a locally dense ligand can outproduce a large quantity distributed thinly. Applied in Chapter 14 to the expression data.

The sensing cell is not necessarily the suffering cell. The messenger diffuses and is transported, and the ligand itself can be exported between cells.^{30, 84} Applied in Chapter 18.

Catalysis and occupancy are separate claims. Where tested with defined mutants, several functions require binding and not catalysis^{2, 3}, so an inhibitor of catalysis is not an inhibitor of the protein. Applied in Chapters 5, 20 and 23.

Species must be named at every crossing. The human enzyme is structurally and functionally distinct from the mouse orthologue in the DNA-binding surface²², and inhibitor potency does not transfer. Applied in Chapters 2, 20 and 21.

Corrections, retractions and failed replications are cited at the point of use. Applied to the retracted mitophagy study⁷⁹, the nucleosome-structure correction³⁵, the chromatin-sequencing erratum³⁹ and the agonist-trial correction.¹⁹⁶

29.3 Observations requiring further work

Table 6 — Observations requiring further work

Observation	What it would take to resolve
The direction of nuclear cGAS's effect on genome stability is disputed	Side-by-side replication in one system with the same mutants and endpoints
The messenger rises in aged cortex without downstream activation	Cell-sorted measurement of enzyme, messenger and downstream activation across age
No cGAS-bound DNA has been recovered from brain	Immunoprecipitation and length-preserving sequencing from sorted populations
Neurons make and export micronuclei; nobody knows if this matters in disease	Longitudinal imaging in ageing and disease models with enzyme-null microglia
Signalling is reported impaired rather than hyperactive in human disease cortex	Replication with cell-type resolution in a larger, better-powered cohort
The enzyme-null mouse ages prematurely	Chronic pharmacological inhibition in ageing animals with the same endpoints
No human loss-of-function phenotype is known for the enzyme	Population sequencing for predicted null alleles and phenotyping of carriers
No compound active on the human enzyme has measured brain exposure	Standard pharmacokinetics on an existing dual-species lead

References and Source Notes

References are numbered in order of first citation in the main text. Primary studies support the mechanistic discussion; official gene records establish nomenclature and orthology, and trial records establish development status only. Corrections, failed replications and disputed detections are identified explicitly, both here and at the point of use. Proposed integrations, the ledger and the experimental programme are original analytical synthesis.

- [1] Volkman HE, Cambier S, Gray EE, Stetson DB. Tight nuclear tethering of cGAS is essential for preventing autoreactivity. *eLife*. 2019. doi:10.7554/eLife.47491.
- [2] Jiang H, Xue X, Panda S, et al. Chromatin-bound cGAS is an inhibitor of DNA repair and hence accelerates genome destabilization and cell death. *EMBO Journal*. 2019. doi:10.15252/emboj.2019102718.
- [3] Chen H, Chen H, Zhang J, et al. cGAS suppresses genomic instability as a decelerator of replication forks. *Science Advances*. 2020. doi:10.1126/sciadv.abb8941.
- [4] Luecke S, Holleufer A, Christensen MH, et al. cGAS is activated by DNA in a length-dependent manner. *EMBO Reports*. 2017. doi:10.15252/embr.201744017.
- [5] Andreeva L, Hiller B, Kostrewa D, et al. cGAS senses long and HMGB/TFAM-bound U-turn DNA by forming protein-DNA ladders. *Nature*. 2017. doi:10.1038/nature23890.
- [6] He S, Li X, Mittra N, et al. Microglial cGAS deletion preserves intercellular communication and alleviates amyloid-beta-induced pathogenesis of Alzheimer's disease. *Advanced Science*. 2025. doi:10.1002/advs.202410910.
- [7] Akhter F, Akhter A, Zhu X, et al. Amyloid-beta glycation induces neuronal mitochondrial dysfunction and Alzheimer's pathogenesis via VDAC1-dependent mtDNA efflux. *PNAS*. 2025. doi:10.1073/pnas.2505046122.
- [8] Li T, Huang T, Du M, et al. Phosphorylation and chromatin tethering prevent cGAS activation during mitosis. *Science*. 2021. doi:10.1126/science.abc5386.
- [9] Terry RD, Masliah E, Salmon DP, et al. Physical basis of cognitive alterations in Alzheimer's disease: Synapse loss is the major correlate of cognitive impairment. *Annals of Neurology*. 1991. doi:10.1002/ana.410300410.
- [10] Udeochu JC, Amin S, Huang Y, et al. Tau activation of microglial cGAS-IFN reduces MEF2C-mediated cognitive resilience. *Nature Neuroscience*. 2023. doi:10.1038/s41593-023-01315-6.
- [11] National Center for Biotechnology Information. CGAS cyclic GMP-AMP synthase [Homo sapiens] — Gene ID 115004. *NCBI Gene*. 2026. Source.
- [12] National Center for Biotechnology Information. STING1 stimulator of interferon response cGAMP interactor 1 [Homo sapiens] — Gene ID 340061. *NCBI Gene*. 2026. Source.

- [13] Ishikawa H, Barber GN. STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling. *Nature*. 2008. doi:10.1038/nature07317.
- [14] Sun L, Wu J, Du F, Chen X, Chen ZJ. Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. *Science*. 2013. doi:10.1126/science.1232458.
- [15] Wu J, Sun L, Chen X, et al. Cyclic GMP-AMP is an endogenous second messenger in innate immune signaling by cytosolic DNA. *Science*. 2013. doi:10.1126/science.1229963.
- [16] Zhang X, Shi H, Wu J, et al. Cyclic GMP-AMP containing mixed phosphodiester linkages is an endogenous high-affinity ligand for STING. *Molecular Cell*. 2013. doi:10.1016/j.molcel.2013.05.022.
- [17] Civril F, Deimling T, de Oliveira Mann CC, et al. Structural mechanism of cytosolic DNA sensing by cGAS. *Nature*. 2013. doi:10.1038/nature12305.
- [18] Kranzusch PJ, Lee ASY, Berger JM, Doudna JA. Structure of human cGAS reveals a conserved family of second-messenger enzymes in innate immunity. *Cell Reports*. 2013. doi:10.1016/j.celrep.2013.05.008.
- [19] Li X, Shu C, Yi G, et al. Cyclic GMP-AMP synthase is activated by double-stranded DNA-induced oligomerization. *Immunity*. 2013. doi:10.1016/j.immuni.2013.10.019.
- [20] Xie W, Lama L, Adura C, et al. Human cGAS catalytic domain has an additional DNA-binding interface that enhances enzymatic activity and liquid-phase condensation. *PNAS*. 2019. doi:10.1073/pnas.1905013116.
- [21] Tao J, Zhang XW, Jin J, et al. Nonspecific DNA binding of cGAS N terminus promotes cGAS activation. *Journal of Immunology*. 2017. doi:10.4049/jimmunol.1601909.
- [22] Zhou W, Whiteley AT, de Oliveira Mann CC, et al. Structure of the human cGAS-DNA complex reveals enhanced control of immune surveillance. *Cell*. 2018. doi:10.1016/j.cell.2018.06.026.
- [23] Liu D, Zhang H, Huang YP, Gao YQ. Investigating the activation mechanism differences between human and mouse cGAS by molecular dynamics simulations. *Journal of Physical Chemistry B*. 2023. doi:10.1021/acs.jpcc.3c02377.
- [24] Du M, Chen ZJ. DNA-induced liquid phase condensation of cGAS activates innate immune signaling. *Science*. 2018. doi:10.1126/science.aat1022.
- [25] Zhou W, Mohr L, Maciejowski J, Kranzusch PJ. cGAS phase separation inhibits TREX1-mediated DNA degradation and enhances cytosolic DNA sensing. *Molecular Cell*. 2021. doi:10.1016/j.molcel.2021.01.024.
- [26] Lueck J, Strom A, Martinez Torres S, et al. Dimerization-dependent gel-like condensation with dsDNA underpins the activation of human cGAS. *Cell Reports*. 2026. doi:10.1016/j.celrep.2025.116920.
- [27] Jiang Z, Shi F, Li J, et al. Crucial role of the cGAS N terminus in mediating flowable and functional cGAS-DNA condensate formation via DNA interactions. *PNAS*. 2025. doi:10.1073/pnas.2411659122.
- [28] Liu S, Cai X, Wu J, et al. Phosphorylation of innate immune adaptor proteins MAVS, STING, and TRIF induces IRF3 activation. *Science*. 2015. doi:10.1126/science.aaa2630.

- [29] Gui X, Yang H, Li T, et al. Autophagy induction via STING trafficking is a primordial function of the cGAS pathway. *Nature*. 2019. doi:10.1038/s41586-019-1006-9.
- [30] Ablasser A, Schmid-Burgk JL, Hemmerling I, et al. Cell intrinsic immunity spreads to bystander cells via the intercellular transfer of cGAMP. *Nature*. 2013. doi:10.1038/nature12640.
- [31] Michalski S, de Oliveira Mann CC, Stafford CA, et al. Structural basis for sequestration and autoinhibition of cGAS by chromatin. *Nature*. 2020. doi:10.1038/s41586-020-2748-0.
- [32] Zhao B, Xu P, Rowlett CM, et al. The molecular basis of tight nuclear tethering and inactivation of cGAS. *Nature*. 2020. doi:10.1038/s41586-020-2749-z.
- [33] Boyer JA, Spangler CJ, Strauss JD, et al. Structural basis of nucleosome-dependent cGAS inhibition. *Science*. 2020. doi:10.1126/science.abd0609.
- [34] Pathare GR, Decout A, Gluck S, et al. Structural mechanism of cGAS inhibition by the nucleosome. *Nature*. 2020. doi:10.1038/s41586-020-2750-6.
- [35] Pathare GR, Decout A, Gluck S, et al. Author Correction: Structural mechanism of cGAS inhibition by the nucleosome. *Nature*. 2026. doi:10.1038/s41586-026-10959-w.
- [36] Kujirai T, Zierhut C, Takizawa Y, et al. Structural basis for the inhibition of cGAS by nucleosomes. *Science*. 2020. doi:10.1126/science.abd0237.
- [37] Guey B, Wischnewski M, Decout A, et al. BAF restricts cGAS on nuclear DNA to prevent innate immune activation. *Science*. 2020. doi:10.1126/science.aaw6421.
- [38] Gentili M, Lahaye X, Nadalin F, et al. The N-terminal domain of cGAS determines preferential association with centromeric DNA and innate immune activation in the nucleus. *Cell Reports*. 2019. doi:10.1016/j.celrep.2019.01.105.
- [39] Gentili M, Lahaye X, Nadalin F, et al. Erratum: The N-terminal domain of cGAS determines preferential association with centromeric DNA and innate immune activation in the nucleus. *Cell Reports*. 2019. doi:10.1016/j.celrep.2019.03.049.
- [40] Liu H, Zhang H, Wu X, et al. Nuclear cGAS suppresses DNA repair and promotes tumorigenesis. *Nature*. 2018. doi:10.1038/s41586-018-0629-6.
- [41] Zhang Q, Tong C, Zhao M, et al. PKC- α -mediated nuclear translocation of cGAS stabilizes beta-catenin and drives metastasis. *Molecular Cell*. 2026. doi:10.1016/j.molcel.2026.05.023.
- [42] Bai J, Liu F. Nuclear cGAS: sequestration and beyond. *Protein and Cell*. 2021. doi:10.1007/s13238-021-00869-0.
- [43] Yang H, Gao B, Wu B, et al. cGAS restricts PARP1-mediated microhomology-mediated end joining by suppressing poly-ADP-ribosylation. *Cell Death and Differentiation*. 2025. doi:10.1038/s41418-025-01637-x.
- [44] Zhang H, Jiang L, Du X, et al. The cGAS-Ku80 complex regulates the balance between two end joining subpathways. *Cell Death and Differentiation*. 2024. doi:10.1038/s41418-024-01296-4.

- [45] Guo H, Han Y, Yao S, et al. Decrotonylation of cGAS K254 prompts homologous recombination repair by blocking its DNA binding and releasing PARP1. *Journal of Biological Chemistry*. 2024. doi:10.1016/j.jbc.2024.107554.
- [46] Cho MG, Kumar RJ, Lin CC, et al. MRE11 liberates cGAS from nucleosome sequestration during tumorigenesis. *Nature*. 2024. doi:10.1038/s41586-023-06889-6.
- [47] Wang X, Zhu Y, Lu W, et al. Microcystin-LR-induced nuclear translocation of cGAS promotes mutagenesis in human hepatocytes by impeding homologous recombination repair. *Toxicology Letters*. 2022. doi:10.1016/j.toxlet.2022.11.015.
- [48] Qin Y, Yuan X, Cui Z, et al. Low dose PFDA induces DNA damage and DNA repair inhibition by promoting nuclear cGAS accumulation in ovarian epithelial cells. *Ecotoxicology and Environmental Safety*. 2023. doi:10.1016/j.ecoenv.2023.115503.
- [49] Cho MG, Gupta GP. Unveiling cGAS mechanisms: insights into DNA damage and immune sensing in cancer. *DNA Repair*. 2025. doi:10.1016/j.dnarep.2025.103868.
- [50] Martinez JC, Morandini F, Rechsteiner C, et al. cGAS-deficient mice display premature aging associated with derepression of LINE1 elements and inflammation. *Nature Aging*. 2026. doi:10.1038/s43587-026-01206-y.
- [51] Liang Q, Seo GJ, Choi YJ, et al. Crosstalk between the cGAS DNA sensor and Beclin-1 autophagy protein shapes innate antimicrobial immune responses. *Cell Host and Microbe*. 2014. doi:10.1016/j.chom.2014.01.009.
- [52] Lei Z, Deng M, Yi Z, et al. cGAS-mediated autophagy protects the liver from ischemia-reperfusion injury independently of STING. *American Journal of Physiology: Gastrointestinal and Liver Physiology*. 2018. doi:10.1152/ajpgi.00326.2017.
- [53] Cai Z, Yang Y, Zhong J, et al. cGAS suppresses beta-cell proliferation by a STING-independent but CEBP-beta-dependent mechanism. *Metabolism*. 2024. doi:10.1016/j.metabol.2024.155933.
- [54] Zierhut C, Yamaguchi N, Paredes M, et al. The cytoplasmic DNA sensor cGAS promotes mitotic cell death. *Cell*. 2019. doi:10.1016/j.cell.2019.05.035.
- [55] Ling X, Song X, Zhang D, et al. Electrostatic bimodality of human cytosolic DNA sensor cGAS-dsDNA condensates revealed by millisecond flashing electrophoretic separation. *Nature Communications*. 2026. doi:10.1038/s41467-026-73740-7.
- [56] Joshi B, Joshi JC, Balaji Raghunathrao VA, et al. Condensation of cGAS induced by Tyr phosphorylation and interaction with MYL6 mediates vascular innate immunity in the lung. *Science Signaling*. 2026. doi:10.1126/scisignal.adu6757.
- [57] Wang W, Chen H, Li J, et al. Co-exposure to environmental lead and hypertension exacerbates anxiety and depression via mtDNA-mediated cGAS phase separation. *Environmental Pollution*. 2026. doi:10.1016/j.envpol.2026.128656.
- [58] Bergman M, Goshtchevsky U, Atlan T, et al. A dual role for cGAS in shaping cellular and organismal responses to genomic instability. *Genes and Development*. 2026. doi:10.1101/gad.352760.125.

- [59] Li H, Zhang Y, Gao S, Yin H. T cell-intrinsic cGAS-CTCF regulation maintains regulatory T cell development and function. *Cell Reports*. 2025. doi:10.1016/j.celrep.2025.116302.
- [60] Yang H, Wang H, Ren J, Chen Q, Chen ZJ. cGAS is essential for cellular senescence. *PNAS*. 2017. doi:10.1073/pnas.1705499114.
- [61] Gluck S, Guey B, Gulen MF, et al. Innate immune sensing of cytosolic chromatin fragments through cGAS promotes senescence. *Nature Cell Biology*. 2017. doi:10.1038/ncb3586.
- [62] Aditi, Downing SM, Schreiner PA, et al. Genome instability independent of type I interferon signaling drives neuropathology caused by impaired ribonucleotide excision repair. *Neuron*. 2021. doi:10.1016/j.neuron.2021.09.040.
- [63] Wu W, Kargbo-Hill SE, Nathan WJ, et al. Neuronal enhancers are hotspots for DNA single-strand break repair. *Nature*. 2021. doi:10.1038/s41586-021-03468-5.
- [64] Madabhushi R, Gao F, Pfenning AR, et al. Activity-induced DNA breaks govern the expression of neuronal early-response genes. *Cell*. 2015. doi:10.1016/j.cell.2015.05.032.
- [65] Podlesniy P, Figueiro-Silva J, Llado A, et al. Low cerebrospinal fluid concentration of mitochondrial DNA in preclinical Alzheimer disease. *Annals of Neurology*. 2013. doi:10.1002/ana.23955.
- [66] Takousis P, Devonshire AS, Redshaw N, et al. A standardised methodology for the extraction and quantification of cell-free DNA in cerebrospinal fluid and application to evaluation of Alzheimer's disease and brain cancers. *New Biotechnology*. 2022. doi:10.1016/j.nbt.2022.10.001.
- [67] West AP, Khoury-Hanold W, Staron M, et al. Mitochondrial DNA stress primes the antiviral innate immune response. *Nature*. 2015. doi:10.1038/nature14156.
- [68] McArthur K, Whitehead LW, Heddleston JM, et al. BAK/BAX macropores facilitate mitochondrial herniation and mtDNA efflux during apoptosis. *Science*. 2018. doi:10.1126/science.aao6047.
- [69] Riley JS, Quarato G, Cloix C, et al. Mitochondrial inner membrane permeabilisation enables mtDNA release during apoptosis. *EMBO Journal*. 2018. doi:10.15252/embj.201899238.
- [70] Kim J, Gupta R, Blanco LP, et al. VDAC oligomers form mitochondrial pores to release mtDNA fragments and promote lupus-like disease. *Science*. 2019. doi:10.1126/science.aav4011.
- [71] Newman LE, Weiser Novak S, Rojas GR, et al. Mitochondrial DNA replication stress triggers a pro-inflammatory endosomal pathway of nucleoid disposal. *Nature Cell Biology*. 2024. doi:10.1038/s41556-023-01343-1.
- [72] Victorelli S, Salmonowicz H, Chapman J, et al. Apoptotic stress causes mtDNA release during senescence and drives the SASP. *Nature*. 2023. doi:10.1038/s41586-023-06621-4.
- [73] Cai D, Chen C, Meekrathok P, et al. Cytosolic TOP3- α facilitates mitochondrial DNA sensing by cGAS. *EMBO Reports*. 2025. doi:10.1038/s44319-025-00614-2.

- [74] Jauhari A, Baranov SV, Suofu Y, et al. Melatonin inhibits cytosolic mitochondrial DNA-induced neuroinflammatory signaling in accelerated aging and neurodegeneration. *Journal of Clinical Investigation*. 2020. doi:10.1172/JCI135026.
- [75] Yang NSY, Zhong WJ, Sha HX, et al. mtDNA-cGAS-STING axis-dependent NLRP3 inflammasome activation contributes to postoperative cognitive dysfunction induced by sevoflurane in mice. *International Journal of Biological Sciences*. 2024. doi:10.7150/ijbs.91543.
- [76] Dwamena A, Asadi Y, Gilstrap E, et al. Proteasomal dysfunction in the mouse forebrain induces mitochondrial DNA release, cGAS-STING signaling activation, and necroptosis. *Journal of Neuropathology and Experimental Neurology*. 2025. doi:10.1093/jnen/nlaf093.
- [77] Deng Y, Yi X, Gong Y, et al. Palmitic acid induces nDNA release to cytosol and promotes microglial M1 polarization via cGAS-STING signaling pathway. *Biochimica et Biophysica Acta: Molecular Cell Research*. 2023. doi:10.1016/j.bbamcr.2022.119385.
- [78] Tigano M, Vargas DC, Tremblay-Belzile S, Fu Y, Sfeir A. Nuclear sensing of breaks in mitochondrial DNA enhances immune surveillance. *Nature*. 2021. doi:10.1038/s41586-021-03269-w.
- [79] Sliter DA, Martinez J, Hao L, et al. Retraction Note: Parkin and PINK1 mitigate STING-induced inflammation. *Nature*. 2025. doi:10.1038/s41586-025-09346-8.
- [80] Du H, Guo L, Fang F, et al. Cyclophilin D deficiency attenuates mitochondrial and neuronal perturbation and ameliorates learning and memory in Alzheimer's disease. *Nature Medicine*. 2008. doi:10.1038/nm.1868.
- [81] Mackenzie KJ, Carroll P, Martin CA, et al. cGAS surveillance of micronuclei links genome instability to innate immunity. *Nature*. 2017. doi:10.1038/nature23449.
- [82] Harding SM, Benci JL, Irianto J, et al. Mitotic progression following DNA damage enables pattern recognition within micronuclei. *Nature*. 2017. doi:10.1038/nature23470.
- [83] MacDonald KM, Nicholson-Puthenveedu S, Tageldeen MM, et al. Antecedent chromatin organization determines cGAS recruitment to ruptured micronuclei. *Nature Communications*. 2023. doi:10.1038/s41467-023-36195-8.
- [84] Yano S, Asami N, Kishi Y, et al. Propagation of neuronal micronuclei regulates microglial characteristics. *Nature Neuroscience*. 2025. doi:10.1038/s41593-024-01863-5.
- [85] Maeda C, Kishi Y, Takeda I, et al. Age-associated neuronal micronuclei formation and transfer to microglia. *Frontiers in Aging Neuroscience*. 2026. doi:10.3389/fnagi.2026.1787252.
- [86] Yano S, Akiyama K, Tsuchiya R, et al. A MATLAB-based program for three-dimensional quantitative analysis of micronuclei reveals that neuroinflammation induces micronuclei formation in the brain. *Scientific Reports*. 2021. doi:10.1038/s41598-021-97640-6.
- [87] Aguado J, Chaggar HK, Gomez-Inclan C, et al. Inhibition of the cGAS-STING pathway ameliorates the premature senescence hallmarks of Ataxia-Telangiectasia brain organoids. *Aging Cell*. 2021. doi:10.1111/ace1.13468.

- [88] Dou Z, Ghosh K, Vizioli MG, et al. Cytoplasmic chromatin triggers inflammation in senescence and cancer. *Nature*. 2017. doi:10.1038/nature24050.
- [89] Zhang H, Li J, Yu Y, et al. Nuclear lamina erosion-induced resurrection of endogenous retroviruses underlies neuronal aging. *Cell Reports*. 2023. doi:10.1016/j.celrep.2023.112593.
- [90] Frost B, Bardai FH, Feany MB. Lamin dysfunction mediates neurodegeneration in tauopathies. *Current Biology*. 2016. doi:10.1016/j.cub.2015.11.039.
- [91] De Cecco M, Ito T, Petrashen AP, et al. L1 drives IFN in senescent cells and promotes age-associated inflammation. *Nature*. 2019. doi:10.1038/s41586-018-0784-9.
- [92] Simon M, Van Meter M, Ablaeva J, et al. LINE1 derepression in aged wild-type and SIRT6-deficient mice drives inflammation. *Cell Metabolism*. 2019. doi:10.1016/j.cmet.2019.02.014.
- [93] Stetson DB, Ko JS, Heidmann T, Medzhitov R. Trex1 prevents cell-intrinsic initiation of autoimmunity. *Cell*. 2008. doi:10.1016/j.cell.2008.06.032.
- [94] Crow YJ, Hayward BE, Parmar R, et al. Mutations in the gene encoding the 3'-5' DNA exonuclease TREX1 cause Aicardi-Goutieres syndrome at the AGS1 locus. *Nature Genetics*. 2006. doi:10.1038/ng1845.
- [95] Sun W, Samimi H, Gamez M, Zare H, Frost B. Pathogenic tau-induced piRNA depletion promotes neuronal death through transposable element dysregulation in neurodegenerative tauopathies. *Nature Neuroscience*. 2018. doi:10.1038/s41593-018-0194-1.
- [96] Guo C, Jeong HH, Hsieh YC, et al. Tau activates transposable elements in Alzheimer's disease. *Cell Reports*. 2018. doi:10.1016/j.celrep.2018.05.004.
- [97] Nicodemus J, Liu CS, Ransom L, et al. Sequence diversity and encoded enzymatic differences of monocistronic L1 ORF2 mRNA variants in the aged normal and Alzheimer's disease brain. *Journal of Neuroscience*. 2025. doi:10.1523/JNEUROSCI.2298-24.2025.
- [98] Mertens J, Paquola ACM, Ku M, et al. Directly reprogrammed human neurons retain aging-associated transcriptomic signatures and reveal age-related nucleocytoplasmic defects. *Cell Stem Cell*. 2015. doi:10.1016/j.stem.2015.09.001.
- [99] Herdy JR, Taylor EE, Karbacher L, et al. Cytoplasmic DNA sensing links LINE-1 expression to neuronal senescence in Alzheimer's disease. *bioRxiv*. 2026. doi:10.64898/2026.07.27.740588.
- [100] Shanbhag NM, Evans MD, Mao W, et al. Early neuronal accumulation of DNA double strand breaks in Alzheimer's disease. *Acta Neuropathologica Communications*. 2019. doi:10.1186/s40478-019-0723-5.
- [101] Zhang X, Liu Y, Huang M, et al. Landscape of double-stranded DNA breaks in postmortem brains from Alzheimer's disease and non-demented individuals. *Journal of Alzheimer's Disease*. 2023. doi:10.3233/JAD-230316.

- [102] Suberbielle E, Djukic B, Evans M, et al. DNA repair factor BRCA1 depletion occurs in Alzheimer brains and impairs cognitive function in mice. *Nature Communications*. 2015. doi:10.1038/ncomms9897.
- [103] Mecocci P, MacGarvey U, Beal MF. Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease. *Annals of Neurology*. 1994. doi:10.1002/ana.410360510.
- [104] Wang J, Markesbery WR, Lovell MA. Increased oxidative damage in nuclear and mitochondrial DNA in mild cognitive impairment. *Journal of Neurochemistry*. 2006. doi:10.1111/j.1471-4159.2005.03615.x.
- [105] Delint-Ramirez I, Konada L, Heady L, et al. Calcineurin dephosphorylates topoisomerase II-beta and regulates the formation of neuronal-activity-induced DNA breaks. *Molecular Cell*. 2022. doi:10.1016/j.molcel.2022.09.012.
- [106] Suberbielle E, Sanchez PE, Kravitz AV, et al. Physiologic brain activity causes DNA double-strand breaks in neurons, with exacerbation by amyloid-beta. *Nature Neuroscience*. 2013. doi:10.1038/nn.3356.
- [107] Gehrke N, Mertens C, Zillinger T, et al. Oxidative damage of DNA confers resistance to cytosolic nuclease TREX1 degradation and potentiates STING-dependent immune sensing. *Immunity*. 2013. doi:10.1016/j.immuni.2013.08.004.
- [108] Crow YJ, Chase DS, Lowenstein Schmidt J, et al. Characterization of human disease phenotypes associated with mutations in TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, ADAR, and IFIH1. *American Journal of Medical Genetics Part A*. 2015. doi:10.1002/ajmg.a.36887.
- [109] Gray EE, Treuting PM, Woodward JJ, Stetson DB. Cutting edge: cGAS is required for lethal autoimmune disease in the Trex1-deficient mouse model of Aicardi-Goutieres syndrome. *Journal of Immunology*. 2015. doi:10.4049/jimmunol.1500969.
- [110] Kawane K, Ohtani M, Miwa K, et al. Chronic polyarthritis caused by mammalian DNA that escapes from degradation in macrophages. *Nature*. 2006. doi:10.1038/nature05245.
- [111] Rodero MP, Tesser A, Bartok E, et al. Type I interferon-mediated autoinflammation due to DNase II deficiency. *Nature Communications*. 2017. doi:10.1038/s41467-017-01932-3.
- [112] Sisirak V, Sally B, D'Agati V, et al. Digestion of chromatin in apoptotic cell microparticles prevents autoimmunity. *Cell*. 2016. doi:10.1016/j.cell.2016.05.034.
- [113] Li LJ, Sun XY, Huang YR, et al. Neuronal double-stranded DNA accumulation induced by DNase II deficiency drives tau phosphorylation and neurodegeneration. *Translational Neurodegeneration*. 2024. doi:10.1186/s40035-024-00427-8.
- [114] Li LJ, Liang SY, Sun XY, et al. Microglial double stranded DNA accumulation induced by DNase II deficiency drives neuroinflammation and neurodegeneration. *Journal of Neuroinflammation*. 2025. doi:10.1186/s12974-025-03333-6.
- [115] Van Acker ZP, Perdok A, Hellemans R, et al. Phospholipase D3 degrades mitochondrial DNA to regulate nucleotide signaling and APP metabolism. *Nature Communications*. 2023. doi:10.1038/s41467-023-38501-w.

- [116] Zenaro E, Pietronigro E, Della Bianca V, et al. Neutrophils promote Alzheimer's disease-like pathology and cognitive decline via LFA-1 integrin. *Nature Medicine*. 2015. doi:10.1038/nm.3913.
- [117] Karlsson M, Zhang C, Mear L, et al. A single-cell type transcriptomics map of human tissues. *Science Advances*. 2021. doi:10.1126/sciadv.abh2169.
- [118] Siletti K, Hodge R, Mossi Albiach A, et al. Transcriptomic diversity of cell types across the adult human brain. *Science*. 2023. doi:10.1126/science.add7046.
- [119] Zhang Y, Chen K, Sloan SA, et al. An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. *Journal of Neuroscience*. 2014. doi:10.1523/JNEUROSCI.1860-14.2014.
- [120] Zhang Y, Sloan SA, Clarke LE, et al. Purification and characterization of progenitor and mature human astrocytes reveals transcriptional and functional differences with mouse. *Neuron*. 2016. doi:10.1016/j.neuron.2015.11.013.
- [121] Sjöstedt E, Zhong W, Fagerberg L, et al. An atlas of the protein-coding genes in the human, pig, and mouse brain. *Science*. 2020. doi:10.1126/science.aay5947.
- [122] Jorstad NL, Close J, Johansen N, et al. Transcriptomic cytoarchitecture reveals principles of human neocortex organization. *Science*. 2023. doi:10.1126/science.adf6812.
- [123] Mathys H, Davila-Velderrain J, Peng Z, et al. Single-cell transcriptomic analysis of Alzheimer's disease. *Nature*. 2019. doi:10.1038/s41586-019-1195-2.
- [124] Xie X, Ma G, Li X, et al. Activation of innate immune cGAS-STING pathway contributes to Alzheimer's pathogenesis in 5xFAD mice. *Nature Aging*. 2023. doi:10.1038/s43587-022-00337-2.
- [125] Chung S, Jeong JH, Park JC, et al. Blockade of STING activation alleviates microglial dysfunction and a broad spectrum of Alzheimer's disease pathologies. *Experimental and Molecular Medicine*. 2024. doi:10.1038/s12276-024-01295-y.
- [126] Jin M, Shiwa H, Tanaka H, et al. Tau activates microglia via the PQBP1-cGAS-STING pathway to promote brain inflammation. *Nature Communications*. 2021. doi:10.1038/s41467-021-26851-2.
- [127] Carling GK, Fan L, Foxe NR, et al. Alzheimer's disease-linked risk alleles elevate microglial cGAS-associated senescence and neurodegeneration in a tauopathy model. *Neuron*. 2024. doi:10.1016/j.neuron.2024.09.006.
- [128] Gulen MF, Samson N, Keller A, et al. cGAS-STING drives ageing-related inflammation and neurodegeneration. *Nature*. 2023. doi:10.1038/s41586-023-06373-1.
- [129] Packer JM, Giammo SG, Wangler LM, et al. Diffuse traumatic brain injury induced stimulator of interferons (STING) signaling in microglia drives cortical neuroinflammation, neuronal dysfunction, and impaired cognition. *Journal of Neuroinflammation*. 2025. doi:10.1186/s12974-025-03451-1.
- [130] Liu Y, Wang A, Chen C, et al. Microglial cGAS-STING signaling underlies glaucoma pathogenesis. *PNAS*. 2024. doi:10.1073/pnas.2409493121.
- [131] Jiang SY, Tian T, Yao H, et al. The cGAS-STING-YY1 axis accelerates progression of neurodegeneration in a mouse model of Parkinson's disease via LCN2-dependent

- astrocyte senescence. *Cell Death and Differentiation*. 2023. doi:10.1038/s41418-023-01216-y.
- [132] Arboleda-Velasquez JF, Lopera F, O'Hare M, et al. Resistance to autosomal dominant Alzheimer's disease in an APOE3 Christchurch homozygote: a case report. *Nature Medicine*. 2019. doi:10.1038/s41591-019-0611-3.
- [133] Naguib S, Lopez-Lee C, Torres ER, et al. The R136S mutation in the APOE3 gene confers resilience against tau pathology via inhibition of the cGAS-STING-IFN pathway. *Immunity*. 2025. doi:10.1016/j.immuni.2025.05.023.
- [134] Huang Y, Fan L, Wong MY, et al. cGAS-mediated type I IFN signaling contributes to disease progression in drug-refractory epilepsy. *Nature Neuroscience*. 2026. doi:10.1038/s41593-026-02384-z.
- [135] Roy ER, Wang B, Wan YW, et al. Type I interferon response drives neuroinflammation and synapse loss in Alzheimer disease. *Journal of Clinical Investigation*. 2020. doi:10.1172/JCI133737.
- [136] Roy ER, Chiu G, Li S, et al. Concerted type I interferon signaling in microglia and neural cells promotes memory impairment associated with amyloid beta plaques. *Immunity*. 2022. doi:10.1016/j.immuni.2022.03.018.
- [137] Jin M, Xu R, Wang L, et al. Type-I-interferon signaling drives microglial dysfunction and senescence in human iPSC models of Down syndrome and Alzheimer's disease. *Cell Stem Cell*. 2022. doi:10.1016/j.stem.2022.06.007.
- [138] Cribbs DH, Berchtold NC, Perreau V, et al. Extensive innate immune gene activation accompanies brain aging, increasing vulnerability to cognitive decline and neurodegeneration: a microarray study. *Journal of Neuroinflammation*. 2012. doi:10.1186/1742-2094-9-179.
- [139] Baruch K, Deczkowska A, David E, et al. Aging-induced type I interferon response at the choroid plexus negatively affects brain function. *Science*. 2014. doi:10.1126/science.1252945.
- [140] Passarella S, Kethiswaran S, Brandes K, et al. Alteration of cGAS-STING signaling pathway components in the mouse cortex and hippocampus during healthy brain aging. *Frontiers in Aging Neuroscience*. 2024. doi:10.3389/fnagi.2024.1429005.
- [141] Woo MS, Mayer C, Binkle-Ladisch L, et al. STING orchestrates the neuronal inflammatory stress response in multiple sclerosis. *Cell*. 2024. doi:10.1016/j.cell.2024.05.031.
- [142] Marques C, Held A, Dorfman K, et al. Neuronal STING activation in amyotrophic lateral sclerosis and frontotemporal dementia. *Acta Neuropathologica*. 2024. doi:10.1007/s00401-024-02688-z.
- [143] Defaye M, Bradaia A, Abdullah NS, et al. Induction of antiviral interferon-stimulated genes by neuronal STING promotes the resolution of pain in mice. *Journal of Clinical Investigation*. 2024. doi:10.1172/JCI176474.
- [144] Liu Y, Gordesky-Gold B, Leney-Greene M, et al. Inflammation-induced, STING-dependent autophagy restricts Zika virus infection in the Drosophila brain. *Cell Host and Microbe*. 2018. doi:10.1016/j.chom.2018.05.022.

- [145] Dorrity TJ, Shin H, Wiegand KA, et al. Long 3'UTRs predispose neurons to inflammation by promoting immunostimulatory double-stranded RNA formation. *Science Immunology*. 2023. doi:10.1126/sciimmunol.adg2979.
- [146] Dai Y, Idorn M, Serrero MC, et al. TMEFF1 is a neuron-specific restriction factor for herpes simplex virus. *Nature*. 2024. doi:10.1038/s41586-024-07670-z.
- [147] Ritchie C, Cordova AF, Hess GT, Bassik MC, Li L. SLC19A1 is an importer of the immunotransmitter cGAMP. *Molecular Cell*. 2019. doi:10.1016/j.molcel.2019.05.006.
- [148] Zhou C, Chen X, Planells-Cases R, et al. Transfer of cGAMP into bystander cells via LRRc8 volume-regulated anion channels augments STING-mediated interferon responses and anti-viral immunity. *Immunity*. 2020. doi:10.1016/j.immuni.2020.03.016.
- [149] Chen Q, Boire A, Jin X, et al. Carcinoma-astrocyte gap junctions promote brain metastasis by cGAMP transfer. *Nature*. 2016. doi:10.1038/nature18268.
- [150] Ge X, Zhu X, Liu W, et al. cGAMP promotes inner blood-retinal barrier breakdown through P2RX7-mediated transportation into microglia. *Journal of Neuroinflammation*. 2025. doi:10.1186/s12974-025-03391-w.
- [151] Reinert LS, Rashidi AS, Tran DN, et al. Brain immune cells undergo cGAS/STING-dependent apoptosis during herpes simplex virus type 1 infection to limit type I IFN production. *Journal of Clinical Investigation*. 2021. doi:10.1172/JCI136824.
- [152] An J, Hendricks N, Wheeler J, et al. Neuronal TDP-43 pathology drives astrocytic interferon response in a mouse model of ALS. *Journal of Neuroinflammation*. 2025. doi:10.1186/s12974-025-03658-2.
- [153] Reinert LS, Lopusna K, Winther H, et al. Sensing of HSV-1 by the cGAS-STING pathway in microglia orchestrates antiviral defence in the CNS. *Nature Communications*. 2016. doi:10.1038/ncomms13348.
- [154] Wang X, Yang C, Wang X, et al. Driving axon regeneration by orchestrating neuronal and non-neuronal innate immune responses via the IFN-gamma-cGAS-STING axis. *Neuron*. 2023. doi:10.1016/j.neuron.2022.10.028.
- [155] Miao G, Fortier TM, Liu H, et al. Microglia promote inflammatory cell death upon neuronal mitochondrial impairment during neurodegeneration. *Nature Structural and Molecular Biology*. 2025. doi:10.1038/s41594-025-01602-9.
- [156] Viengkhou B, Hayashida E, McGlasson S, et al. The brain microvasculature is a primary mediator of interferon-alpha neurotoxicity in human cerebral interferonopathies. *Immunity*. 2024. doi:10.1016/j.immuni.2024.05.017.
- [157] Chakraborty R, Maya S, Testa V, et al. Alpha-synuclein aggregates induce mitochondrial damage and trigger innate immunity to drive neuron-microglia communication. *Nature Communications*. 2026. doi:10.1038/s41467-026-73136-7.
- [158] Yu CH, Davidson S, Harapas CR, et al. TDP-43 triggers mitochondrial DNA release via mPTP to activate cGAS/STING in ALS. *Cell*. 2020. doi:10.1016/j.cell.2020.09.020.
- [159] Hou Y, Wei Y, Lautrup S, et al. NAD⁺ supplementation reduces neuroinflammation and cell senescence in a transgenic mouse model of Alzheimer's disease via cGAS-STING. *PNAS*. 2021. doi:10.1073/pnas.2011226118.

- [160] Sharma M, Rajendrarao S, Shahani N, Ramirez-Jarquin UN, Subramaniam S. Cyclic GMP-AMP synthase promotes the inflammatory and autophagy responses in Huntington disease. *PNAS*. 2020. doi:10.1073/pnas.2002144117.
- [161] Hinkle JT, Patel J, Panicker N, et al. STING mediates neurodegeneration and neuroinflammation in nigrostriatal alpha-synucleinopathy. *PNAS*. 2022. doi:10.1073/pnas.2118819119.
- [162] Sen T, Saha P, Gupta R, et al. Aberrant ER stress induced neuronal-IFN-beta elicits white matter injury due to microglial activation and T-cell infiltration after TBI. *Journal of Neuroscience*. 2019. doi:10.1523/JNEUROSCI.0718-19.2019.
- [163] Packer JM, Bray CE, Beckman NB, et al. Impaired cortical neuronal homeostasis and cognition after diffuse traumatic brain injury are dependent on microglia and type I interferon responses. *Glia*. 2024. doi:10.1002/glia.24475.
- [164] Talbot EJ, Joshi L, Thornton P, et al. cGAS-STING signalling regulates microglial chemotaxis in genome instability. *Nucleic Acids Research*. 2024. doi:10.1093/nar/gkad1184.
- [165] Nelson TJ, Xu Y. Sting and p53 DNA repair pathways are compromised in Alzheimer's disease. *Scientific Reports*. 2023. doi:10.1038/s41598-023-35533-6.
- [166] Naumann M, Wierschin TM, Kretschmer S, et al. RIG-I mediated neuron-specific IFN type I signaling in FUS-ALS induces neurodegeneration and offers new biomarker-driven individualized treatment options for (FUS-)ALS. *Advanced Science*. 2026. doi:10.1002/advs.202417135.
- [167] Hyde VR, Zhou C, Fernandez JR, et al. Anti-herpetic tau preserves neurons via the cGAS-STING-TBK1 pathway in Alzheimer's disease. *Cell Reports*. 2025. doi:10.1016/j.celrep.2024.115109.
- [168] Hall J, Brault A, Vincent F, et al. Discovery of PF-06928215 as a high affinity inhibitor of cGAS enabled by a novel fluorescence polarization assay. *PLoS ONE*. 2017. doi:10.1371/journal.pone.0184843.
- [169] An J, Woodward JJ, Sasaki T, Minie M, Elkon KB. Cutting edge: antimalarial drugs inhibit IFN-beta production through blockade of cyclic GMP-AMP synthase-DNA interaction. *Journal of Immunology*. 2015. doi:10.4049/jimmunol.1402793.
- [170] An J, Woodward JJ, Lai W, et al. Inhibition of cyclic GMP-AMP synthase using a novel antimalarial drug derivative in Trex1-deficient mice. *Arthritis and Rheumatology*. 2018. doi:10.1002/art.40559.
- [171] Wang M, Soorshjani MA, Mikek C, Opoku-Temeng C, Sintim HO. Suramin potently inhibits cGAMP synthase, cGAS, in THP1 cells to modulate IFN-beta levels. *Future Medicinal Chemistry*. 2018. doi:10.4155/fmc-2017-0322.
- [172] Vincent J, Adura C, Gao P, et al. Small molecule inhibition of cGAS reduces interferon expression in primary macrophages from autoimmune mice. *Nature Communications*. 2017. doi:10.1038/s41467-017-00833-9.
- [173] Lama L, Adura C, Xie W, et al. Development of human cGAS-specific small-molecule inhibitors for repression of dsDNA-triggered interferon expression. *Nature Communications*. 2019. doi:10.1038/s41467-019-08620-4.

- [174] Padilla-Salinas R, Sun L, Anderson R, et al. Discovery of small-molecule cyclic GMP-AMP synthase inhibitors. *Journal of Organic Chemistry*. 2020. doi:10.1021/acs.joc.9b02666.
- [175] Song J, Yang RR, Chang J, et al. Discovery and characterization of a novel cGAS covalent inhibitor for the treatment of inflammatory bowel disease. *Acta Pharmacologica Sinica*. 2022. doi:10.1038/s41401-022-01002-5.
- [176] Li J, Xiong M, Liu J, et al. Discovery of novel cGAS inhibitors based on natural flavonoids. *Bioorganic Chemistry*. 2023. doi:10.1016/j.bioorg.2023.106802.
- [177] Kissai M, Chin EN, Martinez-Pena F, et al. Cladophorol-A is an inhibitor of cyclic GMP-AMP synthase. *Bioorganic and Medicinal Chemistry Letters*. 2024. doi:10.1016/j.bmcl.2024.130007.
- [178] Chen M, Lei S, Zhou Z, et al. Design, synthesis, and pharmacological evaluation of spiro[carbazole-3,3'-pyrrolidine] derivatives as cGAS inhibitors for treatment of acute lung injury. *Journal of Medicinal Chemistry*. 2024. doi:10.1021/acs.jmedchem.3c02229.
- [179] Alarcon-Esposito J, Nagiri RK, Wang S, et al. High-throughput screening and initial SAR studies identify a novel submicromolar potent human cGAS inhibitor. *ACS Chemical Biology*. 2025. doi:10.1021/acscchembio.5c00649.
- [180] Cyr P, Fader LD, Burch JD, et al. Discovery of potent and orally bioavailable pyrimidine amide cGAS inhibitors via structure-guided hybridization. *ACS Medicinal Chemistry Letters*. 2024. doi:10.1021/acsmchemlett.4c00471.
- [181] Cyr P, Chefson A, Sietsema DV, et al. Reducing hERG inhibition in the design of potent and bioavailable indazole cGAS inhibitors. *ACS Medicinal Chemistry Letters*. 2025. doi:10.1021/acsmchemlett.5c00641.
- [182] Alarcon-Esposito J, Nagiri RK, Gan L, Sinha SC. Identification and development of cGAS inhibitors and their uses to treat Alzheimer's disease. *Neurotherapeutics*. 2025. doi:10.1016/j.neurot.2025.e00536.
- [183] Huang Y, Liu B, Sinha SC, Amin S, Gan L. Mechanism and therapeutic potential of targeting cGAS-STING signaling in neurological disorders. *Molecular Neurodegeneration*. 2023. doi:10.1186/s13024-023-00672-x.
- [184] Feng W, Aikedan A, Sinha SC, Gan L. Expanding roles of cGAS-STING signaling in neuroinflammation. *Journal of Clinical Investigation*. 2026. doi:10.1172/JCI204550.
- [185] Dai J, Huang YJ, He X, et al. Acetylation blocks cGAS activity and inhibits self-DNA-induced autoimmunity. *Cell*. 2019. doi:10.1016/j.cell.2019.01.016.
- [186] Elkon KB. Aspirin meets cGAS. *Nature Reviews Rheumatology*. 2019. doi:10.1038/s41584-019-0205-y.
- [187] Zeng YF, Wang QG, Qi Z, et al. Aspirin inhibits the cGAS-STING signaling pathway to ameliorate the development of aortic aneurysm and dissection. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2026. doi:10.1161/ATVBAHA.126.324930.
- [188] Wiser C, Kim B, Vincent J, Ascano M. Small molecule inhibition of human cGAS reduces total cGAMP output and cytokine expression in cells. *Scientific Reports*. 2020. doi:10.1038/s41598-020-64348-y.

- [189] Rice GI, Forte GM, Szykiewicz M, et al. Assessment of interferon-related biomarkers in Aicardi-Goutieres syndrome associated with mutations in TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, and ADAR: a case-control study. *Lancet Neurology*. 2013. doi:10.1016/S1474-4422(13)70258-8.
- [190] Montealegre Sanchez GA, Reinhardt A, Ramsey S, et al. JAK1/2 inhibition with baricitinib in the treatment of autoimmune inflammatory interferonopathies. *Journal of Clinical Investigation*. 2018. doi:10.1172/JCI98814.
- [191] Vanderver A, Adang L, Gavazzi F, et al. Janus kinase inhibition in the Aicardi-Goutieres syndrome. *New England Journal of Medicine*. 2020. doi:10.1056/NEJMc2001362.
- [192] Ventus Therapeutics U.S., Inc.; ClinicalTrials.gov. A Phase 2a study of VENT-03 in adult participants with active cutaneous lupus erythematosus with or without systemic lupus erythematosus — NCT07260877. *ClinicalTrials.gov study registration*. 2026. Source.
- [193] ImmuneSensor Therapeutics Inc.; ClinicalTrials.gov. A study of IMSB301 in participants with type I interferonopathies — NCT07364513. *ClinicalTrials.gov study registration*. 2026. Source.
- [194] Han VX, Hayes JP, Sun L, et al. cGAS inhibitor IMSB301 modifies interferon signalling in peripheral mononuclear cells of TREX1 genetic interferonopathy. *Clinical and Translational Immunology*. 2026. doi:10.1002/cti2.70090.
- [195] Meric-Bernstam F, Sweis RF, Hodi FS, et al. Phase I dose-escalation trial of MIW815 (ADU-S100), an intratumoral STING agonist, in patients with advanced/metastatic solid tumors or lymphomas. *Clinical Cancer Research*. 2022. doi:10.1158/1078-0432.CCR-21-1963.
- [196] Meric-Bernstam F, Sweis RF, Hodi FS, et al. Correction: Phase I dose-escalation trial of MIW815 (ADU-S100), an intratumoral STING agonist, in patients with advanced/metastatic solid tumors or lymphomas. *Clinical Cancer Research*. 2023. doi:10.1158/1078-0432.CCR-23-1170.
- [197] Harrington KJ, Champiat S, Brody JD, et al. Phase I and II clinical studies of the STING agonist ulevostinag with and without pembrolizumab in participants with advanced or metastatic solid tumors or lymphomas. *Clinical Cancer Research*. 2025. doi:10.1158/1078-0432.CCR-24-3630.
- [198] Luke JJ, Sweis RF, Hecht JR, et al. Intratumoral or subcutaneous MK-2118, a noncyclic dinucleotide STING agonist, with or without pembrolizumab, for advanced or metastatic solid tumors or lymphomas. *Clinical Cancer Research*. 2025. doi:10.1158/1078-0432.CCR-24-2824.
- [199] Cooper BT, Iams WT, Page DB, et al. Phase Ib study of dazostinag plus pembrolizumab after hypofractionated radiotherapy in patients with select advanced solid tumors. *Cancer Research Communications*. 2025. doi:10.1158/2767-9764.CRC-25-0566.
- [200] Liu Y, Jesus AA, Marrero B, et al. Activated STING in a vascular and pulmonary syndrome. *New England Journal of Medicine*. 2014. doi:10.1056/NEJMoal312625.

- [201] Crow YJ, Manel N. Aicardi-Goutieres syndrome and the type I interferonopathies. *Nature Reviews Immunology*. 2015. doi:10.1038/nri3850.
- [202] Patel S, Blaauboer SM, Tucker HR, et al. The common R71H-G230A-R293Q human TMEM173 is a null allele. *Journal of Immunology*. 2017. doi:10.4049/jimmunol.1601585.
- [203] Van Gool WA, Weinstein HC, Scheltens P, Walstra GJ. Effect of hydroxychloroquine on progression of dementia in early Alzheimer's disease: an 18-month randomised, double-blind, placebo-controlled study. *Lancet*. 2001. doi:10.1016/s0140-6736(01)05623-9.
- [204] Aisen PS, Schafer KA, Grundman M, et al. Effects of rofecoxib or naproxen vs placebo on Alzheimer disease progression: a randomized controlled trial. *JAMA*. 2003. doi:10.1001/jama.289.21.2819.
- [205] ADAPT Research Group; Lyketsos CG, Breitner JC, Green RC, et al. Naproxen and celecoxib do not prevent AD in early results from a randomized controlled trial. *Neurology*. 2007. doi:10.1212/01.wnl.0000260269.93245.d2.
- [206] Butchart J, Brook L, Hopkins V, et al. Etanercept in Alzheimer disease: a randomized, placebo-controlled, double-blind, phase 2 trial. *Neurology*. 2015. doi:10.1212/WNL.0000000000001617.
- [207] Valles-Saiz L, Avila J, Hernandez F. Lamivudine (3TC), a nucleoside reverse transcriptase inhibitor, prevents the neuropathological alterations present in mutant tau transgenic mice. *International Journal of Molecular Sciences*. 2023. doi:10.3390/ijms241311144.
- [208] Zhang Y, Zhang X, Huang J, Sun Z. Lamivudine ameliorates neuropathology in 5xFAD mice via coordinated inhibition of the cGAS-STING pathway with enhancement of mitophagy. *Brain Research Bulletin*. 2026. doi:10.1016/j.brainresbull.2026.112014.
- [209] Martinez de Lagran M, Elizalde-Torrent A, Paredes R, Clotet B, Dierssen M. Lamivudine, a reverse transcriptase inhibitor, rescues cognitive deficits in a mouse model of down syndrome. *Journal of Cellular and Molecular Medicine*. 2022. doi:10.1111/jcmm.17411.
- [210] Magagnoli J, Ambati M, Cummings TH, et al. Association of nucleoside reverse transcriptase inhibitor use with reduced risk of Alzheimer's disease. *Alzheimer's and Dementia*. 2025. doi:10.1002/alz.70180.
- [211] Fundacion FLS de Lucha Contra el Sida; ClinicalTrials.gov. Effect of lamivudine on neurocognitive impairment biomarkers and type-I interferon-stimulated genes in the plasma of patients with mild cognitive impairment — NCT06519357. *ClinicalTrials.gov study registration*. 2026. Source.