

Catching a killer: Mechanisms of programmed cell death and immune activation in Amyotrophic Lateral Sclerosis

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Abstract

In the central nervous system (CNS), execution of programmed cell death (PCD) is crucial for proper neurodevelopment. However, aberrant activation of these pathways in adult CNS leads to neurodegenerative diseases including amyotrophic lateral sclerosis (ALS). How a cell dies is critical, as it can drive local immune activation and tissue damage. Classical apoptosis engages several mechanisms to evoke “immunologically silent” responses, whereas other forms of programmed death such as pyroptosis, necroptosis, and ferroptosis release molecules that can potentiate immune responses and inflammation. In ALS, a fatal neuromuscular disorder marked by progressive death of lower and upper motor neurons, several cell types in the CNS express machinery for multiple PCD pathways. The specific cell types engaging PCD, and ultimate mechanisms by which neuronal death occurs in ALS are not well defined. Here, we provide an overview of different PCD pathways implicated in ALS. We also examine immune activation in ALS and differentiate apoptosis from necrotic mechanisms based on downstream immunological consequences. Lastly, we highlight therapeutic strategies that target cell death pathways in the treatment of neurodegeneration and inflammation in ALS.

KEYWORDS

cell death, innate immunology, neurodegeneration

1 | INTRODUCTION: CELL DEATH IN THE CNS

For the organism to live, some cells must die. Seminal studies in developmental biology have shown that normal development and tissue homeostasis rely upon programmed cell death (PCD). For instance, the death of cells in the apical ectodermal ridge sculpts the digits of developing vertebrate limbs during embryogenesis.^{1,2} Early work mapped morphological features of cells in the process of “orderly” elimination.³ Collectively, these features—cytoplasmic shrinkage, nuclear condensation, fragmentation, and the budding small bodies—were termed “apoptosis.”³

The central nervous system (CNS), comprised of the brain and spinal cord, is sculpted by apoptotic events in neural precursor cells

(NPCs), differentiated post-mitotic neurons, and glial cells.⁴ Only the cells with appropriate connections, location, size, and shape are needed for CNS homeostasis. Superfluous neurons are eliminated, and axonal projections “pruned”. Between birth and adulthood, humans lose an estimated 15% of their neurons.⁵ In transgenic mice lacking effectors of apoptosis (such as Apaf-1 and caspase-3/9), the CNS is the most affected tissue, presenting with a glut of neurons contained in disorganized structures.^{6,7} Collectively, studies describing the process of neurodevelopment highlight the importance of apoptotic cell death in sculpting normal CNS architecture.^{4,8}

Although life begins with elimination of unwanted neurons and is necessary for proper development, aberrant death of distinct CNS regions is a hallmark of neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), and

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Alzheimer's disease (AD). The upstream etiologies of neurodegenerative diseases are diverse and multifactorial.^{9,10} A combination of aging, genetic predisposition, and environmental triggers disrupt neuronal function and initiate these pathologies. Broad signaling networks, such as oxidative stress, excitotoxicity, mitochondrial damage, and ER stress, have all been implicated in driving downstream neuronal cell death in patients and transgenic mouse disease models (reviewed in refs 11–13).

While many studies originally indicated that neurodegeneration culminates in apoptotic neuronal death,¹⁴ close pathological examination of degenerating tissues reveals some puzzling findings. For example, neurodegeneration patient samples display features inconsistent with orderly apoptosis—swollen neurons with punctured plasma membranes, release of intracellular contents and damage-associated molecular patterns (DAMPs), elevated levels of cytokines in cerebrospinal fluid (CSF) and progressive neuroinflammation.^{15–19} These findings challenge our traditional notions of apoptotic death, which is classically considered “immunologically silent.”^{20,21}

Recent advances in the fields of cell death and innate immunology have uncovered novel programmed necrotic pathways—namely necroptosis, pyroptosis, and ferroptosis. Unlike apoptosis, these modes of cell death culminate in plasma membrane disruption (ie, necrosis). Over the last decade, elegant investigations have revealed that these necrotic PCD (programmed cell death) mechanisms lead to coordinated release of pro-inflammatory intracellular molecules (eg, DAMPs and cytokines) that activate the immune system. Further, these modes of cell death often promote tissue damage, inflammation and are closely linked to onset of pathology *in vivo*.^{22–24}

Could activation of these necrotic pathways drive chronic neuroinflammation and neurodegeneration? The molecular mechanisms that initiate activation of glial cells (eg, astrocytes and microglia) and infiltration of peripheral immune cells into the degenerating CNS are not well understood. Further, how neuroinflammation potentiates neuronal demise on a cellular signaling level also remains an open area of investigation. Linking programmed cell death mechanisms to neuroinflammation will advance our basic understanding of neuroimmunology and provide new therapeutic strategies for patients.

This review provides an overview of the different PCD pathways implicated in ALS, differentiating apoptosis from necrotic mechanisms by describing downstream immunological consequences (Figure 1). We examine the specific evidence for immune activation in ALS and investigate the known and proposed roles of apoptotic, necroptotic, and pyroptotic cell death in disease progression. Lastly, we highlight therapeutic strategies that target cell death pathways and discuss proposed crosstalk between apoptosis, necroptosis, and pyroptosis. Ultimately, inhibition of multiple cell death “culprits” may provide the greatest benefit for ALS patients.

2 | ALS AND NEUROINFLAMMATION

Amyotrophic lateral sclerosis (ALS) is a fatal, progressive adult-onset form of neuromuscular disease caused by the aberrant death

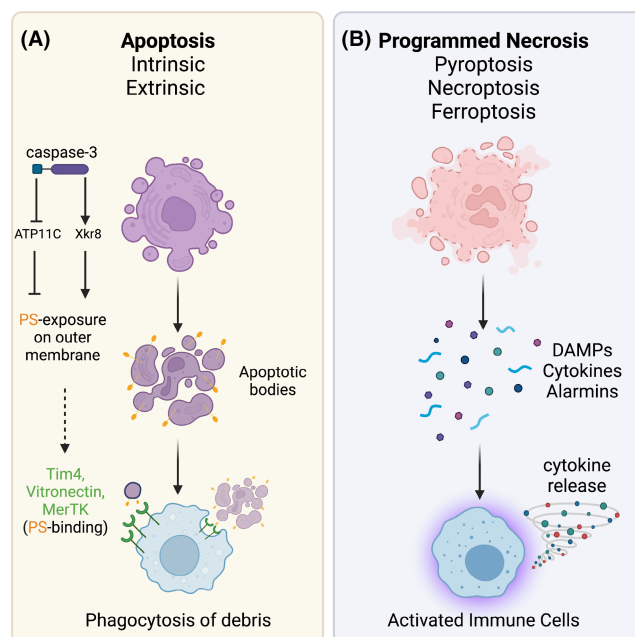


FIGURE 1 Immunological outcomes of apoptosis vs programmed necrotic mechanisms. (A) Apoptosis induced either by the intrinsic pathway or by extrinsic death receptor signaling (eg, TNFR1 and CD95) leads to characteristic morphological changes: nuclear condensation, cell shrinkage, and budding of apoptotic bodies. Caspase-mediated inhibition of the ATP11C flippase and activation of Xkr8 scramblase exposes phosphatidylserine residues (PS) on the outside of the plasma membrane. Macrophages use several receptors (eg, TIM4 and MERTK) to bind exposed PS residues (“eat me” signal) and phagocytose debris. (B) Programmed necrotic mechanisms display morphologic features of cellular swelling and loss of plasma membrane integrity. Necrosis culminates in the release of cytokines, chemokines, DAMPs, and other intracellular molecules. These cellular contents are sensed by immune cells, which engage innate immune pathways (eg, TLRs, RAGE1, and RIGI/MAVS) to promote activation and boost local inflammation

of upper and lower motor neurons in the cerebral cortex and spinal cord (Figure 2). Clinically, these patients present with muscle atrophy, weakness, twitches, spasticity, respiratory failure, and ultimately death.²⁵ Roughly 30% of patients first lose motor neurons in the corticobulbar region of the brainstem, this leads to early “bulbar” symptoms such as hoarse voice, impaired speech, difficulty swallowing, and vocal cord spasms.^{10,25} A small proportion of patients may also present with the related condition frontotemporal dementia (FTD), which shares common genetic features with ALS.^{26,27} FTD is a progressive brain disorder that causes death of cortical neurons affecting personality, behavior, and language. Though the majority (~90%) of ALS is sporadic, several mutations have been identified that cause familial ALS and/or FTD: These include superoxide dismutase (SOD1), TAR DNA-binding protein 43 (TDP-43), C9ORF72 and FUS.²⁶ Myriad signaling processes have been associated with disease progression, including oxidative stress, excitotoxicity, mitochondrial dysfunction, and downstream caspase activation.²⁷ No effective disease modifying therapy is currently available for ALS

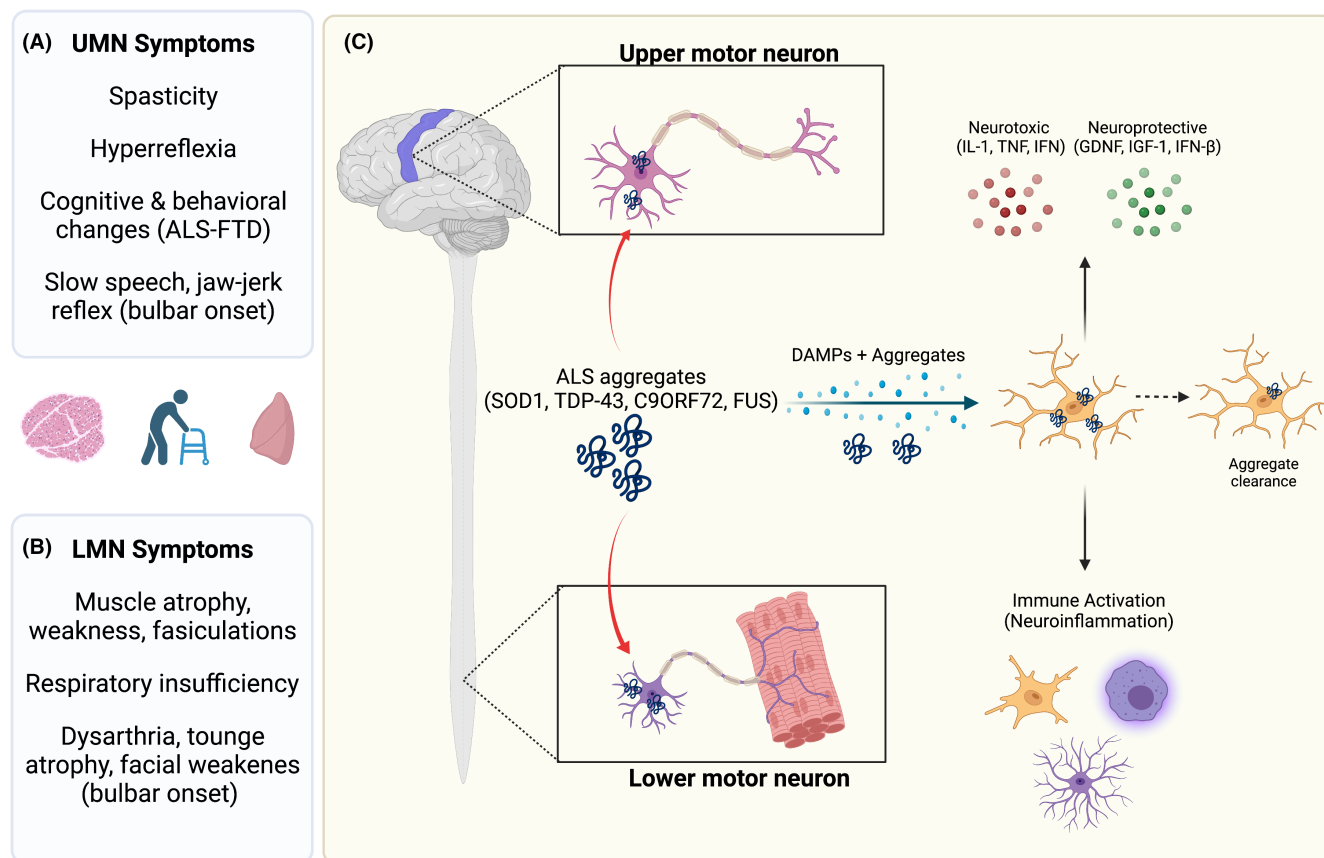


FIGURE 2 Amyotrophic lateral sclerosis (ALS) symptoms and pathogenesis. Patients presenting with ALS classically exhibit both (A) upper motor neuron (UMN) and (B) lower motor neuron (LMN) symptoms. These include spasticity (UMN) and muscle weakness and fasciculations (LMN). Thirty percent of patients display “bulbar” onset symptoms such as slurred speech, difficulty swallowing, and facial weakness. A subset of patients also display concomitant symptoms of frontotemporal dementia (FTD), presenting with cognitive and behavioral changes due to cortical degeneration. Ultimately, ALS patients succumb to respiratory insufficiency. (C) In ALS, both upper and lower motor neurons display pathology culminating in cell death. There are many proposed mechanisms, but aggregation of ALS-associated proteins, such as TDP-43, is considered central to disease pathogenesis. Studies employing SOD1 G93A and other mouse models have shown that expression of protein aggregates in neurons is key to disease onset, but that subsequent neuroinflammation drives disease progression. Microglia, astrocytes, and oligodendrocytes can sense and respond to protein aggregates directly by initiating innate immune and programmed cell death (PCD) pathways. Activation of these cell signaling programs leads to secretion of neurotoxic factors (eg, IL-1 family cytokines), gliosis and recruitment of peripheral immune cells. Glia can also mediate neuroprotective functions in the context of neurodegeneration. For example, microglia can bind TDP-43 using TREM2 to promote aggregate clearance and neuroprotection. Experimental depletion of microglia (or Trem2 $-/-$) worsen disease progression in TDP-43 murine models of ALS

patients. Approved by the FDA in 1996, the anti-glutamatergic drug Riluzole extends the survival of ALS patients by only 3 months.²⁸ The more recently approved Radicava (edaravone), which is a free radical scavenger, has a similarly modest impact on patient survival.^{29,30} Thus, identification of novel therapeutic targets is critical for this patient population.

Neuroinflammation is a hallmark of ALS onset and progression.^{10,16,31} CNS-resident microglia and astrocytes, as well as peripheral immune cells, are highly activated at the level of the motor cortex, ventral horn, and peripheral motor nerve.^{32–35} Gliosis in conjunction with an influx of peripheral immune cells into the CNS can predate ALS symptom onset, suggesting that these cells may impact disease onset and progression.^{16,36} Depending on disease stage, the immune system may mediate both neuroprotective and deleterious functions.³⁶ For example, in a mouse model of ALS, microglia

transcriptionally upregulate both neuroprotective and toxic factors.³⁷ Chemical depletion (PLX3397: a CSF1R and c-kit inhibitor) of microglia worsens disease recovery in a TDP-43 (Δ NLS) mouse model of ALS, indicating that microglia play neuroprotective roles in this setting.^{38,39} A recent study found that microglial triggering receptor expressed on myeloid cell 2 (TREM2) binds TDP-43 directly and contributed to aggregate clearance and neuroprotection in a murine ALS model.⁴⁰ Collectively, this work suggests that the role of neuroinflammation in ALS progression is highly context specific—disease stage, genetic background, and cell type are crucial considerations (Figure 2). Strategies aimed at ablating all immune subsets may thus hold limited promise for persons with ALS.

In 1993, genetic analysis of ALS families led to the identification of superoxide dismutase (SOD1), a reactive oxygen species (ROS) scavenger, as the first gene linked to significantly increased incidence of

familial ALS.⁴¹ Soon after, mouse models carrying transgenes of the identified human SOD1 mutations, G93A, G37R, and G85R, were created to study the mechanisms of motor neuron death.⁴² While SOD1 is ubiquitously expressed in all cell types, seminal work in the SOD1(G93A) model showed that expression of mutant SOD1 in motor neurons determines disease onset, but microglial mutant SOD1 expression potentiates disease progression.⁴³ Similar studies have implicated glial-dependent neuroinflammation in TAR DNA-binding protein 43 (TDP-43) mediated pathogenesis. TDP-43 is a versatile RNA/DNA-binding protein deeply linked to ALS and FTD pathogenesis. Hyperphosphorylated and ubiquitinated TDP-43 deposits are found in the majority (~97%) of sporadic ALS cases, and mutations in the TDP-43 gene underly 5%–10% of familial cases. Several mouse models have been generated to study the role of this protein in mediating neuronal dysfunction and ALS and FTD.^{44–46} These models are characterized by progressive microgliosis and astrogliosis, which correlate with symptom onset and degree of neurologic impairment. Importantly, TDP-43 leads to both caspase-3 activation in neurons and caspase-1 activation and cytokine secretion in microglia and astrocytes.^{45,47} Mouse conditional knockout models of other ALS-risk alleles, such as TANK-binding kinase 1 (TBK1, upstream regulator of necroptosis and interferon signaling), display cell-type dependent phenotypes. For example, knockout of TBK1 in motor neurons of SOD1 mice leads to accelerated disease onset.⁴⁸ However, pan-cell TBK1 knockout prolongs lifespan in these mice, suggesting TBK1 loss in glial populations may dampen neuroinflammation. However, further studies are required to determine which cell-type-specific loss of TBK1 ameliorates disease.⁴⁸

Collective learnings from cellular and mouse models suggest that ALS-associated genes and pathways can uniquely affect neuronal and non-neuronal cells to impact disease progression (Figure 2). Many of the PCD pathways discussed in this review occur in multiple cell types and can be engaged in both neurons and glia. For example, mutant SOD1 and TDP-43 stimulate both caspase-3-dependent apoptosis in motor neurons,¹⁴ and engage inflammasome and caspase-1 signaling (key players in pyroptosis) in microglia and astrocytes.^{45,47,49,50,51,52,53} Mutant C9orf72 promotes cell death in motor neurons, whereas endogenous protein (lacking G₂C₄ repeat expansion) suppresses microglial neuroinflammation.^{54,55} Throughout this review, we will not only outline the contribution of cell death pathways to disease, but also highlight the importance of identifying the specific cell types that execute these programs. We also discuss gaps in knowledge regarding the role of specific inflammatory cell death mechanisms in ALS, and therapeutic strategies targeting these pathways in disease.

3 | APOPTOSIS AND ALS

3.1 | Apoptotic signaling and the immune response

Just as a medical examiner must certify the manner of death following autopsy, so too must the immune system decide whether the destruction of a cell warrants further investigation. Apoptosis

is often described as a “clean” and inconspicuous mode of cell death that does not induce local inflammation.^{23,56,57}

Apoptosis is mediated by the caspase family of cysteine proteases, which cleave hundreds of cellular targets, including the endonucleases that fragment nuclear DNA.^{58–60} There are two roads leading to downstream caspase activation and apoptosis—the extrinsic and intrinsic pathways (Figure 3). The intrinsic apoptotic pathway follows mitochondrial damage and release of cytochrome-c (cyt-c) from the inner mitochondrial membrane. Cyt-c complexes with Apaf-1 and caspase-9 to form an assembly called the “apoptosome.”⁶¹ Active caspase-9 then cleaves and activates caspase-3, the final executioner of apoptosis.^{60–62} Endoplasmic reticulum stress, reactive oxygen species (ROS), DNA damage, toxin exposure, viral infection, and growth factor deprivation can all stimulate intrinsic apoptosis (reviewed in ref. 59). For example, withdrawal of nerve growth factor (NGF) from cultured sympathetic neurons leads to mitochondrial damage, cyt-c release, and intrinsic apoptosis. Microinjection of a neutralizing anti-cyt-c mAb rescues cell death, confirming the importance of this mode of cell death to NGF-withdrawal.^{63–65} Other mitochondrial proteins release from the intermembrane space, such as Smac/DIABLO and Omi/HtrA2, potentiate apoptosis by blocking the inhibitor of apoptosis protein (IAP) family, which block caspase activity. By releasing negative regulation of caspases, Smac and Omi potentiate apoptotic cell death.^{66–68} Mitochondrial stress can also lead to phosphorylation of eIF12a and induction of ATF4 transcription. ATF4 regulates the integrated stress response pathway (ISR), which upregulates apoptotic factors (eg, PUMA, caspases) and suppresses anti-apoptotic molecules.⁶⁹ ATF4 activation in neurons has been linked to cell death and neurodegeneration.^{69,70} Recent work showed that following mitochondrial damage, the mitochondrial proteins OMA1 and DELE1 activate an HR1-eIF2a axis that induces ATF4-mediated ISR.^{71,72} Thus, other mitochondrial molecules beyond cyt-c, such as Smac and Omi, also play roles in amplifying intrinsic apoptotic death.

Extrinsic apoptosis occurs following a ligand (eg, FasL) binding to a death-associated member of the tumor necrosis factor (TNF) receptor family (eg, Fas) on the plasma membrane. Receptor activation induces a cascade that leads to the intracellular recruitment and activation of the initiator caspase-8.⁷³ Caspase-8 then cleaves caspase-3 leading to downstream apoptosis. Crosstalk exists between the extrinsic and intrinsic apoptosis pathways. For example, caspase-8 can cleave the pro-apoptotic BH3 protein BID, which activates the effector proteins BAX and BAK (reviewed in refs 74,75) to cause mitochondrial outer-membrane permeabilization (MOMP) (Figure 3). Upon MOMP, proteins like cytochrome-c (and other pro-apoptotic molecules such as SMAC) are released into the cytosol to further drive intrinsic apoptosis/caspase-3 activation.^{62,76,77} Both intrinsic and extrinsic apoptotic pathways have been implicated in CNS injury and dysfunction.^{78,79}

The “immunologically silent” nature of apoptosis is often attributed to its capacity to contain intracellular components of

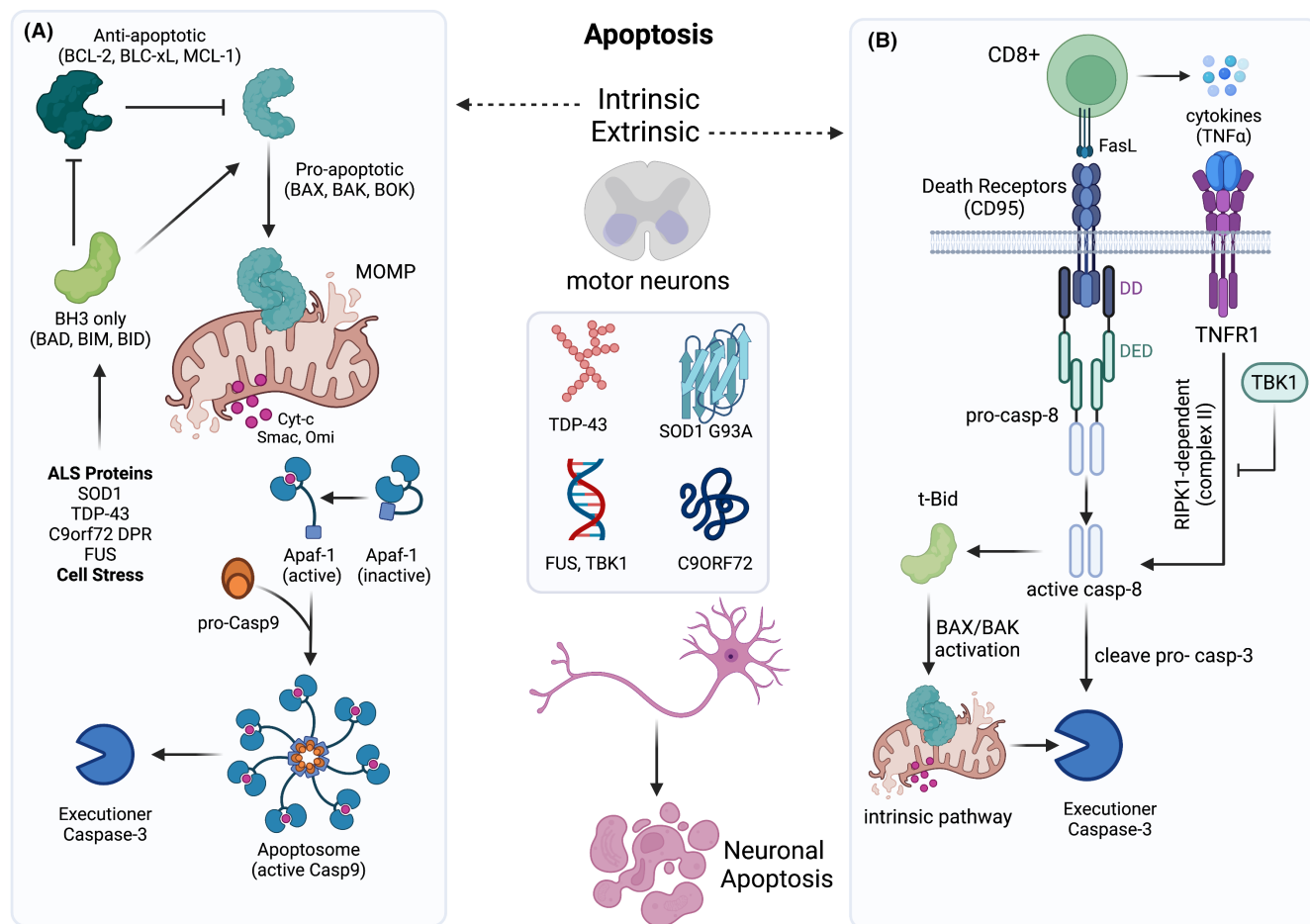


FIGURE 3 Evidence for activation of apoptosis in ALS. Studies using in vitro and in vivo approaches have found that ALS-associated proteins (eg, TDP-43, mSOD1, and toxic C9ORF72 dipeptide repeat proteins) and mutations in fused-in-sarcoma (FUS) can cause activation of intrinsic apoptosis in neurons. (A) Intrinsic apoptosis begins with cellular stress that activates pro-apoptotic BH3-only proteins. BH3 only proteins such as BIM and BID, sequester anti-apoptotic BCL-2 proteins and promote oligomerization of pro-apoptotic family members (eg, BAX) in mitochondrial membrane. Following mitochondrial outer membrane permeabilization (MOMP) mediated by BAX and BAK, cytochrome-c (cyt-c) is released from the intermembrane space into the cytosol. There cyt-c binds and activates Apaf-1, allowing for oligomerization and recruitment of pro-caspase-9 via homotypic CARD-CARD interactions. The apoptosome (cyt-c, Apaf-1, active caspase-9) cleaves executioner caspases to mediate apoptosis. (B) Infiltration of CD8+ T cells into the spinal cord, or mutations in TBK1 can sensitize neurons to extrinsic apoptosis. This mode of cell death is triggered by ligation of a death receptor (TNFR1, Fas, or TRAIL-R1/2) and can lead to apoptosis, necroptosis, or cell survival. Following CD95 ligation (by FasL), CD95 binds FADD using death domains (DD). FADD then recruits caspase-8 via death effector domain (DED) interactions and assembles a caspase activation platform called the death-inducing signaling complex (DISC). This platform leads to caspase-8 activation and thus engages the extrinsic apoptotic pathway. Caspase-8 can also process Bid into t-Bid, which then engages the mitochondrial (intrinsic) pathway of apoptosis. Upon ligation to TNF, TNFR1 recruits the adaptor protein TRADD and RIPK1, which in turn mobilizes additional partners, such as the ubiquitin ligases TRAF2 and cIAP1/2 to form complex I. When RIPK1 is deubiquitinated it allows for release of complex 1, which can then bind FADD and caspase-8 (complex II). This complex can induce apoptosis, or necroptosis in situations where FADD/caspase-8 are inhibited. TBK1 restrains TNFR signaling and downstream RIPK1-dependent apoptosis (RDA). Mutations in TBK1, which are genetically associated with ALS, can lead to loss of this inhibition and enhanced caspase-8 activation

the cell during death, preventing the release of pro-inflammatory DAMPs, alarmins, and cytokines⁸⁰ (Figure 1). Classically, apoptotic cells release “find me” (eg, ATP, UTP, LPC, and S1P) and “eat me” signals to recruit phagocytic myeloid immune cells and promote clearance,⁸¹ reviewed in refs 82–84. For example, a hallmark of apoptotic death is the caspase-3-mediated activation of Xkr8 scramblase. This scramblase flips the membrane lipid phosphatidylserine (PS) from the inner to the outer leaflet of the plasma

membrane, PS exposure allows for identification of apoptotic cells by phagocytes.^{82,85,86} The T-cell immunoglobulin and mucin domain-containing molecule 4 (Tim4) expressed on macrophages can bind PS to promote the phagocytic engulfment of the dying/dead cell and subsequent degradation in a process known as efferocytosis (reviewed in ref. 56). Efferocytosis is LC3-dependent and is as an important component of immune silencing.⁵⁷ PS-binding and LC3-associated phagocytosis alters the macrophage

transcriptional response, promoting expression and secretion of the anti-inflammatory factors TGF- β and IL-10.^{21,87}

CNS-resident microglia can recognize PS on the surface of apoptotic neurons using the vitronectin or MERTK receptors; PS-receptor binding leads to microglial phagocytosis of viable neurons.^{88,89} This mechanism of PS recognition is important for microglial engulfment of not only apoptotic neurons but also virally infected neurons in the CNS.⁹⁰ In cultured neurons, ROS generation (via H₂O₂ treatment) induces reversible PS exposure, which can be recovered and internalized in the absence of microglia.^{91,92} However, stressed neurons co-cultured with microglia are promptly phagocytosed prior to PS-recovery. Interestingly, knockout of the ALS-associated gene progranulin (PGRN) in *C. elegans* and mice led to enhanced rates of neuronal clearance via macrophage and microglial phagocytosis.⁹³ Further, PGRN mutation in mice results in an age-dependent increase in phagocytic microglia, which may relate to elevated neuronal apoptosis that occurs due to aging.⁹⁴ However, the implications of these findings with respect to the role of apoptosis in ALS progression are still unclear.

Rather than just simply sequestering intracellular contents, recent work has shown that apoptosis may inhibit inflammation more directly. Medina and colleagues found that apoptotic lymphocytes and macrophages release anti-inflammatory metabolites while preserving their plasma membrane integrity.⁹⁵ This metabolic “secretome” relied upon caspase-dependent activation of pannexin-1 (PANX1) channels. Metabolites (eg, spermidine, AMP, and Creatine) leaked via PANX1 channels induced immunosuppressive and tissue repair gene programs in neighboring cells. When given exogenously, a cocktail of apoptotic metabolites reduced disease severity in mouse models of inflammatory arthritis and lung-graft rejection.⁹⁵ Tanzer et al⁹⁶ identified a specific “secretome” signature that is dominated by release of ectodomain portions of cell surface proteins.⁹⁶ Receptor shedding may have an anti-inflammatory effect through the generation of soluble decoy receptors. Ultimately, more research is needed to define the extent to which neurons have a unique apoptotic “secretome” and its effects on immune cells and other neurons.

Several apoptotic mechanisms carefully work to silence immune activation and ensure that cells “go quietly.” Nonetheless, inappropriate activation of apoptosis contributes to loss of motor neurons in ALS. The evidence for the activation of this PCD pathway in patient samples and ALS mouse models is detailed below.

3.2 | The role of apoptosis in ALS

Apoptosis was first implicated as a driver of ALS following detection of caspase activation in SOD1 mutant mice and human patient samples (Table 1). Pasinelli et al found elevated caspase-3 expression and activation in SOD1 G37R, G85R, and G93A neurons and glial cells; cleaved caspase-3 immunoreactivity preceded symptom onset and increased with age and disease severity.¹⁴ A concomitant report found that SOD1 (G85R) caused neuronal p53

transcription and increased expression of pro-apoptotic BCL2 family members, Bcl-x and Bax.⁹⁷ A later report also found that overexpression of TDP-43, a pathologic hallmark of ALS, also evoked a p53 transcriptional response in neurons.⁵³ Subsequent work showed that mutated SOD1 binds and depletes the anti-apoptotic protein Bcl-2 from spinal cord mitochondria, allowing for induction of intrinsic apoptosis.⁹⁸ Several other studies have also detected aberrations in the BCL-2 family, such as increased levels of BAX and BIM (pro-apoptotic) in patient spinal cord.^{99,100} Importantly, genetic knockout of pro-apoptotic BCL2 family members (Bim, Bax, and Bak) or pharmacologic caspase inhibition improves disease symptoms and extends lifespan in SOD1 transgenic mice. These studies firmly implicate transcriptional regulators (p53), upstream inducers (BCL-2 family), and downstream executioners (caspase-3) of intrinsic apoptosis in mediating mutant SOD1-driven pathology (Figure 3).

There is also evidence to support a role for T-cell-mediated apoptosis in disease progression. Studies using the SOD1 G93A mouse have showed an influx of CD8+ T cells in both spinal cord and sciatic nerve.^{101,118} CD8+ T cells recognize antigen presented on major histocompatibility complex I (MHC I). Following binding of T-cell receptor (TCR) to MHC I, these cells can release granules filled with perforin and granzyme to trigger apoptosis of the target cell. CD8+ T cells can also release FasL, which binds and clusters Fas receptors on the target cell plasma membrane to mediate extrinsic apoptosis (Figure 3). Coque et al,¹⁰¹ found that in vitro CD8+ T cells carrying SOD1 G93A mutations produce interferon- γ , which elicits the expression of the MHC-I complex in motoneurons. These activated T cells can attack MHCI-positive neurons directly and cause apoptosis dependent on both FasL/CD95 and perforin/granzyme pathways (Figure 3). Depletion of CD8+ cells using anti-CD8 neutralizing antibodies delays disease progression in the SOD1 G93A mutant mouse.¹⁰¹ Very recent work has shown that mutations in SETX (which causes ALS4, a rare juvenile onset form of ALS) drive clonal CD8 T-cell expansion in the periphery and infiltration into the spinal cord.¹¹⁹ This work suggests that CD8-mediated neuronal killing may be a common feature of ALS, upstream of several common and rare ALS mutations (Table 1).

Fewer reports have explored apoptotic signaling in non-SOD1 genetic causes of ALS (Table 1). A recent study developed an in vitro RNA-seq and ATAC-seq platform to explore neuronal cell death in response to mutant c9orf72-driven neuropathology. Using this unbiased approach, it was uncovered that toxic dipeptide repeats (DPRs), transcribed from hexanucleotide repeats in the c9orf72 locus, caused p53 and caspase-3-dependent cell death *in vitro*.⁵⁴ Employing recently developed mouse and *Drosophila* models of c9orf72 DPR toxicity, they found that knockout of p53 or PUMA (a pro-apoptotic BCL2 family member) dampened neuronal loss and improved overall survival. Other work has found that mutations in ALS-risk genes, such as FUS and TBK1, lead to neuronal apoptosis (assessed by caspase-3 activation and TUNEL staining) in vitro and in mouse models.^{121,122} These studies raise the possibility that apoptosis is central to ALS in multiple genetic contexts. Future work

TABLE 1 Selected evidence for programmed cell death mechanisms in ALS

PCD pathway	ALS model/methodology	Result summary	Citation(s)
Apoptosis	SOD1 G93A, G85R, G37R	Caspase-3 activation preceded symptom onset and increased with disease severity in ALS mice	14
	SOD1 G85R, G93A	Mutant SOD1 causes pro-apoptotic dysregulation of BCL-2 family in motor neurons	97-100
	SOD1 G93A and neuron T-cell co-cultures	CD8+ T cells can induce extrinsic apoptosis in cultured neurons; CD8+ depletion rescues SOD1 mice	101
	AAV-PR50 mouse model (FTD/ALS)	The c9orf72 DPR, PR-50, induces p53 and caspase-3-dependent neuronal death	54
Pyroptosis	Human CSF samples	Elevated IL-1 family cytokines in ALS patient CSF and spinal cord samples	102-104
	SOD1 G93A, G85R, G37R mice	Caspase-1 activation in SOD1 spinal cords	14
	SOD1 G93A mouse	Inhibition or genetic KO of caspase-1 delays disease progression	102,104
	Primary mouse microglia cultures and neuronal co-cultures	SOD1 and TDP-43 activate microglial NLRP3 inflammasomes, which can kill cultured neurons	49,104,105, 106
	SOD1 G93A mice	Immunostaining reveals NLRP3/ASC present in microglia, astrocytes, skeletal muscle	50,105,107,108
Necroptosis	Patient genetics	Mutations in negative regulators of RIPK1 pathway, TBK1, and OPTN, are associated with ALS	109-111
	Optn ^{-/-} , SOD1 G93A mice	Knockout of Ripk1 rescues two mouse models of ALS. RIPK1 and MLKL activation are detected in human ALS spinal cord samples	112
	Astrocyte-neuron co-culture	Astrocytes derived from individuals with sporadic ALS killed iPSC-derived motor neurons via necroptosis	113
	Astrocyte-neuron co-culture	Conditional KO of Ripk3 in neurons protects from Tg-SOD1 G93A astrocyte toxicity. SOD1 G93A Ripk3 ^{-/-} mice not protected from disease progression	114
	SOD1 G93A and Optn ^{-/-} mice	Subset of microglia in the SOD1 G93A and Optn ^{-/-} models of ALS rely on RIPK1 signaling to boost neuroinflammation.	115
Ferroptosis	SOD1 G93A mice and human ALS samples	xCT antiporter expression is increased in microglia and regulates neuroinflammation in SOD1 G93A mice	116
	Human ALS samples	Iron and ROS accumulation in patient spinal cords, iron chelation improves motor function	117
	SOD1 G93A mice	Transgenic overexpression of GPX4 increased motor performance and survival in the SOD1 G93A mouse model	

studying temporal activation of caspases in c9orf72, FUS, and TDP-43 animal models, as well as in sporadic ALS patient samples, will shed light on this point.

Unlike apoptosis, necrosis has traditionally been viewed as an “accidental” cause of death.^{22,23} Cellular trauma, exposure to high doses of toxins, or overwhelming shifts in osmotic gradient would cause “bursting” of the plasma membrane and release of intracellular contents. Over the last decade, elegant investigations have identified programmed necrotic mechanisms and pathways—pyroptosis, necroptosis, and ferroptosis (Figure 1). In contrast to apoptotic death, necrotic PCD leads to coordinated release DAMPs and cytokines that activate the immune system (Figure 1). Given that ALS is characterized by cell death and inflammation, whether necrotic PCD mechanisms contribute to disease progression is of intense translational interest. We review these mechanisms and their links to ALS in the subsequent sections.

4 | PYROPTOSIS

4.1 | Pyroptotic signaling and release of inflammatory mediators

Pyroptosis is a form of cell death that occurs downstream of inflammasome activation, classically in immune cells (Figure 4). The term “inflammasome” was coined in 2002 to describe a protein complex in the cytosol of immune cells that senses pathogen-associated molecular patterns (PAMPs) and DAMPs and responds by activating inflammatory caspases.¹²² When “seed” molecules, such as NLRP3 or AIM2, bind PAMPs or DAMPs, they oligomerize and recruit the adaptor apoptosis-associated speck-like protein containing a CARD (ASC). The myriad mechanisms by which inflammasomes recognize microbial and damage-associated molecules are reviewed elsewhere.¹²²⁻¹²⁴

Following adaptor oligomerization, inflammasomes act as scaffolding to recruit and activate caspase-1.^{126,127} Cleaved caspase-1 can then process pro-interleukin (IL)-1 β and pro-IL-18 into their mature forms (Figure 4). In 2015, several studies reported the identification of gasdermin-d (GSDMD) as a key executioner of cell death and regulator of cytokine release from macrophages.¹²⁸⁻¹³⁰ Collectively, this work described how caspase-1 cleaves gasdermin-D (GSDMD) in a linker region separating the N and C-termini. Cleavage at this linker region allows the N-terminus to oligomerize, and form 215 to 310 Å pores in the plasma membrane through which IL-1 cytokines can efflux^{129,131,132} (Figure 4). GSDM-driven pore formation, release of IL-1 family cytokines and eventual cellular swelling and necrosis are collectively termed "pyroptosis".¹³³ Recent work has shown that in addition to IL-1 cytokines, small, neutral, or positively charged molecules (eg, cytochrome-c) can pass directly through GSDMD pores.¹³² The full spectrum of molecules that can traverse GSDMD pores is currently unexplored and may have profound effects on inflammation.

In immune cells, pyroptosis is characterized by the release of IL-1 β and IL-18.^{133,134} IL-1 β is a potent inflammatory mediator, promoting vasodilation, immune cell extravasation, and adaptive immunity.¹³⁴ IL-18 promotes interferon (IFN)- γ production in TH1 cells, NK cells, and cytotoxic T cells, enhances the development of TH2 cells, promotes gut antimicrobial immunity, and local tissue inflammation (reviewed in ref. 135). Alarmins such as IL-1 α and HMGB1, as well as other endogenous DAMPs (eg, nuclear and mitochondrial DNA), are also released by pyroptotic cells following plasma membrane rupture.¹³⁶⁻¹³⁹ Collectively, these molecules bind their cognate cytokine receptors (IL1 or IL18 receptors) or HMGB1 to pattern recognition receptors (PRRs) such as toll-like receptors and RAGE (receptor for advanced glycation endproducts) on immune cells to promote chemotaxis, antigen presentation, and inflammatory transcriptional profiles (eg, NF- κ B programs). NETosis—the process by which neutrophils expel their nuclear chromatin to trap pathogens and promote inflammation, is also found to be GSDMD-dependent.¹⁴⁰ Lastly, studies have revealed

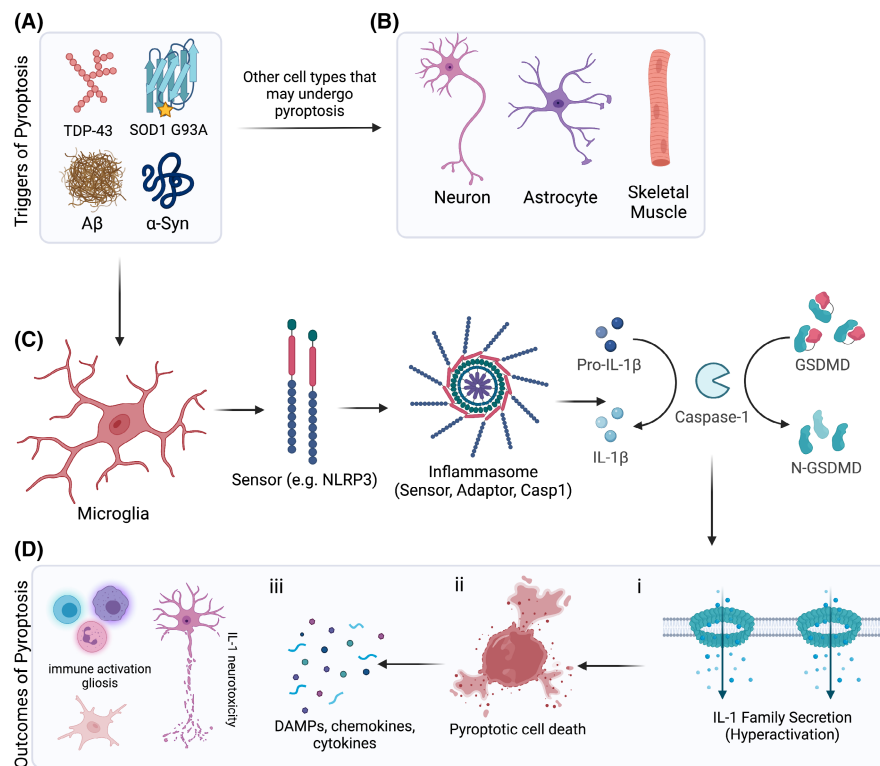


FIGURE 4 Signaling mechanisms and outcomes of pyroptosis in the ALS. (A) Triggers of pyroptosis in the context of neurodegeneration include aggregated proteins such as TDP-43 and mutant SOD1 (ALS), as well as amyloid beta oligomers (AD) and fibrillar alpha-synuclein (PD). There is some evidence to suggest (B) neurons, astrocytes, and skeletal muscles can undergo pyroptosis in the context of ALS. Most of the available evidence points to (C) microglia as the chief cells responding to aggregated protein species by engaging pyroptosis. Neurodegeneration-associated molecules are detected by sensor molecules (eg, NLRP3), which recruit and oligomerize adaptor proteins (eg, ASC). Adaptors, such as ASC and pyrin, contain CARD domains that can recruit caspase-1 as part of the inflammasome complex. Active caspase-1 can process IL-1 family cytokines and cleave the linker region of full-length GSDMD (Asp 275). (D) When released, N-terminal GSDMD undergoes a conformational change enabling binding to acidic lipid residues and pore formation in cellular membranes. (i) If these pores form in the plasma membrane of a living cell they act as conduits for IL-1 release and support a "hyperactivated" phenotype. (ii) If enough GSDMD pores accumulate in the plasma membrane, cellular swelling and overt pyroptotic cell death can occur. (iii) Pyroptosis leads to release of intracellular contents and serves to recruit peripheral immune cells and activate microglia and astrocytes. High levels of IL-1 release can contribute to neurotoxicity and may exacerbate neuronal injury

that pyroptotic cells release active inflammasomes (ie, NLRP3) and ASC specks into the extracellular microenvironment to potentiate inflammasome activation in other immune cells.^{141,142} These studies are beginning to define a pyroptotic “secretome” that serves to initiate and amplify inflammation. Inhibition of specific secretome members may alter the immune response *in vivo*. For example, a recent study utilized proteomics to find that intracellular LPS induced the release of the DAMP galectin-1 from pyroptotic macrophages. Galectin-1-deficiency, or treatment with galectin-1-neutralizing antibodies, reduced inflammation and improved outcomes in a mouse model of LPS-induced sepsis.¹⁴³

The *in vivo* role of pyroptosis has been found to contribute to a variety of conditions, including multiple sclerosis (MS), neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's Disease (PD), as well as in peripheral tissues in anti-tumor immunity, inflammatory bowel disease, viral and parasite infections, sepsis.^{144,145} Recent evidence also points toward an important role for the AIM2 inflammasome, caspase-1, and GSDMD in regulating cell death in neurodevelopment.¹⁴⁶ There are many clues implicating “fiery” cell death as a central culprit driving neurodegeneration. Classically, pyroptotic death in monocytes promotes immune activation in the context of autoimmune disease and infection.¹³² Downstream of inflammasome assembly, innate immune cells engage caspase-1 to produce mature IL-1 α , IL-1 β , and IL-18, these cytokines are then released through GSDMD pores and subsequent cellular necrosis ensues.¹³² Here, we will examine the evidence and therapeutic strategies for targeting pyroptosis, in the context of ALS.

4.2 | Evidence for pyroptosis in ALS

Given that chronic and progressive inflammation is a hallmark of ALS, it was hypothesized that pyroptosis may be involved in this disorder¹⁰³ (Table 1). Clinical studies have found pyroptotic “fingerprints” at the crime scene elevated IL-1 family cytokines in samples of spinal cord and CSF from ALS patients.^{102,104} Inflammasome assembly and cleaved caspase-1 have also been detected via histology for a wide range of CNS diseases, including AD, PD, HD, MS, and stroke (reviewed in ref. 148). While there is evidence from mouse models to implicate pyroptosis as a driver of ALS (Table 1), the upstream triggers, functional relevance and cell types involved are less clear.

Early reports showed that cleaved caspase-1 was increased in spinal cord samples from SOD1 transgenic animals (containing human G93A, G85R, and G37R mutations). This activation was detected several months prior to disease onset and induction of neuronal apoptosis.¹⁴ Subsequent work has shown that total caspase-1 knockout or pharmacologic inhibition of caspase-1 delays disease onset in SOD1 transgenic mice, have not clarified this issue of cell-type specificity.^{102,104} In these studies, caspase-1 activation was assessed by immunoblots of spinal cord lysates. However, the identity of the cells (ie, neuronal vs glial) expressing activated caspase-1 remains unclear.

Work in PD, AD, and EAE models suggests that protein aggregates can activate microglial NLRP3 inflammasomes directly, driving downstream caspase-1 and IL-1 processing.¹⁴⁸⁻¹⁵⁴ Supporting the notion that pyroptosis occurs chiefly in microglia, several studies have shown that SOD1 and TDP-43 protein aggregates induce NLRP3/ASC oligomerization and IL-1 β secretion from mouse microglia *in vitro*.^{49,104,105} Co-culture of TDP-43-treated microglia with motor neurons causes neuronal death.⁴⁷ This phenotype was dependent on microglial TLR4 and NLRP3 expression, suggesting that TDP-43 may activate an inflammatory cascade in microglia to drive non-cell autonomous neuronal death. Very recent work established and profiled primary monocyte-derived cells taken from 30 sporadic ALS patients; these “microglial-like” cells had cytoplasmic TDP-43 inclusions, impaired phagocytosis, elevated cytokine secretion and greatly enhanced NLRP3 inflammasome activation relative to cells taken from healthy control patients.¹⁰⁶

Given that pyroptosis was discovered in peripheral monocytes, it is tempting to completely segregate this pathway to microglia in the CNS. However, the situation may be more complex (Figure 4B). Several studies that immunostained SOD1 G93A spinal cords found early increases in the levels of NLRP3 that did not colocalize with microglia.^{50,105,107,108} Using both the SOD1 G93A mouse model and patient samples, Johann et al⁵⁰ identified the cellular source of NLRP3 to be astrocytes rather than microglia.⁵⁰ In subsequent studies, Johann and colleagues have suggested that inflammasome expression on skeletal muscle and in thalamic neurons may be contributing to motor and cognitive deficits found in ALS-FTD patients.¹⁰⁸ In addition, other work has suggested that the NLRC4 and AIM2 inflammasomes are also increased in SOD1 G93A mice, though the tissue expression patterns and relevance of these inflammasomes to disease are unclear.¹⁵⁵ Several studies also indicate that neurons can express active inflammasome components (AIM2 and NLRP3) and undergo pyroptosis following exposure to TLR agonists, oxygen-glucose deprivation, or exogenous amyloid β .¹⁵⁶⁻¹⁵⁸ Though these studies were able to colocalize inflammasomes with neuronal markers, these studies did not examine neuronal caspase-1 or GSDMD activity, use ALS-associated stimuli, and were limited to *in vitro* assessment.¹⁵⁷

4.3 | Unexplored avenues linking pyroptosis to ALS

The investigation of pyroptosis in mediating ALS (and neurodegeneration more broadly) is limited by several factors. Though neuroinflammation is a common marker of all forms of ALS, currently the temporal and spatial expression of inflammasomes and caspase-1 has only been studied in the SOD1 G93A mouse model *in vivo*. Future studies examining expression of additional pyroptosis components (eg, NLRP3, ASC, and GSDMD) in c9orf72 and TDP-43 driven animal models and in ALS patient tissues may shed light on the relevance of this pathway to disease. Classically, complete knockouts (or chemical inhibitors) have been used to assess the role of pyroptosis in neurodegeneration. For example, deficiency in caspase-1 ameliorates disease progression in mouse models of PD and

ALS (SOD1 G93A).^{102,104,149} Though informative, these studies do not shed light on which cell type is employing this cell death pathway. Coupling conditional knockout strategies (eg, Cxc3r1 or Syn1a driven Cre expression) of pyroptosis components (eg, NLRP3, ASC, and GSDMD) with ALS disease models will give the field a more detailed understanding of how and where pyroptosis is activated *in vivo*. The molecular mechanisms leading to inflammasome activation also remain to be fully elucidated. It would be important to define whether specific ALS-associated proteins trigger NLRP3 or other intracellular inflammasome components at the structural level.

Recent work has shown that macrophages can possess active inflammasomes and GSDMD pores in the absence of overt necrotic cell death (ie, pyroptosis). These “hyperactivated” cells use GSDMD channels to secrete high levels of IL-1 family cytokines but maintain cellular viability.¹⁵⁸ Future work assessing whether neurodegeneration proteins (eg, SOD1 and TDP-43) cause hyperactivation or pyroptotic death of glial cells will add nuance to our understanding of how this pathway contributes to neurologic disease.

5 | NECROPTOSIS

5.1 | Necroptotic signaling and DAMP release

Necroptosis is another recently identified cell death pathway that has been linked to neurodegenerative diseases.^{159,160,161} While the caspase family of proteases drive apoptosis (caspase-8, caspase-3/7) and pyroptosis (caspase-1/11), necroptosis was originally identified as a caspase-independent form of cell death. Rather, the receptor-interacting serine/threonine kinases (RIP kinases) control necroptosis (Figure 5). As in extrinsic apoptosis, necroptosis is classically initiated by ligation of death receptors, such as tumor necrosis factor receptor 1 (TNFR1).¹⁵⁹ *In vitro*, death receptor ligation combined with caspase inhibition induces the recruitment and deubiquitylation of RIPK1.^{159,162} RIPK1 is then autophosphorylated and can recruit RIPK3 via interactions between homotypic RHIM domains present on both proteins.^{160,163} RIPK3 then oligomerizes and phosphorylates the pseudokinase Mixed Lineage Kinase-Like (MLKL) (Figure 4). Phosphorylated-MLKL, the executioner of necroptosis, translocates to the plasma membrane to induce membrane rupture and cell death.¹⁶⁴⁻¹⁶⁶ (Figure 5).

Upstream engagement of the receptors TLR3 and TLR4, IFNAR, and ZBP1 can also result in RIPK3 activation and p-MLKL-mediated necroptosis.¹⁶⁷⁻¹⁶⁹ Signaling through these receptors concomitantly activates inflammatory transcription factors (such as NF- κ B and p38) and expression of the pro-survival gene *CFLAR* (FLIP_L).¹⁷⁰ FLIP_L can form a heterodimer with caspase-8, blocking its ability to cleave caspase-3.^{24,171} Active caspase-8 can also cleave and inactivate RIPK1 and RIPK3 and inhibit downstream MLKL-driven death. Thus, only in settings where the catalytic activity of caspase-8 is disrupted (via chemical inhibition or binding to FLIP_L) can necroptosis ensue.²⁴ Caspase-8 inactivation may provide an important mechanism for necroptosis induction in the CNS diseases. Cortical tissue samples from MS patients showed reduced levels of the active caspase

8 subunit and correspondingly reduced caspase 8 activity.¹⁷² The expression of FLIP_L was also substantially elevated in MS samples.¹⁷² In the CNS, FLIP_L is expressed in many cell types including microglia, oligodendrocyte precursor cells, and mature oligodendrocytes.¹⁷³ Induction of this molecule (potentially downstream of NF- κ B or p38) may be an important step in licensing necroptosis during disease.

There are several additional regulatory proteins that serve to repress RIPK1 function and block necroptosis. TBK1 phosphorylates and thereby inhibits RIPK1 autophosphorylation, while the M1 ubiquitin-binding protein optineurin (OPTN) negatively regulates RIPK1 activation while it is bound to death receptors (eg, TNFR1) via TRADD.^{24,175,176} (Figure 5). Mutations causing haploinsufficiency of TBK1 are a major genetic cause of ALS and frontotemporal dementia, accounting for 10.8% of all cases in patients presenting with comorbid ALS and FTD.^{109,110} Interestingly, mutations in OPTN lead to development of either hereditary glaucoma or ALS.¹¹¹ In Asian populations, mutations in *OPTN* account for 4% of familial ALS cases, whereas this frequency is much lower in European populations at 1.5%.^{111,177} Both *Tbk1*^{+/−} and *Optn*^{−/−} mice develop motor neuron disease that resembles ALS.^{24,112,176} That mutations in two negative regulators RIPK1 activation lead to the development of ALS in humans and animal models, thereby linking necroptotic cell death to disease pathogenesis.

In contrast to the “natural causes” death associated with apoptosis, cells that undergo necroptosis are more akin to a car crash. Transcriptional programs induced by ligation of TLRs or IFNARs (eg, p38 and NF- κ B pathways) combined with p-MLKL-mediated release of intracellular contents lead to high levels of inflammation. Many DAMPs, such as ATP, LDH, and HMGB1, are released following necroptosis (reviewed in ref. 178). A recent study used a mass-spectrometry approach to characterize the “secretome” of necroptotic cells. They found that necroptosis induced a large release of lysosomal components, which were not detected in media containing apoptotic cells.⁹⁶ The *in vivo* relevance of these specific secreted components has not yet been characterized. It will be interesting to assess how specific DAMPs affect innate and adaptive immune responses. Very recent work has shown that both GSDMD and MLKL rely on the action of another protein, NINJIN-1 (NINJ1), to mediate ultimate plasma membrane rupture (PMR) and release of large intracellular molecules downstream of both pyroptosis and necroptosis.^{179,180} DAMP-release is MLKL and NINJ1 dependent, indicating that pore formation and PMR is critical for recruiting immune cells.¹⁸¹ Conformational change of this transmembrane molecule allows for PMR downstream of either GSDMD or p-MLKL pore formation. Thus, inhibition of NINJ1 prevents PMR, attenuates release of DAMPs like LDH, and reduces local inflammation.¹⁷⁹

The *in vivo* role of necroptosis has been proposed to contribute to a variety of conditions, including stroke, atherosclerosis, pancreatitis, inflammatory bowel disease, and certain malignancies.^{23,178,181} Regarding the role of necroptosis in neurodegeneration, several studies have implicated RIPK1 signaling in ALS, PD, AD, and multiple sclerosis (MS).^{23,24,161,182} In the following sections, we will examine the evidence and therapeutic rationale for targeting necroptosis in the setting of ALS.

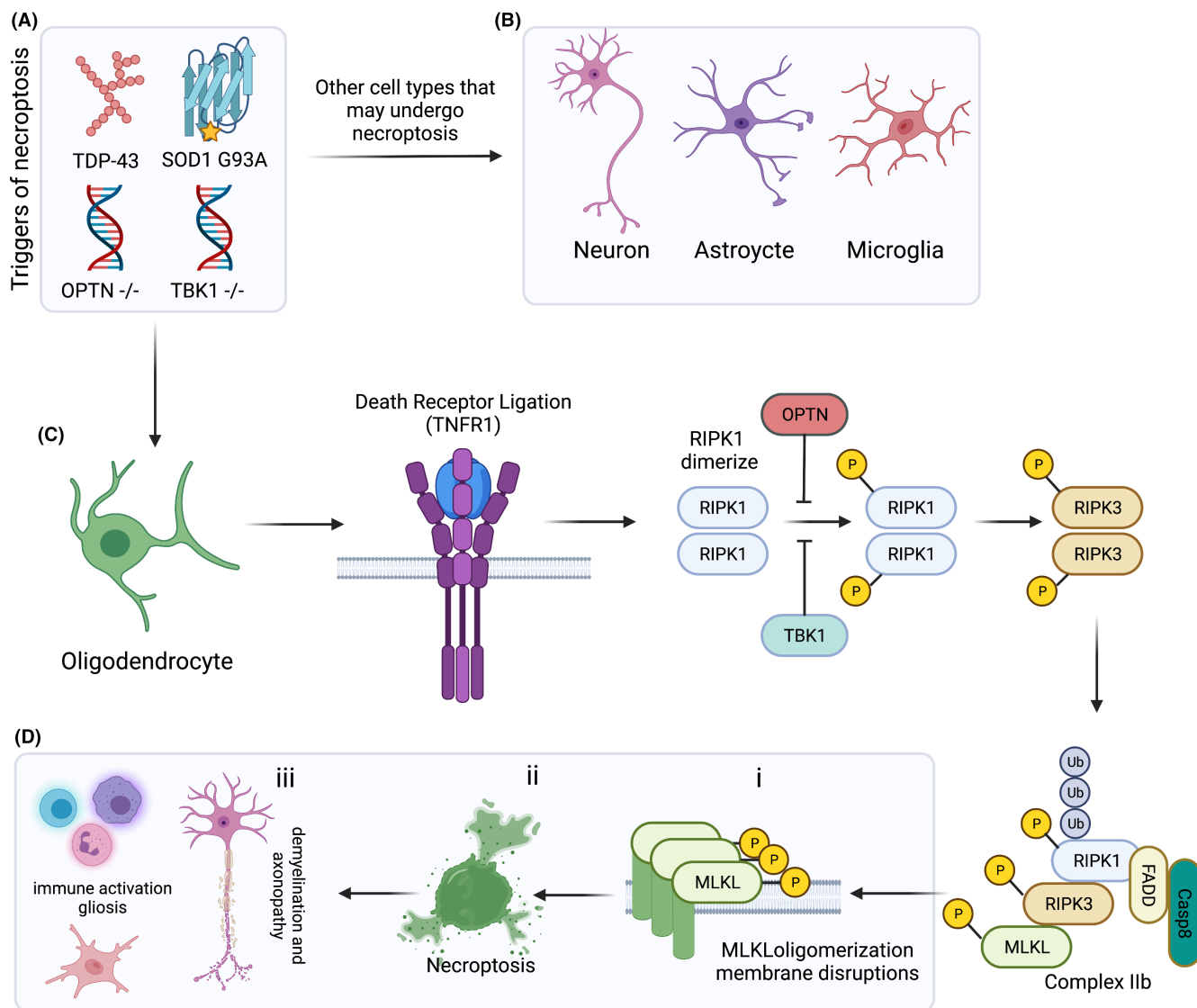


FIGURE 5 Necroptotic signaling mechanisms and outcomes in ALS. (A) Triggers of necroptosis in the context of ALS include aggregated proteins such as TDP-43 and mutant SOD1 (ALS), as well as mutations in negative repressors of the RIPK1 pathway such as optineurin (OPTN) and TBK1. There is evidence to suggest (B) neurons, astrocytes, and microglia undergo necroptosis in the context of ALS. However, the strongest evidence points to (C) oligodendrocytes as the chief cells executing necroptosis in ALS. Stimulation of TNFR1 by TNF promotes the formation of an intracellular signaling complex where the death domains (DD) of trimerized TNFR1 binds DD-containing proteins: TRADD and receptor-interacting serine/threonine-protein kinase 1 (RIPK1). TRADD helps to recruit a number of RIPK1 regulators, including OPTN and TBK1. In situations of caspase-8 downregulation or inhibition, RIPK1 is autophosphorylated and dimerizes via its C-terminal death domain. This dimerization promotes RIPK3 activation and the subsequent formation of the necrosome (complex IIb) comprised of RIPK1, FADD, Casp8, RIPK3, and mixed-lineage kinase domain-like pseudokinase (MLKL). (D) This complex enables (i) p-MLKL to insert and oligomerize in plasma membrane and (ii) execute overt necroptotic cell death. As in other forms of programmed necrosis, (iii) release of DAMPs and intracellular contents serves to recruit and activate peripheral immune cells and promote gliosis. In the case of necroptosis in oligodendrocytes, this leads to demyelination and perhaps contributes to the progressive axon loss observed in ALS

5.2 | Necroptotic molecules driving death and inflammation in ALS

The necroptosis pathway is brutal; during ALS progression, no tissue or cell type is left unaffected. Current evidence suggests that necroptosis can detriment motor neuron health in several ways: by (i) driving oligodendrocyte death and axonal demyelination; (ii) promoting cell autonomous neuronal death via p-MLKL membrane

disruption; and (iii) enhancing neuroinflammatory transcriptional programs in microglia (Figure 5; Table 1).

5.2.1 | Axonal attack

Axonal degeneration that precedes pathology at the cell body is commonly observed in patients suffering from ALS, MS, AD, and

PD.¹⁸³ In ALS, axons undergo 'Wallerian-like' degeneration, which is an axonopathy that resembles the "dying back" of processes seen after nerve transection.¹⁸³ Motor neurons, which have the longest axons of any neurons in the body, are especially susceptible to attack. Growing evidence suggests that axonal degeneration in ALS begins before the onset of clinical motor symptoms and is driven by oligodendrocyte dysfunction.¹⁸⁴

Necroptosis was first implicated in ALS pathogenesis by Ito et al,¹¹² where they demonstrated RIPK1-dependent axonal loss in Optn-deficient mice.¹¹² OPTN encodes a ubiquitin-binding protein that inhibits RIPK1, mutations in this gene are found in a small subset of ALS patients.¹¹¹ Optn knockout mice exhibited swollen large-diameter axons, reductions in the total number of motor axons in ventrolateral white matter and abnormal myelination patterns that preceded the loss of motor neuron soma.¹¹² Conditional ablation of Optn in oligodendrocytes sensitizes these cells to TNF-induced necroptosis, and phenocopies the Wallerian-like degeneration seen in total Optn $-/-$ mice. Necroptosis of Optn $-/-$ oligodendrocytes can be rescued by pharmacologic Ripk1 inhibition (Nec-1s) or kinase dead mutations in Ripk1 or Ripk3.¹¹² Thus, the loss of Optn induces necroptosis in oligodendrocytes to mediate axonopathy.

Oligodendrocyte cell death has also been described in the SOD1 G93A mouse model, prior to disease onset.¹⁸⁵ Reduced numbers and dysfunctional oligodendrocytes were also detected in spinal cord and motor cortex of human ALS patients.¹⁸⁵ Ito and colleagues found that chemical RIPK1 inhibition or RIPK3 knockout restored myelination defects in SOD1 G93A mice and delayed onset of motor behavior defects. Finally, increased levels of phosphorylated RIPK1, RIPK3, and MLKL were detected in sporadic ALS patient spinal cords—though the cell type expressing these necroptotic molecules was not identified.¹¹² Parallel findings in the cuprizone model of MS highlight the role of necroptosis in mediating degeneration of oligodendrocytes and non-cell autonomous axon loss in neurons.^{112,173}

5.2.2 | Bodily harm

Necroptosis may also contribute more directly to neuronal cell death. In a humanized co-culture ALS model, astrocytes derived from individuals with sporadic ALS (sALS astrocytes) killed iPSC-derived motor neurons via activation of neuronal necroptosis.¹¹³ In this in vitro system, inhibition of motor neuron RIPK1 by lentiviral shRNA transduction, or Nec-1 treatment, reduced sALS astrocyte-induced cell death.¹¹³ A subsequent study found that conditional deletion of *Ripk3* in cultured mouse motor neurons also protected them from SOD1^{G93A} astrocyte-induced neurotoxicity.¹¹⁴ However, *Ripk3* knockout in the SOD1 G93A background failed to provide survival, behavioral or neuropathological improvement.¹¹⁴ Thus, the benefit of *Ripk3* and necroptotic silencing in vivo remains unclear.

Though there is less evidence that neuronal necroptosis contributes to ALS, it is an area that deserves more attention. Prior work has found that acidosis can activate necroptosis in cultured neurons,

and that both necroptosis and apoptosis are implicated in neuronal death following cerebral ischemia.^{186,187} A recent report has found that in aged mice, Ripk1 and p-MLK levels are increased in cortical and hippocampal neurons relative to young controls.¹⁸⁸ Ripk1 inhibition (using nec-1s) and Mkl1 knockout mice were protective against age-induced neuroinflammation and cell death. Interestingly, necroptotic molecules were detected only in the neuronal soma and not in axonal processes.¹⁸⁸ Thus, necroptosis of oligodendrocytes may mediate an early axonal dysfunction in ALS, which may be followed by a delayed necroptosis at the level of the neuronal cell body. Future work using neuron-specific (eg, Syn1a) conditional knockouts of Ripk1, Ripk3, and Mkl1 may elucidate the role of necroptosis in mediating cell autonomous death in preclinical ALS models.

5.2.3 | Inflammatory evidence

In addition to initiating necroptotic death, RIPK1 can also alter innate immune signaling and promote inflammatory transcriptional programs in immune cells.²⁴ Studies examining mouse models of AD have elucidated a role for microglial RIPK1 in increasing neuroinflammation, which is independent of p-MLKL and overt necroptotic death.^{189,190} Optn $-/-$ mouse show an increase in activated microglia in the mouse spinal cord.¹¹² RNA-sequencing analysis of these Optn $-/-$ microglia revealed a module of approximately 1300 genes—enriched in pro-inflammatory microglial markers such as CD14 and CD86—that were differentially expressed compared to wild-type cells. This inflammatory signature was Ripk1-dependent and could be reversed by nec-1s treatment.¹⁸⁹ Following this analysis, a recent study identified a subset of microglia in the SOD1(G93A) and Optn $-/-$ models of ALS that rely on RIPK1 signaling. These RIPK1-regulated inflammatory microglia (RRIMs) have increased TNF α and NF- κ B signaling and express pro-inflammatory cytokines such as IL-1 α .¹¹⁵ RRIM transcription is dampened by nec-1s treatment and is distinct from the classic disease-associated microglia (DAMs).¹¹⁵ How RRIMs and DAMs cooperate to mediate motor neuron cell death is likely a rich area of future investigation.

5.3 | Unanswered questions and limitations

Several recent reports have called into question the role of necroptosis in mediating ALS progression.¹⁹⁰ Two independent groups have recently found that Ripk1 or Mkl1 knockout did not affect disease onset, motor symptoms, neuroinflammation, or survival in SOD1 G93A mutant mice.¹⁹¹⁻¹⁹⁴ These studies contradict prior work implicating RIPK1 and necroptosis as central mediators of motor neuron loss and neuroinflammation in SOD1 G93A mice.^{112,113,115} Adding to the confusion, neither of the recent studies detected appreciable accumulation of RIPK1, RIPK3, or p-MLKL in human ALS or mouse SOD1 G93A spinal cords.¹⁹¹⁻¹⁹⁴ The discrepancies between these reports are unclear, though it may be due to use of different antibody reagents, immunolabelling

protocols or variable levels of perfusion to remove circulating blood cells, which are rich in necroptotic markers.¹⁸⁰ Given that RIPK1 inhibitors are advancing to phase II trials in human ALS patients, elucidating the role of this pathway in disease progression is paramount. Future studies assessing the role of necroptosis in other ALS disease models (eg, C9ORF72 mutation) coupled with consistent experimental conditions, reporting of antibody reagents, and genetic backgrounds of knockout transgenic mice will shed light on this important issue.

6 | FERROPTOSIS IN ALS

Ferroptosis is a necrotic form of PCD, distinct from pyroptosis or necroptosis. Specifically, it is an iron-dependent, oxidative cell death mechanism first described by Dixon et al.¹⁹⁵ While the term “ferroptosis” was coined in 2012, similar processes have been observed years earlier in conditions where glutathione metabolism is disrupted.^{196,197} Ferroptosis is triggered by inactivation of the system x_c^- cystine/glutamate antiporter (xCT), leading to depletion of intracellular glutathione and downstream ROS elevation.¹⁹⁵ The xCT antiporter is highly expressed on activated microglia, and xCT levels were increased in both spinal cord and isolated microglia from mutant SOD1 ALS transgenic mice.¹¹⁶ Expression of xCT was also detectable in spinal cord post-mortem tissues of patients with ALS, which correlated with high levels of neuroinflammation. In ALS mice, xCT deletion led to decreased production of pro-inflammatory microglial factors nitric oxide, TNF α and IL6. This work suggests that increased ferroptosis (via genetic knockout of xCT) may serve to limit microglial inflammation.¹¹⁶ In SOD1 G93A mice, xCT deletion accelerated disease onset, but slowed progression and increased overall survival of motor neurons.¹¹⁶

In vitro, ferroptosis can also be activated by chemical or genetic inhibition of the glutathione-dependent lipid hydroperoxidase GPX4 (reviewed in refs 200,201). Glutathione depletion or GPX4 inhibition ultimately leads to the iron-dependent accumulation of lipid peroxides which kill the cell via uncontrolled lipid peroxidation.²⁰⁰ Accordingly, ferroptosis is dependent on expression of ACSL4 and LPCAT3—the enzymes that generate the membrane lipids susceptible to peroxidation.²⁰² Unlike the pore-forming molecules in pyroptosis or necroptosis, the executioner of ferroptosis is overwhelming ROS-driven lipid peroxidation causing cell failure and membrane rupture. There have been some reports implicating iron accumulation and ferroptosis in neurologic disease and ALS.^{117,193,203} For example, Wang et al found that transgenic overexpression of GPX4 increased motor performance and survival in the SOD1 G93A mouse model.^{193,194} Further, edaravone (an ROS scavenger approved for ALS treatment) reduces ferroptosis in cultured cells, protecting against both xCT and GPX4 inactivation.²⁰⁴ How ferroptosis in motor neurons or glial cells contributes to disease progression remains unclear. Recent work suggests that ferroptotic motor neuron death may be contributing to disease,^{193,194} while earlier studies suggest that ferroptosis may also limit microglial neuroinflammation

later in disease progression.¹¹⁶ The role of this PCD pathway in different disease phases and cell types in the CNS has yet to be adequately interrogated.

This review has focused on the role of apoptosis, pyroptosis, and necroptosis in driving motor neuron death and inflammation ALS. Going forward, the precise signaling, immunologic consequences, and cell-specific roles of ferroptosis (reviewed in refs 196,198) will be exciting to study in the context of ALS and neurologic disease.

7 | NEW LEADS: POTENTIAL THERAPEUTIC STRATEGIES TARGETING PROGRAMMED CELL DEATH AND INFLAMMATION IN ALS

7.1 | Caspase inhibition as a therapeutic strategy

There is considerable evidence suggesting that caspase activity promotes cell death and inflammation in mouse models of ALS and neurodegeneration.^{14,54,79,151,203,204,205} That caspase-1 and caspase-3 regulate distinct cell death pathways (pyroptosis and apoptosis, respectively), suggesting that a pan-caspase inhibition strategy may have clinical benefit. For example, the administration of pan-caspase inhibitor Z-VAD-FMK delayed onset and mortality in SOD1 G93A mice.²⁰⁵ Similarly, administration of recombinant XIAP (a mammalian protein blocking caspase-3, caspase-7, and caspase-9) also slowed disease progression.²⁰⁴

While the use of caspase inhibitors has contributed to a basic understanding of cell death and inflammation, many of these compounds have failed to progress to clinical trials. This is due to several factors, including an adverse safety profile, poor pharmacokinetic (PK) and pharmacodynamic (PD) properties, low target specificity, and selectivity within the caspase family.^{208,209} Efforts to target individual caspase family members have failed both *in vitro* and in preclinical contexts.²¹⁰ For these reasons, current studies using caspase inhibitors to treat neurologic disease have focused on the animal models rather than clinical trial data. Despite the central role of caspases in regulating neuronal demise, elegant work in innate immunology and cell death have revealed alternative therapeutic strategies. Several of these targets are currently being pursued in clinical trials for the treatment of ALS.

7.2 | Targeting apoptosis

Recently, a randomized double-blind trial showed that the combination of Sodium Phenylbutyrate and Taurursodiol (PB-TURSO) slowed motor decline in ALS patients, as measured by the clinical ALSFRS-R score.²¹¹ Though the precise mechanism of action is unclear, Taurursodiol exerts anti-apoptotic properties *in vitro* by blocking the translocation of the pro-apoptotic BCL2 family member BAX to mitochondrial membranes.²¹¹ Sodium phenylbutyrate, a histone deacetylase inhibitor, reduces endoplasmic reticulum (ER) stress which

is upstream of apoptosis. ER stress has been shown to promote BAX-translocation, leading to calcium overload, caspase activation, and apoptosis.²¹² While targeting caspase-3 may be pharmacologically off limits, drug combinations (eg, PB-TURSO) able to alter upstream mediators of apoptosis may attenuate disease progression.

7.3 | Targeting pyroptosis

The NLRP3 inflammasome has been implicated in preclinical models of ALS, AD, PD, HD, MS, and stroke.^{150,213,214,215,216,217} Inhibitors of NLRP3 are advancing into clinical trials for treatment of peripheral autoimmune conditions, such as cryopyrin-associated periodic syndrome, liver and lung fibrosis, gout, and COVID-19.²¹⁶ To date, NLRP3 inhibitors have not been tested in patients with neurodegenerative disease. However, several companies (eg, NodThera, Inflazome, Ventus Therapeutics, Genentech, and Novartis) are developing molecules that can cross the blood-brain barrier (BBB). As these molecules progress through phase I testing, it will be important to plan future trials evaluating efficacy in patients suffering from neurologic illness.

GSDMD activation downstream of inflammasome signaling leads to release of IL-1 family cytokines and pyroptosis. In theory, inhibition of GSDMD would block both cytokine and DAMP release downstream of NLRP3 or other inflammasomes. Recently, several groups have discovered GSDMD inhibitors (Hu et al,²¹⁸; Humphries et al,²¹⁹; Rathkey et al,²²⁰). All these compounds—disulfiram, dimethyl-fumarate (DMF), and necrosulfonamide (NSA)—are FDA approved for other indications and have excellent safety profiles. One such molecule, DMF also targets Nrf2 pathway and is currently used to treat MS. DMF reduces GSDMD activation and slows disease progression in the EAE mouse model of MS (Humphries et al, 2020). Whether these molecules have efficacy in preclinical ALS models is currently untested. Further, these molecules are non-selective cysteine reactive compounds that have many targets in vivo. For example, NSA blocks GSDMD but also targets the pore-forming molecule MLKL (Rathkey et al,²²⁰; Samson et al,²²¹). Strategies that selectively target GSDMD are in their infancy but may represent exciting new avenues for patients suffering from neurologic disease.

7.4 | Targeting necroptosis

Genetic knockout or pharmacological inhibition of RIPK1 has produced neuroprotective outcomes in some preclinical mouse models of ALS (SOD1 G93A and Optn ^{-/-}). The BBB penetrant RIPK1 inhibitor, DNL747 (Denali/Sanofi), has progressed through phase I trials in ALS patients. A modified successor compound, DNL788, is currently being tested in a phase II HIMALAYA trial. Given the controversy surrounding the preclinical efficacy of RIPK inhibition in mouse models, results in these trials may have definitive implications for the utility of targeting necroptosis in neurodegenerative disease.

8 | CONCLUSION: OPEN QUESTIONS

Studies mapping the contribution of programmed necrotic mechanisms and subsequent immune activation to neurodegenerative diseases including ALS are still in their infancy. Many questions remain regarding the cell types involved, precise signaling mechanisms and whether inhibition of these pathways can alter disease progression.

Focusing on ALS, we have highlighted evidence implicating these PCD pathways in disease pathophysiology. However, almost no studies have examined whether multiple pathways are concurrently activated in the same cell, and the extent to which these mechanisms are compensatory in the CNS. For example, recent work has shown that a delicate balance exists between apoptosis and pyroptosis in peripheral immune cells. In macrophages that are deficient in GSDMD (the executioner of pyroptosis), inflammatory caspase-1 can activate caspase-3 and lead to apoptosis.²²² Other work has shown that in pyroptotic cells, activated GSDMD can puncture mitochondrial membranes, causing release of mtDNA, cyt-c, and apoptosis.^{223,224} Proteases such as caspase-8 exert control over multiple cell death pathways—negatively regulating necroptosis, promoting caspase-3 processing and apoptosis, and cleaving GSDMD to release its pyroptotic N-terminal domain.^{24,58,225}

How is this cell death crosstalk preserved in glia and motor neurons? Can one pathway compensate for another downstream of challenge with an ALS-associated protein? Would therapeutic strategies need to block multiple PCD signaling nodes to spare a motor neuron or limit neuroinflammation? Future studies interrogating multiple cell death pathways in a cell-type-specific manner may start to address these issues. Recent work has shown that ALS-associated proteins like TDP-43 can engage innate immune signaling, such as the cGAS-STING pathway, in neurons and glia.²²⁶ How do these innate immune molecules (eg, cGAS-STING and MAVs) influence cell death decisions and promote neuroinflammation? Do they sit upstream or downstream of PCD mechanisms? Recent advances in generating iPSC-derived neurons and glial cells, as well as traditional conditional knockout approaches, are helpful tools to dissect this complicated biology.

Characterizing the immunologic sequelae of different cell death pathways in the CNS is another exciting frontier. What does a dying neuron release, and how are these products sensed by the immune system? Do pyroptotic, necroptotic, or ferroptotic cells have distinct CNS “secretomes”? These questions are of basic and translational interest. The types of molecules released downstream of PCD induction may play important biological roles in signaling to the immune system (ie, DAMPs and alarmins) or in promoting neurodegeneration. Spread of aggregated proteins, such as alpha-synuclein and TDP-43, are hallmarks of neurodegenerative disease. The mechanisms by which these aggregates traverse the CNS are still poorly understood. Could activation of pore-forming molecules (eg, GSDMs and MLKL) and induction of necrotic PCD amplify the spread of these protein species from dying to healthy neurons? Future work using cell death deficient animals and examining distribution and burden of TDP-43 or other protein aggregates (eg, alpha-synuclein or tau) in models of

neurodegeneration may shed light on this point. Studies constructing molecular “fingerprints” for different cell death pathways may also aid in biomarker discovery. Detection of neurofilament-light (Nf-L) in the CSF has recently been shown to correlate well with disease progression and prognosis in ALS and neurodegeneration.²²⁵ Though this intracellular neuronal protein is presumably released during motor neuron death, identification other proteins or metabolites may offer greater resolution in measuring treatment response and disease progression.

Cell death is a defining event in both normal development and disease pathophysiology. Advances in innate immunology, and cell death have mapped novel PCD pathways that are now implicated in ALS and neurodegeneration more broadly. Examining these pathways in detail may offer novel therapeutic strategies for patients and give biologists a greater understanding of how cells in the brain live and die.

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CONFLICT OF INTERESTS

IMC serves on scientific advisory boards of GSK pharmaceuticals and Limm therapeutics. His laboratory also receives sponsored research support from Abbvie/Allergan pharmaceuticals.

DATA AVAILABILITY STATEMENT

We do not have any data available.

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REFERENCES

- Nelson CE, Morgan BA, Burke AC, et al. Analysis of Hox gene expression in the chick limb bud. *Development (Cambridge, England)*. 1996;122(5):1449-1466. doi:10.1242/dev.122.5.1449
- Zhu J, Nakamura E, Nguyen MT, Bao X, Akiyama H, Mackem S. Uncoupling sonic hedgehog control of pattern and expansion of the developing limb bud. *Dev Cell*. 2008;14:624-632. doi:10.1016/j.devcel.2008.01.008
- Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer*. 1972;26(4):239-257. doi:10.1038/bjc.1972.33
- Kuan CY, Roth KA, Flavell RA, Rakic P. Mechanisms of programmed cell death in the developing brain. *Trends Neurosci*. 2000;23:291-297. doi:10.1016/s0166-2236(00)01581-2
- Peter R. H. Synaptic density in human frontal cortex — developmental changes and effects of aging. *Brain Res*. 1979;163(2):195-205. doi:10.1016/0006-8993(79)90349-4
- Honarpour N, Du C, Richardson JA, Hammer RE, Wang X, Herz J. Adult Apaf-1-deficient mice exhibit male infertility. *Dev Biol*. 2000;218:248-258. doi:10.1006/dbio.1999.9585
- Kuida K, Haydar TF, Kuan C-Y, et al. Reduced apoptosis and cytochrome c-mediated caspase activation in mice lacking caspase 9. *Cell*. 1998;94(3):325-337. doi:10.1016/S0092-8674(00)81476-2
- Nijhawani D, Honarpour N, Wang X. Apoptosis in neural development and disease. *Annu Rev Neurosci*. 2000;23:73-87. doi:10.1146/annurev.neuro.23.1.73
- Gan L, Cookson MR, Petrucelli L, la Spada AR. Converging pathways in neurodegeneration, from genetics to mechanisms. *Nat Neurosci*. 2018;21:1300-1309. doi:10.1038/s41593-018-0237-7
- Hardiman O, Al-Chalabi A, Chio A, et al. Amyotrophic lateral sclerosis. *Nat Rev Dis Primers*. 2017;3:17071. doi:10.1038/nrdp.2017.71
- Bock FJ, Tait SWG. Mitochondria as multifaceted regulators of cell death. *Nat Rev Mol Cell Biol*. 2020;21:85-100. doi:10.1038/s41580-019-0173-8
- Murali Mahadevan H, Hashemiaghdam A, Ashrafi G, Harbauer AB. Mitochondria in neuronal health: from energy metabolism to Parkinson's disease. *Adv Biol*. 2021;5:2100663. doi:10.1002/adbi.202100663
- Uttara B, Singh AV, Zamboni P, Mahajan RT. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Curr Neuropharmacol*. 2009;7(1):65-74. doi:10.2174/157015909787602823
- Pasinelli P, Houseweart MK, Brown RH, Cleveland DW. Caspase-1 and -3 are sequentially activated in motor neuron death in Cu, Zn superoxide dismutase-mediated familial amyotrophic lateral sclerosis. *Proc Natl Acad Sci USA*. 2000;97(25):13901-13906. doi:10.1073/pnas.240305897
- Kwon HS, Koh SH. Neuroinflammation in neurodegenerative disorders: the roles of microglia and astrocytes. *Transl Neurodegener*. 2020a;9:42. doi:10.1186/s40035-020-00221-2
- Liu J, Wang F. Role of neuroinflammation in amyotrophic lateral sclerosis: cellular mechanisms and therapeutic implications. *Front Immunol*. 2017;8:1005. doi:10.3389/fimmu.2017.01005
- McGeer EG, McGeer PL. Neuroinflammation in Alzheimer's disease and mild cognitive impairment: a field in its infancy. *J Alzheimers Dis*. 2010;19:355-361. doi:10.3233/JAD-2010-1219
- McGeer PL, McGeer EG. Inflammation and neurodegeneration in Parkinson's disease. *Parkinsonism Relat Disord*. 2004;10:S3-S7.
- Texidó L, Hernández S, Martín-Satué M, et al. Sera from amyotrophic lateral sclerosis patients induce the non-canonical activation of NMDA receptors “in vitro”. *Neurochem Int*. 2011;59:954-964. doi:10.1016/j.neuint.2011.07.006
- Birge RB, Ucker DS. Innate apoptotic immunity: the calming touch of death. *Cell Death Differ*. 2008;15:1096-1102. doi:10.1038/cdd.2008.58
- Szondy Z, Sarang Z, Kiss B, Garabuczi É, Köröskényi K. Anti-inflammatory mechanisms triggered by apoptotic cells during their clearance. *Front Immunol*. 2017;8:909. doi:10.3389/fimmu.2017.00909
- Green DR. The cell's dilemma, or the story of cell death: an entertainment in three acts. *FEBS J*. 2016;283:2568-2576. doi:10.1111/febs.13658
- Heckmann BL, Tummers B, Green DR. Crashing the computer: apoptosis vs. necroptosis in neuroinflammation. *Cell Death Differ*. 2019;26:41-52. doi:10.1038/s41418-018-0195-3
- Yuan J, Amin P, Ofengeim D. Necroptosis and RIPK1-mediated neuroinflammation in CNS diseases. *Nat Rev Neurosci*. 2019;20:19-33. doi:10.1038/s41583-018-0093-1
- Masrori P, van Damme P. Amyotrophic lateral sclerosis: a clinical review. *Eur J Neurol*. 2020;27:1918-1929. doi:10.1111/ene.14393
- Al-Chalabi A, van den Berg LH, Veldink J. Gene discovery in amyotrophic lateral sclerosis: implications for clinical management. *Nat Rev Neurol*. 2017;13:96-104. doi:10.1038/nrneurol.2016.182
- Ferraiuolo L, Kirby J, Grierson AJ, Sendtner M, Shaw PJ. Molecular pathways of motor neuron injury in amyotrophic lateral

- sclerosis. *Nat Rev Neurol*. 2011;7:616-630. doi:[10.1038/nrneuro.2011.152](https://doi.org/10.1038/nrneuro.2011.152)
28. Bensimon G, Lacomblez L, Meininger V. A Controlled trial of riluzole in amyotrophic lateral sclerosis. *N Engl J Med*. 1994;330:585-591. doi:[10.1056/NEJM199403033300901](https://doi.org/10.1056/NEJM199403033300901)
 29. Abe K, Aoki M, Tsuji S, et al. Safety and efficacy of edaravone in well defined patients with amyotrophic lateral sclerosis: a randomised, double-blind, placebo-controlled trial. *Lancet Neurol*. 2017;16:505-512. doi:[10.1016/S1474-4422\(17\)30115-1](https://doi.org/10.1016/S1474-4422(17)30115-1)
 30. Vu M, Tortorice K, Zacher J, et al. Assessment of use and safety of edaravone for amyotrophic lateral sclerosis in the veterans affairs health care system. *JAMA Netw Open*. 2020;3:e2014645. doi:[10.1001/jamanetworkopen.2020.14645](https://doi.org/10.1001/jamanetworkopen.2020.14645)
 31. Kwon HS, Koh SH. Neuroinflammation in neurodegenerative disorders: the roles of microglia and astrocytes. *Transl Neurodegener*. 2020b;9:42. doi:[10.1186/s40035-020-00221-2](https://doi.org/10.1186/s40035-020-00221-2)
 32. Lee J, Hyeon SJ, Im H, Ryu H, Kim Y, Ryu H. Astrocytes and microglia as non-cell autonomous players in the pathogenesis of ALS. *Exp Neurobiol*. 2016;25:233-240. doi:[10.5607/en.2016.25.5.233](https://doi.org/10.5607/en.2016.25.5.233)
 33. Philips T, Robberecht W. Neuroinflammation in amyotrophic lateral sclerosis: role of glial activation in motor neuron disease. *Lancet Neurol*. 2011;10:253-263. doi:[10.1016/S1474-4422\(11\)70015-1](https://doi.org/10.1016/S1474-4422(11)70015-1)
 34. Sussmuth S, Brettschneider J, Ludolph A, Tumani H. Biochemical markers in CSF of ALS patients. *Curr Med Chem*. 2008;15:1788-1801. doi:[10.2174/092986708785133031](https://doi.org/10.2174/092986708785133031)
 35. Volpe CMO, Nogueira-Machado JA. Is innate immunity and inflammasomes involved in pathogenesis of Amyotrophic Lateral Sclerosis (ALS)? *Recent Pat Endocr Metab Immune Drug Discov*. 2015;9:40-45. doi:[10.2174/1872214809666150407111420](https://doi.org/10.2174/1872214809666150407111420)
 36. Komine O, Yamanaka K. Neuroinflammation in motor neuron disease. *Nagoya J Med Sci*. 2015;77(4):537-549.
 37. Chiu IM, Morimoto ETA, Goodarzi H, et al. A neurodegeneration-specific gene-expression signature of acutely isolated microglia from an amyotrophic lateral sclerosis mouse model. *Cell Rep*. 2013;4:385-401. doi:[10.1016/j.celrep.2013.06.018](https://doi.org/10.1016/j.celrep.2013.06.018)
 38. Clarke BE, Patani R. The microglial component of amyotrophic lateral sclerosis. *Brain*. 2020;143:3526-3539. doi:[10.1093/brain/awaa309](https://doi.org/10.1093/brain/awaa309)
 39. Spiller KJ, Restrepo CR, Khan T, et al. Microglia-mediated recovery from ALS-relevant motor neuron degeneration in a mouse model of TDP-43 proteinopathy. *Nat Neurosci*. 2018;21:329-340. doi:[10.1038/s41593-018-0083-7](https://doi.org/10.1038/s41593-018-0083-7)
 40. Xie M, Liu YU, Zhao S, et al. TREM2 interacts with TDP-43 and mediates microglial neuroprotection against TDP-43-related neurodegeneration. *Nat Neurosci*. 2022;25:26-38. doi:[10.1038/s41593-021-00975-6](https://doi.org/10.1038/s41593-021-00975-6)
 41. Rosen DR, Siddique T, Patterson D, et al. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature*. 1993;362:59-62. doi:[10.1038/362059a0](https://doi.org/10.1038/362059a0)
 42. Gurney ME, Pu H, Chiu AY, et al. Motor neuron degeneration in mice that express a human Cu, Zn superoxide dismutase mutation. *Science*. 1994;1979(264):1772-1775. doi:[10.1126/science.8209258](https://doi.org/10.1126/science.8209258)
 43. Boillée S, Yamanaka K, Lobsiger CS, et al. Onset and progression in inherited ALS determined by motor neurons and microglia. *Science*. 2006;312:1389-1392. doi:[10.1126/science.1123511](https://doi.org/10.1126/science.1123511)
 44. Walker AK, Spiller KJ, Ge G, et al. Functional recovery in new mouse models of ALS/FTLD after clearance of pathological cytoplasmic TDP-43. *Acta Neuropathol*. 2015;130:643-660. doi:[10.1007/s00401-015-1460-x](https://doi.org/10.1007/s00401-015-1460-x)
 45. Wils H, Kleinberger G, Janssens J, et al. TDP-43 transgenic mice develop spastic paralysis and neuronal inclusions characteristic of ALS and frontotemporal lobar degeneration. *Proc Natl Acad Sci USA*. 2010;107:3858-3863. doi:[10.1073/pnas.0912417107](https://doi.org/10.1073/pnas.0912417107)
 46. Xu Y-F, Zhang Y-J, Lin W-L, et al. Expression of mutant TDP-43 induces neuronal dysfunction in transgenic mice. *Mol Neurodegener*. 2011;6:73. doi:[10.1186/1750-1326-6-73](https://doi.org/10.1186/1750-1326-6-73)
 47. Zhao W, Beers DR, Bell S, et al. TDP-43 activates microglia through NF- κ B and NLRP3 inflammasome. *Exp Neurol*. 2015;273:24-35. doi:[10.1016/j.expneurol.2015.07.019](https://doi.org/10.1016/j.expneurol.2015.07.019)
 48. Gerbino V, Kaunga E, Ye J, et al. The loss of TBK1 kinase activity in motor neurons or in all cell types differentially impacts ALS disease progression in SOD1 mice. *Neuron*. 2020;106:789-805.e5. doi:[10.1016/j.neuron.2020.03.005](https://doi.org/10.1016/j.neuron.2020.03.005)
 49. Deora V, Lee JD, Albornoz EA, et al. The microglial NLRP3 inflammasome is activated by amyotrophic lateral sclerosis proteins. *Glia*. 2020;68:407-421. doi:[10.1002/glia.23728](https://doi.org/10.1002/glia.23728)
 50. Johann S, Heitzer M, Kanagaratnam M, et al. NLRP3 inflammasome is expressed by astrocytes in the SOD1 mouse model of ALS and in human sporadic ALS patients. *Glia*. 2015;63:2260-2273. doi:[10.1002/glia.22891](https://doi.org/10.1002/glia.22891)
 51. de Marco G, Lomartire A, Mandili G, et al. Reduced cellular Ca²⁺ availability enhances TDP-43 cleavage by apoptotic caspases. *Biochim Biophys Acta Mol Cell Res*. 2014;1843:725-734. doi:[10.1016/j.bbamcr.2014.01.010](https://doi.org/10.1016/j.bbamcr.2014.01.010)
 52. Roberts K, Zeineddine R, Corcoran L, Li W, Campbell IL, Yerbury JJ. Extracellular aggregated Cu/Zn superoxide dismutase activates microglia to give a cytotoxic phenotype. *Glia*. 2013;61:409-419. doi:[10.1002/glia.22444](https://doi.org/10.1002/glia.22444)
 53. Vogt MA, Ehsaei Z, Knuckles P, et al. TDP-43 induces p53-mediated cell death of cortical progenitors and immature neurons. *Sci Rep*. 2018;8:8097. doi:[10.1038/s41598-018-26397-2](https://doi.org/10.1038/s41598-018-26397-2)
 54. Maor-Nof M, Shipony Z, Lopez-Gonzalez R, et al. p53 is a central regulator driving neurodegeneration caused by C9orf72 poly(PR). *Cell*. 2021;184:689-708.e20. doi:[10.1016/j.cell.2020.12.025](https://doi.org/10.1016/j.cell.2020.12.025)
 55. Zhu Q, Jiang J, Gendron TF, et al. Reduced C9ORF72 function exacerbates gain of toxicity from ALS/FTD-causing repeat expansion in C9orf72. *Nat Neurosci*. 2020;23:615-624. doi:[10.1038/s41593-020-0619-5](https://doi.org/10.1038/s41593-020-0619-5)
 56. Boada-Romero E, Martinez J, Heckmann BL, Green DR. The clearance of dead cells by efferocytosis. *Nat Rev Mol Cell Biol*. 2020;21:398-414. doi:[10.1038/s41580-020-0232-1](https://doi.org/10.1038/s41580-020-0232-1)
 57. Green DR, Oguin TH, Martinez J. The clearance of dying cells: table for two. *Cell Death Differ*. 2016;23:915-926. doi:[10.1038/cdd.2015.172](https://doi.org/10.1038/cdd.2015.172)
 58. Degterev A, Boyce M, Yuan J. A decade of caspases. *Oncogene*. 2003;22:8543-8567. doi:[10.1038/sj.onc.1207107](https://doi.org/10.1038/sj.onc.1207107)
 59. Julien O, Wells JA. Caspases and their substrates. *Cell Death Differ*. 2017;24:1380-1389. doi:[10.1038/cdd.2017.44](https://doi.org/10.1038/cdd.2017.44)
 60. Yuan J. The *C elegans* cell death gene ced-3 encodes a protein similar to mammalian interleukin-1 β -converting enzyme. *Cell*. 1993;75:641-652. doi:[10.1016/0092-8674\(93\)90485-9](https://doi.org/10.1016/0092-8674(93)90485-9)
 61. Li P, Nijhawan D, Budihardjo I, et al. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell*. 1997;91:479-489. doi:[10.1016/S0092-8674\(00\)80434-1](https://doi.org/10.1016/S0092-8674(00)80434-1)
 62. Kluck RM, Bossy-Wetzel E, Green DR, Newmeyer DD. The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis. *Science*. 1997;275:1132-1136. doi:[10.1126/science.275.5303.1132](https://doi.org/10.1126/science.275.5303.1132)
 63. Deckwerth TL, Elliott JL, Knudson CM, Johnson EM, Snider WD, Korsmeyer SJ. BAX is required for neuronal death after trophic factor deprivation and during development. *Neuron*. 1996;17:401-411. doi:[10.1016/S0896-6273\(00\)80173-7](https://doi.org/10.1016/S0896-6273(00)80173-7)
 64. Mesner PW, Winters TR, Green SH. Nerve growth factor withdrawal-induced cell death in neuronal PC12 cells resembles that in sympathetic neurons. *J Cell Biol*. 1992;119:1669-1680. doi:[10.1083/jcb.119.6.1669](https://doi.org/10.1083/jcb.119.6.1669)
 65. Neame SJ, Rubin LL, Philpott KL. Blocking cytochrome c activity within intact neurons inhibits apoptosis. *J Cell Biol*. 1998;142(6):1583-1593. doi:[10.1083/jcb.142.6.1583](https://doi.org/10.1083/jcb.142.6.1583)
 66. Maas C, Verbrugge I, de Vries E, et al. Smac/DIABLO release from mitochondria and XIAP inhibition are essential to limit clonogenicity

- of Type I tumor cells after TRAIL receptor stimulation. *Cell Death Differ.* 2010;17:1613-1623. doi:[10.1038/cdd.2010.39](https://doi.org/10.1038/cdd.2010.39)
67. Suzuki Y, Takahashi-Niki K, Akagi T, Hashikawa T, Takahashi R. Mitochondrial protease Omi/HtrA2 enhances caspase activation through multiple pathways. *Cell Death Differ.* 2004;11:208-216. doi:[10.1038/sj.cdd.4401343](https://doi.org/10.1038/sj.cdd.4401343)
 68. Vucic D, Deshayes K, Ackerly H, et al. SMAC negatively regulates the anti-apoptotic activity of melanoma inhibitor of apoptosis (ML-IAP). *J Biol Chem.* 2002;277:12275-12279. doi:[10.1074/jbc.M112045200](https://doi.org/10.1074/jbc.M112045200)
 69. Galehdar Z, Swan P, Fuerth B, Callaghan SM, Park DS, Cregan SP. Neuronal apoptosis induced by endoplasmic reticulum stress is regulated by ATF4-CHOP-mediated induction of the Bcl-2 homology 3-only member PUMA. *J Neurosci.* 2010;30:16938-16948. doi:[10.1523/JNEUROSCI.1598-10.2010](https://doi.org/10.1523/JNEUROSCI.1598-10.2010)
 70. Baleriola J, Walker CA, Jean YY, et al. Axonally synthesized ATF4 transmits a neurodegenerative signal across brain regions. *Cell.* 2014;158:1159-1172. doi:[10.1016/j.cell.2014.07.001](https://doi.org/10.1016/j.cell.2014.07.001)
 71. Fessler E, Eckl E-M, Schmitt S, et al. A pathway coordinated by DELE1 relays mitochondrial stress to the cytosol. *Nature.* 2020;579:433-437. doi:[10.1038/s41586-020-2076-4](https://doi.org/10.1038/s41586-020-2076-4)
 72. Guo X, Aviles G, Liu Y, et al. Mitochondrial stress is relayed to the cytosol by an OMA1-DELE1-HRI pathway. *Nature.* 2020;579:427-432. doi:[10.1038/s41586-020-2078-2](https://doi.org/10.1038/s41586-020-2078-2)
 73. Brunner T, Mogil RJ, LaFace D, et al. Cell-autonomous Fas (CD95)/Fas-ligand interaction mediates activation-induced apoptosis in T-cell hybridomas. *Nature.* 1995;373:441-444. doi:[10.1038/373441a0](https://doi.org/10.1038/373441a0)
 74. Czabotar PE, Lessene G, Strasser A, Adams JM. Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy. *Nat Rev Mol Cell Biol.* 2014;15:49-63. doi:[10.1038/nrm3722](https://doi.org/10.1038/nrm3722)
 75. Kalkavan H, Green DR. MOMP, cell suicide as a BCL-2 family business. *Cell Death Differ.* 2018;25:46-55. doi:[10.1038/cdd.2017.179](https://doi.org/10.1038/cdd.2017.179)
 76. Du C, Fang M, Li Y, Li L, Wang X. Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition. *Cell.* 2000;102:33-42. doi:[10.1016/S0092-8674\(00\)00008-8](https://doi.org/10.1016/S0092-8674(00)00008-8)
 77. Muñoz-Pinedo C, Guío-Carrión A, Goldstein JC, Fitzgerald P, Newmeyer DD, Green DR. Different mitochondrial intermembrane space proteins are released during apoptosis in a manner that is coordinately initiated but can vary in duration. *Proc Natl Acad Sci USA.* 2006;103(31):11573-11578. doi:[10.1073/pnas.0603007103](https://doi.org/10.1073/pnas.0603007103)
 78. D'Amelio M, Cavallucci V, Cecconi F. Neuronal caspase-3 signaling: not only cell death. *Cell Death Differ.* 2010;17:1104-1114. doi:[10.1038/cdd.2009.180](https://doi.org/10.1038/cdd.2009.180)
 79. Eldadah BA, Faden AL. Caspase pathways, neuronal apoptosis, and CNS injury. *J Neurotrauma.* 2000;17:811-829. doi:[10.1089/neu.2000.17.811](https://doi.org/10.1089/neu.2000.17.811)
 80. Bertheloot D, Latz E, Franklin BS. Necroptosis, pyroptosis and apoptosis: an intricate game of cell death. *Cell Mol Immunol.* 2021;18:1106-1121. doi:[10.1038/s41423-020-00630-3](https://doi.org/10.1038/s41423-020-00630-3)
 81. Hamon Y, Broccardo C, Chambenoit O, et al. ABC1 promotes engulfment of apoptotic cells and transbilayer redistribution of phosphatidylserine. *Nat Cell Biol.* 2000;2:399-406. doi:[10.1038/35017029](https://doi.org/10.1038/35017029)
 82. Ravichandran KS. Find-me and eat-me signals in apoptotic cell clearance: progress and conundrums. *J Exp Med.* 2010;207:1807-1817. doi:[10.1084/jem.20101157](https://doi.org/10.1084/jem.20101157)
 83. Reddien PW, Horvitz HR. CED-2/CrkII and CED-10/Rac control phagocytosis and cell migration in *Caenorhabditis elegans*. *Nat Cell Biol.* 2000;2:131-136. doi:[10.1038/35004000](https://doi.org/10.1038/35004000)
 84. Wu YC, Horvitz HR. C. elegans phagocytosis and cell-migration protein CED-5 is similar to human DOCK180. *Nature.* 1998;392:501-504. doi:[10.1038/33163](https://doi.org/10.1038/33163)
 85. Asano K, Miwa M, Miwa K, et al. Masking of phosphatidylserine inhibits apoptotic cell engulfment and induces autoantibody production in mice. *J Exp Med.* 2004;200:459-467. doi:[10.1084/jem.20040342](https://doi.org/10.1084/jem.20040342)
 86. Krahling S, Callahan MK, Williamson P, Schlegel RA. Exposure of phosphatidylserine is a general feature in the phagocytosis of apoptotic lymphocytes by macrophages. *Cell Death Differ.* 1999;6:183-189. doi:[10.1038/sj.cdd.4400473](https://doi.org/10.1038/sj.cdd.4400473)
 87. Fadok VA, Bratton DL, Konowal A, Freed PW, Westcott JY, Henson PM. Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. *J Clin Invest.* 1998;101:890-898. doi:[10.1172/JCI1112](https://doi.org/10.1172/JCI1112)
 88. Neher JJ, Emmrich JV, Fricker M, Mander PK, Théry C, Brown GC. Phagocytosis executes delayed neuronal death after focal brain ischemia. *Proc Natl Acad Sci USA.* 2013;110:E4098-E4107. doi:[10.1073/pnas.1308679110](https://doi.org/10.1073/pnas.1308679110)
 89. Scott RS, McMahon EJ, Pop SM, et al. Phagocytosis and clearance of apoptotic cells is mediated by MER. *Nature.* 2001;411:207-211. doi:[10.1038/35075603](https://doi.org/10.1038/35075603)
 90. Tufail Y, Cook D, Fourgeaud L, et al. Phosphatidylserine exposure controls viral innate immune responses by microglia. *Neuron.* 2017;93:574-586.e8. doi:[10.1016/j.neuron.2016.12.021](https://doi.org/10.1016/j.neuron.2016.12.021)
 91. Brown GC, Neher JJ. Eaten alive! Cell death by primary phagocytosis: "phagoptosis". *Trends Biochem Sci.* 2012;37:325-332. doi:[10.1016/j.tibs.2012.05.002](https://doi.org/10.1016/j.tibs.2012.05.002)
 92. Nomura K, Vilalta A, Allendorf DH, Hornik TC, Brown GC. Activated microglia desialylate and phagocytose cells via neuraminidase, galectin-3, and mer tyrosine kinase. *J Immunol.* 2017;198:4792-4801. doi:[10.4049/jimmunol.1502532](https://doi.org/10.4049/jimmunol.1502532)
 93. Kao AW, Eisenhut RJ, Martens LH, et al. A neurodegenerative disease mutation that accelerates the clearance of apoptotic cells. *Proc Natl Acad Sci USA.* 2011;108:4441-4446. doi:[10.1073/pnas.1100650108](https://doi.org/10.1073/pnas.1100650108)
 94. Yin F, Banerjee R, Thomas B, et al. Exaggerated inflammation, impaired host defense, and neuropathology in progranulin-deficient mice. *J Exp Med.* 2010;207:117-128. doi:[10.1084/jem.20091568](https://doi.org/10.1084/jem.20091568)
 95. Medina CB, Mehrotra P, Arandjelovic S, et al. Metabolites released from apoptotic cells act as tissue messengers. *Nature.* 2020;580:130-135. doi:[10.1038/s41586-020-2121-3](https://doi.org/10.1038/s41586-020-2121-3)
 96. Tanzer MC, Frauenstein A, Stafford CA, Phulphagar K, Mann M, Meissner F. Quantitative and dynamic catalogs of proteins released during apoptotic and necroptotic cell death. *Cell Rep.* 2020;30:1260-1270.e5. doi:[10.1016/j.celrep.2019.12.079](https://doi.org/10.1016/j.celrep.2019.12.079)
 97. González de Aguilar JL, Gordon JW, René F, et al. Alteration of the Bcl-x/Bax ratio in a transgenic mouse model of amyotrophic lateral sclerosis: evidence for the implication of the p53 signaling pathway. *Neurobiol Dis.* 2000;7:406-415. doi:[10.1006/nbdi.2000.0295](https://doi.org/10.1006/nbdi.2000.0295)
 98. Pasinelli P, Belford ME, Lennon N, et al. Amyotrophic lateral sclerosis-associated SOD1 mutant proteins bind and aggregate with Bcl-2 in spinal cord mitochondria. *Neuron.* 2004;43:19-30. doi:[10.1016/j.neuron.2004.06.021](https://doi.org/10.1016/j.neuron.2004.06.021)
 99. Ekegren T, Grundström E, Lindholm D, Aquilonius SM. Upregulation of Bax protein and increased DNA degradation in ALS spinal cord motor neurons. *Acta Neurol Scand.* 1999;100:317-321. doi:[10.1111/j.1600-0404.1999.tb00403.x](https://doi.org/10.1111/j.1600-0404.1999.tb00403.x)
 100. Hetz C, Thielen P, Fisher J, et al. The proapoptotic BCL-2 family member BIM mediates motoneuron loss in a model of amyotrophic lateral sclerosis. *Cell Death Differ.* 2007;14:1386-1389. doi:[10.1038/sj.cdd.4402166](https://doi.org/10.1038/sj.cdd.4402166)
 101. Coque E, Salsac C, Espinosa-Carrasco G, et al. Cytotoxic CD8+ T lymphocytes expressing ALS-causing SOD1 mutant selectively trigger death of spinal motoneurons. *Proc Natl Acad Sci USA.* 2019;116:2312-2317. doi:[10.1073/pnas.1815961116](https://doi.org/10.1073/pnas.1815961116)

102. Maier A, Deigendesch N, Müller K, et al. Interleukin-1 antagonist anakinra in amyotrophic lateral sclerosis—a pilot study. *PLoS One*. 2015;10:e0139684. doi:[10.1371/journal.pone.0139684](https://doi.org/10.1371/journal.pone.0139684)
103. McCauley ME, Baloh RH. Inflammation in ALS/FTD pathogenesis. *Acta Neuropathol*. 2019;137:715-730. doi:[10.1007/s00401-018-1933-9](https://doi.org/10.1007/s00401-018-1933-9)
104. Meissner F, Molawi K, Zychlinsky A. Mutant superoxide dismutase 1-induced IL-1 β accelerates ALS pathogenesis. *Proc Natl Acad Sci USA*. 2010;107:13046-13050. doi:[10.1073/pnas.1002396107](https://doi.org/10.1073/pnas.1002396107)
105. Gugliandolo A, Giacompo S, Bramanti P, Mazzon E. NLRP3 inflammasome activation in a transgenic amyotrophic lateral sclerosis model. *Inflammation*. 2018;41:93-103. doi:[10.1007/s10753-017-0667-5](https://doi.org/10.1007/s10753-017-0667-5)
106. Quek H, Cuní-López C, Stewart R, et al. ALS monocyte-derived microglia-like cells reveal cytoplasmic TDP-43 accumulation, DNA damage, and cell-specific impairment of phagocytosis associated with disease progression. *J Neuroinflammation*. 2022;19(1):58. doi:[10.1186/s12974-022-02421-1](https://doi.org/10.1186/s12974-022-02421-1)
107. Heitzer M, Kaiser S, Kanagaratnam M, et al. Administration of 17 β -estradiol improves motoneuron survival and down-regulates inflammasome activation in male SOD1(G93A) ALS mice. *Mol Neurobiol*. 2017;54:8429-8443. doi:[10.1007/s12035-016-0322-4](https://doi.org/10.1007/s12035-016-0322-4)
108. Lehmann S, Esch E, Hartmann P, et al. Expression profile of pattern recognition receptors in skeletal muscle of SOD1(G93A) amyotrophic lateral sclerosis (ALS) mice and sporadic ALS patients. *Neuropathol Appl Neurobiol*. 2018;44:606-627. doi:[10.1111/nan.12483](https://doi.org/10.1111/nan.12483)
109. Cirulli ET, Lasseigne BN, Petrovski S, et al. Exome sequencing in amyotrophic lateral sclerosis identifies risk genes and pathways. *Science*. 2015;347:1436-1441. doi:[10.1126/science.aaa3650](https://doi.org/10.1126/science.aaa3650)
110. Freischmidt A, Müller K, Ludolph AC, Weishaupt JH, Andersen PM. Association of mutations in TBK1 with sporadic and familial amyotrophic lateral sclerosis and frontotemporal dementia. *JAMA Neurol*. 2017;74:110-113. doi:[10.1001/jamaneurol.2016.3712](https://doi.org/10.1001/jamaneurol.2016.3712)
111. Maruyama H, Morino H, Ito H, et al. Mutations of optineurin in amyotrophic lateral sclerosis. *Nature*. 2010a;465:223-226. doi:[10.1038/nature08971](https://doi.org/10.1038/nature08971)
112. Ito Y, Ofengeim D, Najafov A, et al. RIPK1 mediates axonal degeneration by promoting inflammation and necroptosis in ALS. *Science*. 2016;353:603-608. doi:[10.1126/science.aaf6803](https://doi.org/10.1126/science.aaf6803)
113. Re DB, le Verche V, Yu C, et al. Necroptosis drives motor neuron death in models of both sporadic and familial ALS. *Neuron*. 2014;81:1001-1008. doi:[10.1016/j.neuron.2014.01.011](https://doi.org/10.1016/j.neuron.2014.01.011)
114. Dermentzaki G, Politi KA, Lu L, et al. Deletion of Ripk3 prevents motor neuron death in vitro but not in vivo. *Eneuro*. 2019;6:ENEURO.0308-18.2018. doi:[10.1523/ENEURO.0308-18.2018](https://doi.org/10.1523/ENEURO.0308-18.2018)
115. Mifflin L, Hu Z, Dufort C, et al. A RIPK1-regulated inflammatory microglial state in amyotrophic lateral sclerosis. *Proc Natl Acad Sci USA*. 2021;118:e2025102118. doi:[10.1073/pnas.2025102118/-/DCSupplemental](https://doi.org/10.1073/pnas.2025102118/-/DCSupplemental)
116. Mesci P, Zaïdi S, Lobsiger CS, et al. System xC⁻ is a mediator of microglial function and its deletion slows symptoms in amyotrophic lateral sclerosis mice. *Brain*. 2015;138:53-68. doi:[10.1093/brain/awu312](https://doi.org/10.1093/brain/awu312)
117. Moreau C, Danel V, Devedjian JC, et al. Could conservative iron chelation lead to neuroprotection in amyotrophic lateral sclerosis? *Antioxid Redox Signal*. 2018;29:742-748. doi:[10.1089/ars.2017.7493](https://doi.org/10.1089/ars.2017.7493)
118. Nardo G, Trolese MC, Verderio M, et al. Counteracting roles of MHCI and CD8⁺ T cells in the peripheral and central nervous system of ALS SOD1G93A mice. *Mol Neurodegener*. 2018;13:42. doi:[10.1186/s13024-018-0271-7](https://doi.org/10.1186/s13024-018-0271-7)
119. Rolfes L, Schulte-Mecklenbeck A, Schreiber S, et al. Amyotrophic lateral sclerosis patients show increased peripheral and intrathecal T-cell activation. *Brain Commun*. 2021;3:fcab157. doi:[10.1093/braincomms/fcab157](https://doi.org/10.1093/braincomms/fcab157)
120. Marazzi I, Campisi L, Chizari S, et al. Clonally expanded CD8 T cells characterize Amyotrophic Lateral Sclerosis. *Mapp Intimacies*. 2021;4. doi:[10.21203/rs.3.rs-798338/v1](https://doi.org/10.21203/rs.3.rs-798338/v1)
121. Suzuki H, Matsuoka M. Overexpression of nuclear FUS induces neuronal cell death. *Neuroscience*. 2015;287:113-124. doi:[10.1016/j.neuroscience.2014.12.007](https://doi.org/10.1016/j.neuroscience.2014.12.007)
122. Yu H, Cleveland DW. Tuning apoptosis and neuroinflammation: TBK1 restrains RIPK1. *Cell*. 2018;174:1339-1341. doi:[10.1016/j.cell.2018.08.035](https://doi.org/10.1016/j.cell.2018.08.035)
123. Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-1 β . *Mol Cell*. 2002;10:417-426. doi:[10.1016/s1097-2765\(02\)00599-3](https://doi.org/10.1016/s1097-2765(02)00599-3)
124. Broz P, Dixit VM. Inflammasomes: mechanism of assembly, regulation and signalling. *Nat Rev Immunol*. 2016;16:407-420. doi:[10.1038/nri.2016.58](https://doi.org/10.1038/nri.2016.58)
125. Man SM, Karki R, Kanneganti TD. Molecular mechanisms and functions of pyroptosis, inflammatory caspases and inflammasomes in infectious diseases. *Immunol Rev*. 2017;277:61-75. doi:[10.1111/imr.12534](https://doi.org/10.1111/imr.12534)
126. Boucher D, Monteleone M, Coll RC, et al. Caspase-1 self-cleavage is an intrinsic mechanism to terminate inflammasome activity. *J Exp Med*. 2018;215:827-840. doi:[10.1084/jem.20172222](https://doi.org/10.1084/jem.20172222)
127. Broz P, Monack DM. Newly described pattern recognition receptors team up against intracellular pathogens. *Nat Rev Immunol*. 2013;13:551-565. doi:[10.1038/nri3479](https://doi.org/10.1038/nri3479)
128. Kayagaki N, Stowe IB, Lee BL, et al. Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling. *Nature*. 2015;526:666-671. doi:[10.1038/nature15541](https://doi.org/10.1038/nature15541)
129. Liu X, Zhang Z, Ruan J, et al. Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores. *Nature*. 2016;535:153-158. doi:[10.1038/nature18629](https://doi.org/10.1038/nature18629)
130. Shi J, Zhao Y, Wang K, et al. Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. *Nature*. 2015;526:660-665. doi:[10.1038/nature15514](https://doi.org/10.1038/nature15514)
131. Ruan J, Xia S, Liu X, Lieberman J, Wu H. Cryo-EM structure of the gasdermin A3 membrane pore. *Nature*. 2018;557:62-67. doi:[10.1038/s41586-018-0058-6](https://doi.org/10.1038/s41586-018-0058-6)
132. Xia S, Zhang Z, Magupalli VG, et al. Gasdermin D pore structure reveals preferential release of mature interleukin-1. *Nature*. 2021;593:607-611. doi:[10.1038/s41586-021-03478-3](https://doi.org/10.1038/s41586-021-03478-3)
133. Lieberman J, Wu H, Kagan JC. Gasdermin D activity in inflammation and host defense. *Sci Immunol*. 2019;4:1-9. doi:[10.1126/sciimmunol.aav1447](https://doi.org/10.1126/sciimmunol.aav1447)
134. Kuida K, Lippke JA, Ku G, et al. Altered cytokine export and apoptosis in mice deficient in interleukin-1 β converting enzyme. *Science*. 1995;267:2000-2003. doi:[10.1126/science.7535475](https://doi.org/10.1126/science.7535475)
135. Li P, Allen H, Banerjee S, et al. Mice deficient in IL-1 β converting enzyme are defective in production of mature IL-1 β and resistant to endotoxic shock. *Cell*. 1995;80:401-411. doi:[10.1016/0092-8674\(95\)90490-5](https://doi.org/10.1016/0092-8674(95)90490-5)
136. Dinarello CA, Novick D, Kim S, Kaplanski G. Interleukin-18 and IL-18 binding protein. *Front Immunol*. 2013;4:289. doi:[10.3389/fimmu.2013.00289](https://doi.org/10.3389/fimmu.2013.00289)
137. Dombrowski Y, Peric M, Koglin S, et al. Cytosolic DNA triggers inflammasome activation in keratinocytes in psoriatic lesions. *Sci Transl Med*. 2011;3:82ra38. doi:[10.1126/scitranslmed.3002001](https://doi.org/10.1126/scitranslmed.3002001)
138. Eigenbrod T, Park J-H, Harder J, Iwakura Y, Núñez G. Cutting edge: critical role for mesothelial cells in necrosis-induced inflammation through the recognition of IL-1 α released from dying cells. *J Immunol*. 2008;181:8194-8198. doi:[10.4049/jimmunol.181.12.8194](https://doi.org/10.4049/jimmunol.181.12.8194)
139. Lamkanfi M, Sarkar A, Vande Walle L, et al. Inflammasome-dependent release of the alarmin HMGB1 in endotoxemia. *J Immunol*. 2010;185:4385-4392. doi:[10.4049/jimmunol.1000803](https://doi.org/10.4049/jimmunol.1000803)
140. Orzalli MH, Prochera A, Payne L, Smith A, Garlick JA, Kagan JC. Virus-mediated inactivation of anti-apoptotic Bcl-2 family

- members promotes Gasdermin-E-dependent pyroptosis in barrier epithelial cells. *Immunity*. 2021;54:1447-1462.e5. doi:[10.1016/j.immuni.2021.04.012](https://doi.org/10.1016/j.immuni.2021.04.012)
141. Sollberger G, Choidas A, Burn GL, et al. Gasdermin D plays a vital role in the generation of neutrophil extracellular traps. *Sci Immunol*. 2018;3:eaar6689. doi:[10.1126/sciimmunol.aar6689](https://doi.org/10.1126/sciimmunol.aar6689)
 142. Baroja-Mazo A, Martín-Sánchez F, Gomez AI, et al. The NLRP3 inflammasome is released as a particulate danger signal that amplifies the inflammatory response. *Nat Immunol*. 2014;15:738-748. doi:[10.1038/ni.2919](https://doi.org/10.1038/ni.2919)
 143. Franklin BS, Bossaller L, de Nardo D, et al. The adaptor ASC has extracellular and "prionoid" activities that propagate inflammation. *Nat Immunol*. 2014;15:727-737. doi:[10.1038/ni.2913](https://doi.org/10.1038/ni.2913)
 144. Russo AJ, Vasudevan SO, Méndez-Huergo SP, et al. Intracellular immune sensing promotes inflammation via gasdermin D-driven release of a lectin alarmin. *Nat Immunol*. 2021;22:154-165. doi:[10.1038/s41590-020-00844-7](https://doi.org/10.1038/s41590-020-00844-7)
 145. McKenzie BA, Dixit VM, Power C. Fieery cell death: pyroptosis in the central nervous system. *Trends Neurosci*. 2020;43:55-73. doi:[10.1016/j.tins.2019.11.005](https://doi.org/10.1016/j.tins.2019.11.005)
 146. Shi J, Gao W, Shao F. Pyroptosis: gasdermin-mediated programmed necrotic cell death. *Trends Biochem Sci*. 2017;42:245-254. doi:[10.1016/j.tibs.2016.10.004](https://doi.org/10.1016/j.tibs.2016.10.004)
 147. Lammert CR, Frost EL, Bellingier CE, et al. AIM2 inflammasome surveillance of DNA damage shapes neurodevelopment. *Nature*. 2020;580(7805):647-652. doi:[10.1038/s41586-020-2174-3](https://doi.org/10.1038/s41586-020-2174-3)
 148. Voet S, Srinivasan S, Lamkanfi M, Loo G. Inflammasomes in neuroinflammatory and neurodegenerative diseases. *EMBO Mol Med*. 2019;11:e10248. doi:[10.15252/emmm.201810248](https://doi.org/10.15252/emmm.201810248)
 149. Gordon R, Albornoz EA, Christie DC, et al. Inflammasome inhibition prevents -synuclein pathology and dopaminergic neurodegeneration in mice. *Sci Transl Med*. 2018;10:465. doi:[10.1126/scitranslmed.aah4066](https://doi.org/10.1126/scitranslmed.aah4066)
 150. Heneka MT, Kummer MP, Stutz A, et al. NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. *Nature*. 2013;493:674-678. doi:[10.1038/nature11729](https://doi.org/10.1038/nature11729)
 151. Heneka MT, McManus RM, Latz E. Inflammasome signalling in brain function and neurodegenerative disease. *Nat Rev Neurosci*. 2018;19(10):610-621. doi:[10.1038/s41583-018-0055-7](https://doi.org/10.1038/s41583-018-0055-7)
 152. McKenzie BA, Mamik MK, Saito LB, et al. Caspase-1 inhibition prevents glial inflammasome activation and pyroptosis in models of multiple sclerosis. *Proc Natl Acad Sci USA*. 2018;115:E6065-E6074. doi:[10.1073/pnas.1722041115](https://doi.org/10.1073/pnas.1722041115)
 153. Rui W, Xiao H, Fan Y, et al. Systemic inflammasome activation and pyroptosis associate with the progression of amnesic mild cognitive impairment and Alzheimer's disease. *J Neuroinflammation*. 2021;18:280. doi:[10.1186/s12974-021-02329-2](https://doi.org/10.1186/s12974-021-02329-2)
 154. Song L, Pei L, Yao S, Wu Y, Shang Y. NLRP3 inflammasome in neurological diseases, from functions to therapies. *Front Cell Neurosci*. 2017;11:63. doi:[10.3389/fncel.2017.00063](https://doi.org/10.3389/fncel.2017.00063)
 155. Pasinelli P, Borchelt DR, Houseweart MK, Cleveland DW, Brown RH. Caspase-1 is activated in neural cells and tissue with amyotrophic lateral sclerosis-associated mutations in copper-zinc superoxide dismutase. *Proc Natl Acad Sci USA*. 1998;95:15763-15768. doi:[10.1073/pnas.95.26.15763](https://doi.org/10.1073/pnas.95.26.15763)
 156. Adamczak SE, de Rivero Vaccari JP, Dale G, et al. Pyroptotic neuronal cell death mediated by the AIM2 inflammasome. *J Cereb Blood Flow Metab*. 2014;34:621-629. doi:[10.1038/jcbfm.2013.236](https://doi.org/10.1038/jcbfm.2013.236)
 157. Han C, Yang Y, Guan Q, et al. New mechanism of nerve injury in Alzheimer's disease: β -amyloid-induced neuronal pyroptosis. *J Cell Mol Med*. 2020;24:8078-8090. doi:[10.1111/jcmm.15439](https://doi.org/10.1111/jcmm.15439)
 158. Pronin A, Pham D, An W, et al. Inflammasome activation induces pyroptosis in the retina exposed to ocular hypertension injury. *Front Mol Neurosci*. 2019;12:36. doi:[10.3389/fnmol.2019.00036](https://doi.org/10.3389/fnmol.2019.00036)
 159. Degterev A, Huang Z, Boyce M, et al. Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury. *Nat Chem Biol*. 2005;1:112-119. doi:[10.1038/nchembio711](https://doi.org/10.1038/nchembio711)
 160. Degterev A, Hitomi J, Germscheid M, et al. Identification of RIP1 kinase as a specific cellular target of necrostatins. *Nat Chem Biol*. 2008;4:313-321. doi:[10.1038/nchembio.83](https://doi.org/10.1038/nchembio.83)
 161. Morrice JR, Gregory-Evans CY, Shaw CA. Necroptosis in amyotrophic lateral sclerosis and other neurological disorders. *Biochim Biophys Acta Mol Basis Dis*. 2017;1863:347-353. doi:[10.1016/j.bbadis.2016.11.025](https://doi.org/10.1016/j.bbadis.2016.11.025)
 162. Evavold CL, Ruan J, Tan Y, Xia S, Wu H, Kagan JC. The pore-forming protein gasdermin D regulates interleukin-1 secretion from living macrophages. *Immunity*. 2018;48:35-44.e6. doi:[10.1016/j.immuni.2017.11.013](https://doi.org/10.1016/j.immuni.2017.11.013)
 163. Orozco S, Yatim N, Werner MR, et al. RIPK1 both positively and negatively regulates RIPK3 oligomerization and necroptosis. *Cell Death Differ*. 2014;21:1511-1521. doi:[10.1038/cdd.2014.76](https://doi.org/10.1038/cdd.2014.76)
 164. Zhang Y, Su SS, Zhao S, et al. RIP1 autophosphorylation is promoted by mitochondrial ROS and is essential for RIP3 recruitment into necrosome. *Nat Commun*. 2017;8:14329. doi:[10.1038/ncomm14329](https://doi.org/10.1038/ncomm14329)
 165. Murphy JM, Czabotar PE, Hildebrand JM, et al. The pseudokinase MLKL mediates necroptosis via a molecular switch mechanism. *Immunity*. 2013;39:443-453. doi:[10.1016/j.immuni.2013.06.018](https://doi.org/10.1016/j.immuni.2013.06.018)
 166. Sun L, Wang H, Wang Z, et al. Mixed lineage kinase domain-like protein mediates necrosis signaling downstream of RIP3 kinase. *Cell*. 2012;148:213-227. doi:[10.1016/j.cell.2011.11.031](https://doi.org/10.1016/j.cell.2011.11.031)
 167. Zhao J, Jitkaew S, Cai Z, et al. Mixed lineage kinase domain-like is a key receptor interacting protein 3 downstream component of TNF-induced necrosis. *Proc Natl Acad Sci USA*. 2012;109:5322-5327. doi:[10.1073/pnas.1200012109](https://doi.org/10.1073/pnas.1200012109)
 168. Kaiser WJ, Sridharan H, Huang C, et al. Toll-like receptor 3-mediated necrosis via TRIF, RIP3, and MLKL. *J Biol Chem*. 2013;288:31268-31279. doi:[10.1074/jbc.M113.462341](https://doi.org/10.1074/jbc.M113.462341)
 169. Lin J, Kumari S, Kim C, et al. RIPK1 counteracts ZBP1-mediated necroptosis to inhibit inflammation. *Nature*. 2016;540:124-128. doi:[10.1038/nature20558](https://doi.org/10.1038/nature20558)
 170. Newton K, Wickliffe KE, Maltzman A, et al. RIPK1 inhibits ZBP1-driven necroptosis during development. *Nature*. 2016;540:129-133. doi:[10.1038/nature20559](https://doi.org/10.1038/nature20559)
 171. Yeh WC, Itie A, Elia AJ, et al. Requirement for Casper (c-FLIP) in regulation of death receptor-induced apoptosis and embryonic development. *Immunity*. 2000;12:633-642. doi:[10.1016/s1074-7613\(00\)80214-9](https://doi.org/10.1016/s1074-7613(00)80214-9)
 172. Pasparakis M, Vandenabeele P. Necroptosis and its role in inflammation. *Nature*. 2015;517:311-320. doi:[10.1038/nature14191](https://doi.org/10.1038/nature14191)
 173. Ofengeim D, Ito Y, Najafov A, et al. Activation of necroptosis in multiple sclerosis. *Cell Rep*. 2015;10:1836-1849. doi:[10.1016/j.celrep.2015.02.051](https://doi.org/10.1016/j.celrep.2015.02.051)
 174. 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. *J Neurosci*. 2014;34:11929-11947. doi:[10.1523/JNEUROSCI.1860-14.2014](https://doi.org/10.1523/JNEUROSCI.1860-14.2014)
 175. Nakazawa S, Oikawa D, Ishii R, et al. Linear ubiquitination is involved in the pathogenesis of optineurin-associated amyotrophic lateral sclerosis. *Nat Commun*. 2016;7:12547. doi:[10.1038/ncomm12547](https://doi.org/10.1038/ncomm12547)
 176. Xu D, Jin T, Zhu H, et al. TBK1 suppresses RIPK1-driven apoptosis and inflammation during development and in aging. *Cell*. 2018;174:1477-1491.e19. doi:[10.1016/j.cell.2018.07.041](https://doi.org/10.1016/j.cell.2018.07.041)
 177. Toth RP, Atkin JD. Dysfunction of optineurin in amyotrophic lateral sclerosis and glaucoma. *Front Immunol*. 2018;9:1017. doi:[10.3389/fimmu.2018.01017](https://doi.org/10.3389/fimmu.2018.01017)
 178. Kaczmarek A, Vandenabeele P, Krysko DV. Necroptosis: the release of damage-associated molecular patterns and its physiological relevance. *Immunity*. 2013;38:209-223. doi:[10.1016/j.immuni.2013.02.003](https://doi.org/10.1016/j.immuni.2013.02.003)

179. Kayagaki N, Kornfeld OS, Lee BL, et al. NINJ1 mediates plasma membrane rupture during lytic cell death. *Nature*. 2021;591:131-136. doi:[10.1038/s41586-021-03218-7](https://doi.org/10.1038/s41586-021-03218-7)
180. Wang Y, Shao F. NINJ1, rupturing swollen membranes for cataplastic cell lysis. *Mol Cell*. 2021;81:1370-1371. doi:[10.1016/j.molcel.2021.03.005](https://doi.org/10.1016/j.molcel.2021.03.005)
181. Hanson B. Necroptosis: a new way of dying? *Cancer Biol Ther*. 2016;17:899-910. doi:[10.1080/15384047.2016.1210732](https://doi.org/10.1080/15384047.2016.1210732)
182. Iannielli A, Bido S, Folladori L, et al. Pharmacological inhibition of necroptosis protects from dopaminergic neuronal cell death in Parkinson's disease models. *Cell Rep*. 2018;22:2066-2079. doi:[10.1016/j.celrep.2018.01.089](https://doi.org/10.1016/j.celrep.2018.01.089)
183. Conforti L, Gilley J, Coleman MP. Wallerian degeneration: an emerging axon death pathway linking injury and disease. *Nat Rev Neurosci*. 2014;15:394-409. doi:[10.1038/nrn3680](https://doi.org/10.1038/nrn3680)
184. Zhou T, Ahmad TK, Gozda K, Truong J, Kong J, Namaka M. Implications of white matter damage in amyotrophic lateral sclerosis (Review). *Mol Med Rep*. 2017;16:4379-4392. doi:[10.3892/mmr.2017.7186](https://doi.org/10.3892/mmr.2017.7186)
185. Kang SH, Li Y, Fukaya M, et al. Degeneration and impaired regeneration of gray matter oligodendrocytes in amyotrophic lateral sclerosis. *Nat Neurosci*. 2013;16:571-579. doi:[10.1038/nn.3357](https://doi.org/10.1038/nn.3357)
186. Naito MG, Xu D, Amin P, et al. Sequential activation of necroptosis and apoptosis cooperates to mediate vascular and neural pathology in stroke. *Proc Natl Acad Sci USA*. 2020;117:4959-4970. doi:[10.1073/pnas.1916427117/-/DCSupplemental](https://doi.org/10.1073/pnas.1916427117/-/DCSupplemental)
187. Wang Y-Z, Wang J-J, Huang Y, et al. Tissue acidosis induces neuronal necroptosis via ASIC1a channel independent of its ionic conduction. *ELife*. 2015;4:e05682. doi:[10.7554/eLife.05682](https://doi.org/10.7554/eLife.05682)
188. Thadathil N, Nicklas EH, Mohammed S, Lewis TL, Richardson A, Deepa SS. Necroptosis increases with age in the brain and contributes to age-related neuroinflammation. *Geroscience*. 2021;43:2345-2361. doi:[10.1007/s11357-021-00448-5](https://doi.org/10.1007/s11357-021-00448-5)
189. Ofengeim D, Mazzitelli S, Ito Y, et al. RIPK1 mediates a disease-associated microglial response in Alzheimer's disease. *Proc Natl Acad Sci USA*. 2017;114:E8788-E8797. doi:[10.1073/pnas.1714175114](https://doi.org/10.1073/pnas.1714175114)
190. Yang S-H, Lee DK, Shin J, et al. Nec-1 alleviates cognitive impairment with reduction of A β and tau abnormalities in APP/PS1 mice. *EMBO Mol Med*. 2017;9:61-77. doi:[10.15252/emmm.201606566](https://doi.org/10.15252/emmm.201606566)
191. Chevin M, S  bire G. Necroptosis in ALS: a hot topic in-progress. *Cell Death Discov*. 2021;7:79. doi:[10.1038/s41420-021-00458-4](https://doi.org/10.1038/s41420-021-00458-4)
192. Dominguez S, Varfolomeev E, Brendza R, et al. Genetic inactivation of RIP1 kinase does not ameliorate disease in a mouse model of ALS. *Cell Death Differ*. 2021;28:915-931. doi:[10.1038/s41418-020-00625-7](https://doi.org/10.1038/s41418-020-00625-7)
193. Wang M, Chen X, Zhang Y. Biological functions of gasdermins in cancer: from molecular mechanisms to therapeutic potential. *Front Cell Dev Biol*. 2021;9:638710. doi:[10.3389/fcell.2021.638710](https://doi.org/10.3389/fcell.2021.638710)
194. Wang T, Perera ND, Chiam MDF, et al. Necroptosis is dispensable for motor neuron degeneration in a mouse model of ALS. *Cell Death Differ*. 2020;27:1728-1739. doi:[10.1038/s41418-019-0457-8](https://doi.org/10.1038/s41418-019-0457-8)
195. Nakazawa D, Desai J, Steiger S, et al. Activated platelets induce MLKL-driven neutrophil necroptosis and release of neutrophil extracellular traps in venous thrombosis. *Cell Death Discov*. 2018;4:71. doi:[10.1038/s41420-018-0073-2](https://doi.org/10.1038/s41420-018-0073-2)
196. Dixon SJ. Ferroptosis: bug or feature? *Immunol Rev*. 2017;277:150-157. doi:[10.1111/imr.12533](https://doi.org/10.1111/imr.12533)
197. Dixon SJ, Lemberg KM, Lamprecht MR, et al. Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell*. 2012;149:1060-1072. doi:[10.1016/j.cell.2012.03.042](https://doi.org/10.1016/j.cell.2012.03.042)
198. Armenta DA, Dixon SJ. Investigating nonapoptotic cell death using chemical biology approaches. *Cell Chem Biol*. 2020;27:376-386. doi:[10.1016/j.chembiol.2020.03.005](https://doi.org/10.1016/j.chembiol.2020.03.005)
199. Jiang X, Stockwell BR, Conrad M. Ferroptosis: mechanisms, biology and role in disease. *Nat Rev Mol Cell Biol*. 2021;22:266-282. doi:[10.1038/s41580-020-00324-8](https://doi.org/10.1038/s41580-020-00324-8)
200. Cao JY, Dixon SJ. Mechanisms of ferroptosis. *Cell Mol Life Sci*. 2016;73:2195-2209. doi:[10.1007/s00018-016-2194-1](https://doi.org/10.1007/s00018-016-2194-1)
201. Hirschhorn T, Stockwell BR. The development of the concept of ferroptosis. *Free Radic Biol Med*. 2019;133:130-143. doi:[10.1016/j.freeradbiomed.2018.09.043](https://doi.org/10.1016/j.freeradbiomed.2018.09.043)
202. Doll S, Proneth B, Tyurina YY, et al. ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition. *Nat Chem Biol*. 2017;13:91-98. doi:[10.1038/nchembio.2239](https://doi.org/10.1038/nchembio.2239)
203. Rao SS, Portbury SD, Lago L, et al. The iron chelator deferiprone improves the phenotype in a mouse model of tauopathy. *J Alzheimers Dis*. 2020;77:753-771. doi:[10.3233/JAD-200551](https://doi.org/10.3233/JAD-200551)
204. Homma T, Kobayashi S, Sato H, Fujii J, Edaravone, a free radical scavenger, protects against ferroptotic cell death in vitro. *Exp Cell Res*. 2019;384:111592. doi:[10.1016/j.yexcr.2019.111592](https://doi.org/10.1016/j.yexcr.2019.111592)
205. Hartmann A, Hunot S, Michel PP, et al. Caspase-3: a vulnerability factor and final effector in apoptotic death of dopaminergic neurons in Parkinson's disease. *Proc Natl Acad Sci USA*. 2000;97:2875-2880. doi:[10.1073/pnas.040556597](https://doi.org/10.1073/pnas.040556597)
206. Inoue H. The crucial role of caspase-9 in the disease progression of a transgenic ALS mouse model. *EMBO J*. 2003;22:6665-6674. doi:[10.1093/emboj/cdg634](https://doi.org/10.1093/emboj/cdg634)
207. Li M, Ona VO, Gu  gan C, et al. Functional role of caspase-1 and caspase-3 in an ALS transgenic mouse model. *Science*. 2000;288:335-339. doi:[10.1126/science.288.5464.335](https://doi.org/10.1126/science.288.5464.335)
208. Linton SD. Caspase inhibitors: a pharmaceutical industry perspective. *Curr Top Med Chem*. 2005;5:1697-1717. doi:[10.2174/156802605775009720](https://doi.org/10.2174/156802605775009720)
209. MacKenzie SH, Schipper JL, Clark AC. The potential for caspases in drug discovery. *Curr Opin Drug Discov Devel*. 2010;13(5):568-576.
210. Dhani S, Zhao Y, Zhivotovsky B. A long way to go: caspase inhibitors in clinical use. *Cell Death Dis*. 2021;12:949. doi:[10.1038/s41418-021-04240-3](https://doi.org/10.1038/s41418-021-04240-3)
211. Paganoni S, Macklin EA, Hendrix S, et al. Trial of sodium phenylbutyrate-taurursodiol for amyotrophic lateral sclerosis. *N Engl J Med*. 2020;383:919-930. doi:[10.1056/NEJMoa1916945](https://doi.org/10.1056/NEJMoa1916945)
212. Szegezdi E, Logue SE, Gorman AM, Samali A. Mediators of endoplasmic reticulum stress-induced apoptosis. *EMBO Rep*. 2006;7:880-885. doi:[10.1038/sj.embor.7400779](https://doi.org/10.1038/sj.embor.7400779)
213. Franke M, Bieber M, Kraft P, Weber ANR, Stoll G, Schuhmann MK. The NLRP3 inflammasome drives inflammation in ischemia/reperfusion injury after transient middle cerebral artery occlusion in mice. *Brain Behav Immun*. 2021;92:221-231. doi:[10.1016/j.bbi.2020.12.009](https://doi.org/10.1016/j.bbi.2020.12.009)
214. Inoue M, Shinohara ML. NLRP3 inflammasome and MS/EAE. *Autoimmune Dis*. 2013;2013:1-8. doi:[10.1155/2013/859145](https://doi.org/10.1155/2013/859145)
215. Wang S, Yang H, Yu L, et al. Oridonin attenuates A β 1-42-induced neuroinflammation and inhibits NF- κ B pathway. *PLoS One*. 2014;9:e104745. doi:[10.1371/journal.pone.0104745](https://doi.org/10.1371/journal.pone.0104745)
216. Zahid A, Li B, Kombe AJK, Jin T, Tao J. Pharmacological inhibitors of the NLRP3 inflammasome. *Front Immunol*. 2019;10:2538. doi:[10.3389/fimmu.2019.02538](https://doi.org/10.3389/fimmu.2019.02538)
217. Zhang Z, Daniels R, Schluesener HJ. Oridonin ameliorates neuropathological changes and behavioural deficits in a mouse model of cerebral amyloidosis. *J Cell Mol Med*. 2013;17:1566-1576. doi:[10.1111/jcmm.12124](https://doi.org/10.1111/jcmm.12124)
218. Hu JJ, Liu X, Xia S, et al. FDA-approved disulfiram inhibits pyroptosis by blocking gasdermin D pore formation. *Nat Immunol*. 2020;21(7):736-745. doi:[10.1038/s41590-020-0669-6](https://doi.org/10.1038/s41590-020-0669-6)
219. Humphries F, Shmuel-Galia L, Ketelut-Carneiro N, et al. Succination inactivates gasdermin D and blocks pyroptosis. *Science (New York, N.Y.)*. 2020;369(6511):1633-1637. doi:[10.1126/science.abb9818](https://doi.org/10.1126/science.abb9818)
220. Rathkey JK, Zhao J, Liu Z, et al. Chemical disruption of the pyroptotic pore-forming protein gasdermin D inhibits inflammatory cell

- death and sepsis. *Sci Immunol*. 2018;3:aat2738. doi:[10.1126/sciimmunol.aat2738](https://doi.org/10.1126/sciimmunol.aat2738)
221. Samson AL, Zhang Y, Geoghegan ND, et al. MLKL trafficking and accumulation at the plasma membrane control the kinetics and threshold for necroptosis. *Nat Commun*. 2020;11(1):3151. doi:[10.1038/s41467-020-16887-1](https://doi.org/10.1038/s41467-020-16887-1)
 222. Tsuchiya K, Nakajima S, Hosojima S, et al. Caspase-1 initiates apoptosis in the absence of gasdermin D. *Nat Commun*. 2019;10:2091. doi:[10.1038/s41467-019-09753-2](https://doi.org/10.1038/s41467-019-09753-2)
 223. Taabazuing CY, Okondo MC, Bachovchin DA. Pyroptosis and apoptosis pathways engage in bidirectional crosstalk in monocytes and macrophages. *Cell Chem Biol*. 2017;24:507-514.e4. doi:[10.1016/j.chembiol.2017.03.009](https://doi.org/10.1016/j.chembiol.2017.03.009)
 224. Torre-Minguela C, Gómez AI, Couillin I, Pelegrín P. Gasdermins mediate cellular release of mitochondrial DNA during pyroptosis and apoptosis. *FASEB J*. 2021;35:e21757. doi:[10.1096/fj.202100085R](https://doi.org/10.1096/fj.202100085R)
 225. Demarco B, Grayczyk JP, Bjanes E, et al. Caspase-8-dependent gasdermin D cleavage promotes antimicrobial defense but confers susceptibility to TNF-induced lethality. *Sci Adv*. 2020;6:eabc3465. doi:[10.1126/sciadv.abc3465](https://doi.org/10.1126/sciadv.abc3465)
 226. Yu CH, Davidson S, Harapas CR, et al. TDP-43 triggers mitochondrial dna release via mPTP to activate cGAS/STING in ALS. *Cell*. 2020;183:636-649.e18. doi:[10.1016/j.cell.2020.09.020](https://doi.org/10.1016/j.cell.2020.09.020)
 227. Verde F, Otto M, Silani V. Neurofilament light chain as biomarker for amyotrophic lateral sclerosis and frontotemporal dementia. *Front Neurosci*. 2021;15:679199. doi:[10.3389/fnins.2021.679199](https://doi.org/10.3389/fnins.2021.679199)

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