Apoptotic cell death in disease – A consensus view of the NCCD 2022

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Abstract

Apoptosis is a form of regulated cell death (RCD) that is precipitated by proteases of the caspase family. Pharmacological and genetic strategies that experimentally delay apoptosis in mammalian systems have elucidated the key contribution of this process not only to (post-)embryonic development and adult tissue homeostasis, but also to the etiology of multiple human disorders. Consistent with this notion, while defects in the molecular machinery for apoptotic cell death impair organismal development and promote oncogenesis, the unwarranted activation of apoptosis promotes cell loss and tissue damage in the context of various neurological, cardiovascular, renal, hepatic, infectious, oncological and inflammatory conditions. Here, the Nomenclature Committee on Cell Death (NCCD) gathered to critically summarize abundant preclinical literature mechanistically linking the core apoptotic apparatus to organismal homeostasis in the context of disease.

Introduction

The health and homeostasis of multicellular organisms depend on the tight balance between cell proliferation and cell death. In this context, a large body of experimental evidence has demonstrated the existence of a form of regulated cell death (RCD) that is executed by a genetically encoded machinery and, hence can be manipulated by genetic or pharmacological means {Galluzzi, 2018, 29362479}. Over the past decades, multiple variants of RCD have been characterized at the genetic, biochemical, functional and immunological level {Jiang, 2021, 33495651; Del Re, 2019, 31364924; Bok, 2020, 31636403; Broz, 2020, 31690840; Weinlich, 2017, 27999438; Galluzzi, 2017, 27748397}. For instance, programmed cell death (PCD) has been functionally defined as an RCD modality that is activated under purely physiological conditions (i.e., in absence of perturbations of extracellular or intracellular homeostasis) in the context of embryonic/post-embryonic development or adult tissue homeostasis {Galluzzi, 2018, 29362479, Gudipaty, 2018, 30089222}. Conversely, stress-driven RCD is invariably initiated in the context of failing adaptation to shifts in extracellular or intracellular homeostasis, *de facto* constituting an organismal program for the elimination of excessively damaged and/or potentially harmful cells {Galluzzi, 2018, 29362479; Bedoui, 2020, 32873928}. From a biochemical perspective, an increasing number of RCD modalities have been defined by the Nomenclature Committee on Cell Death (NCCD) based on the mechanistic involvement of specific molecular components {Galluzzi, 2018, 29362479, Galluzzi, 2012, 21760595}. For instance, apoptotic cell death has most recently been defined as a form of RCD that is precipitated by proteases of the caspase family {Kesavardhana, 2020. 32017655}, and hence can be at least retarded by pharmacological of genetic strategies that inhibit caspase activation {Galluzzi, 2018, 29362479}. Mitochondrial permeability transition (MPT)-driven necrosis, necroptosis, ferroptosis, pyroptosis, parthanatos, entotic cell death, NETotic cell death, lysosome-dependent cell death, and autophagy-dependent cell death represent additional variants of RCD that impinge on precise molecular events and hence can be manipulated with pharmacological or genetic interventions {Galluzzi, 2018, 29362479; Jiang, 2021, 33495651; Del Re, 2019, 31364924; Bok, 2020, 31636403; Broz, 2020, 31690840; Weinlich, 2017, 27999438}.

Along with the identification of key RCD regulators and the advent of modern tools for genetic engineering, a large experimental effort has been devoted to elucidating the role of RCD in the physiopathology of multicellular organisms {Green, 2019, 31100266}. Thus, various studies in animals (mostly rodents) genetically altered to lack or overexpress components of the apoptotic apparatus (either at the whole body levels or in selected cell types) have provided formal proof of the relevance, but not always the exquisite requirement, of apoptosis for embryogenesis, embryonic/post-embryonic development or adult tissue homeostasis {Singh, 2019, 30655609}. Along similar lines, pharmacological and genetic tools aimed at altering apoptotic signaling in preclinical disease models have elucidated the mechanistic contribution of apoptosis to the etiology of various conditions associated with the loss of post-mitotic cells including a panel of neurological, cardiovascular, renal, hepatic and inflammatory disorders {Singh, 2019, 30655609}. Such an intensive wave of investigation, which has now spanned across more than three decades, have pointed to the apoptotic machinery as a major target for the development of novel therapeutic interventions {Spetz, 2020, 32334819}, not only for the induction of cell death in the context of lost tissue homeostasis (e.g., for oncological indication) {Carneiro, 2020, 32203277}, but also for the inhibition of cell death in the context of ischemic, degenerative and inflammatory conditions {Anderton, 2020, 32641743; Li, 2021, 34037273}. However, while at least one drug deliberately designed to induce apoptosis is currently approved for use in humans, namely the BCL2 apoptosis regulator (BCL2) inhibitor venetoclax for the treatment of chronic lymphocytic leukemia and small lymphocytic lymphoma {Jain, 2019, 31141631}, no agents specifically conceived to inhibit the apoptotic apparatus have been fully licensed for use in the clinical practice so far. The pan-caspase

inhibitor emricasan has indeed received fast-track designation by the US Food and Drug Administration (FDA) for the treatment of non-alcoholic steatohepatitis in 2016 but has demonstrated inconsistent clinical activity {Frenette, 2019, 29913280; Garcia-Tsao, 2020, 31870950; Harrison, 2020, 31887369}, and – as of now– remains unapproved.

The lack of clinically approved apoptosis inhibitors and the inconclusive efficacy of emricasan in recent trials relate to several aspects of (apoptotic and non-apoptotic) RCD that have only recently begun to emerge (**Figure 1**). First, while detecting cell death as well as biomarkers of specific RCD variants *in vitro* is relatively straightforward {Galluzzi, 2009, 19373242}, precisely quantifying cell death *in vivo* remains challenging, at least in part owing to the rapid disposal of cell corpses by efferocytosis {Boada-Romero, 2020, 32251387; Rothlin, 2021, 33188303}. This suggests that the actual contribution of cell death to the etiology of various human disorders is difficult to quantify by observational approaches. Second, while for a long time specific RCD variants were considered as virtually inter-independent entities, it has recently become clear that the molecular machinery for RCD is composed of highly interconnected modules characterized by elevated redundancy, backup pathways and feedback loops {Bedoui, 2020, 32873928; Kist, 2021, 33439509; Bedoui, 2020, 32873928; Doerflinger, 2020, 32735843}. Thus, molecules that inhibit one specific RCD variant may ultimately be unable to confer actual cytoprotection but instead may only alter the kinetic and biochemical manifestations of death by engaging a different RCD variant. For instance, while caspase 8 (CASP8) is a major signal transducer in death receptor (DR)-driven extrinsic apoptosis (see below), it intrinsically inhibits DR-driven necroptosis {O'Donnell, 2011, 22037414; Kaiser, 2011, 21368762}, suggesting that pan-caspase inhibition in the context of DR signaling may promote necroptotic over apoptotic cell death {Brumatti, 2016, 27194727}. Third, even in the hypothetical scenario of agents that would simultaneously inhibit all (known and unknown) active RCD pathways, loss of cellular homeostasis in the context of failing adaptation to stress generally involve degenerative processes that at some stage cannot be reverted, such as widespread mitochondrial permeabilization {Tait, 2010, 20683470; Chipuk, 2021, 33887204; Bock, 2020, 31636403}. In this setting, cell death may simply occur as a consequence of an irremediable degeneration of cellular functions that can no longer be manipulated pharmacologically or genetically {Green and Victor, 2012, 22995729}. Supporting these latter notions and introducing the concept of essential *vs.* accessory aspects of cell death{Galluzzi, 2015, 25236395}, and accumulating literature indicate that so-called apoptotic caspases - namely CASP3, CASP6, CASP7, CASP8 and CASP9 – which have long been considered as the essential executioner of apoptosis, mainly control the kinetic of apoptotic cell death and its immunological manifestations (but not whether death will ultimately occur, at least in mammalian systems), pointing to the entire caspase family as to a major regulator of organismal homeostasis via the control of inflammatory responses {Davidovich, 2014, 25153241; Galluzzi, 2016, 26885855}. Along similar lines, multiple components of the core apoptotic machinery, including caspases and multiple members of the BCL2 family regulate a variety of non-apoptotic functions beyond inflammation, such as Ca^{2+} signaling and terminal differentiation {Glab, 2020, 32247577; Gross, 2017, 28234359; Hollville, 2018, 29199140; Nakajima, 2017, 28524858}. Finally, there is a hitherto unclarified degree of heterogeneity in the regulation of RCD at distinct anatomical sites (linked to microenvironmental features) and in the context of diverse pathophysiological states (*e.g.*, in young versus aged individuals).

All these issues should be kept under attentive consideration also in the context of the present review, in which the NCCD aims at critically discussing a large amount of preclinical data in support of a key role for the apoptotic machinery in mammalian disease. Specifically, attention should be placed on interpreting the results of the genetic and pharmacological experiments presented herein in the context of the aforementioned connectivity amongst different RCD variants and the discrimination between essential vs accessory aspects of cell death. Our objective is not only to provide a critical summary of

the existing literature, but also to offer an updated framework for the interpretation of these findings in view of currently accepted models of RCD signaling.

Intrinsic apoptosis in disease

A considerable amount of genetic data demonstrates that the molecular machinery for intrinsic apoptosis (described in **Box 1** and **Figure 2**) is involved in embryonic/post-embryonic development and adult tissues homeostasis. Moreover, a large number of preclinical studies in animal models of disease indicates that intrinsic apoptosis provides an etiological contribution to various disorders involving the loss of post-mitotic tissues, including neurological, cardiac, renal, hepatic, autoimmune/inflammatory, oncological and infectious conditions.

Below, we will detail the impact of the pro-apoptotic BCL2 proteins, the anti-apoptotic BCL2 proteins, the apoptosome and apoptotic caspases in disease. The effect of these intrinsic apoptosis players on health is detailed in **Box 2**, **Box 3** and **Box 4.**

Neurological disorders. Intrinsic apoptosis factors have also been involved in the pathophysiology of numerous neurological diseases (**Figure 3**). In a mouse model of amyotrophic lateral sclerosis (ALS), deletion of BCL2-associated X protein (*Bax*) results in limited neuronal cell death coupled to attenuated motor dysfunction and neuromuscular degeneration {Gould, 2006, 16928866}. Additional ablation of BCL2-antagonist/killer 1 (*Bak1*) further enhances neuroprotection, ultimately resulting in improved overall survival {Reyes, 2010, 20890041}. Similar protective effects have been observed in mice lacking the BH3-only protein BCL2 like 11 (BCL2L11, best known as BIM) as well as in transgenic mice expressing BCL2, X-linked inhibitor of apoptosis (XIAP), or the baculoviral pan-caspase inhibitor p35 {Hetz, 2007, 17510659; Kostic, 1997, 9228005; Vukosavic, 2000, 11124989; Inoue, 2003, 14657037; Wootz, 2006, 16566922}. Of note, p35 and XIAP expression delay disease onset and progression, respectively {Inoue, 2003, 14657037}, supporting the relevance of apoptotic caspases at multiple stages of ALS pathogenesis. In line with this notion, intracerebroventricular administration of the pan-caspase inhibitor Z-VAD-FMK protects mice from ALS {Li, 2000, 10764647}. Moreover, a link between CASP9 expression levels and severity of the disease has been documented in a cohort of patients with ALS {Ilzecka, 2012, 22048794}. *Bax* deletion also attenuates neuromuscular dysfunctions in a mouse model of congenital muscular dystrophy (another neurodegenerative disease affecting motoneurons) {Girgenrath, 2004, 15578095}, while BCL2 overexpression limits neuromuscular disease progression in some (but not all) mouse models of progressive motor neuronopathy and muscular dystrophy {Davies, 2011, 21199860; Dominov, 2005, 15757977; Sagot, 1995, 7472523}.

Multiple components of the molecular machinery for intrinsic apoptosis, including BCL2-associated agonist of cell death (BAD), BAX, BCL2 binding component 3 (BBC3, best known as PUMA), BH3 interacting domain death agonist (BID), harakiri, BCL2 interacting protein (contains only BH3 domain) (HRK), and caspases, have been shown to drive neuronal death in the context of Alzheimer's disease (AD) and Parkinson's diseases (PD) {Kudo, 2012, 22592316;Bove, 2014, 24686337;Vila, 2001, 11226327;Kim, 2011, 22043283;Ma, 2016, 26612350;Jiang, 2012, 23019260;Biswas, 2005, 16187218;Akhter, 2018, 29499358;Imaizumi, 1999, 10075695;Louneva, 2008, 18818379;Rohn, 2002, 12505426;Hartmann, 2000, 10688892;Zhang, 2021, 34430820}. Thus, overexpression of BCL2 decreases the appearance of early pathological markers of AD such as amyloid precursor protein (APP) and microtubule-associated protein tau (MAPT, best known as tau) cleavage, which depend on caspases {Rissman, 2004, 15232619; Gervais, 1999, 10319819;Chu, 2017, 28138159}, resulting in attenuated

neurological defects {Rohn, 2008, 18354008; Kumasaka, 2009, 20411026}. Accordingly, pharmacological inhibition of CASP3 reduces early synaptic failure in mouse models of AD, ultimately improving cognitive defects {D'Amelio, 2011, 21151119}. Moreover, expression of a mutated form of amyloid β (an APP cleavage product) or administration of pan-caspase inhibitors attenuates synaptic defects in models of AD, an effect only partially recapitulated by CASP3-specific inhibitors {Park, 2020, 32610140}. Deletion of *Bcl2l11* also confers protection to dopaminergic neurons in experimental PD imposed by inhibition of mitochondrial complex I, an effect that depends on restricted BAX activation {Perier, 2007, 17483459}. Moreover, genetic deletion or downregulation of *Casp3*, as well CASP3 inhibition via transgenic, neuron-restricted expression of p35 or XIAP protects mice against pharmacologically-driven PD, attenuating both dopaminergic neuron alterations and behavioral deficits {Yamada, 2010, 20937256; Viswanath, 2001, 11739563; Crocker, 2003, 12667469; Liu, 2013, 23675438}. Similar findings have been observed in animals treated with a membrane-permeable variant of BCL2-like 1 (BCL2L1, best known as BCL-X_L) {Dietz, 2008, 17995935}. Finally, pharmacological inhibition of CASP3 confers neuroprotection to rats developing Huntington's disease (HD) {Toulmond, 2004, 14744804;Leyva, 2010, 21095569;Chen, 2000, 10888929}. That said, the precise mechanisms whereby components of the molecular apparatus for intrinsic apoptosis influence neurodegeneration need to be further explored, as pharmacological inhibition of myeloid cell leukemia sequence 1 (MCL1) reportedly improves disease outcome in a mouse model of AD by stimulating mitophagy {Cen, 2020, 33184293}, while *Mcl1* haploinsufficiency accelerates the degeneration and dysfunctionality of motor neurons in mice {Ekholm-Reed, 2019, 30963113}. Finally, although *Bax* deletion prevents the depletion of cerebellar granule neurons in a transgenic model of inherited prion disease {Chiesa, 2005, 15618403}, the direct contribution of BAX to neurotoxicity during prion disorders is a matter of controversy {Steele, 2007, 18032675}.

Pro-apoptotic BCL2 family and caspases also contribute to axonal degeneration and neuronal cell death in animal models of brain trauma, degeneration, or neurotoxicity {Pemberton, 2021, 33162554; Ray, 2011, 21373949}. Thus, BAX-or BID-deficient mice, as well as transgenic mice overexpressing BCL2, display increased survival of cortical or hippocampal neurons after experimental traumatic brain injury, as compared wild type mice {Tehranian, 2008, 18627254; Tehranian, 2006, 16782076; Bermpohl, 2006, 16395279; Raghupathi, 1998, 9809516}. Moreover, transgenic BCL2 overexpression protects mouse neurons against the detrimental effects of transection of the sciatic nerve {Farlie, 1995, 7753817}. Along similar lines, BAX deficiency enhances the survival of oligodendrocytes in mice subjected to spinal cord injury {Dong, 2003, 14507967}, while the oligodendrocyte-specific expression of p35 protects animals against motor dysfunction after contusive thoracic spinal cord injury {Tamura, 2005, 15846791}. Both neuroprotection and functional improvements have been observed in rat or mice model of traumatic spinal cord injury upon local administration of pan-caspase inhibitors (Z-VAD-FMK) as well as specific inhibitors of CASP3 (Z-DEVD-FMK) or CASP9 (Z-LEHD-FMK) {Barut, 2005, 16099247; Colak, 2005, 15796358; Li, 2000, 10938439}. Of note, in rats, post-traumatic neuroprotection can further be improved by combined inactivation of poly (ADP-ribose) polymerase family, member 1 (PARP1) and CASP3 {Zhao, 2019, 30904799}, suggesting a potential involvement for PARP1-dependent parthanatos in the process.

Deletion of *Bax* (but not of *Bbc3*, *Bcl2l11* and/or *Bid*), as well as *Bax* haploinsufficiency, prevents the death or degeneration of retinal ganglion cells in mice subjected to optic nerve injury {Donahue, 2020, 31673950; Libby, 2005, 16103918; Harder, 2011, 21762490; Harder, 2013, 22996683}. Moreover, the demise of injured retinal ganglion cells is exacerbated in mice with a conditional loss of *Bcl2l1* {Harder, 2012, 22836101} and decreased in transgenic mice expressing XIAP {Visuvanathan, 2021, 34363035} or BCL-X_L {Donahue, 2021, 34376637} in the eye, or in rodents treated with the CASP9 inhibitor Z-

DEVD-FMK {Liu, 2015, 25588462; Sanchez-Migallon, 2016, 26780312}, an XIAP-derived cell-permeant peptide {Avrutsky, 2020, 32576823}, or a CASP3-targeting small-interfering RNA (siRNA) {Ishikawa, 2012, 22642649; Tawfik, 2021, 33907045}. Moreover, transgenic XIAP expression protects the retina in various animal models of retinal disease, degeneration, or ischemia {Wassmer, 2020, 32735323; Wassmer, 2017, 28335619; Renwick, 2006, 16307001; McKinnon, 2002, 12027563; Zadro-Lamoureux, 2009, 19060276; Yao, 2011, 20926819; Avrutsky, 2020, 32576823}, while BCL-X_L inhibitor alleviates pathogenic neovascularization during diabetic retinopathy {Crespo-Garcia, 2021, 33548171}. Of note, CASP7 seems to play a crucial role in retinal ganglion cell death, as demonstrated in a model of optic injury in *Casp7*$^{-/-}$ mice {Choudhury, 2015, 26306916}. Finally, it is interesting to note that both pro-survival (BCL2) and pro-apoptotic (BAK1, BAX and BIM) BCL2 family members contribute to retinal neovascularization in response to experimental ischemic retinopathy {Wang, 2005, 15708569; Wang, 2011, 21047504; Grant, 2020, 32427589}. These observations may indicate that factors released by dying cells are key for neovascularization in the retina.

Deletion of *Bax*, *Hrk* or *Casp3* as well as transgenic overexpression of XIAP prevents neuronal loss and/or axon degeneration in mouse models of trophic factor deprivation including nerve growth factor (NGF) withdrawal {Deckwerth, 1996, 8816704; Unsain, 2013, 23954782; Unsain, 2013, 23954782; Imaizumi, 2004, 15084651}. Conversely, *Bcl2l11* or *Bbc3* deletion does not limit hippocampal neuronal injury upon experimental excitotoxicity {Theofilas, 2009, 19104441; Bunk, 2020, 33155994}. Moreover, while *in vivo* pharmacological inhibition of BAX or delivery of an XIAP fusion protein protects neurons against death induced by glutamate or kainic acid {Niu, 2017, 28392146; Li, 2006, 16336964}, kainic acid-mediated neurodegeneration cannot be rescued by the CASP3 inhibitor DEVD-CHO {Tzeng, 2013, 24313976}. Contrasting results have also been documented in rodents engineered to express p35 in neurons and subjected to experimental excitotoxicity {Viswanath, 2000, 10681443; Tomioka, 2002, 12480175}. Conversely, BIM appears to be activated during excitotoxicity {Concannon, 2010, 20351066}, and *Bcl2l11*$^{-/-}$ rodents display attenuated neurodegeneration after experimental seizures induced by administration of kainic acid into the amygdala, at least in part because of decreased neuronal death in the hippocampus (but not in the neocortex) {Murphy, 2010, 19779495}. Moreover, data from knockout mice suggest that experimental seizure-induced neuronal death involves BAD, BCL2 interacting killer (BIK), BCL2 modifying factor (BMF), or PUMA {Foley, 2018, 29171006; Moran, 2013, 23618904; Engel, 2010, 19890018; Engel, 2010, 20362645} and can be prevented by BCL2-like 2 (BCL2L2; best known as BCL-W) {Murphy, 2007, 17702891}. Confirming a certain degree of functional redundancy, phorbol-12-myristate-13-acetate-induced protein 1 (PMAIP1, best known as NOXA) and BID seem to be dispensable for RCD driven by excitotoxicity, as shown in kainic acid-treated animals {Ichikawa, 2017, 28079889; Engel, 2010, 20646170}. Taken together, these observations indicate that some (but not all) components of the molecular machinery for intrinsic apoptosis etiologically contribute to excitotoxicity. The fact that caspases do not appear to be involved points to non-apoptotic mechanisms (e.g., deregulated Ca^{2+} fluxes) as to major etiological determinants in this setting.

Intrinsic apoptosis has also been involved in neuronal apoptosis post-ischemic injury, in both developing and adult brains. In a rodent model of neonatal hypoxia-ischemia, neuroprotection can be documented upon deletion of *Bax* {Gibson, 2001, 11778654}, simultaneous deletion of *Bcl2l11* and *Bad* {Ness, 2006, 16780816}, transgenic overexpression of a specific apoptotic peptidase activating factor 1 (APAF1)-inhibitory protein or XIAP {Gao, 2010, 19910549; Wang, 2004, 15207275}, as well as by the administration of a BAX-inhibiting peptide or the caspase inhibitors Q-VD-OPh or TRP-601 { Han, 2014, 24525800; Sun, 2015, 26823794; Chauvier, 2011, 21881605}. Along similar lines, *Xiap*$^{-/-}$ mice are sensitized to neonatal hypoxia-ischemia injury {West, 2009, 19570023}. Apparently at odds with

these findings, *Casp3*$^{-/-}$ mice display increased vulnerability to such experimental perturbation, possibly due to complementary overactivation of CASP3-independent pathways {West, 2006, 16480886}. Of note, the absence of CASP3, BAX, or PUMA (but not NOXA, BIM, or HRK) also confers neuroprotection to newborn mice acutely exposed to ethanol {Ghosh, 2009, 19535997; Young, 2003, 14502238; Young, 2005, 15927478}, while that of BAX is neuroprotective in newborn mice exposed to isoflurane {Slupe, 2021, 33434191}. In this context, it is interesting to note that BAX-dependent neuronal RCD also contributes to microglia activation during the recovery of the developing brain from acute alcohol exposure {Ahlers, 2015, 25856413}, pointing to an etiological role for microglial cells activated by dead neurons.

Pharmacological inhibition of BAX protects adult rodents against neuron death induced by global brain ischemia {Hetz, 2005, 16219766}, which is in line with the pronounced neuroprotection observed in *Bax*$^{-/-}$ mice subjected to distinct experimental brain injuries, including middle cerebral artery occlusion {D'Orsi, 2015, 25632145}. A similar protection against experimental ischemic insults has been observed in mice deficient for BMF {Pfeiffer, 2014, 25299781}, BID {Plesnila, 2001, 11742085;Yin, 2002, 12200426;Plesnila, 2002, 11867899} or CASP3 {Le, 2002, 12415117}. Conversely, NOXA seems to be dispensable for neuronal damage induced by experimental ischemic stroke {Pfeiffer, 2014, 25299781}. Moreover, the absence of BID fails to protect mice from ischemia-reperfusion, although it limits the associated inflammatory response {Martin, 2016, 26869884}. Transgenic overexpression of BCL2, BCL-X_L or XIAP as well as inhibition of CASP9 (by administration of Z-LEHD-FMK or XIAP-derived peptides) or CASP3 (by exposure to Z-DEVD-FMK or transgenic expression pf p35) ameliorates neuronal survival upon global ischemia, focal ischemia or stroke {Kitagawa, 1998, 9836775;Cao, 2002, 12097494;Kilic, 2002, 12505420;Akpan, 2011, 21677173;Fan, 2006, 16293346;Trapp, 2003, 12812761;Zhu, 2007, 18052985;Zhao, 2005, 15789032;Chen, 1998, 9634557;Gao, 2010, 19910549;Karatas, 2009, 19889988;Endres, 1998, 9498840;Gottron, 1997, 9245499;Shibata, 2000, 10974017;Sung, 2007, 17945431;Braun, 2007, 17585906;Sun, 2015, 26170924}. In apparent contrast with this result, pharmacological inhibition of BCL2 and BCL-X_L with ABT-737 protects rats against neuronal death upon experimental brain ischemia, an effect that has been ascribed to the inhibition of a pro-apoptotic truncated form of BCL-X_L {Ofengeim, 2012, 22366758}. Of note, various examples of caspase-independent neuronal death after cerebral ischemia have also been reported {Lapchak, 2003, 12493605; Osman, 2016, 26734998; Zhan, 2001, 11333363}

Cardiovascular conditions. *Bax*$^{-/-}$*Bak1*$^{-/-}$ mice subjected to experimental myocardial infarction display considerably reduced infarct size as compared to their wild-type littermates, although such an effect was in part ascribed to limited activation of MPT-driven necrosis rather than apoptosis {Whelan, 2012, 22493254; Karch, 2013, 23991283; Hochhauser, 2007, 17406056}. Likewise, deletion of *Bbc3* preserves cardiomyocyte integrity and cardiac function in mice subjected to ischemia-reperfusion {Toth, 2006, 16399862}, ultimately translating in increased survival {Gao, 2016, 27160138}. A similar degree of protection against myocardial ischemia-reperfusion has been reported in mice with a conditional deletion of *Casp3* and *Casp7* in the cardiac tissue {Inserte, 2016, 26924441}, in transgenic mice overexpressing BCL2 {Brocheriou, 2000, 11045426; Kristen, 2013, 23410819; Chen, 2001, 11299236} as well as in mice and rat exposed to XIAP mimicking peptides {Souktani, 2009, 19233193} and to a BCL-X_L-derived peptide {Ono, 2005, 15621482}. In this context, pan-caspase inhibition appears to be more effective in protecting rats than mice against myocardial ischemic injury {Mersmann, 2008, 18805622; Yaoita, 1998, 9462530; Huang, 2000, 10940367}. The reason behind such species-specific effects remains to be deciphered.

The role of intrinsic apoptosis in non-ischemic heart conditions is debated. In a mouse model of cardiomyopathy based on desmin (*Des*) deletion, the myocardiocyte-specific overexpression of BCL2 reduces cardiac lesions and hypertrophy coupled to ameliorated cardiac functionality {Weisleder, 2004, 14715896}. However, despite improved survival, these mice show increased levels of necrosis due to the activation of alternative RCD pathways {Maloyan, 2010, 20360253}. Moreover, *Casp3*$^{-/-}$ mice display enhanced vulnerability to experimental cardiomyopathy, at least in part reflecting the inefficient activation of pro-survival AKT serine/threonine kinase 1 (AKT1) signaling {Khalil, 2012, 22949508}. As an alternative explanation, the absence of CASP3 may foster RCD-driven inflammation as a consequence of superior type I interferon (IFN) release {Rodriguez-Ruiz, 2019, 31646105; White, 2014, 25525874; Rongvaux, 2014, 25525875}, knowing that experimental data linking deregulated type I IFN release and cardiac conditions have recently emerged {King, 2017, 22949508}.

As for therapeutic interventions, cardioprotective effects have been achieved by inhibition of CASP3 in rodent models of myocardial dysfunction induced by endotoxin {Fauvel, 2001, 11247771}, burn injury {Carlson, 2007, 17431085} or hypoxia {Araki, 2000, 10800082}. Moreover, pharmacological inhibition of BAX prevents cardiotoxicity induced by doxorubicin in zebrafish and mice without affecting the antineoplastic activity of the agent {Amgalan, 2020, 32776015}. Along similar lines, the endothelial cell-specific expression of B cell leukemia/lymphoma 2 related protein A1a (BCL2A1A) favors survival in a model of allogeneic heart transplantation {Smyth, 2017, 28120329}. Finally, the mechanistic links between intrinsic apoptosis and atherosclerosis remain a matter of debate. Indeed, while *Casp3* deletion favors plaque development in mouse models of atherosclerosis {Grootaert, 2016, 27847551}, the absence of DNA fragmentation factor subunit beta (DFFB, best known as CAD) {Chao, 2016, 28007744} protects mice against the disease. Similarly, while conditional deletion of *Mcl1* in myeloid cells is pro-atherogenic {Fontaine, 2019, 31601924}, genetic or pharmacological inhibition of BCL-X_L reduces atherosclerosis via a mechanism involving the depletion of platelets {Lee, 2021, 33441028}. Moreover, the macrophage or leukocyte-specific deletion of *Bcl2l11* in mice has modest effects on plaque development, especially in the early phase of atherosclerosis {Temmerman, 2017, 28596542; Thorp, 2009, 18988889}. As the etiology of atherosclerosis involves a major inflammatory component, these apparently discrepant results may reflect (at least in part) the key role of some components of the apoptotic machinery in the control of inflammatory responses.

Renal disorders. Germinal or kidney-specific deletion of *Bax* attenuates acute kidney damage in mice subjected to experimental renal ischemia/reperfusion {Wei, 2013, 23466994}. A similar nephroprotection has been observed in *Bid*$^{-/-}$ mice {Wei, 2006, 16106037}, as well as in transgenic mice specifically expressing BCL-X_L in the kidney {Chien, 2007, 17998875}. Moreover, the simultaneous deletion of *Bax* and *Bak1* in kidney proximal tubules limits tubular apoptosis and ameliorates kidney inflammation and fibrosis in a mouse model of renal fibrosis based on unilateral ureteral obstruction {Mei, 2017, 28317867; Jang, 2015, 26180237}. Lending further support to the notion that intrinsic apoptosis contributes to the etiology of renal conditions, *Casp3* deletion reduces microvascular rarefaction and renal fibrosis in mice subjected to experimental ischemia-reperfusion injury {Yang, 2018, 29925521}, resulting in better long-term outcomes {Lan, 2021, 34338031}. Moreover, the lack of CASP3 increases the survival of mice with chronic kidney disease caused by a congenital mutation in cystin 1 (*Cys1*) {Tao, 2008, 18272845}. In this setting, CASP3-deficient mice display increased CASP7 and decreased BCL2 expression, which is in line with recent clinical evidence of constitutive BCL2 downregulation in patients with polycystic kidney disease {Duplomb, 2017, 28973148}. In support of this observation, inhibition of caspases by transgenic p35 overexpression in the tubular epithelium protects mice against experimental glomerulonephritis, although the same nephroprotective effect cannot be observed in mouse models of nephrosis {Inoue, 2012, 22785176}. Moreover, administration of pan-

caspase inhibitors attenuates kidney damage and improves renal functionality after a variety of experimental insults to kidneys, as observed in animal models of renal ischemia {Daemen, 1999, 10487768; Bral, 2019, 31770375}, polycystic kidney disease {Tao, 2005, 15863619}, glomerulonephritis {Yang, 2003, 12753292}, lupus nephritis {Seery, 2001, 11509582} and diabetic renal disease {Wen, 2020, 32104028}. That said, these studies do not rule out the involvement of non-apoptotic RCD pathways in the etiology of acute and chronic kidney injury {Belavgeni, 2020, 32302582; von Mässenhausen, 2018, 29961062}. Indeed, some of the nephroprotective effects of pan-caspase inhibitors have been linked to decreased post-RCD inflammation rather than the sole inhibition of apoptosis {Daemen, 1999, 10487768; Guo, 2004, 15579512}. Moreover, Z-VAD-FMK aggravates (rather than ameliorates) renal function in a mouse model of cisplatin nephrotoxicity, by a mechanism involving the abrogation of cytoprotective autophagy {Herzog, 2012, 22896037}. Similarly, Z-VAD-FMK is ineffective in mouse models of osmotic nephrosis and contrast-induced acute kidney injury {Linkermann, 2013, 23833261}, potentially linked to the ability of Z-VAD-fmk to inhibit CASP8 (and hence promote necroptosis).

Hepatic diseases. Abundant evidence ascribes a pathogenic role to apoptosis in acute liver injuries, as well as to alcohol-related and alcohol-unrelated chronic liver disorders. However, hepatocytes are well known for expressing high levels of BID, which connects DR signaling to mitochondrial outer membrane permeabilization (MOMP) upon CASP8-dependent cleavage {Yin, 1999, 10476969} and complicates distinguishing between the intrinsic and extrinsic pathways in this respect. Here, we will discuss studies with animal models of liver injury unrelated to overt signaling engaged by the Fas cell surface death receptor (FAS; also known as CD95 or APO-1) or the TNF receptor superfamily member 1A (TNFRSF1A, best known as TNF-R1) (which instead will be discussed in the next section).

Distinct preclinical models of hepatic ischemia-reperfusion injury have shown that deletion of *Bcl2l11* and/or *Bid* as well as overexpression of BCL2 or administration of pharmacological pan-caspase inhibition mediate robust hepatoprotective effects {DuBray, 2015, 25483735; Selzner, 2002, 11830333; Cursio, 2000, 11112076}. A similar improvement in hepatocyte survival and liver functionality has been observed in rodents specifically expressing a mutated variant of BID in the liver and subjected to warm ischemia/reperfusion injury {Riddle-Taylor, 2007, 17893612}. As for other models of liver injury, *Bcl2l11*$^{-/-}$ mice are protected against viral hepatitis {Lauer, 2012, 22156338}. Moreover, ablation of *Bbc3* but not that of BCL2-related ovarian killer (*Bok*) or inhibition of PUMA limits liver injury in mice exposed to the hepatotoxic agent acetaminophen {Chen, 2019, 30552702; Naim, 2021, 33807047}. Also, pre-treatment with Z-VAD-FMK improves the survival of mice subjected to extensive hepatectomy {Yoshida, 2007, 17559362}, while administration of recombinant cytochrome c, somatic (CYCS) protected liver homeostasis in a rat model of hemorrhagic shock and resuscitation, through a mechanism involving reduced oxidative stress {Powell, 2017, 27602909}.

There is contrasting evidence on the role of BID in the etiology of liver conditions unrelated to overt FAS and TNF-R1 signaling. In a model of alcohol-related liver disease, the lack of BID confers some protection against ethanol-induced fibrosis, although mice display persisting signs of inflammation and steatosis {Roychowdhury, 2012, 22273278}. Moreover, mice with a hepatocyte-specific deletion or depletion of *Bid* present reduced liver inflammation and fibrosis when subjected to a choline-deficient diet to cause non-alcoholic steatohepatitis (NASH) {Eguchi, 2016, 26555271}. However, while BID deficiency fails to ameliorate liver injury and fibrosis upon bile duct ligation (as a model of obstructive cholestasis and chronic liver disease) {Nalapareddy, 2009, 19661444}, administration of a *Bcl2l11*-targeting antisense confers significant hepatoprotective effects {Higuchi, 2001, 11714870}. The reasons underlying such an apparent discrepancy remain to be elucidated. Of note, in the same experimental

model, the liver-specific overexpression of MCL1 but not BCL2 protects animals from hepatic damage {Kahraman, 2009, 19051025; Mitchell, 2011, 20856227}, suggesting the potential implication of mechanisms other than core apoptotic signaling. To add a layer of complexity, conditional deletion of *Xiap* in hepatocytes does not result in liver injury, steatosis, or fibrosis, possibly due to compensatory effects of other inhibitor of apoptosis protein (IAPs) {He, 2021, 34025452}. That said, *Xiap*$^{-/-}$ and *Casp3*$^{-/-}$ mice subjected to diet-induced hepatic steatosis and/or fibrosis, display exacerbated and attenuated liver damage, respectively {Zilu, 2019, 31841118; Thapaliya, 2014, 24795036}. Finally, genetic co-deletion of *Mcl1* and transformation-related protein 53 (*Trp53*) {Weng, 2011, 21146511} as well as conditional deletion of *Bcl2l1* or *Mcl1* promote fibrosis and/or carcinogenesis, two commonly final stages of liver disease {Hikita, 2012, 22414765}. In this latter study, the additional deletion of *Bak1* limited hepatotoxicity, which is in line with evidence indicating that *Bid* and/or *Bok* deletion protects mice against experimentally-induced hepatocarcinogenesis {Rabachini, 2018, 29229991; Wree, 2015, 25909884; Orlik, 2015, 25951810}.

Supporting the etiological contribution of caspase activation to liver disease, administration of pan-caspase inhibitors (e.g., emricasan, VX-166) reduces liver injury, inflammation and fibrosis in mice fed with a diet rich in fat or deficient for methionine and choline {Barreyro, 2015, 24750664; Witek, 2009, 19676126}. Along similar lines, emricasan reportedly attenuates portal pressure, fibrogenesis and hepatic inflammation, and preserves liver function in rodent models of chronic carbon tetrachloride (CCl_4)-mediated cirrhosis or cholestasis driven by bile duct ligation {Gracia-Sancho, 2019, 31304452; Eguchi, 2018, 29728708; Canbay, 2004, 14617689}. Preliminary anti-inflammatory effects coupled with improved liver function have also been observed in patients with NASH-related cirrhosis treated with emricasan {Garcia-Tsao, 2019, 30063802; Frenette, 2019, 29913280}. However, follow-up clinical studies failed to observe beneficial effects on portal pressure and clinical outcome {Garcia-Tsao, 2020, 31870950; Harrison, 2020, 31887369; Frenette, 2021, 33038432}. At least in part, these findings may reflect the complex interconnection between multiple RCD variants involved in the pathogenesis of NASH. Supporting this possibility, specific pharmacological inhibition of CASP9 with Z-LEHD-FMK aggravates (rather than ameliorates) experimental acute liver injury imposed by CCl_4 as suppresses cytoprotective autophagy {Guo, 2016, 27580936}. Along similar lines, the administration of CASP3-specific inhibitors that abrogate both its pro-apoptotic and pro-pyroptotic activity protects mice against acute liver injury caused by bile duct ligation {Xu, 2021, 32457417}. Additional pharmacological and genetic studies specifically targeting intrinsic apoptosis (over other RCD pathways controlled by caspases) are awaited to formally prove the involvement of this pathway in the etiology of hepatic conditions.

Hematologic malignancies and solid cancers. The impact of intrinsic apoptosis in preventing oncogenesis has been demonstrated in multiple animal models of induced hematological and solid tumors. In particular, a wide range of evidence demonstrates that overexpression of BCL2 or BCL-X_L accelerates the onset of leukemia and lymphoma induced by MYC proto-oncogene, bHLH transcription factor (MYC) {Hogstrand, 2012, 22393362; Finch, 2006, 16904610; Swanson, 2004, 15153484; Strasser, 1990, 2250704}. Accordingly, the pharmacological inhibition of these anti-apoptotic BCL2 proteins is effective against MYC-driven tumors also when combined with p53 deficiency {Kelly, 2014, 24395247; Vandenberg, 2013, 23341542; Kelly, 2013, 22814621; Mason, 2008, 19004807}, although such effect could be related to specific function of p53 in RCD (e.g., {Yin, 2021, 33723373;Bowen, 2021, 33574585;Liang, 2021, 33110215}). Of note, when analyzing the impact of endogenous protein, it was shown that the absence of BCL-X_L but non BCL2 limits the development of lymphoma in transgenic mice expressing MYC under the IgH enhancer (Eμ-myc mice) {Kelly, 2011, 21998213; Kelly, 2007, 17317859}, thus supporting the therapeutic use of BCL-X_L inhibitors against these blood cancers.

Along similar lines, MCL1 overexpression {Campbell, 2010, 20631380} or *Mcl1* ablation {Grabow, 2016, 26947081;Xiang, 2010, 20484815;Kelly, 2014, 24395247}, respectively, accelerates and suppresses MYC-driven lymphomagenesis. Lending further support to the relevance of MCL1, a prophylactic effect was observed with *Mcl1* haploinsufficiency {Kelly, 2014, 24395247; Xiang, 2010, 20484815}, B cell-specific deletion of *Mcl1* {Grabow, 2016, 26962682} or co-deletion of *Mcl1* and *Tpr53* {Kelly, 2014, 24395247}. Of note, MCL1 (but not BCL-X_L) also contributes to the development of thymic lymphoma in p53-deficient mice {Grabow, 2014, 25368374}, which possibly explains the limited effect of the inhibition ABT-737 in these models of tumorigenesis {Luk'ianchikova, 1976, 1063464}. Finally, the contribution of most BCL2 proteins including BCL-W and BCL2A1 in MYC-induced myeloid leukemogenesis has been demonstrated in a model of mice reconstituted with genetically-modified bone marrow cells overexpressing these factors {Beverly, 2009, 19137012}. Accordingly, ablation of *Bcl2l2* limits the development of MYC-mediated B cell lymphoma {Adams, 2017, 28094768}.

In support of the relevance of the intrinsic apoptosis in tumorigenesis, the development of MYC-driven lymphoma and leukemia is accelerated in mice deficient for BAX {Eischen, 2001, 11604501}, BIM {Egle, 2004, 15079075;Delbridge, 2015, 24858047}, BAD {Frenzel, 2010, 19965635}, BMF {Frenzel, 2010, 19965635} or PUMA {Hemann, 2004, 15192153;Michalak, 2009, 19148184}. In particular, in these studies, it was reported that *Bcl2l11* haploinsufficiency accelerates the appearance of lymphoma and that such effect was reversed via the full ablation of *Bcl2l1* {Delbridge, 2015, 24858047}. In this context, the presence of all prosurvival BCL2 protein is shown to limit the impact of BIM in Eμ-Myc transgenic mice {Merino, 2012, 22081075}, while the combined ablation of *Bcl2l11* and *Tpr53* or *Bbc3* and *Tpr53* - but less *Bbc3* and cyclin-dependent kinase inhibitor 1A (P21) (*Cdkn1a*, coding for a cell cycle regulator best known as p21) - accelerates MYC-driven lymphomagenesis {Shang, 2012, 22446994. Garrison, 2008, 18573879}. This is in line with the evidence that loss of *Bax* or *Bcl2l11* augmented lymphomagenesis in p53-deficient mice {Delbridge, 2016, 27621418; Knudson, 2001, 11212265}. Of note, PUMA seems to exert a strong tumor-suppressive role in blood cancer, as shown by the evidence that *Bbc3* deletion exacerbates the development of MYC-driven B-cell lymphomas and that Emu-Myc tumors developing in PUMA-proficient mice display downregulates expression of PUMA {Garrison, 2008, 18573879}. On the contrary, the role of NOXA and BIK in this tumorigenic model is debated {Michalak, 2009, 19148184; Happo, 2012, 22573037}. Along similar lines, while CASP2 suppresses MYC-induced murine lymphomagenesis {Ho, 2009, 19279217}, the tumor-suppressive role of apoptosome components (**Box 1**) is questioned, as shown in lethally irradiated mice reconstituted with Eμ-Myc transgenic APAF-deficient or CASP9-deficient fetal liver cells {Scott, 2004, 14709542}.

Concerning other experimental animal models of induced hematological malignancies, the ablation of *Bbc3* accelerates the development of both myelodysplasia, as shown in transgenic mice expressing a nucleoporin 98 (Nup98)-homeobox D13 (Hoxd13) fusion gene {Guirguis, 2016, 26742432}, and lymphoma induced by gamma radiations {Michalak, 2010, 20679396; Labi, 2010, 20679395}. Concerning NOXA, the loss of *Pmaip1* augments the development of chronic lymphocytic leukemia in T cell lymphoma breakpoint 1 (TCL1) transgenic mice {Slinger, 2016, 27479816}, but has no impact on lymphomagenesis induced by gamma radiations {Michalak, 2010, 20679396}. Moreover, conditional deletion of *Bcl2l11* in B cells accelerates murine mantle cell lymphoma-driven cyclin D1 (CCND1) overexpression {Katz, 2014, 24352880}. Also, upregulated expression of MCL1 and/or BCL2 favors the development of acute myeloid leukemia driven by lysine (K)-specific methyltransferase 2A (KMT2A, best known as MLL) fusion proteins {Anstee, 2019, 30470795;Glaser, 2012, 22279045} and plasmacytoma driven by ABL proto-oncogene 1, non-receptor tyrosine kinase (ABL1) {Vandenberg, 2014, 24986687}. Likewise, the loss of one *Mcl1* allele suppresses the development of T lymphocyte

non-Hodgkin's lymphoma, as shown in models based on sequential low-dose irradiation or the expression of a transgene encoding an IL2 inducible T cell kinase (ITK)–spleen tyrosine kinase (SYK) fusion protein {Spinner, 2016, 27055871}. Finally, the absence of CASP2 exacerbates lymphomagenesis in the ataxia telangiectasia mutated (ATM)-deficient mice {Puccini, 2013, 24248351}.

Significant work demonstrated the tumor suppressor role of the intrinsic apoptosis in most solid tumors. Thus, BCL2 overexpression accelerates the development of MYC-induced mammary tumorigenesis {Jager, 1997, 9362445}. A similar tumor predisposition has been described for the loss of *Bax*, *Bbc3*, *Bcl2l11,* or *Casp2* in distinct models of breast oncogenesis induced by C3(1)/SV40 T-antigen, MYC, or erb-b2 receptor tyrosine kinase 2 (ERBB2, best known as HER2) {Shibata, 1999, 10329616; Jamerson, 2004, 15354213; Bean, 2013, 23532334; Parsons, 2013, 23645210}. At odds with these results, BCL2 overexpression in the mammary gland suppresses the development of breast tumors driven by the administration of dimethylbenz(a)anthracene {Murphy, 1999, 10597264}. The conditional deletion of *Bcl2l1* in intestinal epithelial cells (IECs) delays the development of colorectal cancer driven by inflammation {Scherr, 2016, 27537525}, which is in line with the evidence that *Bbc3* deletion exacerbates colorectal tumorigenesis as shown in a mouse model of intestinal oncogenesis driven by colitis or APC, WNT signaling pathway regulator (APC) {Qiu, 2009, 19491259}. Moreover, a tumor-suppressive effect is ascribed to BAX and CASP2 respectively in murine models of the brain {Garcia, 2013, 22710714; Yin, 1997, 9024662} and lung {Terry, 2015, 25301067} oncogenesis. Of note, there is evidence of a certain tissue-specificity in the epigenetic regulation of *Bcl2* and *Mcl1*, such as the epigenetic mechanism centered on the deubiquitinase BRCA1 associated protein 1 (BAP1) {He, 2019, 31000662} a tumor suppressor often mutated in cancer {Carbone, 2020, 32690542}, which can explain the contribution of the intrinsic apoptosis in tumorigenesis of specific tissues.

Cancer-specific contributions have been attributed to particular BCL2 proteins. So, for instance, deletion of *Bax* accelerates the development of MYC-induced pancreatic tumors {Dansen, 2006, 16464852}, a result that is not recapitulated by ablation of *Bak1* or *Casp3* {Dansen, 2006, 16464852; Radziszewska, 2009, 19213729}. Likewise, BOK seems to be crucial in hepatocarcinogenesis, as demonstrated in a mouse model of diethylnitrosamine-induced liver cancer with a $Bok^{-/-}$ genetic background {Rabachini, 2018, 29229991}. Using the same model, a predisposition to hepatic cancer was demonstrated also for the deletion of *Bbc3* or *Casp2* {Shalini, 2016, 27518436; Qiu, 2011, 21725994}. Moreover, the overexpression of BCL2 is shown to limit transforming growth factor-alpha (TGFA)-driven hepatic tumorigenesis {Pierce, 2002, 12000706; Vail, 2001, 11212255}. Finally, the transgenic overexpression of BCL-X_L (but not BCL2) and the keratinocyte-specific deletion of *Bcl2l1* respectively accelerates and limits chemically- and/or ultraviolet B (UVB)-induced skin tumorigenesis {Pena, 1998, 9605754; Schenkel, 2008, 19035317; Rossiter, 2001, 11325830; Kim, 2009, 19309000}. Confirming the role of intrinsic apoptosis in this model, exacerbated chemically-induced skin tumorigenesis is reported in transgenic mice overexpressing BCL-X_L {Pena, 1998, 9605754} and in mice deficient for CASP3 or CAD {Liu, 2015, 25866249; Yan, 2009, 19541853}.

Autoimmune and inflammatory diseases. Contrasting evidence links intrinsic apoptosis to the development and progression of autoimmune diseases {Hughes, 2006, 16394656; Sionov, 2015, 26405162}. In mouse models of rheumatoid arthritis, ablation of *Bcl2l11*, *Bid* or *Bad*, but *Bax* and *Bak1*, accelerates the emergence and increases both the duration and severity of the disease {Scatizzi, 2006, 17009248; Li, 2020, 33270017; Scatizzi, 2007, 17509138}. Consistent with these findings, administration of a BIM mimetic also suppresses inflammatory arthritis in mice {Scatizzi, 2010, 20112357}. Of note, in a collagen-induced arthritis model, expression of a *Bad* variant that can no longer be activated by phosphorylation is sufficient to exacerbate disease progression {Li, 2020, 33270017}.

Along similar lines, mice deficient for BAX as well as transgenic mice expressing XIAP display increased severity of autoimmune encephalomyelitis induced by immunization with myelin oligodendrocyte glycoprotein (MOG) {Moore, 2008, 18687476; Lev, 2004, 15050683}. Comparable results have been obtained in mouse models of autoimmune encephalomyelitis genetically engineered for the hematopoietic cell-specific deletion of *Bcl2l11*, the neuron-specific overexpression of BCL2, or the oligodendrocyte-specific expression of p35 {Ludwinski, 2009, 19411758; Offen, 2000, 11303781; Hisahara, 2000, 10654933}. In apparent contrast with these results, distinct preclinical models of type 1 (autoimmune) or type 2 (non-autoimmune) diabetes reveal that deletion of *Bax* alone or together with *Bak*1 {Sun, 2016, 27137932;White, 2020, 32620813}, *Bcl2l11* alone or together with *Bbc3* {Krishnamurthy, 2015, 25948683;Ren, 2014, 24658302;Ren, 2014, 24760140;Ludwinski, 2009, 19411758}, *Bmf* {Pfeiffer, 2015, 27551471} or *Hrk* {Cunha, 2012, 22773666} increases the resistance of pancreatic β cells to destruction. Moreover, the absence of BIM prevents the emergence of type 1 diabetes in non-obese diabetic (NOD) mice {Krishnamurthy, 2015, 25948683; Ludwinski, 2009, 19411758}. Likewise, CASP3 deficiency protects mice against the onset of type 1 diabetes by limiting genetically or pharmacologically-induced pancreatic β cell apoptosis {Radziszewska, 2009, 19213729; Liadis, 2005, 15831467}. Confirming the therapeutic relevance of strategies modulating specific members of the BCL2 protein family, phospho-BAD BH3 mimetics reportedly reverse type 1 diabetes by restoring pancreatic β cell functionality {Ljubicic, 2015, 25640178}34141087. It seems unlikely, however, that such a beneficial effect reflects *bona fide* intrinsic apoptotic signaling.

While pan-caspase inhibition reportedly protects rats against severe acute pancreatitis {Yasuda, 2007, 17292420}, activation of intrinsic apoptosis appears to attenuate the severity of this disease by limiting inflammation, as shown *in vivo* in a pancreatitis mouse model lacking XIAP {Liu, 2017, 28300832}. These data reinforce the notion that inhibiting apoptotic cell death may exacerbate unwarranted inflammatory reactions that contribute to the pathology of various disorders. In line with this notion, chronic colitis driven by dextran sulfate sodium in mice manifests with increased (rather than decreased) severity in BID- or BIM-deficient hosts as compared to their wild-type littermates, at least in part owing to immune deregulation {Leucht, 2013, 23668821; Wicki, 2018, 29495595}. Similarly, inhibition of BCL2 and/or BCL-X_L reduces inflammation and ameliorates experimental colitis {Weder, 2018, 29745420; Lutz, 2015, 25845418}, an effect can be abrogated by co-deletion of *Bcl2l11* {Lutz, 2015, 25845418}. Apparently at odds with these findings, specific ablation of *Bid* in IECs or macrophages attenuates experimental colitis, possibly due to non-apoptotic functions of BID {Yeretssian, 2011, 21552281}. Moreover, PUMA-deficient mice display reduced levels of apoptosis amongst IECs but not inflammation in an experimental model of colitis {Dirisina, 2011, 21699775}. Corroborating the specific relevance of PUMA for intestinal homeostasis, *Bbc3*$^{-/-}$ (but not *Bax*$^{-/-}$*Bak1*$^{-/-}$) mice are protected against the gastrointestinal side effects of radiotherapy, at least in part due to increased survival of progenitor and stem cell compartments {Qiu, 2008, 18522850; Kirsch, 2010, 20019247}. Moreover, the absence of PUMA confers protection to IECs in mouse models of hypertensive gastropathy {Tan, 2014, 24625987}, ulcerative colitis (UC) {Qiu, 2011, 21490394} and intestinal ischemia/reperfusion {Wu, 2007, 17127703}. In this latter model, transgenic BCL2 expression limited IEC death {Coopersmith, 1999, 10070044}. In this context, it is interesting to note that CASP3 or CASP7-deficient mice display an altered gut microbiome {Brinkman, 2011, 22012254}, which may play a hitherto unexplored role in multiple disorders beyond intestinal conditions. Finally, BIM, BID, BAD and caspases have all been shown to influence survival in mouse models of septic shock {Schwulst, 2008, 18197142;Chung, 2010, 20023601; Yan, 2018, 29795446; Oberholzer, 2006, 16453149; Oberholzer, 2006, 16453149; Lamkanfi, 2009, 19168786} as well as in patients with severe sepsis {Weber, 2008, 18925930}.

Infectious Diseases. The activation of RCD constitutes a protective mechanism against most microbe infection by eliminating infected cells and helping mount the anti-infection immunity. In line with this notion, mice with *Bcl2l1* haploinsufficiency displayed impaired pathogenicity and improved survival rate against the Japanese encephalitis virus (JEV) challenge, as compared with the wild-type mice. This was attributed to compromised viral propagation within JEV-infected cells succumbing to intrinsic apoptosis {Suzuki, 2018, 30261081}. Moreover, there is evidence of a contribution of BAX and BAK1 to the response to murine cytomegalovirus (MCMV) infection. In particular, MCMV encodes for inhibitors of BAK (m41.1 protein) and BAX (m38.5 protein), promoting viral replication by inhibiting the induction of intrinsic apoptosis in infected cells {Handke, 2013, 23302869, Fleming, 2013, 23468630;Fleming, 2013, 23468630, Manzur, 2009, 18949000}. Supporting the requirement of the inhibition of intrinsic apoptosis for optimal *in vivo* MCMV dissemination, the titers of m41.1-deficient viruses was elevated in salivary glands and other organs in *Bak1*$^{-/-}$ mice as compared to wild-type animals {Handke, 2013, 23302869, Fleming, 2013, 23468630}. Intrinsic apoptosis has also protective role from bacterial infection, as demonstrated by the lethality of *Bbc3*$^{-/-}$ mice to *Streptococcus pneumoniae* infection {Garrison, 2010, 21203486}. Such an effect has been attributed to insufficient immune-mediated bacterial clearance caused by deregulated neutrophil lifespan due to the absence PUMA-mediated apoptosis.

However, at least in in specific contexts, excessive activation of the intrinsic apoptosis is also reported to drive, rather than prevent, microbe pathogenicity and lethality. As an example, specific ablation of *Xiap* increases the susceptibility of mice to *Shigella* infection, manifested with coalescing necrotic areas and a high bacterial burden in the liver coupled to an inefficient immune-mediated resolution of bacterial infection {Andree, 2014, 25056906}. Moreover, *Bcl2l11*$^{-/-}$*Pmaip1*$^{-/-}$ mice display high resistance to the challenge with high-doses of *Listeria monocytogenes*, as shown by a decreased bacterial burden and low apoptosis induction in the spleen {Margaroli, 2016, 27064265}. As yet another example, conditional deletion of *Casp3* in the intestinal epithelium conferred protection from pathogenic *Salmonella enterica*, and this was attributed to a shortage of death-induced nutrients sustaining bacterial growth {Anderson, 2021, 34349263}. Finally, *Casp3*$^{-/-}$ mice subjected to intracranial inoculation of Reovirus type 3 (strain Dearing) display limited injuries in the central nervous system (CNS) and enhanced survival {Beckham, 2010, 20626234}. Notably, there is evidence of a role of specific apoptotic players in the response to the infection with the human herpes simplex virus 1 (HSV-1). Thus, on the one hand, a significant accumulation in total leukocyte and CD8$^+$ T cells was observed in *Bcl2l11*$^{-/-}$ and *Bbc3*$^{-/-}$ mice infected with HSV-1 {Fischer, 2008, 18287039}, which is in line with an immunological role of these proteins {Pellegrini, 2004, 15504823}. On the other hand, *Pmaip1*$^{-/-}$, *Bad*$^{-/-}$ and *Bid*$^{-/-}$ mice are able to mount a normal CD8$^+$ T cell immune response to HSV-1 infection {Fischer, 2008, 18287039}. Finally, the overexpression of BCL2 in the hematopoietic compartment increased the survival of mice infected with Ebolavirus {Bradfute, 2010, 20028660}.

Others. Pro-apoptotic BCL2 proteins and caspases have been also involved in disorders affecting other tissues/organs, such as the skeletal muscle and lungs. For instance, the conditional ablation of *Bax* and *Bak1* protects the mouse skeletal muscle against pressure-induced injury {Tam, 2018, 29487339}. Similar results have been obtained in rats receiving Z-VAD-FMK after being subjected to muscular compression or blunt injury {Stratos, 2012, 22089165; Teng, 2011, 21540338}. Moreover, deletion of *Casp3* or CASP3 inhibition with Ac-DEVD-CHO limits muscular damage and atrophy in experimental models of plaster-mediated immobilization {Talbert, 2013, 23471945; Zhu, 2013, 23401051}. In mouse models of catabolic disorders, muscle wasting due to protein degradation is decreased by lentiviral expression of XIAP {Wang, 2007, 17315041; Hu, 2010, 20431038}, although it is unlikely that such an effect reflects the inhibition of intrinsic apoptosis. Finally, *Casp3*$^{-/-}$ mice are protected against

denervation-induced muscular atrophy {Plant, 2009, 19390003}, while expression of a dominant-negative variant of CASP9 improves the neuromuscular activity in a transgenic mouse model of slow-channel syndrome {Zhu, 2014, 23943790}.

In a mouse model of oxidant-induced lung injury, the tissue-specific ablation of *Bax* and *Bak1* but not *Bcl2l11*, *Bid*, *Bbc3*, or *Pmaip1* protects lung epithelia from degeneration {Budinger, 2011, 20959557}. Among the anti-apoptotic BCL2 proteins, BCL2A1 seems to exert a crucial role in this setting, as *Bcl2a1* deletion aggravates lung injury in mice subjected to hyperoxia {He, 2005, 15841185}, while lung-specific overexpression of BCL2 does not confer protection to mice exposed to excessive oxygen supply {Metrailler-Ruchonnet, 2010, 20382751}. That said, the cytoprotective effect of BCL2A1 is not extended to acute lung inflammation and peritonitis {Gangoda, 2021, 34304242}. Intrinsic apoptosis has also been involved in pulmonary fibrosis {Kang, 2007, 17209037}. Thus, *Bid*$^{-/-}$ mice display decreased levels of pulmonary fibrosis after intratracheal bleomycin administration {Budinger, 2006, 16537427}. In the same model of fibrotic pulmonary damage, similar protection is reported in mice deleted for *Bcl2* {Gu, 2021, 34413485} or in animals treated with BCL2 {Gu, 2021, 34413485} or caspase {Kuwano, 2001, 11159011; Wang, 2000, 10893213} inhibitors. Along similar lines, in mice, ablation of *Bid* limits acute lung injury induced by exposure to lipopolysaccharide {Wang, 2007, 17641050}. Moreover, CASP3 depletion with specific short-hairpin RNAs (shRNAs) protects the lungs of mice subjected to pulmonary ischemia/reperfusion {Zhang, 2010, 19969310}, a protection further strengthened by the combined inhibition of apoptosis and necroptosis {Wang, 2020, 33162831}. Of note, BCL2 overexpression or caspase inhibition also protects rodents subjected to lung transplantation {Cooke, 2005, 15818317; Quadri, 2005, 15643988}. This is in line with the notion that Z-VAD-FMK delivery to rodents ameliorates lung injuries developing as a consequence of severe acute pancreatitis or lipopolysaccharide administration {Liu, 2016, 27324074; Kawasaki, 2000, 10934162} but not as a result of pneumovirus infection {van den Berg, 2015, 25780096}. In this latter setting, lung damage is actually exacerbated by Z-VAD-FMK, perhaps as a consequence of accrued inflammation downstream of necroptotic RCD {van den Berg, 2015, 25780096}.

Extrinsic apoptosis in disease

The molecular apparatus for extrinsic apoptosis (described in **Box 5** and illustrated in **Figure 4**) plays a critical role in embryonic/post-embryonic development and adult tissues homeostasis, as detailed in **Box 6** and **Box 7**. Moreover, various components of the extrinsic pathway of apoptosis have been involved in the etiology of multiple human disorders, although (1) with a considerable degree of context-dependency, and (2) with an effect not necessarily linked to the activation of apoptosis, as outlined below.

Neurological diseases. Although numerous studies involve FAS and TNF-R1 signaling in the pathogenesis of multiple neurological diseases, the precise role of extrinsic apoptosis remains unclear (**Figure 5**). Loss-of-function mutations of FAS ligand (*Faslg*) as well as *Fas* silencing prevent motoneuron loss in mouse models of ALS based on mutant superoxide dismutase 1, soluble (SOD1) {Locatelli, 2007, 17503505; Petri, 2006, 17049562}, but the survival of *gld* mice was only mildly improved in this setting. Moreover, the lack of tumor necrosis factor (TNF) does not affect motoneuron loss and mouse survival in this model {Gowing, 2006, 17079668}, while ligation of the TNF receptor superfamily member 1B (TNFRSF1B, best known as TNF-R2) appears to mediate neuroprotective effects {Tortarolo, 2015, 25940956}. As an additional layer of complexity, TNF also mediates neuroprotective functions in wobbler mice (yet another model of ALS), at least in part by promoting the

upregulation of ADAM metallopeptidase domain 8 (ADAM8) {Bartsch, 2010, 20826683}. Moreover, CASP8 has not yet been formally involved in the pathogenesis of ALS, and alternative variants of FAS-driven RCD may play a predominant role in this setting. As an example, FAS ligation reportedly triggers the demise of motoneurons in mouse models of ALS by aggravating endoplasmic reticulum stress {Bernard-Marissal, 2012, 22492046}. Also, cleavage of BID by CASP1 (and not CASP8) appears to contribute to neurodegeneration in transgenic mice bearing mutant *Sod1* {Guegan, 2002, 12213439}. However, the precise contribution of endoplasmic reticulum stress and CASP1 in ALS and other motoneuron disorders remains to be elucidated.

The ability of TNF signaling to precipitate neurodegenerative conditions involves not only the induction of extrinsic apoptosis but also the activation of an inflammatory response. In distinct murine models of AD, deletion of *Tnf*, modification of its untranslated region (UTR) as well as pharmacological or genetic TNF inhibition reduces plaque formation as it limits brain inflammation, resulting in attenuated neurological deficits {Kalovyrna, 2020, 32457323; Paouri, 2017, 28826177; Paouri, 2017, 28442538; Tweedie, 2012, 22642825; McAlpine, 2009, 19320056; MacPherson, 2017, 28237313; Gabbita, 2015, 26436670; Gabbita, 2012, 22632257}. Mechanistic studies in mice and monkeys revealed that TNF activation stimulates the protein activator of interferon-induced protein kinase EIF2AK2 (PRKRA) network {Lourenco, 2013, 24315369}, which is linked to PD in humans {Camargo, 2008, 18243799}. Moreover, TNF signaling has been shown to favor the activation of microglia during neurodegeneration, culminating in neuroinflammation and neuronal loss {Bhaskar, 2014, 24141019}. Similar results have been obtained in mouse models of AD upon genetic and pharmacological inhibition of TNF-R1 {Steeland, 2018, 29472246; He, 2007, 17724122}. However, AD-associated neuroinflammation seems to depend on the activation by TNF of necroptosis rather than extrinsic apoptosis {Jayaraman, 2021, 34625123; Xu, 2021, 34646380}. Moreover, some studies point to TNF-R2 (rather than TNF-R1) as an etiological determinant of neurodegeneration {Dong, 2016, 27791020; Jiang, 2014, 24824215}. Enhanced AD pathogenesis has also been documented in mice bearing a co-deletion of *Tnfrsf1a* and *Tnfrsf1b* {Montgomery, 2011, 21835156}, a phenotype that appears to impinge on a complex network of mutual interactions between TNF-R1 and TNF-R2 signaling {Montgomery, 2013, 23567638}. Such a network also appears to contribute to PD pathogenesis. Thus, genetic ablation *Tnf* or *Tnfrsf1a* plus *Tnfrsf1b*, as well as pharmacological inhibition of TNF, protects dopaminergic neurons in murine models of PD imposed by the administration of 1-metil 4-fenil 1,2,3,6-tetraidro-piridina (MPTP) or 6-hydroxydopamine {Ferger, 2004, 15140182; Sriram, 2002, 12205053; Zhou, 2011, 21831964; McCoy, 2006, 16971520}. Notably, in the abovementioned experimental settings, TNF has been endowed with the ability to induce neuronal death *in vivo* by promoting microglia activation {Sriram, 2006, 16581975} with a complex interaction between TNF-R1 and TNF-R2 signaling {Dong, 2016, 27791020}. Importantly, clinical evidence from AD patients treated with the TNF blockers infliximab or etanercept supports the inhibition of TNF as a strategy to prevent and treat AD {Shi, 2011, 21668921; Tobinick, 2008, 18184433}. Conversely, a dominant-negative variant of TNF fails to protect mice against neuronal degeneration and neuroinflammation in a model of HD {Alto, 2014, 24824433}, suggesting that this therapeutic approach may not be viable in patients with HD.

Some studies suggest that TRAIL signaling is also implicated in the onset and progression of AD {Cantarella, 2015, 25472798; Uberti, 2007, 16936710}. Specifically, in a mouse model of AD, neutralization of TNF superfamily member 10 (*TNFSF10*, beast known as TRAIL) results in decreased neuroinflammation and improved cognitive defects {Cantarella, 2015, 25472798}. However, these findings have not been extensively validated. Similarly, the impact of FAS-FASLG signaling on neurodegenerative conditions is debated. Indeed, *lpr* and (to a lesser extent) *grd* mice are particularly susceptible to neuronal degeneration driven by MPTP {Landau, 2005, 16129703}. However, FASLG

(but not FAS) deficiency appears to confer neuroprotection to MPTP-treated mice {Gao, 2015, 25779632; Hayley, 2004, 14985447}. Such an apparent discrepancy may originate from the pleiotropic role of FAS in apoptosis and inflammation.

CASP8 activation has been detected in the brain of both AD {Rohn, 2001, 11741396} and HD {Sanchez, 1999, 10197541} patients as well as in the dopaminergic neurons of MPTP-treated mice and PD patients, a setting in which BID cleavage has also been documented {Viswanath, 2001, 11739563}. This is in line with the ability of the broad-spectrum caspase inhibitor Q-VD-OPH to inhibit BID cleavage and mediate neuroprotection in MPTP-treated rodents {Yang, 2004, 15474362}. Of note, CASP8 also promotes microglia activation and hence drives neuroinflammation, both of which characterize PD and AD {Viceconte, 2015, 25586882; Fricker, 2013, 23386613; Burguillos, 2011, 21389984}. In this context, genetic or pharmacological CASP8 inhibition attenuates neurotoxicity by reducing microglial activation and extending survival, at least in part by stimulating the necroptotic demise of activated microglial cells {Viceconte, 2015, 25586882; Fricker, 2013, 23386613; Burguillos, 2011, 21389984}. Consistent with this notion, *Casp8* deletion in myeloid cells protects mice from MPTP-mediated neurotoxicity {Kavanagh, 2015, 26405176}, suggesting that CASP8 inhibitors may be harnessed for the treatment of neurodegenerative conditions. Corroborating this possibility, a pharmacological inhibitor of TNFR1-associated death domain protein (TRADD) protects mice from a mouse model of AD-like proteinopathy driven by mutant tau {Xu, 2020, 32968279}. However, pharmacological inhibition of CASP8 only partially prevents neuronal alteration in other models of AD {Park, 2020, 32610140} and exacerbates dopaminergic neuronal necrosis in mice developing PD upon MPTP administration {Hartmann, 2001, 11264300}. Moreover, rare *CASP8* loss-of-function variants have been associated with AD in a large cohort of patients {Rehker, 2017, 28985224}. Thus, the precise contribution of CASP8 signaling to neurodegenerative disorders remains to be formally elucidated.

DR signaling has also been shown to contribute to neuronal death and inflammation in preclinical models of CNS trauma. In a compression model of spinal cord injury, mice with dysfunctional FAS (*i.e.*, *lpr* mice) as well as mice treated with FAS blockers display reduced post-traumatic neuronal degeneration and inflammation coupled to considerable functional improvement {Yu, 2011, 22038545; Casha, 2005, 16202410; Demjen, 2004, 15004554}, a beneficial effect also involving limited engagement of intrinsic apoptosis {Yu, 2009, 19120440}. Likewise, the myeloid cell-specific deletion of *Faslg* favors neuronal regeneration and functional recovery in mice experiencing spinal cord damage {Letellier, 2010, 20153221}. A similar functional improvement after the spinal injury is observed in mice with conditional deletion of *Tnf* in macrophages and neutrophils but not microglia {Ellman, 2020, 33153044}. Moreover, neuroprotection and limited neuroinflammation have been documented in *lpr* mice subjected to traumatic brain injury {Ziebell, 2011, 21871613} as well as in mice subjected to experimental spondylotic myelopathy and exposed to FASLG-neutralizing agents {Yu, 2011, 21490053}. Of note, studies on mice bearing a *Fas* and *Tnfrsf1a* co-deletion reveal at least some redundancy between FAS and TNF-R1 signaling in the context of experimental brain trauma {Yang, 2010, 20205514; Bermpohl, 2007, 17406655; Longhi, 2013, 23611870; Khuman, 2011, 20940727; Quintana, 2005, 16267827}. According to this notion, TNF inhibition reduces damage in mice or rats experiencing spinal cord injury {Mironets, 2018, 29610439; Baratz, 2015, 25879458; Chen, 2011, 21224756}, also limiting the appearance of signs of autonomic dysreflexia, a cardiovascular disease associated with high-level spinal cord injury {O'Reilly, 2021, 33397170; Mironets, 2018, 29610439}. Interestingly, some of these studies point to a neuroprotective function for TNF-R2 {Longhi, 2013, 23611870; Yang, 2010, 20205514; Quintana, 2005, 16267827}, which is in line with at least some results from models of ALS {Tortarolo, 2015, 25940956; Montgomery, 2013, 23567638}. Moreover, independent studies question a pure detrimental effect of TNF signaling in these experimental settings {Ellman, 2016, 28070141; Oshima, 2009, 19616519; Kim,

2001, 11517251; Scherbel, 1999, 10411942}. In particular, TNF seems to support, at least in part, regeneration and long-term functional recovery in rodents exposed to traumatic brain injury {Oshima, 2009, 19616519; Kim, 2001, 11517251; Scherbel, 1999, 10411942}. Conversely, TRAIL neutralization stands out as a promising strategy to promote neuronal regeneration and functional recovery in mice with spinal cord injuries {Cantarella, 2010, 20107429; Fang, 2020, 32848609}. In this context, injured neurons seem to undergo Fas associated via death domain (FADD)- and CASP8-dependent RCD {Sobrido-Camean, 2018, 29666570}. Thus, *Casp8* deletion or transgenic expression of a FADD inhibitor protects mice after spinal cord injury {Sung, 2013, 23935974; Krajewska, 2011, 21957448}. Similarly, transgenic expression of a dominant negative mutant of FADD (FADD-DN) limits motoneuron loss in mice undergoing axotomy {Ugolini, 2003, 13679421}.

Some components of the molecular apparatus for the extrinsic pathway have also been associated with disorders of the visual system, again in the context of exacerbated cell death and inflammation. Thus, in rodent models of optic nerve injury, deletion of *Tnfrsf1a* or inhibition of CASP8 with Z-IETD-FMK prevents the degeneration of retinal ganglion cells {Monnier, 2011, 21775595; Tezel, 2004, 14697498}. Moreover, the lack of TNF-R1 (but not TNF-R2) attenuates neurodegeneration in a mouse model of retinal ischemia, despite neuronal survival being only mildly affected {Fontaine, 2002, 11917000}. Along similar lines, transgenic overexpression of soluble FASLG (which inhibits FAS signaling) {Krishnan, 2016, 27849168}, deletion of *Tnf* {Nakazawa, 2006, 17151265} as well inhibition of FAS {Krishnan, 2019, 31570110} or TNF {Cueva Vargas, 2015, 26338321; Roh, 2012, 22802951} protects mice against retinal ganglion cell death driven by glaucoma. Similar neuroprotective effects have been documented upon the conditional deletion of *Casp8* in astrocytes or Z-IETD-FMK administration {Yang, 2021, 33434617}. In this context, the specific ablation of *Casp8* from endothelial cells reduces retinal neovascularization in a mouse model of oxygen-induced retinopathy {Tisch, 2019, 31454332}, which is in line with the evidence CASP8 inhibition prevents experimental neovascularization of the cornea {Tian, 2020, 32023953}. Finally, in mice, TRAIL neutralization protects the retinal tissue from damages associated with AD {Burgaletto, 2021, 34611142}

In models of focal ischemia driven by middle cerebral artery occlusion, *lpr* mice and *gld* mice display decreased infarct size and neuroinflammation, respectively, {Meng, 2016, 27283206; Niu, 2012, 21802508; Martin-Villalba, 1999, 10234013}. Robust neuroprotection has also been observed in *lpr* mice subjected to neonatal hypoxia-ischemia {Graham, 2004, 15350969}, as well as in *lpr* and *gld* mice subjected to hyperoxia {Dzietko, 2008, 19107989}. FAS inhibition or FASLG neutralization exerts neuroprotective effects in an experimental murine model of stroke {Ullah, 2018, 30301943; Martin-Villalba, 2001, 11464212}. Along similar lines, TRAIL neutralization limits brain injury in rodents subjected to middle cerebral artery occlusion {Xu, 2017, 26971954;Martin-Villalba, 1999, 10234013} or transient ischemia-reperfusion {Cui, 2010, 20359534}. Moreover, despite some contention in this respect {Clausen, 2016, 27384243;Lambertsen, 2009, 19193879;Murakami, 2005, 15935078;Bruce, 1996, 8673925}, abrogation of TNF signaling by genetic or pharmacological means prevents brain injury in rodent models of intracerebral hemorrhage {Lei, 2013, 23962089} and focal cerebral ischemia {Yli-Karjanmaa, 2019, 31440125;Madsen, 2016, 26661199;Wu, 2016, 26374550;Clausen, 2014, 25498129;Arango-Davila, 2015, 25350870;Lu, 2014, 24120040;Nawashiro, 1997, 9183285;Kanazawa, 2019, 31540164;Lin, 2021, 34073455}. Further corroborating an etiological contribution of DR signaling, transgene-driven expression of CASP8 and FADD like apoptosis regulator (CFLAR; best known as c-FLIP) attenuates brain damage after middle cerebral artery occlusion {Xiaohong, 2019, 31387178; Taoufik, 2007, 17581950}. This is in line with the ability of CASP8 to drive BID activation upon focal cerebral ischemia {Yin, 2002, 12200426}, as well as with the neuroprotective effects afforded by pharmacological CASP8 inhibitors to rodents experiencing subarachnoid hemorrhage {Ke, 2020,

31960814} or focal cerebral ischemia {Shabanzadeh, 2015, 26539914; Inoue, 2006, 16632840}. Importantly, FADD and CASP8 expression and/or activation have also been associated with ischemic stroke in humans {Muhammad, 2018, 30354994; Rodhe, 2016, 27566702}.

Of note, TNF appears to protect mice against experimental seizures, not only through the engagement of TNF-R2 but also upon TNF-R1 signaling {Taoufik, 2008, 18413601;Lu, 2008, 18189316;Balosso, 2005, 15852477;Patel, 2017, 28497109;Marchetti, 2004, 15155767;Thompson, 2004, 15046874;Bruce, 1996, 8673925} and consequent modulation of NF-κB {Zhang, 2013, 23627756;Dolga, 2008, 18823372}. Conversely, *lpr* mice {Ettcheto, 2015, 25119776}, mice with neuron-specific deletion of *Tnfrsf1a* {Papazian, 2021, 34565380} as well as rodents treated with Z-IETD-FMK {Krajewska, 2011, 21957448;Li, 2006, 16774749;Henshall, 2001, 11493022} display a relative insensitivity to experimental seizures, pointing to a detrimental role for apoptotic DR signaling. The precise mechanisms through which TNF-R1 signaling promotes anti-apoptotic and anti-inflammatory effects in the context of excitotoxic insults remain unclear. Finally, The FASLG and TRAIL signaling have been associated with alcohol-related neuronal cell death {Qin, 2021, 33806288;Liu, 2021, 32139808}

Cardiovascular disorders. Preclinical models of ischemic and non-ischemic conditions argue in favor of the involvement of FASLG, TRAIL and TNF signaling in the onset and progression of myocardial infarction and other heart diseases. In particular, both *lpr* mice, as well as hearts isolated therefrom, display reduced cardiomyocyte death and infarct area upon experimental ischemia-reperfusion {Lee, 2003, 12414449; Jeremias, 2000, 10952962}. Nonetheless, no protection against ischemia-reperfusion could be documented in *Fas*$^{-/-}$ or *Fasl*$^{-/-}$ mouse hearts {Tekin, 2006, 16456239}. However, supporting the therapeutic relevance of DR inhibition for the management of myocardial infarction, FASLG-neutralizing antibodies as well as a peptide blocking FAS signaling mediate cardioprotection, limit inflammation, and improve cardiac function in mice experiencing cardiac ischemia-reperfusion {Boisguerin, 2020, 31147690; Shiraishi, 2002, 12218072; Covinhes, 2020, 33093627}. Likewise, TRAIL blockade protects monkeys, pigs, and rats against experimental infarction by favoring cardiomyocyte survival and reducing inflammation {Wang, 2020, 32321866}, which is in line with the predictive value of TRAIL as a biomarker for heart failure in patients {Mattisson, 2017, 29208468; Stenemo, 2018, 28967680}. In this context, TRAIL has also been ascribed with apoptosis-independent roles in cardiomyocyte growth and heart hypertrophy {Tanner, 2019, 31473246} as well as in angiogenesis and neovascularization upon hindlimb ischemia {Di Bartolo, 2015, 26572549}.

Similar to neurological conditions, while TNF-R2 signaling appears to mediate cardioprotective effects, the engagement of TNF-R1 drives cardiac hypertrophy, inflammation and cardiomyocyte loss {Hamid, 2009, 19255345;Zhang, 2013, 23704873;Kelly, 2010, 19953003;Monden, 2007, 17416608;Luo, 2006, 17071609;Gouweleeuw, 2021, 33444731;Guo, 2017, 28572508;Higuchi, 2004, 15051641}. The opposite outcome of TNF-R1 *vs.* TNF-R2 signaling has been invoked to explain the clinical failure of TNF blockers agents in patients with chronic heart failure {Mann, 2004, 15023878}, despite encouraging preliminary findings {Deswal, 1999, 11222463; Bozkurt, 2001, 11222463}, as well as cardiotoxic effects associated with the use of TNF blockers in patients with rheumatoid arthritis {Generali, 2019, 30413926}. Confirming the involvement of extrinsic apoptosis in cardiac diseases, the cardiomyocyte-specific deletion of *Fadd* in mice results in improved cardiomyocyte survival and heart functionality after ischemia/reperfusion {Fan, 2013, 24058479}. Along similar lines, *Cflar* haploinsufficiency increases infarct area and aggravates cardiac dysfunction in mice subjected to myocardial infarction, while the cardiomyocyte-specific overexpression of c-FLIP attenuates such effects {Xiao, 2012, 22202974; Liu, 2021, 33895078}. A similar degree of cardioprotection has been observed in a mouse model of ischemia/reperfusion upon shRNA-mediated CASP8 depletion {Liang, 2014, 25060909} or upon

exposure to the CASP8 inhibitor Q-LETD-OPh {Fauconnier, 2011, 21788490}. That said, combined pharmacological inhibition of apoptosis and necroptosis exert a higher cardioprotective effect than monotherapy against myocardial ischemia-reperfusion injury {Koshinuma, 2014, 24113863} suggesting the involvement of multiple RCD pathways in cardiovascular disorders.

FASLG neutralization has been reported to improve cardiomyocyte survival and cardiac function also in a model of cirrhotic cardiomyopathy {Nam, 2014, 24712830}, which is in contrast with the cardioprotective effect of TRAIL and TNF documented in mice developing cardiomyopathy upon apolipoprotein E (*ApoE*) {Toffoli, 2012, 21197620} or desmin (*Des*) {Papathanasiou, 2015, 26280121} deletion, respectively. Both FASLG deficiency and administration of CASP8 inhibitors decrease tissue inflammation and aneurysm formation in mice subjected to $CaCl_2$-induced abdominal aortic aneurysms {Liu, 2019, 30428004}. Moreover, the cardiomyocyte-specific expression of soluble FAS (which competitively inhibits FASLG) protects mice from a genetic form of myocarditis {Niu, 2006, 16678847}. Finally, deletion of *Tnfrsf1b* results in increased cardiomyocyte death and hypertrophy induced by isoproterenol {Tanner, 2021, 34527710}. On the contrary, deletion of *Tnfrsf1a* (but not *Tnfrsf1b*) is cardioprotective in murine models of vascular thrombosis {Pircher, 2012, 23079185}, and heart failure based on angiotensin II administration {Duerrschmid, 2013, 23337087}. Similar cardioprotection to angiotensin II is reported after *Tnfrsf1a* silencing {Woods, 2021, 33303682}. In line with these findings, $Cflar^{+/-}$ mice display increased sensitivity to cardiac injury upon angiotensin II administration {Li, 2010, 20975036}.

FASLG and TNF signaling also promote cardiac maladaptation and hypertrophy in models of pressure overload {Jobe, 2009, 19666842; Sun, 2007, 17353445; Badorff, 2002, 17353445; Stamm, 2001, 11568081; Miao, 2020, 33270628}. Consistent with this notion, TNF inhibition {Mattos, 2020, 32592722}, transgenic c-FLIP overexpression {Giampietri, 2008, 18398344} or conditional *Cflar* knockout {Huang, 2014, 25087120} limits experimental heart hypertrophy driven by hypertension. Moreover, treatment with etanercept reduces cardiac fibrosis in a diet-induced obesity mouse model {Hsu, 2021, 33916242}. Conversely, both FAS and TNF receptor superfamily member 10b (TNFRSF10B, best known as TRAIL-R2) protect mice against atherosclerosis, at least in part by modulating TNF superfamily member 11 (TNFSF11, best known as RANKL) signaling {Di Bartolo, 2013, 24040204;Di Bartolo, 2011, 21965021; Watt, 2011, 21324463; Zadelaar, 2005, 15927188;Yang, 2004, 15178561}, while the impact of TNF on experimental atherosclerosis remains a matter of debate {Xanthoulea, 2009, 19582157; Xanthoulea, 2008, 18628255; Zhang, 2007, 17442899; Secchiero, 2006, 17000905; Branen, 2004, 15345516}. Finally, inhibition of FASLG signaling upon transgenic expression of soluble FAS or pharmacological inhibition of the TNF signaling prevents cardiotoxicity induced by doxorubicin {Miyata, 2010, 20035047;Niu, 2009, 19066339; Clayton, 2021, 33719511}

Renal conditions. FASLG, TNF and TRAIL reportedly support the development of acute kidney injury by driving the activation of both extrinsic apoptosis and inflammation. Thus, loss-of-function mutations in *Faslg*, inhibition or depletion of FASLG {Furuichi, 2012, 22479266;Ko, 2011, 21436290;Hamar, 2004, 15466709} as well as *Fas* {Du, 2006, 16970799} *or Tnf* {Hou, 2016, 27752902} silencing, TNF neutralization {Adachi, 2014, 24407718;Choi, 2009, 19917350}, or TRAIL blockade {Adachi, 2013, 24610963} mediate nephroprotective effects in multiple models of renal ischemia/reperfusion. An experimental setting involving chimeric mice reconstituted with splenocytes from *gld* mice reveals a particular impact of FASLG signaling in the hematopoietic compartment on ischemic acute kidney injury {Ko, 2011, 21436290}. However, some redundancy between DRs has also been reported. Indeed, while one study suggests that FASLG neutralization is more effective than *Tnfrsf1a* deletion in preventing renal inflammation and cell death after acute kidney injury {Furuichi, 2012, 22479266}, another one indicates

that the neutralization of TNF but not FASLG prevents tubular apoptosis and renal atrophy upon ischemia/reperfusion injury {Adachi, 2014, 24407718}.

TRAIL blockade reportedly protects mice against renal damage after full-thickness scald burn {Leng, 2014, 25031778}, while TNF inhibition limits nephrotoxicity, in mice treated with cisplatin {Ramesh, 2002, 12235115}, and acute tubulointerstitial nephritis, in patients administered with checkpoint inhibitors {Lin, 2021, 33643693}. TNF neutralization also results in reduced tubulointerstitial fibrosis and renal injury in a model of unilateral ureteral obstruction {Misaki, 2009, 19541932; Misseri, 2005, 15507546}. At odds with these findings, *Tnf*$^{-/-}$ mice show increased fibrosis at later stages of ureteral obstruction {Morimoto, 2008, 18840428}. This apparent discrepancy may reflect the distinct contribution of TNF-R1 and TNF-R2 signaling to different stages of renal fibrosis driven by ureteral obstruction {Guo, 1999, 10564241}. Conversely, experiments with *lpr* mice subjected to unilateral ureteric ligation demonstrate a limited impact of FAS signaling {Hughes, 1999, 10409294}. Finally, the involvement of CASP8 in acute kidney injury is debated. Thus, while *Casp8* and *Casp3* silencing protects kidneys against damage induced by experimental renal ischemia, alongside increasing survival {Zhang, 2006, 17198267; Du, 2006, 16970799}, the same nephroprotective effect cannot be observed by pan-caspase inhibition with Z-VAD-FMK {Linkermann, 2012, 22237751}, potentially linked to the ability of the latter to favor necroptosis. In line with this notion, chemical receptor interacting serine/threonine kinase 1 (RIPK1) inhibitors, as well as *Ripk3* deletion, exert robust nephroprotection in mouse models of ischemia/reperfusion {Linkermann, 2012, 22237751; Linkermann, 2013, 23818611}. However, co-deletion of *Casp8* and *Ripk3* does not extend the beneficial effects of the *Ripk3*$^{-/-}$ genotype and is associated with a more pronounced demise of tubular epithelial cells by intrinsic apoptosis {Sung, 2019, 30175514}.

Deregulated DR activation has also been associated with chronic kidney disorders, but formal evidence involving CASP8-mediated apoptotic RCD is lacking. In particular, the conditional deletion of *Tnf* from macrophages {Awad, 2015, 26061548}, as well as the administration of TNF inhibitors {Awad, 2015, 26061548; Omote, 2014, 24647715; Moriwaki, 2007, 17767370; Cheng, 2021, 33564432}, appears to mediate beneficial effects in models of diabetic nephropathy. Conversely, the impact of TRAIL on this renal condition remains a matter of debate {Cartland, 2014, 24667560; Lorz, 2008, 18287563; Toffoli, 2020, 32857135}, as that of TNF on polycystic kidney disease {Roix, 2013, 24160989; Li, 2008, 18552856}. As for glomerular inflammation, *gld* mice, as well as wild-type mice treated with TNF blockers, display increased protection against crescentic glomerulonephritis {Tarzi, 2012, 21918502; Khan, 2005, 15840028; Zaenker, 2004, 15648440; Le Hir, 1998, 9881962}. Indeed, balanced TNF-R1 and TNF-R2 signaling appears to be critical for mice to resist experimentally induced glomerulonephritis {Wen, 2020, 31736350; Taubitz, 2013, 23869211; Pfeifer, 2012, 22449555; Vielhauer, 2005, 15841213; Ryffel, 1998, 10319026; Muller, 2019, 30389199}, potentially explaining apparently discrepant findings obtained with TNF-targeting measures.

Hepatic disorders. TNF-deficient mice, as well as rodents treated with TNF inhibitors, present attenuated liver injury and apoptosis upon experimental ischemia/reperfusion, resulting in improved survival {Mahmoud, 2012, 22311349; Hernandez-Alejandro, 2012, 22221603; Rudiger, 2002, 11781294}, a beneficial effect that not always could be recapitulated in *lpr* and *gld* mice {Rudiger, 2002, 11781294}. Similarly, FAS inhibition, FASLG neutralization, as well as administration of low-dose TNF (as a pre-conditioning maneuver) have been shown to protect the liver against ischemia/reperfusion injury by quenching hepatic cell apoptosis and/or inflammation {Al-Saeedi, 2018, 29374146; Nakajima, 2008, 18561025; Teoh, 2003, 12500196}. Hepatoprotection from ischemia/reperfusion has also been observed in mice deficient for TRAIL {Fahrner, 2014, 24804996}, as well as upon the knockdown of

CASP8 or CASP3, the co-deletion of *Casp8* and *Ripk1*, and the transgenic expression of CASP8-insensitive BID mutant {Contreras, 2004, 15300206; Kolachala, 2019, 31334443; Riddle-Taylor, 2007, 17893612}.

Lpr mice {Williams, 2013, 23628456}, *Tnfsf10*$^{-/-}$ mice {Badmann, 2011, 21654829}, as well as animals exposed to TRAIL blockers {Chen, 2020, 31676378}, are protected against acetaminophen-induced liver damage, in line with the notion that FAS signaling exacerbates acetaminophen hepatotoxicity {Tinel, 2004, 14999684}. Along similar lines, the hepatocyte-specific deletion of *Cflar* enhances liver injury and fibrosis induced by CCl_4 and thioacetamide {Schattenberg, 2012, 22700824}. Moreover, a large body of evidence demonstrates that the abrogation of extrinsic apoptosis signaling protects rodents against fulminant hepatitis induced by FASLG and TNF. This has been achieved with strategies including (but not limited to) FADD blockade {Schuchmann, 2003, 12500197;Seino, 2001, 11685033}, hepatocyte-specific *Cflar* {Schattenberg, 2011, 21703207} or *Casp8* {Kang, 2004, 15322156;Liedtke, 2011, 21878202;Ni, 2016, 27616656} ablation, and *Casp8* silencing {Zender, 2003, 12810955}. Consistent with this notion, *Bid*$^{-/-}$ mice resist fatal hepatitis and hepatocellular apoptosis induced by FAS or TNF {Yin, 1999, 10476969; Lazic, 2014, 24681344; Kaufmann, 2009, 19119023}, a protection that is enhanced by concomitant loss of BIM or CASP8 {Kaufmann, 2009, 19119023} and can be recapitulated by the lack of deficiency of modulator of apoptosis 1 (MOAP1), an activator of BID downstream of FAS signaling {Tan, 2016, 27320914}. Conditional deletion of *Bak* and *Bax1 or Bbc3*, as well as overexpression of BCL2, protects hepatocytes from FAS-induced fulminant death, although inhibition of liver injury in this setting requires concomitant caspase inhibition {Hikita, 2011, 21425311; Rodriguez, 1996, 8642244; Lacronique, 1996, 8564847; Tan, 2021, 33980818}. The differential sensitivity of BAD-deficient mice to TNF-induced hepatitis is controversial {Yan, 2013, 23332762; Ottina, 2015, 25611386}. Conversely, mice deficient for CASP3 or treated with CASP3 or CASP8 inhibitors display reduced sensitivity to FAS-mediated hepatocyte apoptosis {Woo, 1999, 10528193; Bajt, 2001, 11559023}. Of note, some degree of mutual compensation between caspases and alternative mechanisms of caspase activation have emerged from studies in hepatocytes responding to FAS agonists {Zheng, 2000, 11062535}. Finally, FAS and TNF-R1 signaling, as well as FADD activation, have been involved in liver regeneration following partial hepatectomy {Sudo, 2008, 18948191; Desbarats, 2000, 10932231; Sudo, 2008, 18948191; Knight, 2005, 15592751; Taira, 2001, 11805393}. In this context, the liver-specific deletion of *Casp8* results in deregulated hepatocyte proliferation upon hepatectomy coupled to the initiation of an inflammatory response {Ben Moshe, 2007, 17385212}. However, it has been suggested that CASP8 modulates liver regeneration by balancing NF-κB activation and necroptosis rather than inducing apoptosis {Freimuth, 2013, 23728913}.

Gld mice chronically fed with ethanol display reduced liver injury, steatosis and inflammation as compared to their wild-type counterparts, but exhibit signs of incipient fibrosis {Isayama, 2016, 27102767}. Some degree of protection against alcohol-induced liver damage has also been documented in mice deficient for TRAIL-R2 {Verma, 2016, 26632633} or TNF-R1 (but not TNF-R2) {Yin, 1999, 10500078}, as well as in mice receiving a TRAIL-neutralizing antibody {Mundt, 2005, 16227360}. Moreover, the hepatocyte-specific ablation of *Casp8* limits hepatic steatosis in animal models of ethanol administration, although it fails to prevent apoptotic RCD {Hao, 2017, 29072704}. Conversely, apoptosis driven in hepatocytes by chronic ethanol exposure can be abolished by systemic inhibition of CASP3 with Ac-DEVD-FMK {Zhou, 2001, 11438480}, suggesting that ethanol-induced liver injury is involved both intrinsic and extrinsic apoptosis.

The liver-restricted overexpression of FAS induces hepatic steatosis and insulin resistance in mice subjected to a high-fat diet (HFD) {Item, 2017, 28883393}. In the same experimental setting,

hepatoprotection has been linked to the hepatocyte-specific ablation of *Fas*, germline deletion of *Bid* as well as with the administration of pharmacological BID inhibitors {Item, 2017, 28883393}. Moreover, *Tnf* deletion {Kakino, 2018, 28922680;Salles, 2012, 22464148}, whole-body deletion of *Tnfrsf1a* alone or in combination with *Tnfrsf1b* {Kanuri, 2011, 20801629;Tomita, 2006, 16174657} as well as inhibition of TNF {De Sousa Rodrigues, 2019, 31892368;Ilan, 2016, 27818591;Koca, 2008, 18066656} or TNF-R1 {Wandrer, 2020, 32235829} significantly reduce hepatic steatosis, fibrosis, damage, and metabolic alterations in different diet-induced or genetic models of non-alcoholic fatty liver disease (NAFLD). In apparent contrast with these findings, the hepatocyte-specific deletion of *Tnfrsf1a* fails to protect mice from diet-driven NASH {Bluemel, 2020, 32952340}. Moreover, *Tnfrsf1a* deletion accelerates disease progression from steatosis to steatohepatitis in mice fed an HFD {Lambertucci, 2018, 29860102}. Taken together, these findings underscore the pleiotropic and context-dependent effects of TNF signaling in NAFLD. The impact of TRAIL in NAFLD is also debated. Indeed, contrasting evidence from experiments with animals deficient for TRAIL or treated with recombinant TRAIL ascribes either a detrimental or a beneficial role to TRAIL signaling in NAFLD induced by HFD {Bernardi, 2018, 29167318; Hirsova, 2017, 29124251; Cartland, 2017, 28507343}.

The absence of TRAIL-R2 has been shown to promote hepatic inflammation and fibrosis in a genetic mouse model of cholestasis {Krishnan, 2020, 32240619}. Similarly, *lpr* mice {Gujral, 2004, 15382126; Canbay, 2002, 12360492; Miyoshi, 1999, 10464144} as well as TNF-deficient {Osawa, 2013, 23755201; Gabele, 2009, 18996089} and TRAIL-deficient {Takeda, 2008, 18667695; Kahraman, 2008, 18220275} mice display reduced hepatocyte apoptosis and fibrogenesis after experimental cholestasis induced by bile duct ligation. In line with these results, expression of phosphorylation-mimicking FADD mutant results in attenuated HFD-induced hepatomegaly and steatosis {Zhuang, 2016, 27357657}. Moreover, experiments based on the hepatocyte-specific deletion of *Cflar* or transgenic overexpression of c-FLIP revealed a role for the latter as a suppressor of hepatic steatosis and inflammation induced by an HFD {Wang, 2017, 28218919}. In this setting, administration of a small c-FLIP-derived peptide exerts therapeutic effectiveness against steatohepatitis, both in mice and in monkeys {Wang, 2017, 28218919}. Moreover, the hepatocyte-specific deletion of *Cflar* in mice results in enhanced cholestatic liver injury and inflammatory responses upon bile duct ligation {Gehrke, 2018, 29191940}. Also, the hepatocyte-specific deletion of *Casp8* protects mice against liver injury in models of cholestatic hepatitis based on the administration of 3,5-diethoxycarbonyl-1,4-dihydrocollidine {Chaudhary, 2013, 23928400}, as well as in models of steatosis based on a methionine- and choline-deficient diet {Hatting, 2013, 23339067}. A similar hepatoprotection against obstructive cholestasis has been documented in mice with a hepatocyte-specific *Casp8* deletion {Cubero, 2018, 30144553}. Finally, decreased BID cleavage has been associated with attenuated liver injury in mouse models of chronic cholestasis {Vogel, 2006, 16401474}.

Hematologic malignancies and solid cancers. Experiments in *Lpr* mice demonstrate that FAS dysfunctions accelerate lymphomagenesis driven by MCY but not by Moloney murine leukemia virus {Zornig, 1995, 7784089}, which is in line with the requirement of FAS for effective MYC-induced apoptosis {Hueber, 1997, 9360929}. Suppression of MYC-induced tumorigenesis is also reported in transgenic mice expressing FADD-DN {Hueber, 2004, 15382083} but not c-FLIP {Hogstrand, 2012, 22393362}. Similarly, the ablation of *Fas* exacerbates the development of lymphoma in *Trp53*$^{+/-}$ mice, resulting in decreased animal survival {Embree-Ku, 2002, 12512872}. Of note, in this model, deletion of *Tnf* or *Tnfrsf1a* has no impact on oncogenesis {Kuprash, 2008, 18442881}, ruling out a role for the TNF signaling in the development of lymphoma, at least on p53 deficiency. On the contrary, TRAIL seems to exert a tumor-promoting function in lymphomagenesis. Thus, the ablation of one *Tnfrsf10b* allele delays the development of lymphoma in Emu-myc mice {Finnberg, 2008, 18079962}. Moreover,

deficiency in TRAIL (but not TRAIL-R) accelerates the development of lymphoma and other tumors in mice with haploinsufficiency for *Tpr53* {Zerafa, 2005, 16237043; Yue, 2005, 15514675}.

The role of FAS and TRAIL in the development of colorectal cancer is debated. For instance, the loss of *FAS* enhances APC-induced but not inflammation-induced intestinal tumorigenesis {Guillen-Ahlers, 2010, 20140201; Park, 2010, 20049944; Fingleton, 2007, 17510409}. Along similar lines, while the ablation of *Tnfrsf10b* in mice does not impact tumorigenesis induced by APC {Yue, 2005, 15514675}, the administration of TRAIL suppresses oncogenesis in a mouse model of colitis-associated cancer {Kim, 2018, 29416724}. Despite some contentious in this respect {Lopetuso, 2016, 27956796;Craven, 2015, 25581824;Nyboe Andersen, 2014, 24938563;Chang, 2012, 22052015}, TNF seems to contribute colorectal cancerogenesis. Indeed, the abrogation of the TNF signaling through administration of TNF blockers {Ba, 2021, 34675913; Yang, 2020, 33768208; Kim, 2010, 20736334; Onizawa, 2009, 19179628; Rao, 2006, 16397216} or ablation of *Tnf* {Oshima, 2014, 23975421} or *Tnfrsf1a* {Oshima, 2014, 23975421; Popivanova, 2008, 18219394} limits tumor development, as shown in an animal model of colorectal cancer induced by colitis, APC or wingless-type MMTV integration site family, member 1 (WNT1).

With regard to other tumor types, both TNF and FAS display a pro-oncogenic role in hepatic and ovary oncogenesis. Thus, conditional deletion of *Fas* in hepatocytes delays chemically-induced hepatocarcinogenesis, while *Fas* ablation suppresses the development of ovary tumors in phosphatase and tensin homolog (PTEN)-deficient/Kirsten rat sarcoma viral oncogene (KRAS) mutated mice {Chen, 2010, 20505730}. Likewise, TNF neutralization limits the onset of hepatic cancer driven by experimental induced cholestatic hepatitis {Pikarsky, 2004, 15329734}. In line with this evidence, *Casp8*$^{-/-}$ mice are protected against the development of inflammation-imposed liver cancer {Liedtke, 2011, 21878202}, although a more recent study shows a tumor-suppressive functions of CASP8 in early tumorigenesis (but not tumor progression) exerted by modulating the DNA damage response {Boege, 2017, 28898696}. On the contrary, loss of *Tnfrsf10b* exacerbates chemically-induced hepatocarcinogenesis {Finnberg, 2008, 18079962}, suggesting oncosuppressive functions for TRAIL in this cancer type.

Confirming the protumorigenic effect of TNF, the ablation of *Tnf* or *Tnfrsf1a* or the blockade of TNF in mice confers a certain degree of protection against chemically-induced skin cancerogenesis {Rodriguez, 2020, 33202705; Schioppa, 2011, 21670304; Arnott, 2004, 14661063; Scott, 2003, 12748306; Suganuma, 1999, 10493498; Moore, 1999, 10395330}. On the contrary, the impact of genetic and pharmacological inhibition of TNF in UVB-induced skin cancer is debated {Caliskan, 2021, 31868056; Singh, 2016, 26586792}. Of note, the comparison between *Tnfrsf1a*$^{-/-}$ and *Tnfrsf1b*$^{-/-}$ mice reveals a primary role of TNF-R1 in chemically-induced skin oncogenesis {Arnott, 2004, 14661063}. Lending further ground to this evidence, TNF-R1 deficiency suppresses the development of skin cancer induced by NF-kB inhibition {Lind, 2004, 15044707}. A similar tumor supportive role for TNF-R1 has been described in mice models of N-methyl N-nitrosurea/testosterone-induced prostate cancer {Galheigo, 2016, 27018768} and of methylcholanthrene (MCA)-induced fibrosarcoma {Sobo-Vujanovic, 2016, 26896171}. As opposed to TNF-R1, TNF-R2 shows tumor-suppressive function, at least against the development of fibrosarcoma imposed by MCA {Sobo-Vujanovic, 2016, 26896171} and of breast cancer induced by WNT1 {He, 2021, 33383310}. As for other tumor types, in mice, TNF deficiency impairs tumor growth in HER2-driven mammary tumorigenesis {Sangaletti, 2010, 20924115}. Moreover, TNF neutralization suppresses chemical-induced oral {Chadwick, 2021, 34650923} and urethane-induced pulmonary {Karabela, 2011, 22241960} oncogenesis. Along similar lines, TNF overexpression in the airway epithelium promotes KRAS-driven lung tumorigenesis {Gong, 2016, 27853654}.

Preclinical evidence confirms a certain degree of tumor type-specificity for the role of TRAIL in oncogenesis. Thus, on the one hand, transgenic expression of TRAIL in the skin delays chemical-induced carcinogenesis {Kedinger, 2011, 21463519}, an effect recapitulated in mice lacking TRADD {Chio, 2012, 22561347} but not in TRAIL-R-deficient mice {Grosse-Wilde, 2008, 18079967}, which show enhanced lymph node metastasis. On the other hand, *Tnfsf10*$^{-/-}$ mice as well as mice treated with TRAIL blockers display increased susceptibility to develop MCA-induced fibrosarcoma {Takeda, 2002, 11805143; Cretney, 2002, 11801676}. Moreover, malignant cell-specific ablation of *Tnfrsf10b* limits tumor growth and improves the survival in a mouse model of KRAS-driven lung and pancreatic tumorigenesis {von Karstedt, 2015, 25843002}, while systemic ablation of *Tnfsf10* had no impact on HER-2 driven breast oncogenesis {Zerafa, 2005, 16237043}.

Autoimmune and inflammatory diseases. The impact of DRs in the etiology of autoimmune disease is rather heterogeneous. On the one hand, TRAIL signaling appears to prevent or protect rodents against autoimmune encephalomyelitis {Chyuan, 2018, 29403497;Ikeda, 2010, 20921531;Cretney, 2005, 16174101;Razmara, 2009, 19147815;Aktas, 2005, 15882642;Hilliard, 2001, 11145715}, autoimmune arthritis {Zauli, 2010, 20185810;Lamhamedi-Cherradi, 2003, 12577054;Song, 2000, 10748228;Park, 2017, 29017854;Chyuan, 2018, 28392572;Jin, 2010, 19933369} and type I diabetes {Kang, 2010, 21047948;Mi, 2003, 12882912;Lamhamedi-Cherradi, 2003, 12577054;Bossi, 2015, 25759846;Di Bartolo, 2011, 21965021;Lamhamedi-Cherradi, 2003, 12941766}. On the other hand, the engagement of FAS and TNF-R1 is generally associated with the development of autoimmune conditions. Indeed, both *lpr* and *gld* mice, as well as TNF-R1-deficient mice, are protected against experimental encephalomyelitis {Bachmann, 1999, 10329594; Malipiero, 1997, 9464800; Waldner, 1997, 9317104; Sabelko, 1997, 9317103}. Similar results have been obtained in mice with *Tnf* deletion in the monocytes and macrophages (but not microglial cells) {Wolf, 2017, 28330904} as well as in mice subjected to TNF neutralization or TNF-R1 inhibition {Williams, 2018, 30206422; Williams, 2014, 24587232; Nomura, 2011, 20036293; Steeland, 2017, 29057962; Brambilla, 2011, 21908877; Korner, 1997, 9295034; Korner, 1995, 7479938;Richter, 2021, 34305946}. That said, FAS-independent mechanisms also appear to support the pathogenesis of experimental autoimmune encephalomyelitis {Dittel, 1999, 10352252; Bachmann, 1999, 10329594}, with some studies pointing to a protective role for FAS-dependent RCD amongst lymphocytes {Suvannavejh, 2000, 10642601}. Moreover, FAS ligation seems to differentially contribute to the initiation of and recovery from autoimmune encephalomyelitis {Wang, 2013, 23011975;Sabelko-Downes, 1999, 10209037}. In particular, FASLG expression in astrocytes appears to promote recovery from experimental autoimmune encephalomyelitis, as shown by persisting demyelination and paralysis of mice with an astrocyte-restricted *Fasl* deletion {Wang, 2013, 23011975}. Finally, at least in some studies, *Tnf* deletion or TNF neutralization fails to attenuate the severity of autoimmune encephalomyelitis once the disease is established {Batoulis, 2014, 24111507; Liu, 1998, 9427610}.

As for other autoimmune disorders, mice with dysfunctional FASLG or TNF signaling are protected against arthritis induced by collagen type II {Tu-Rapp, 2004, 15380040; Shen, 2019, 30745461; Moore, 2014, 25344414; Zalevsky, 2007, 17641054}. Similar protection is observed in mice transplanted with mesenchymal stem cells engineered to express TNF inhibitors {Zhao, 2021, 34627365}. In keeping with this evidence, the myeloid cell-specific deletion of *Fas* or the administration of antibody simultaneously targeting TNF and chemokine (C-X-C motif) ligand 10 (CXCL10) results in accelerated disease resolution in a model of rheumatoid arthritis induced by K/BxN serum transfer {Huang, 2014, 24431281; Kang, 2021, 33453429}. Moreover, the administration of FASLG promotes autoantibody-induced arthritis, although by ligation of TRAIL-R2 rather than FAS {Jeong, 2021, 34223817}. Notably, in these settings, TNF-R2 displays anti-inflammatory effects, suppressing inflammatory arthritis {Fu, 2021,

34185706}. Likewise, abrogation of FAS signaling by genetic or pharmacological means confers protection against autoimmune diabetes in specific animal models including NOD mice {Itoh, 1997, 9254659; Su, 2000, 10679090; Chervonsky, 1997, 9094710; Vence, 2004, 15504959; Mohamood, 2007, 17591957; Jeong, 2010, 20004692}. However, whether the impact of FAS on the pathogenesis of autoimmune diabetes depends on pancreatic β cell death {Itoh, 1997, 9254659} or inflammation (*e.g.*, in the context of insulitis) remains a matter of debate {Vence, 2004, 15504959}. Moreover, some studies targeting FAS proved to be therapeutically ineffective {Trivedi, 2019, 31552143; Choi, 2009, 19755672; Thomas, 1999, 10415060}. TNF neutralization is therapeutically effective only in a limited fraction of patients with inflammatory bowel disease {Biemans, 2020, 32237087; Almon, 2021, 32501868}. This is in line with the evidence that *Tnfrsf1a* deletion exacerbates colitis in the interleukin 10 (IL10)-deficient mice {Liu, 2020, 33086075}. Similar protection has been ascribed to the TRAIL signaling in a dextran sodium sulfate-induced colitis model {Lin, 2021, 33932348; Chyuan, 2019, 31076664}. Finally, FASLG and TNF signaling has been suggested to contribute to the pathogenesis of acute pancreatitis {Pinhu, 2014, 24566874; Randhi, 2021, 34049483}. A similar detrimental role has been proposed for TNF signaling in autoimmune neuritis {Mao, 2010, 20035831;Taylor, 2007, 17196669;Bao, 2003, 12609491}, despite some contention {Lu, 2007, 17428547}, as well as in spondyloarthritis {Kaaij, 2021, 34561228} and psoriasis {Chen, 2021, 34494306}. Conversely, FAS and TRAIL-R2 appear to mediate beneficial effects in autoimmune thyroiditis {Yu, 2011, 21225479; Fang, 2008, 18810759; Wei, 2004, 15585889; Wang, 2009, 19008314; Wang, 2005, 16123163} At least in part, these findings reflect the pleiotropic effects of whole-body/systemic inhibition of DRs signaling, which concomitantly involves both the target (*i.e.*, parenchymal) and the perpetrator (*i.e.*, immune cells) of damage.

Some experimental evidence links CASP8 activation to autoimmune disorders. In a mouse model of autoimmune encephalomyelitis, the oligodendrocyte-specific deletion of *Fadd* reduces demyelination coupled to limited immune cell infiltration in the spinal cord {Mc Guire, 2010, 21068410}. Likewise, experimental autoimmune encephalomyelitis can be prevented by transgenic expression of FADD-DN in T cells {Sun, 2005, 16177127}. Reinforcing this evidence, active CASP8 has been documented in the microglia of patients with multiple sclerosis {Zhang, 2018, 30372424}. Moreover, transgenic expression of FADD-DN or *Casp8* ablation in pancreatic β cells protects mice from autoimmune diabetes {Allison, 2005, 15972661}. Conversely, BID appears to be dispensable for the development of diabetes in NOD mice {Mollah, 2011, 21644000}. Moreover, contrasting observations on the impact of extrinsic apoptosis on the development and resolution of autoimmune rheumatoid arthritis have been reported. Indeed, *Cflar* deletion results in increased disease severity but limited disease resolution in mice experiencing arthritis upon intraperitoneal injection of K/BxN serum {Huang, 2017, 28511285}. In the same model, deletion of *Casp8* in myeloid cells at large favors disease resolution, while deletion of *Casp8* in DCs accelerates disease onset {Dominguez, 2017, 28978351}. Further experiments are required to unveil the reasons for such cell type specificity and formally elucidate the impact of extrinsic apoptosis in this pathological condition.

Infectious Diseases. The extrinsic apoptosis is reported to act as an anti-infection mechanism. *Lpr* mice *gld* and *Bid*$^{-/-}$ mice exhibit delayed clearance of *Citrobacter rodentium* and increased intestinal pathology{Pearson, 2013, 24025841}. These effects were attributed to the inhibition of extrinsic apoptosis of infected enterocytes promoted by specific virulence proteins, such as the N-acetylglucosamine transferase NleB1, which prevents the association of FADD to the extrinsic apoptosis complexes{Pearson, 2013, 24025841, Li, 2013, 23955153}. Along similar lines, *Fas*$^{-/-}$ mice survived less than the wild-type mice to *Listeria monocytogenes*, succumbing by neurolisteriosis promoted by an impaired loss of monocytes due to upregulated expression of c-FLIP by the bacterial protein InlB {Uchiyama, 2017, 28674179}. In support of this result, monocytes isolated from *Cflar*$^{-/-}$ mice displayed

resistance to apoptosis, while conditional deletion of *Cflar* in myeloid cells improved *Listeria monocytogenes* clearance and animal survival {Maudet, 2022, 35296858}. The FAS signaling also conferred protection from infection of (i) human herpes simplex virus 2 (HSV-2), as demonstrated by a decreased monocyte loss and immune cell recruitment at the infection site in HSV-2-infected *Fas*$^{-/-}$ and *Faslg*$^{-/-}$ mice {Krzyzowska, 2013, 23922974}, and (ii) *Citrobacter rodentium* or lymphocytic choriomeningitis virus, as demonstrated by increased neutrophil fraction in mice with conditional deletion of *Fas* in the myeloid compartment {O'donnell, 2015, 25473101}.

Concerning the apoptotic signaling downstream of DRs, mice lacking kinase activity of RIPK1 failed to control systemic *Yersinia* infection, rapidly succumbing to RIPK1-dependent apoptosis {Peterson, 2017, 28855241}. In line with this evidence, *Ripk3*$^{-/-}$*Casp8*$^{-/-}$ but not *Ripk3*$^{-/-}$ mice died from *Toxoplasma gondii* infection due to acute toxoplasmosis, a result supporting the anti-infection role of CASP8-mediated apoptosis {Delaney, 2019, 31147458}. Moreover, the deletion of Z-DNA binding protein 1 (*Zbp1*), an essential cytoplasmic DNA sensor involved in the activation of both mixed lineage kinase domain like pseudokinase (MLKL)-dependent necroptosis and RIPK1/FADD-dependent apoptosis, as well as *Mlkl*$^{-/-}$*Fadd*$^{-/-}$ mice failed to control Influenza A virus (IAV) infection, succumbing to lethal respiratory failure. These results put forward an essential role of both apoptosis and necroptosis in IAV clearance {Thapa, 2016, 27746097, Oltean, 2021, 33976111, Nogusa, 2016, 27321907}. Similarly, combined activation of apoptosis and other RCD pathways contributed to mice response to *Burkholderia thailandensis* infection {Place, 2021, 34154417}.

Experimental evidence also suggests a detrimental role of extrinsic apoptosis during certain infections. Mice deficient for both TNF-R1 and TNF-R2 display decreased sensitivity to lipopolysaccharides, suggesting a critical role of TNF in tissue injury during gram-negative bacterial infection {Alikhani, 2003, 14551216}. Along similar lines, *Tnfrsf1a*$^{-/-}$ mice were more resistant than their wild-type counterparts to the cytopathic effects of TNF during Sindbis Virus infection, as evidenced by reduced mortality and delayed paralysis {Sarid, 2001, 11753570}. Moreover, ablation of *Ripk1* protected mice from acute liver injury by infection of *Listeria monocytogenes* {Qian, 2020, 32106368}, while knockout of *Fas* or *Faslg* reduced the effect of toxin A-induced enteritis in mice infected by *Clostridium difficile*, which has been attributed to a reduction in enterocyte loss{Kim, 2007, 17854595}. Additionally, the infectious spleen and kidney necrosis virus (ISKNV) induced tissue damages in zebrafish via a mechanism involving the activation of the extrinsic apoptosis by a viral protein encoding for a TRADD interactor {He, 2012, 22615868}. Of note, in this study, the absence of CASP8 protected zebrafish from ISKNV infection. Finally, *Ripk3*$^{-/-}$*Casp8*$^{-/-}$ mice exhibited high levels of protection from a lethal cytokine shock and tissue damage driven by TNF and IFN-γ, mirroring that of SARS-CoV-2 {Karki, 2021, 33278357}. This evidence suggests that the combination of several types of RCD can mediate infection-associated pathogenesis.

Others. TNF is reported to impair myogenesis in a mouse model of skeletal muscle regeneration upon hindlimb suspension {Langen, 2004, 14769817}. In addition, *Tnfsf10* silencing improves muscle regeneration in mice with acute skeletal muscle injury due to local injection of BaCl2 {Kim, 2020, 32645396}. An inhibitory role in myogenesis is also ascribed to FADD, at least in response to frozen-induced muscle injury {Zhang, 2014, 26303234}. In apparent contrast with this result, co-deletion of *Tnfrsf1a* and *Tnfrsf1aìb* limits muscle regeneration upon injury induced by cardiotoxin {Chen, 2007, 17151142; Chen, 2005, 16079187}, suggesting the relevance of a balance between the TNF-R1 and TNF-R2 signaling in this model. Finally, TRAIL neutralization increases muscular strength in a mouse model of Duchenne muscular dystrophy {Dufresne, 2018, 29699580}, while some findings associate TRAIL and FASLG signaling to myositis {Alger, 2011, 21769834; Kondo, 2009, 19740320}.

The activation of DRs has also been involved in the pathogenesis of acute lung injury. *Fas* silencing as well as TNF neutralization protects mice from lung injury induced by ischemia-reperfusion {Del Sorbo, 2016, 26963318; An, 2007, 17786556}. Along similar lines, deletion of *Tnfrsf1a* or pharmacological inhibition of TNF-R1 or CASP8 attenuates edema formation and improves alveolar epithelial functions in a mice model of acute lung injury induced by acid inhalation {Patel, 2013, 23487422; Wilson, 2017, 28243236}. A similar protective effect was provided by pharmacological and/or genetic abrogation of the FASLG or TNF signaling in a lipopolysaccharide-induced mouse model of acute lung injury {Bohr, 2020, 32798665; Lai, 2019, 31037135; Proudfoot, 2018, 29382797;Bohr, 2017, 28639789; Weifeng, 2016, 26990441; Cakarova, 2009, 19590023; Matute-Bello, 2004, 15013988}, although in one study FAS signaling is shown to contribute to the resolution of acute lung injury by promoting the depletion of macrophages {Janssen, 2011, 21471090}. Using distinct mouse models of acute lung damage following sepsis induction, it has been shown that the abrogation of FASLG and TNF signaling, including the silencing of *Fadd*, resulted in decreased pulmonary apoptosis and disease amelioration, in some cases coupled to temporary survival benefit for the animals (e.g., {Qian, 2021, 33435767; Weckbach, 2013, 23425737; Thakkar, 2011, 21451443; Perl, 2007, 17600273; Perl, 2005, 16314469; Messer, 2013, 23247118; Matsuda, 2009, 19201926}. On the contrary, the impact of DR in hyperoxic lung injury and bleomycin-induced pulmonary fibrosis is debated. Thus, on the one hand, FAS deficiency or ablation of *Tnf* exacerbates oxidant-induced lung injury and/or inflammation in newborn mice {Ehrhardt, 2016, 27016588; Mao, 2008, 18587053}. On the other hand, TNF inhibition in rodents confers a certain degree of protection against hyperoxic lung damage {Guthmann, 2009, 19916860; Kaya, 2016, 27309384; Wolthuis, 2009, 18650784}. Moreover, deficiency in TNF-R1 (but not TNF-R2) improved survival in mice subjected to an excessive oxygen supply, although without decreasing inflammation {Pryhuber, 2000, 10781441}. In support of this result, specific ablation of *Fas* in murine fibroblasts or T cells exacerbates pulmonary fibrosis induced by bleomycin {Redente, 2020, 33290280; Hao, 2004, 15148335}. However, the level of bleomycin-induced pulmonary fibrosis is diminished in *lpr* or *gld* mice {Aoshiba, 2000, 10934108} and remains unmodified in mice treated with FAS neutralizing agents {Kuwano, 1999, 10393694}. Likewise, contrasting findings support a role for TNF {Redente, 2014, 24325577; Oikonomou, 2006, 17205112; Kuroki, 2003, 12496444} and TRAIL {Collison, 2019, 30732588; McGrath, 2012, 22496351} in both the resolution and the onset of pulmonary fibrosis after administration of bleomycin. Of note, TNF neutralization attenuates and enhances interstitial pulmonary fibrosis induced respectively by nitrogen mustard {Malaviya, 2015, 26243812} and rituximab {Tan, 2015, 25809984}. Finally, FAS, TNF and/or TRAIL have been involved in infectious or non-infectious lung disorders, including (but not limited to) infection with respiratory syncytial virus (RSV) {Santos, 2021, 33303545;Morris, 2020, 33080861;Nguyen, 2016, 27036916;van den Berg, 2011, 21743025;Lopez, 2009, 20007588;Bem, 2010, 19635930;Neuzil, 1996, 8615393}, adenovirus type 1 respiratory {Pant, 2020, 32560900;Adkins, 2018, 29908447}, allergic reaction and asthma {Li, 2017, 28619762;Starkhammar, 2015, 26494305;Tisato, 2014, 25506835;Faustino, 2014, 24569802;Yilmaz, 2013, 24063972;Sharma, 2012, 22175699;Hwang, 2010, 20194815;Weckmann, 2007, 17934471;Chuang, 2006, 16565865;Broide, 2001, 11245629;Whitehead, 2017, 28758900;Maillet, 2011, 21297077;Choi, 2005, 16159621}, idiopathic pneumonia syndrome {Hildebrandt, 2008, 18342780} as well as in chronic lung diseases (e.g., chronic obstructive pulmonary disease {Gong, 2016, 27853654;Wu, 2015, 26609227;Haw, 2016, 26555706}).

Concluding remarks

Abundant preclinical evidence with experimental disease models demonstrates that the intrinsic and the extrinsic pathways of apoptosis not only contribute to embryonic and post-embryonic development and to adult tissues homeostasis, but are also implicated in the etiology of multiple disorders including

various cardiovascular, hepatic, neurological and renal disorders as well as multiple infectious, autoimmune, inflammatory and oncological conditions {Singh, 2019, 30655609}. However, despite a high potential as targets for therapeutic interventions and a considerable research effort dedicated to develop effective modulatory approaches, the success of intrinsic or extrinsic apoptosis-targeting agents in clinical settings is so far very limited.

Rather than mitigating the enthusiasm about the clinical potential of apoptotic modulators, this unsuccess suggests the need for a substantial change in the experimental design and result interpretation, at different levels (**Figure 1**). One major issue is that studies evaluating the impact of apoptotic cell death on disease has not always addressed the interlink between the core components of the intrinsic and extrinsic apoptotic machinery or their potential interaction with other RCD pathways. Also, they have not always explored (and thus tried to prevent or overcome) the potential activation of alternative RCD modalities as a mechanism to compensate for the inhibition of apoptotic RCD. Along with this, individual apoptotic players or signaling cascades exert a variety of functions beyond cell death induction, including (but not limited to) inflammation (*e.g.*, multiple activated caspases), terminal differentiation (*e.g.*, pro-and anti-apoptotic BCL2 proteins), cell proliferation and survival (*e.g.*, DR engagement), whose relevance is often dependent on cell/tissue type (as it is related to a variable expression levels and activation status of RCD players and their regulators) and the intensity and duration of the initiating stimulus (as they can direct to a distinct biological outcome, as exemplified by DR ligation). On the one hand, this pleiotropy results in a variable (even including an antagonistic protective vs. promoting) impact of apoptosis on distinct human diseases, also explaining the considerable degree of context-dependency observed for its experimental modulation. On the one hand, the pathogenic effect of core components of the apoptotic machinery is often mediated by such apoptosis-unrelated functions (e.g., inflammation), which highlights unexplored targets for the development of new therapeutic agents or approaches.

Investigating the molecular cascade of apoptotic cell death in the context of the functional interconnection between apoptotic and non-apoptotic (RCD) pathways may thus drive a suitable development, and ultimately lead to clinical use, of specific apoptosis-modulatory regimens.

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Table 1. XXX.

Abbreviations.

Legends to Figures

Figure 1. **Principal causes of the therapeutic failure of intrinsic or extrinsic apoptosis inhibitors**.

Figure 2. **Molecular machinery for the intrinsic apoptosis.**

Figure 3. **Impact of intrinsic apoptosis players on neurological disorders.**

Figure 4. **Molecular machinery for the extrinsic apoptosis.**

Figure 5. **Impact of extrinsic apoptosis players on neurological disorders.**

References

Box 1. Principle of intrinsic apoptosis.

Intrinsic apoptosis is a type of regulated cell death (RCD) initiated by perturbations of the extracellular or intracellular microenvironment including (but not limited to) DNA damage, endoplasmic reticulum or oxidative stress, growth factor withdrawal, microtubular alteration, whose critical step is mitochondrial outer membrane permeabilization (MOMP) {Tait, 2010, 20683470;Galluzzi, 2016, 28357340;Dadsena, 2021, 33704419;Bock, 2020, 31636403}. MOMP is modulated by the activity of multiple pro-apoptotic and anti-apoptotic members of the BCL2, apoptosis regulator (BCL2) protein family). {Czabotar, 2014, 24355989; Shamas-Din, 2013, 23545417; Kalkavan, 2018, 29053143;Birkinshaw, 2017, 28396106}. In response to apoptotic stimuli, MOMP leads to the sequential activation of the initiator caspase 9 (CASP9) and executioner CASP3 and CASP7 {Julien, 2017, 28498362;Shalini, 2015, 25526085;Green, 2022, 35232877;Kumar, 2022, 34940803;Kesavardhana, 2020, 32017655}Two functionally-distinct classes of pro-apoptotic BCL2 proteins have been identified. The first class encompasses the apoptotic activators BCL2 associated X, apoptosis regulator (BAX), BCL2 antagonist/killer 1 (BAK1), and BOK, BCL2 family apoptosis regulator (BOK) {Moldoveanu, 2020, 31570337}. Once activated by apoptotic stimuli, BAX, BAK and BOK induce MOMP by generating pores across the outer mitochondrial membrane (OMM) {Llambi, 2016, 26949185;Bleicken, 2013, 24100034;Bleicken, 2013, 23442864} These pro-apoptotic factors promote the release into the cytosol of several apoptogenic factors including cytochrome c, somatic (CYCS) and diablo IAP-binding mitochondrial protein (DIABLO; also known as second mitochondrial activator of caspases, SMAC). The former exerts apoptogenic activity by associating with apoptotic peptidase activating factor 1 (APAF1) and pro-CASP9 to generate a complex known as apoptosome, leading to sequential activation of CASP9 and the executors CASP3 and CASP7{Dorstyn, 2018, 29765111}. The latter contributes to CASP3/CASP7 activation by associating with X-linked inhibitor of apoptosis (XIAP) and other members of the inhibitor of apoptosis (IAP) protein family {Shiozaki, 2004, 15337122}.

The second class of pro-apoptotic BCL2 proteins (known as BH3-only proteins) include BCL2 associated agonist of cell death (BAD), BCL2 binding component 3 (BBC3; best known as p53-upregulated modulator of apoptosis, PUMA), BCL2 interacting killer (BIK), BCL2 like 11 (BCL2L11; best known as BCL2-interacting mediator of cell death, BIM), Bcl2 modifying factor (BMF), BH3 interacting domain death agonist (BID), harakiri, BCL2 interacting protein (HRK, also known as DP5), and phorbol-12-myristate-13-acetate-induced protein 1 (PMAIP1; best known as {Kale, 2018, 29149100;Giam, 2008, 19641498}). Of them, BID, BIM, PUMA, and NOXA promote BAX and BAK activation through a direct interaction with these proteins at the mitochondria{Gavathiotis, 2008, 18948948;Gavathiotis, 2010, 21070973;Kim, 2009, 19917256;Wei, 2001, 11326099;Kim, 2006, 17115033;Dai, 2011, 21727192;Chen, 2015, 26344567}, while BAD, BIK, BMF and HRK activate BAX and BAK indirectly by associating with anti-apoptotic BCL2 family members, thereby hampering the inhibitory binding of the latter to BAX and BAK.{O'Neill, 2016, 27056669;Letai, 2002, 12242151}. The anti-apoptotic members of the BCL2 family encompasses BCL2, apoptosis regulator (BCL2), BCL2 like 1 (BCL2L1; best known as BCL-X_L), MCL1, BCL2 family apoptosis regulator (MCL1), BCL2 like 2 (BCL2L2; best known as BCL-W), and BCL2 related protein A1 (BCL2A1) {Czabotar, 2014, 24355989; Shamas-Din, 2013, 23545417; Kalkavan, 2018, 29053143;Birkinshaw, 2017, 28396106}.

Box 2. Impact of pro-apoptotic BCL2 proteins on health.

Deletion of BCL2-associated X protein (*Bax*), BCL2-antagonist/killer 1 (*Bak1*) or BCL2-related ovarian killer (*Bok*) does not significantly affect mice development {Knudson, 1995, 7569956;Lindsten, 2000, 11163212;Ke, 2012, 22281706}, with the exception of a mild lymphocyte and neuron accumulation in *Bax*$^{-/-}$ mice associated with infertility due to germ cell death in the context of seminiferous tubule malformation {Knudson, 1995, 7569956;Deckwerth, 1996, 8816704}. Of note, a recent study has demonstrated that such defects in germ cells occur in the fetal period {Nguyen, 2020, 33199844}, supporting the requirement for intrinsic apoptosis in testicular development {Russell, 2002, 11906913;Rodriguez, 1997, 9171341}. Subsequent studies confirmed the role of BAX in neurogenesis, in particular the development of hippocampal and cerebellar neurons, cortical interneurons and astrocytes {White, 1998, 9454852;Fan, 2001, 11413548;Jung, 2008, 18337425;Sun, 2004, 15590937;Chang, 2007, 17438128;Southwell, 2012, 23041929}. Accordingly, *Bax*$^{-/-}$ mice exhibit impaired neurological functions manifesting with increased anxiety, depression-like traits, compromised social and sexual behavior, and impaired spatial representation and olfactory system function {Jyotika, 2007, 17525992;Luedke, 2013, 23142367;Krahe, 2015, 26363094}. These mice also show accelerated medulloblastoma formation {Garcia, 2013, 22710714}, which is in line with the oncosuppressive activity of apoptotic (and non-apoptotic) regulated cell death (RCD) {Hanahan, 2011, 21376230}.

Ablation of *Bok* does not compromise the relatively normal development of BAK- or BAX-deficient mice, although *Bax*$^{-/-}$*Bok*$^{-/-}$ mice exhibit an increased number of mature oocytes {Ke, 2013, 23744350}. On the contrary, co-deletion of *Bax* and *Bak1* causes perinatal death in the vast majority (more than 90%) of mice, mainly due to multiple developmental abnormalities and feeding difficulties {Lindsten, 2000, 11163212}. Importantly, the developmental defects of *Bax*$^{-/-}$*Bak1*$^{-/-}$ mice are exacerbated by *Bok* deletion, underscoring not only some functional redundancy between BAX, BAK1 and BOK, but also a crucial role of pro-apoptotic BCL2 family members in the development of the central nervous system (CNS) and hematopoietic compartment {Ke, 2018, 29775594}. However, since *Bax*$^{-/-}$*Bak1*$^{-/-}$ and *Bax*$^{-/-}$*Bak1*$^{-/-}$ *Bok*$^{-/-}$ mice reach adulthood {Ke, 2018, 29775594;Lindsten, 2000, 11163212}, additional systems must be at play to compensate for defective apoptosis in other organs. In this context, it is interesting to note that the developmental defects of *Bax*$^{-/-}$*Bak1*$^{-/-}$ mice can be further aggravated by deletion of autophagy related 5 (*Atg5*) {Arakawa, 2017, 28574506}, which is involved in autophagy as well as in non-canonical vesicular pathways like LC3-associated phagocytosis {Rybstein, 2018, 29476153; Galluzzi, 2019, 31199916}. However, whether autophagy-dependent cell death compensates for the apoptotic defects of *Bax*$^{-/-}$*Bak1*$^{-/-}$ mice remains to be formally determined {Miller, 2020, 32334815; Fairlie, 2020, 32334814}.

Further corroborating the relevance of intrinsic apoptosis for proper development, surviving *Bax*$^{-/-}$*Bak1*$^{-/-}$ mice display phenotypes related to defective programmed cell death (PCD), including webbed feet (due to the incomplete removal of interdigital webs), imperforate vagina and cleft {Lindsten, 2000, 11163212}. CNS issues exhibited by these animals include limited neural stem cell pool {Lindsten, 2003, 14657169} as well as impaired function of the motor {Gu, 2017, 28472660} and visual {Hahn, 2003, 12882813;Hahn, 2005, 15955981} system. Tissue-specific ablation of *Bax* and *Bak1*, confirmed the crucial role of these proteins in the hematopoietic system, and specifically in the homeostasis and functionality of B cells{Takeuchi, 2005, 16055554}, T cells {Biswas, 2010, 20813900} megakaryocytes {Kodama, 2012, 22790873} and platelets {Pleines, 2018, 29784641}. In line with this notion, mice reconstituted with fetal liver cells from *Bax*$^{-/-}$*Bak1*$^{-/-}$ mice display massive lymphadenopathy and defective T cell proliferation, and the severity of these defects is even more pronounced when *Bak1*$^{-/-}$ *Bax*$^{-/-}$*Bok*$^{-/-}$ fetal liver cells are used for reconstitution, an experimental setting that also reveal signs of autoimmunity {Ke, 2015, 26492371;Rathmell, 2002, 12244308;Jones, 2007, 17692540}. Similarly, mice

reconstituted with a *Bak1*$^{-/-}$*Bax*$^{-/-}$ hematopoietic compartment develop a fatal systemic lupus erythematosus (SLE)-like autoimmune disease {Mason, 2013, 23349374}. Moreover, the inducible co-deletion of *Bax* and *Bak1* in lymphocytes of adult mice result in the development of severe autoimmune glomerular nephritis {Takeuchi, 2005, 16055554}. Finally, conditional knockout mouse models reveal a crucial contribution of BAX and BAK1 to endothelial homeostasis {Watson, 2016, 27471260;Grant, 2020, 32427589}, but little impact on cardiac and intestinal functions, as shown by absence of hyperplasia {Whelan, 2012, 22493254;Kirsch, 2010, 20019247}. These results demonstrate that multidomain pro-apoptotic BCL2 proteins are essential for the normal development of multiple tissues but not for organismal survival.

Amongst BH3-only proteins, BCL2 like 11 (BCL2L11, best known as BIM) appears the most critical for embryonic development and tissue homeostasis, as shown by the fact that approximately half of *Bcl2l11*$^{-/-}$ mice die during embryogenesis {Bouillet, 1999, 10576740}. Surviving *Bcl2l11*$^{-/-}$ mice display severe defects in the hematopoietic system including lymphoid hyperplasia and marked splenomegaly, and spontaneously develop systemic autoimmunity often resulting in fatal kidney disease {Bouillet, 1999, 10576740}, a condition that can be accelerated by depletion of immunosuppressive CD4+CD25+FOXP3+ regulatory T (T_{REG}) cells {Wang, 2017, 28152566}. These mice also display deregulated T cell development and homeostasis {Hutcheson, 2007, 17869640;Chougnet, 2011, 21098226;Bouillet, 2002, 11859372;Enders, 2003, 14517273;Zhan, 2011, 21742968} and hence orchestrate defective cellular {Pellegrini, 2003, 14623954;Hildeman, 2002, 12121658;Pellegrini, 2004, 15504823} and humoral {Fischer, 2007, 17720882;Sugimoto-Ishige, 2020, 32889526;Oliver, 2004, 15520248} immune responses. When not embryonic lethal, *Bcl2l11* deletion has also been associated with the impaired development of granulocytes {Villunger, 2003, 12433687}, mammary glands {Mailleux, 2007, 17276340;Schuler, 2016, 26045049}, gastric epithelium {Ohgushi, 2005, 16260615} and retina {Doonan, 2007, 17913922}, and with reduced adiposity {Wali, 2018, 29053141}. Of note, systemic deletion of *Bax* or *Bak1* exacerbates the hematopoietic deregulation of BIM-deficient mice {Hutcheson, 2005, 15967824}. Conditional knockout systems confirmed the key role of BIM in the homeostasis of the hematopoietic system {Liu, 2018, 29623080;Herold, 2014, 25299771;Huntington, 2009, 19454543;Ludwig, 2020, 31993851}, and pointed to the involvement of BIM also in the survival and differentiation of hippocampal neurons {Bunk, 2010, 21364616}. Of note, myeloid cell-specific deletion of *Bcl2l11* induce an SLE-like disease that resembles the pathology developing in surviving *Bcl2l11*$^{-/-}$ mice, which is associated with fatal glomerulonephritis {Tsai, 2017, 29114065}.

As opposed to *Bcl2l11*$^{-/-}$ mice, mice lacking BH3 interacting domain death agonist (BID), phorbol-12-myristate-13-acetate-induced protein 1 (PMAIP1, best known as NOXA) or BCL2 binding component 3 (BBC3, best known as PUMA) display normal embryonic development {Yin, 1999, 10476969;Leonard, 2001, 11412905;Villunger, 2003, 14500851;Jeffers, 2003, 14585359}. In these studies, a decreased apoptotic competence linked to *Bid* knockout could be documented in hepatocytes {Yin, 1999, 10476969}, pancreatic cells {McKenzie, 2008, 18252892;Yin, 1999, 10476969} and possibly neurons {Engel, 2010, 20646170;Zinkel, 2003, 12533511}. Moreover, *Bid*$^{-/-}$ mice display a deregulated myeloid compartment resulting in increased likelihood for leukemogenesis {Zinkel, 2003, 12533511}, as well as some cardiac dysfunctions {Salisbury-Ruf, 2018, 30281024}. Subsequent conditional deletion studies confirmed the relevance of BID in the homeostasis and functionality of hepatic and T cells {Wree, 2015, 25909884;Tischner, 2012, 22257939;Lazic, 2014, 24681344}. Of note, some degree of apoptotic impairment could also be documented in *Bbc3*$^{-/-}$ mice, which display increased resistance to genotoxins {Villunger, 2003, 14500851;Jeffers, 2003, 14585359;Erlacher, 2005, 16118324;Wang, 2021, 34193827}, and *Pmaip1*$^{-/-}$ mice, which show limited stress-induced erythropoiesis {Wensveen, 2013, 23975731}. Moreover, germline *Bbc3* or *Pmaip1* deletion affects humoral immune responses {Clybouw,

2011, 21868573;Wensveen, 2012, 22144184} and increases the abundance of multiple cell types in the retina {Harder, 2011, 21762490}. Interestingly, studies in *Bbc3*$^{-/-}$ mice reveal a role for PUMA-mediated apoptosis in radiation-driven lymphomagenesis and hepatocarcinogenesis {Labi, 2010, 20679395;Michalak, 2010, 20679396;Michalak, 2009, 19148184; Qiu, 2011, 21725994} (see the main text), potentially reflecting the ability of apoptotic cells to secrete mitogenic and immunosuppressive molecules such as prostaglandin E2 (PGE_2) {Huang, 2010, 21725296; Bottcher, 2018, 29429633}, as well as in radiation-induced intestinal damage {Qiu, 2008, 18522850}. On the contrary, using *Bbc3*$^{-/-}$ mice it was shown that neuronal defects induced by the hyperactivation of transformation related protein 53 (TRP53, best known a p53) in neural crest cells occur in a PUMA-independent fashion {Bowen, 2021, 33574585}.

Co-deletion of multiple genes coding for BH3-only proteins confirmed the pronounced relevance of BIM for development and underscored some degree of functional redundancy in the system. On the one hand, *Bbc3*$^{-/-}$*Pmaip1*$^{-/-}$ mice develop normally, showing a mild decrease in sensitivity to genotoxins {Michalak, 2008, 18259198}. Moreover, whole-body deletion of *Bid*, *Bbc3* or *Bbc3* plus *Pmaip1* in *Bcl2l11*$^{-/-}$ mice does not significantly increase the lethality rate and the severity of hematopoietic defects imposed by the lack of BIM {Erlacher, 2006, 17178918;Gray, 2012, 22960223;Happo, 2010, 20829369;Kaufmann, 2009, 19119023}. On the other hand, *Bcl2l11*$^{-/-}$*Bbc3*$^{-/-}$*Bid*$^{-/-}$and *Bcl2l11*$^{-/-}$*Bbc3*$^{-/-}$*Bid*$^{-/-}$*Pmaip1*$^{-/-}$ mice displayed increased incidence of developmental defects, including webbed feet, imperforate vagina and supernumerary neurons as compared to *Bax*$^{-/-}$*Bak1*$^{-/-}$ mice but a lower perinatal lethality {Ren, 2010, 21127253;Chen, 2015, 26344567}, suggesting that the elevated lethality of the latter may involve alterations in processes other than apoptosis, such as Ca^{2+} signaling {Rong, 2008, 17680735; Marchi, 2020, 32559424}.

Along similar lines, mice lacking BCL2-associated agonist of cell death (*Bad*), BCL2 interacting killer (*Bik*), BCL2 modifying factor (*Bmf*) and harakiri, BCL2 interacting protein (contains only BH3 domain) (*Hrk*) are viable and develop normally {Ranger, 2003, 12876200;Imaizumi, 2004, 15084651;Labi, 2008, 18299399;Coultas, 2004, 14749373}. That said, BAD-deficient mice display a prolonged platelet lifespan {Kelly, 2010, 20431598}, while *Bmf*$^{-/-}$ mice are characterized by mild lymphadenopathy, vaginal atresia {Labi et al. 2008 18299399;Hubner, 2010, 19841067} as well as minor defects in mammary gland development and oogenesis {Schuler, 2016, 26045049;Vaithiyanathan, 2016, 26917450}. Of note, defective gland development has been also observed in transgenic mice expressing a *Bad* variant that abolishes activating BAD phosphorylation {Githaka, 2021, 34011960}. Again, co-deletion of these BH3-only protein coding-genes does not cause significant embryonic lethality or developmental abnormalities, although accrued lymphomagenesis has been observed in *Bik*$^{-/-}$*Pmaip1*$^{-/-}$ mice {Happo, 2012, 22573037}, and increased spontaneous tumorigenesis has been documented in *Bad*$^{-/-}$*Bmf*$^{-/-}$ mice {Baumgartner, 2013, 22430207}. Conversely, the absence of some of these BH3-only proteins aggravates the defects imposed by the *Bcl2l11*$^{-/-}$ genotype. This applies to: (1) *Bad*, whose co-deletion with *Bcl2l11* promotes accrued lymphocyte accumulation {Kelly, 2010, 20431598}, (2) *Bik*, whose co-deletion with *Bcl2l11* drives male infertility due to defective spermatogenesis {Coultas, 2005, 16270031}, a phenotype resembling that of BAX-deficient mice, and (3) *Bmf*, whose co-deletion with *Bcl2l11* considerably increases the incidence of developmental defects, vaginal atresia, lymphadenopathy, autoimmune glomerulonephritis, and spontaneous tumorigenesis {Labi, 2014, 24632712;Hubner, 2010, 19841067;Woess, 2015, 25698446}.

Box 3. Impact of anti-apoptotic BCL2 proteins on health.

While myeloid cell leukemia sequence 1 (*Mcl1*) deletion in mice induces embryonic lethality at the blastocyst stage prior to implantation {Rinkenberger, 2000, 10640272;Kuida, 1998, 9708735}, embryos lacking BCL2-like 1 (BCL2L1, best known as BCL-X_L) die after implantation (approximately at embryonic day 13.5) as consequence of massive cell depletion in the developing central nervous system (CNS) and hematopoietic system {Motoyama, 1995, 7878471}. Deletion of BCL2-associated X protein (*Bax*) or caspase 9 (*Casp9*) considerably limits neuronal cell death imposed by the *Bcl2l1*$^{-/-}$ genotype, but some degree of neuronal malformations persist, especially in *Bcl2l1*$^{-/-}$*Casp9*$^{-/-}$ mice {Zaidi, 2001, 11150333;Shindler, 1997, 9096145}. Similarly, deletion of BCL2 like 11 (*Bcl2l11*) rescues hematopoietic (but not neuronal) cell death in BCL-X_L-deficient mice {Akhtar, 2008, 18606610}. On the contrary, *Bcl2*$^{-/-}$ mice develop normally but exhibit severe post-natal defects including alterations of the CNS, hematopoietic system and the thymus, and succumb to polycystic kidney disease at a young age {Bouillet, 2001, 11709185;Veis, 1993, 8402909;Nakayama, 1993, 8372353;Kamada, 1995, 7812968;Michaelidis, 1996, 8755480;Manzl, 2013, 23454286;Carpinelli, 2012, 22874999}, a series of problems that can be rescued by *Bcl2l11* co-deletion {Bouillet, 2001, 11709185}. Confirming an elevated degree of functional redundancy, deletion of B cell leukemia/lymphoma 2 related protein A1a (*Bcl2a1a*) or BCL2-like 2 (*Bcl2l2*) does not cause developmental defects {Hamasaki, 1998, 9841913;Print, 1998, 9770502;Ross, 1998, 9500547}. However, *Bcl2a1a*$^{-/-}$ mice display minor defects in the hematopoietic compartment {Hamasaki, 1998, 9841913;Xiang, 2001, 11733571;Schenk, 2017, 28085150;Tuzlak, 2017, 28085151}. Moreover, the absence of BCL2L2 (best known as BCL-W) results in male infertility due to defective spermatogenesis {Print, 1998, 9770502;Ross, 1998, 9500547;Russell, 2001, 11420255}. A role of BCL-W in B cell survival and lymphomagenesis (see below) has also been reported {Adams, 2017, 28094768}.

As opposed to homozygous deletion, haploinsufficiency for *Mcl1* or *Bcl2l1* did not result in defects in normal mouse development {Motoyama, 1995, 7878471; Rinkenberger, 2000, 10640272}. However, *Mcl1*$^{+/-}$ mice display decreased amounts of various cell types from the hematopoietic system {Brinkmann, 2017, 28800129;Delbridge, 2015, 25847014}, and poor hematopoietic recovery from stress, which can be rescued by BCL2 binding component 3 (*Bbc3*) deletion {Delbridge, 2015, 25847014}. Moreover, the loss of one *Bcl2l1* allele limits male fertility due to defects in germ cell development {Kasai, 2003, 14623242} and platelet half-life {Mason, 2007, 17382885}. Of note, while combined haploinsufficiency for *Mcl1* and *Bcl2*, for *Mcl1* and *Bcl2a1a* or for *Bcl2l1* and *Bcl2* does not affect normal mice development {Schenk, 2020, 32555150;Grabow, 2018, 30232009;Ke, 2020, 32170090}, *Mcl1*$^{+/-}$*Bcl2l1*$^{+/-}$ mice display embryonic or early post-natal lethality, a defect that can be rescued by deletion of a single *Bcl2l11* allele {Grabow, 2018, 30232009}. These observations suggest that physiological development during embryogenesis requires a correct balance between pro- and anti-apoptotic BCL2 proteins.

Conditional knockout studies confirmed the relevance of pro-survival BCL2 family members in specific tissues at precise developmental stages. In this context, MCL1 turned out to favor the development and/or maintenance of most (but not all) hematopoietic cell populations including stem cells {Opferman, 2005, 15718471}, B and T lymphocytes {Opferman, 2003, 14668867;Pierson, 2013, 23852275;Vikstrom, 2010, 20929728;Tripathi, 2013, 23558951}28972012{Dunkle, 2010, 20057504}}, natural killer (NK) cells {Sathe, 2014, 25119382}, neutrophils {Dzhagalov, 2007, 17062731;Steimer, 2009, 19064728}, mast cells and basophils {Lilla, 2011, 22001390}, as well as plasma cells {Slomp, 2018, 30524962; Peperzak, 2013, 23377201}. Of note, accumulating evidence suggests that the survival of some hematopoietic cells can rely on the combined activity of multiple anti-apoptotic BCL2 family member

{Carrington, 2017, 28362427}. Conditional deletion of *Bcl2l1* alone or in combination with *Mcl1* demonstrated some degree of functional redundancy between BCL-X_L and MCL1 in developing lymphocytes {Dzhagalov, 2008, 18566418;Malin, 2010, 19946273} and megakaryocytes {Debrincat, 2012, 22374700;Josefsson, 2011, 21911424;Wagner, 2000, 11044408;Mason, 2007, 17382885}. Conversely, BCL2 and BCL2A1 appear to be critical for the survival of B cells and neutrophils {Vikstrom, 2016, 27560714; Sochalska, 2016, 26450454; Schenk, 2020, 32555150} but not megakaryocytes and platelets {Debrincat, 2015, 25880088}. Data from chimeric mice generated by transplanting stem cells deficient for pro-survival BCL2 family member in blastocysts largely confirm the role of these proteins in physiological hematopoiesis {Motoyama, 1995, 7878471;Ma, 1995, 7761398;Matsuzaki, 1997, 9028316;Villunger, 2003, 12433687}. Of note, BCL2 is reported to contribute to the homeostasis and development of the mouse epidermis {Geueke, 2021, 34342114}. Along similar lines, MCL1 and BCL-X_L have also been shown to participate to the development or homeostasis of other tissues including the myocardium {Thomas, 2013, 24165322;Wang, 2013, 23788622}, the CNS {Arbour, 2008, 18550749;Germain, 2011, 21139567;Malone, 2012, 22357134;Harder, 2012, 22836101;Nakamura, 2016, 27194326;Savitt, 2005, 16033881;Fogarty, 2016, 27665712;Fogarty, 2019, 30361616;Veleta, 2021, 33293647}, the hepatic parenchyma {Weng, 2011, 21146511;Hikita, 2009, 19676108;Takehara, 2004, 15480996;Vick, 2009, 19127517;Boege, 2017, 28898696}, vascular endothelium {Watson, 2016, 26943318}, as well as the intestinal {Healy, 2020, 32179094}, mammary {Walton, 2001, 11731240}, lung {Staversky, 2010, 19880821} and renal {Brinkmann, 2020, 33236795} epithelium.

As a general principle, these studies indicate that the severity of the phenotype imposed by the conditional deletion of genes coding for anti-apoptotic BCL2 family members largely depends on the specific gene and tissue. For instance, conditional deletion of *Mcl1* in mouse hematopoietic stem cells {Opferman, 2005, 15718471}, erythroid cells {Turnis, 2021, 33512417} or T_{REG} cells {Teh, 2020, 32612106} is lethal. In this latter case, lethality is ascribed to multiorgan autoimmunity caused by the depletion of the pool of T_{REG} cells {Teh, 2020, 32612106}. Similarly, the megakaryocyte-specific deletion of *Mcl1* and *Bcl2l1* provokes embryonic or perinatal lethality {Debrincat, 2012, 22374700}, which can be rescued by *Bax* and BCL2 antagonist/killer 1 (*Bak1*) co-deletion {Kodama, 2012, 22790873}. Similar findings have been obtained upon the ablation of *Mcl1* from the CNS or the myocardium, and that of *Bcl2l1* from the respiratory epithelium, although these experiments did not include rescue approaches {Staversky, 2010, 19880821;Wang, 2013, 23788622;Arbour, 2008, 18550749;Germain, 2011, 21139567}. The functional cooperation between MCL1 and BCL-X_L appears to be particularly relevant in the CNS and liver {Hikita, 2009, 19676108;Fogarty, 2019, 30361616}. Of note, the requirement of MCL1 and BCL-X_L for physiological neurogenesis appears to fluctuate over time, although neurodevelopmental defects imposed by the deletion of *Mcl1* or *Bcl2l1* can be rescued by *Bax* co-deletion {Fogarty, 2019, 30361616;Shindler, 1997, 9096145}. Along similar lines, the detrimental effects of the hepatocyte-specific ablation of *Bcl2l1* or *Mcl1* can be rescued by deletion of *Bax* and *Bak1* as well as by that of of *Bcl2l11* and/or BH3 interacting domain death agonist (*Bid*) {Hikita, 2009, 19839062;Kodama, 2013, 23986435}. These observations demonstrate that organogenesis and adult tissue homeostasis depends on the functional balance between both pro and anti-apoptotic factors. Further substantiating this notion, deletion of *Bcl2l1* from keratinocyte precursors limits skin oncogenesis driven by ultraviolet B (UVB) rays and chemical carcinogens {Kim, 2009, 19309000}. Conversely, the hepatocyte-specific deletion of *Mcl1* promotes hepatic carcinogenesis {Weber, 2010, 20099303}, as does the deletion of *Mcl1* in intestinal epithelial cells (IECs) {Healy, 2020, 32179094}. These latter findings may appear counterintuitive, as pre-malignant cells are expected to be more susceptible to succumb to environmental stress in the absence of *Mcl1* or *Bcl2l1*. However, both hepatic and intestinal carcinogenesis involve a robust inflammatory component that is exacerbated by tissue damage and cell death {Coussens, 2013, 23329041}.

Box 4. Impact of the apoptosome and apoptotic caspases on health

In mice, the whole-body deletion of apoptotic peptidase activating factor 1 (*Apaf1*) or caspase 9 (*Casp9*) is associated with early embryonic lethality as a consequence of inhibited PCD {Honarpour, 2000, 10656767;Yoshida, 1998, 9753321}. Severe abnormalities in APAF1-deficient embryos include the presence of webbed feet, craniofacial malformations, incomplete neural tube closure and/or excessive brain growth and exencephaly resulting in alteration of the central nervous system (CNS) including visual and olfactory systems {Cecconi, 1998, 9753320;Yoshida, 1998, 9753321;Nonomura, 2013, 24369835;Long, 2013, 23892366;Ohsawa, 2010, 20624980}. Similar defects in the developing brain result from *Casp9* deletion {Yoshida, 1998, 9753321;Hakem, 1998, 9708736;Kuida, 1998, 9708735}, a phenotype that is not exacerbated by *Casp2* co-deletion {Marsden, 2004, 15210727}. On the contrary, deletion of *Casp9* did not rescue neuronal defects due p53 hyperactivation in neural crest cells {Bowen, 2021, 33574585}.

Of note, clinical evidence linking *APAF*1, *CASP9* and *CASP3* mutation and neural tube defects in humans has also been reported {Spellicy, 2018, 29358613;Zhou, 2018, 29352212}. Mice lacking cytochrome c, somatic (CYCS) die at midgestation {Li, 2000, 10830166}, while the deletion of cytochrome c, testis (*Cyct*), which is specifically expressed in male gonads is associated with normal development but male infertility {Narisawa, 2002, 12101247}. The neuron-specific ablation of *Cycs* also results in postnatal cell death {Pinto, 2019, 30191381}. Confirming that the detrimental effects of *Cycs* deletion results from impaired apoptosis, mice expressing a mutant CYCS that retains the ability to shuttle electrons as a component of the mitochondrial respiratory chain but is unable to assemble the apoptosome exhibit perinatal lethality and developmental brain defects similar to APAF1- and CASP9-deficient mice {Hao, 2005, 15907471}.

Importantly, mouse strain appears to significantly influence the impact of core components of the apoptotic machinery on embryonic development. Thus, while genetic deletion of *Casp3* in 129S1/SvImJ mice results in embryonic or early postnatal lethality due the severe defects in brain development that are only partially rescued by *Bcl2l1* co-deletion, *Casp3*$^{-/-}$ C57BL/6 mice develop normally and survive into adulthood {Woo, 1998, 9512515;Kuida, 1996, 8934524;Leonard, 2002, 12152782;Roth, 2000, 10618441}. A similar mouse strain discrepancy has been documented for *Apaf1* {Matsumoto, 2020, 32979334;Okamoto, 2006, 16294213}. However, adult *Casp3*$^{-/-}$ C57BL/6 mice appear to exhibit defects in complex brain functions including attention and (in males) social behavior, possibly due to the non-apoptotic role of CASP3 in neuronal development {Lo, 2016, 26783106;Lo, 2015, 25653368}, as well as ear and vestibular dysfunction including hearing loss {Takahashi, 2001, 11251216;Morishita, 2001, 11374883;Parker, 2010, 21116635;Armstrong, 2015, 25827332;Makishima, 2011, 21988729} and abnormalities in the kidney and spleen {Suzuki, 2020, 31440817}. Such a discrepancy has been ascribed to the compensatory activation of CASP7 in C57BL/6 mice {Houde, 2004, 15525783}. Indeed, co-deletion of *Casp3* and *Casp7* is lethal in the C57BL/6 background, although death is caused by severe cardiac rather cerebral defects {Lakhani, 2006, 16469926}. Such phenotypic differences may originate from some degree of substrate selectivity exhibited by CASP3 vs. CASP7 {McComb, 2019, 31392262;Lamkanfi, 2009, 19168786;Walsh, 2008, 18723680;Yoshida, 2021, 33417971}.

Approximately 5% of APAF1-deficient mice also develop normally and survive into adulthood, although males are often sterile due to defective spermatogenesis {Honarpour, 2000, 10656767}, which is reminiscent of the phenotype of mice deficient for BCL2-associated X protein (*Bax*), BCL2-antagonist/killer 1 (*Bak1*) and BCL2-related ovarian killer (*Bok*) (*i.e.*, *Bak1*$^{-/-}$*Bax*$^{-/-}$*Bok*$^{-/-}$ mice) {Ke, 2018, 29775594}. Of note, rare adult *Apaf1*$^{-/-}$ male mice that retain fertility display expansion of the lateral

brain ventricles coupled to behavioral abnormalities and growth retardation {Okamoto, 2006, 16294213}. Conversely, the rare mice expressing a CYCS variant specifically deficient in apoptotic functions that survive into adulthood exhibit impaired lymphocyte homeostasis {Hao, 2005, 15907471}. Confirming some degree of functional redundancy in the intrinsic apoptotic cascade, whole-body deletion of diablo, IAP-binding mitochondrial protein (*Diablo*, coding for a pro-apoptotic factor also known as SMAC) alone or along with HtrA serine peptidase 2 (*Htra2*) does not result in developmental defects in mice {Okada, 2002, 11971981;Martins, 2004, 15509788}, while the *Diablo*$^{-/-}$*Casp3*$^{-/-}$ genotypes accrues the perinatal lethality observed in *Casp3*$^{-/-}$ mice {Hui, 2011, 21597464}. Mice lacking the X-linked inhibitor of apoptosis (XIAP, the main target of the pro-apoptotic activity of SMAC and HTRA2) are also viable and develop normally, possibly due to functional compensation by other members of the inhibitor of apoptosis protein (IAP) family {Olayioye, 2005, 15540113;Harlin, 2001, 11313486}, but exhibit mild defects in late pregnancy that do not compromise lactation {Olayioye, 2005, 15540113}. *Xiap*$^{-/-}$ mice also show deregulated innate immune responses {Prakash, 2010, 20427267}, most likely linked to the modulatory role of XIAP in inflammation and necroptosis {Damgaard, 2012, 22607974}, or to the inability of these animals to resolve infections {Hsieh, 2014, 25190756}. Accordingly, loss-of-function mutations in *XIAP* are associated with X-linked lymphoproliferative syndrome type 2 {Salzer, 2008, 18520160; Yang, 2012, 22228567}.

The myocardium-specific deletion of *Casp3* and *Casp7* affects physiological heart development in mice resulting in myocyte hypertrophy {Cardona, 2015, 26121671}. Along similar lines, transgenic mice expressing the baculoviral pan-caspase inhibitor p35 in the CNS or dendritic cells (DCs) display postnatal growth retardation, hydrocephalus and lethality {Yoshida, 2021, 33417971} or chronic lymphocyte activation and signs of systemic autoimmunity {Chen, 2006, 16497935}, respectively. Conversely, the impact of APAF1, CASP9 and CASP3 in hematopoiesis remains debated. Specific ablation of *Apaf1* or *Casp9* from the hematopoietic system in mice reconstituted with hematopoietic stem cells deficient for these factors does not expand the lymphocyte compartment {Marsden, 2002, 12374983}. Likewise, no hematopoietic defects emerge upon the whole-body deletion of *Casp3* or *Casp9* {Lakhani, 2006, 16469926}, in line with the notion that mice lacking *Casp9* in the hematopoietic system display a proper generation and functionality of megakaryocytes and platelets {White, 2012, 22294729}. Moreover, the clearance of *Casp9*$^{-/-}$ thymocytes seems to occur in a caspase-independent fashion {van Delft, 2010, 19911005}. Apparently at odds with these observations, *Casp3*$^{-/-}$ mice display increased numbers of splenic B cells manifesting increased proliferative attitude {Woo, 2003, 12970760}, as well as a deregulated activity in bone marrow stromal stem cells that attenuates osteogenic differentiation {Miura, 2004, 15599395}. Moreover, two studies report that mouse bone marrow chimeras deficient for APAF1 or CASP9 display an hematopoietic defect and/or reduced survival {Lu, 2014, 25349173;White, 2014, 25525874}. A similar debate revolves around the requirement for APAF1 and caspase activity in thymocyte selection and/or T cell responses {Tong, 2018, 29596528;Hara, 2002, 11859117;Nagasaka, 2010, 19960021;Doerfler, 2000, 10754300;Izquierdo, 1999, 9878059}. Taken together, these findings suggest that physiological hematopoiesis may operate at least in part via non-apoptotic regulated cell death (RCD) pathways, at least in some mouse strains. Similar observations have been obtained for the development of murine postmitotic neurons and chicken motoneurons in animals genetically deleted of *Apaf1*, *Casp9* or *Casp3* {Oppenheim, 2008, 18256270;Oppenheim, 2001, 11425902;Yaginuma, 2001, 11520178;Honarpour, 2001, 11566499}. However, neither the degree of functional redundancy exhibited by CASP3, CASP6 and CASP7, nor the potential for APAF1-independent CASP3 activation has been formally excluded in these studies, most of which involved single genetic alterations and otherwise observational approaches.

Box 5. Principles of extrinsic apoptosis.

Extrinsic apoptosis is a regulated cell death (RCD) variant triggered by perturbations of the extracellular microenvironment through the engagement of two types of cell surface receptors: the dependence receptor and the death receptor. The former are activated by the decrease in the availability of a specific ligand on which these receptors depend, while the latter by the binding of a cognate ligand{Gibert, 2015, 26627011;Aggarwal, 2012, 22053109;Wajant, 2002, 12040174;Mehlen, 2011, 21266712}. The dependence receptors include (but are not limited to) the DCC netrin 1 receptor (DCC) and distinct types of unc-5 netrin receptors (UNC5A, UNC5B, UNC5C, and UNC5D), all of which are bound by netrin 1 (NTN1), and the neurotrophic receptor tyrosine kinase 3 (NTRK3) and patched 1 (PTCH1), which are, respectively, ligated by neurotrophin and sonic hedgehog (SHH). The activation of dependence receptors stimulates a hitherto poorly characterized signaling cascades often dependent on caspase activation, leading to the induction of cell death{Brisset, 2021, 34542930;Negulescu, 2018, 29776009}. The principal death receptors which will be discussed in the review are the Fas cell surface death receptor (FAS; also known as CD95 or APO-1), the TNF receptor superfamily member 1A (TNFRSF1A; best known as TNF-R1), the TNF receptor superfamily member 10a (TNFRSF10A; best known as TRAILR1 or DR4) and the TNF receptor superfamily member 10b (TNFRSF10B; best known as TRAILR2 or DR5). FAS is activated by the binding of FAS ligand (FASLG; also known as CD95L or APO-1L), which is primarily expressed in the hematopoietic compartment by effector immune cells as well as in immune-privilege tissue/organs such as the testis and eye, and in gut epithelia {Wajant, 2002, 12040174}. TNF-R1 is activated by ligation of soluble tumor necrosis factor (TNF), a functionally-pleiotropic cytokine expressed in spleen, thymus and other adult tissues {Aggarwal, 2012, 22053109}. Of note, while the soluble form of TNF preferentially binds to TNF-R1, the transmembrane form mainly interacts with the TNF receptor superfamily member 1B (TNFRSF1B, best known as TNF-R2), which lacks of the death domain {Wallach, 2018, 28847899}. Finally, TRAIL-R1 and TRAIL-R2 are specifically activated by the binding of TNF superfamily member 10 (TNFSF10; best known as TRAIL), which is expressed by a variety of immune cell subtypes of the innate and adaptive response, including monocytes, macrophages and effector cells {von Karstedt, 2017, 28536452}. Of note, mice express only one TRAIL receptor: TRAIL-R2.

Under specific circumstances, the engagement of these death receptors promotes the assembly of multiprotein complexes such as the death-inducing signaling complex (DISC) and complex II, resulting in the activation of caspase 8 (CASP8) and apoptosis {Boldin, 1996, 8681376;Dickens, 2012, 22542855;Muzio, 1996, 8681377}. The DISC, which is assembled on the cytoplasmic tail of ligated FAS or TRAIL-Rs, comprises of Fas (TNFRSF6)-associated via death domain (FADD), CASP8, and distinct isoforms of CASP8 and FADD like apoptosis regulator (CFLAR; best known as c-FLIP), including the two alternative splicing variants, the long form ($cFLIP_L$) and the short form (cFLIPs)).{Boldin, 1995, 7536190;Chinnaiyan, 1995, 7538907;Kischkel, 2000, 10894161;Scott, 2009, 19118384;Chan, 2000, 10875917;Fu, 2016, 26853147}. Of note, c-FLIPs are catalytically-inactive CASP8-like molecules acting as a modulator of caspases. The complex II is a cytosolic complex assembled upon TNF-R1 ligation and in condition of reduced pro-survival signals and protein synthesis as for instance upon administration of inhibitor of apoptosis protein (IAP) inhibitors and cycloheximide{Brenner, 2015, 26008591}. It consists of FADD and CASP8 in association with TNFR1-associated death domain protein (TRADD) or receptor interacting serine/threonine kinase 1 (RIPK1), which is primarily involved in the modulation of necroptosis {Galluzzi, 2017, 27959630}. Upon its recruitment to these complexes, CASP8 is activated by a process involving CASP8 oligomerization and autoproteolysis. CASP8 then acts as the executor of the extrinsic apoptosis by favoring the proteolytic cleavage of CASP3 or CASP7 {Tummers,

2017, 28462525}. This occurs either directly, as in thymocytes and mature lymphocytes, or indirectly, as in hepatocytes, pancreatic β cells, and most cancer cells.{Barnhart, 2003, 14563117;Strasser, 1995, 8557033;Yin, 1999, 10476969;Li, 1998, 9727492;Luo, 1998, 9727491}. In this latter case, executioner caspases are activated by a mechanism involving the cleavage of BH3 interacting domain death agonist (BID) by CASP8 {Gross, 1999, 9873064;Huang, 2016, 27053107}, followed by the same steps of the intrinsic apoptosis: BCL2-associated X protein (BAX)/BCL2-antagonist/killer 1 (BAK1) activation, mitochondrial outer membrane permeabilization (MOMP), apoptosome assembly, and CASP9-CASP3 activation.

Of note, the ligation of death receptor by the respective ligand does not necessarily culminate in the activation of the extrinsic apoptosis. Indeed, the engagement of FAS, TRAILRs and TNF-R1 can also result in the activation of pro-survival pathways often dependent on the NF-κB signaling {von Karstedt, 2017, 28536452;Hayden, 2014, 24958609}, or, alternatively, in the initiation of inflammatory responses, the promotion of processes including cell differentiation/activation (as is the case of lymphocytes), and the activation or inhibition of other RCD variants, including necroptosis and pyroptosis {Bertheloot, 2021, 33785842}. Of note, these outcomes are all modulated by non-apoptotic activities of FADD and/or CASP8.

Box 6. Impact of death receptors on health.

A large body of data demonstrates that death receptor (DR) signaling is crucial for organismal development and/or the maintenance of adult tissue homeostasis. Mouse strains with spontaneous mutations in Fas (TNF receptor superfamily member 6) (*Fas*) - the so-called *lpr* or *lpr-like* mice – or Fas ligand (TNF superfamily, member 6) (*Fasl*) - the so-called *gld* mice - are viable but display chronic, benign lymphoproliferative and autoimmune disorders manifesting with progressive lymphocyte accumulation, massive splenomegaly, lymphadenopathy and mild systemic lupus erythematosus (SLE)-like symptoms {Takahashi, 1994, 7511063;Watanabe-Fukunaga, 1992, 1372394;Lynch, 1994, 7889405;Roths, 1984, 6693832;Matsuzawa, 1990, 2406366}. Such disorders appear earlier and with increased severity in $Fas^{-/-}$ or $Fasl^{-/-}$ mice {Adachi, 1996, 8700897;Adachi, 1995, 7581453;Senju, 1996, 8671629}, in the latter case leading to premature death {Karray, 2004, 14764677}. while symptoms are attenuated when *Fas* or *Fasl* mutations are heterozygous {Matsuzawa, 1990, 2406366}. Of note, these lymphoproliferative and autoimmune disorders are not accompanied by impaired thymocyte development {Adachi, 1995, 7581453}, pointing to some complementation by other signaling pathways. Intriguingly, transgenic overexpression of myeloid cell leukemia sequence 1 **(**MCL1) in the lymphocyte compartment of *lpr* mice exacerbates the onset of autoimmune symptoms {Anstee, 2017, 27813531}, possibly due to the key role of MCL1 in the development and homeostasis of multiple hematopoietic cells. FAS or FASLG deficiency also perturbs the homeostasis or function of other mouse tissues, including (but not limited to) the liver {Adachi, 1995, 7581453}, kidney {Karray, 2004, 14764677}, retina {Davies, 2003, 12824272}, pancreas {Schumann, 2007, 17299038} and intestinal epithelium {Trumpi, 2016, 26700225}.

Conditional deletion of *Fas* and *Fasl* in specific immune cell subsets as well as transgenic expression of FAS in lymphocytes confirms the crucial role of FAS-FASLG signaling in the homeostasis of lymphocytes and dendritic cells (DCs) {Hao, 2004, 15148335;Fukuyama, 1998, 9558084;Komano, 1999, 10383935;Stranges, 2007, 17509906;Rathmell, 1995, 17509906}. In this context, experiments in BH3-only protein BCL2 like 11 (*Bcl2l11*) *lpr* mice demonstrate some degree of cooperation between FAS and BIM in preserving the functionality of the immune system {Hughes, 2008, 18275830}. However, abrogating FAS-FASLG signaling ultimately has heterogeneous organismal consequences. Thus, lymphoproliferative disorders imposed by *Fasl* deletion in hematopoietic cells result in pulmonary fibrosis {Hao, 2004, 15148335} but confer protection to autoimmune diabetes {Mohamood, 2007, 17591957}. Moreover, transgenic expression of FASLG in the lung resulted in alveolar developmental defects and postnatal lethality {De Paepe, 2008, 18535181} Of note, relatively more recent studies appear to suggest that key role of FAS-FASGL signaling in hematopoietic homeostasis relies on non-apoptotic rather than apoptotic effects {Bosque, 2007, 17062728;Cruz, 2016, 28008916;La, 2009, 19794494;Yi, 2018, 29880309}. Finally, FAS appears to mediate robust oncosuppressive effects. Indeed, both *gld* mice as well as *lpr* mice lacking the T cell compartment have increased incidence of B cell lymphoma {Zhang, 2004, 15583018;Peng, 1996, 9064331}.

As for the other DRs, mice lacking TNF receptor superfamily member 10b (TNFRSF10B, best known as TRAIL-R2) or its ligand TNF superfamily member 10 (*TNFSF10*, beast known as TRAIL) are viable, fertile, and do not develop spontaneous autoimmune diseases {Sedger, 2002, 12209637;Diehl, 2004, 15589175;Finnberg, 2005, 15713653;Lamhamedi-Cherradi, 2003, 12577054}. Moreover, these mice exhibit normal immune system development and function {Lehnert, 2014, 25217163;McGrath, 2011, 21562052;Sacks, 2008, 18354179;Cretney, 2003, 12900523}, as well as signs of enhanced innate immunity {Diehl, 2004, 15589175}. Along similar lines, the whole-body deletion of the DR ligand tumor necrosis factor (*Tnf*) does not affect mouse development and fertility {Marino, 1997,

9223320;Pasparakis, 1996, 8879212}. However *Tnf*$^{a/-}$ mice often show early hearing loss and, despite presenting a functional immune system, some degree of immunodeficiency manifesting with a high susceptibility to spontaneous bacterial infection, which has been ascribed to multiple defects including defective lymphoid organ architecture as well as deficient granuloma and germinal center formation {Marino, 1997, 9223320;Pasparakis, 1996, 8879212;Pasparakis, 1997, 9177215;Korner, 1997, 9368616;Oishi, 2013, 23996384}. Impaired response to pathogens has been documented in *Tnf*$^{+/-}$ mice {Marino, 1997, 9223320} as well as in mice lacking TNF receptor superfamily member 1A (TNFRSF1A, best known as TNF-R1) {Pfeffer, 1993, 8387893;Pasparakis, 1997, 9177215;Rothe, 1993, 8395024}. Conversely, mice overexpressing TNF in cardiomyocytes suffer from lethal dilated cardiomyopathy, demonstrating that balanced TNF signaling is key the homeostasis of the cardiac tissue {Kubota, 1997, 9220311; Kubota, 1997, 9314845}. Of note, while *Tnfsf10* deletion enhances the severity of lymphoproliferative and autoimmune disorders in *Fasl*$^{-/-}$ mice, culminating in death via autoimmune thrombocytopenia {Sedger, 2010, 20185587}, the lack of TNF attenuates the lymphoproliferative phenotype, ultimately improving *gld* mice survival {Korner, 2000, 10620607}. These findings confirm the pleiotropy and redundancy of DR signaling, encompassing not only apoptotic and non-apoptotic regulated cell death (RCD)-related effects, but also various pro-survival modules.

Multiple clinical observations support the role of FAS-FASLG signaling in human hematopoiesis {Meynier, 2019, 30565243;Rieux-Laucat, 2018, 29911256}. Most human patients with autoimmune lymphoproliferative syndrome (ALPS) - a primary immunodeficiency manifesting with lymphadenopathy, splenomegaly as well as deregulated number, development and function of lymphocytes - or ALPS-like syndromes bear loss-of-function mutations in *FAS* or *FASLG* {Del-Rey, 2006, 16627752;Fisher, 1995, 7540117;Rieux-Laucat, 1995, 7539157;Magerus-Chatinet, 2009, 19176318;Rensing-Ehl, 2014, 24894771;Price, 2014, 24398331;Bi, 2007, 17605793}. Of note, ALPS patients also display an increased incidence of non-Hodgkin and Hodgkin lymphoma {Venkataraman, 2010, 20216376}. While no mutations of *TRAIL*, *TNFRSF10A* or *TNFRSF10B* have been so far associated to human autoimmune disease, autosomal dominant mutations in *TNFRSF1A* have been identified in patients affected by TNF receptor-associated periodic syndrome (TRAPS), whose symptoms include severe abdominal pain, arthralgias, and myalgias {Haas, 2006, 16401480; Tsuji, 2019, 31429073;McDermott, 1999, 10199409}.

Box 7. Impact of extrinsic apoptosis complexes and caspases on health.

Contrarily to death receptors (DRs) and their ligands, most (but not all) the signal transducers of the extrinsic apoptotic pathway are essential for embryonic development in mice. Thus, deletion of Fas (TNFRSF6)-associated via death domain (*Fadd*), caspase 8 (*Casp8*) or CASP8 and FADD-like apoptosis regulator (*Cflar*) is embryonic lethal at mid-gestation as a consequence of severe cardiac defects or cardiac deformation, erythrocyte accumulation and/or spontaneous intra-abdominal hemorrhage {Yeh, 1998, 9506948;Imtiyaz, 2009, 19203997;Alvarez-Diaz, 2016, 27523270;Zhang, 2011, 21368761;Varfolomeev, 1998, 9729047;Sakamaki, 2002, 12404118;Yeh, 2000, 10894163}. Of note, CASP8-deficient mice also exhibit neural tube defects {Sakamaki, 2002, 12404118}. A similar embryonic lethality has also been documented in mice expressing a mutant form of FADD deficient in its death domain {Imtiyaz, 2009, 19203997}. On the contrary, ablation of other components of DR-associated signaling complexes such as TNFRSF1A associated via death domain (TRADD) and receptor interacting serine/threonine kinase 1 (RIPK1) has contrasting outcomes. Thus, while *Tradd*$^{-/-}$ mice develop normally and do not display major hematopoietic defects {Chen, 2008, 18719121;Ermolaeva, 2008, 18641654;Pobezinskaya, 2008, 18641653}, *Ripk1*$^{-/-}$ mice die early after the birth due to severe multiorgan hyperinflammation {Kelliher, 1998, 9529147;Roderick, 2014, 25246544}. These results are attributed to the pleiotropic contribution of RIPK1 and TRADD to a variety of processes beyond apoptosis, notably inflammation and necroptotic regulated cell death (RCD).

Indeed, it has been formally demonstrated that embryonic lethality in *Casp8*$^{-/-}$ and *Fadd*$^{-/-}$ mice is paradoxically due to excessive necroptosis, reflecting the ability of CASP8 to limiting such variant of regulated necrosis downstream of DR activation {O'Donnell, 2011, 22037414; Kaiser, 2011, 21368762}. Accordingly, deletion of genes encoding key components of the necroptotic machinery such as RIPK3 or mixed lineage kinase domain like pseudokinase (MLKL) rescues lethality and developmental defects in FADD- or CASP8-deficient embryos {Alvarez-Diaz, 2016, 27523270;Dillon, 2012, 22675671;Dillon, 2014, 24813850;Oberst, 2011, 21368763;Kaiser, 2011, 21368762;Rickard, 2014, 24813849;Zhao, 2017, 28445730}. Of note, *Casp8*$^{-/-}$*Ripk3*$^{-/-}$ mice develop progressive lymphoproliferative disorders that resemble those imposed by the absence of FAS or FASLG {Oberst, 2011, 21368763;Kaiser, 2011, 21368762}. Moreover, perinatal lethality in *Cflar*$^{-/-}$ or *Ripk1*$^{-/-}$ mice appears to depend on illicit activation of both extrinsic apoptosis and necroptosis. Indeed, these animals can be rescued form death by additional co-deletion of *Fadd* and *Ripk3*, *Casp8* and *Ripk3*, or *Fadd* and *Mlkl* {Dillon, 2012, 22675671;Kaiser, 2011, 21368762;Alvarez-Diaz, 2016, 27523270;Dillon, 2014, 24813850;Alvarez-Diaz, 2016, 27523270;Kaiser, 2014, 24821786;Rickard, 2014, 24813849}. Likewise, mice with a specific *Ripk1* mutation preventing CASP8-mediated cleavage are viable and develop into adulthood {Lalaoui, 2020, 31827281;Newton, 2019, 31511692;Zhang, 2019, 30867408}. As an additional layer of complexity, although the deletion of *Tradd* rescues *Ripk1*$^{-/-}$*Ripk3*$^{-/-}$ embryo from perinatal lethality, triple knockout mice die postnatally {Anderton, 2019, 30185824;Dowling, 2019, 30741936}. Moreover, TRADD deficiency does not revert the lethal phenotype of FADD-deficient mice {Dowling, 2019, 30741936}. Additional studies confirm the importance of the interconnectivity between multiple RCD pathways for mammalian development and homeostasis. For instance, conditional overexpression of baculoviral pan-caspase inhibitor p35 in cardiomyocytes corrects the defects in cardiac development exhibited by *Casp8*$^{-/-}$ mice but fails to prevent embryonic death {Yajima, 2005, 16245150}. Moreover, genetic deletion of BCL2 binding component 3 (*Bbc3*) partially ameliorates the developmental defects of *Fadd*$^{-/-}$ embryos, suggesting a role for BBC3 (best known as PUMA) as necroptosis amplifier {Chen, 2018, 29581256}. Along with this, transgenic mice with non-cleavable CASP8 develop normally {Kang, 2008, 18684943}, but mutations in CASP8 catalytic site result in embryonic lethality during early/mid-gestation due to

accrued necroptosis {Fritsch, 2019, 31748744;Newton, 2019, 31511692}. Surprisingly, genetic ablation of *Mlkl* or *Mlkl* plus *Fadd* does not rescue lethality in these mice, but results in perinatal death dependent on inflammation and possibly pyroptosis {Tummers, 2020, 32428502;Newton, 2019, 31723262}. These observations point to the central role for CASP8 in the regulation of multiple RCD variants and inflammatory processes.

The tissue-specific deletion of *Fadd* or *Casp8* in mouse endothelial cells results in an embryonic lethal phenotype that resembles that of germline *Fadd* or *Casp8* deletion {Fan, 2016, 27584790;Kang, 2004, 15322156}. Conversely, the absence of FADD in cardiomyocytes or cardiac progenitor cells appears to have no impact on embryonic development {Fan, 2016, 27584790}. Again, abrogation of necroptosis rescued the lethal phenotype of endothelial cell-specific *Fadd* or *Casp8* deletion {Fan, 2016, 27584790}, lending additional support to inhibitory role of FADD and CASP8 in necroptotic RCD. FADD, CASP and CFLAR (best known as c-FLIP) have also been involved in hematopoietic homeostasis. However, the abrogation of FADD in specific immune cell subsets in mice via distinct experimental approaches encompassing conditional deletion, reconstitution with $Fadd^{-/-}$ embryonic stem cells (in C57BL/6 $Rag1^{-/-}$ mice) or transgenic expression of dominant-negative FADD variant does drive lymphoproliferative disorders. Instead, FADD appears to be critical for the proliferation and/or development of T lymphocytes {Newton, 1998, 9450996;Zornig, 1998, 9550704;Zhang, 1998, 9521326;Zhang, 2014, 24901044;Newton, 2000, 10698935;Newton, 2000, 10698935;Kabra, 2001, 11353862;Zhang, 2005, 16116191;Osborn, 2010, 20615958;Zhang, 2014, 25078620;Walsh, 1998, 9586634;Newton, 2001, 11250157} and B cells {Imtiyaz, 2006, 16709845}. Similar conclusions have been drawn from the phenotype of mice with a lymphocyte-specific ablation of *Casp8* or *Cflar* {Salmena, 2003, 12654726;Beisner, 2005, 16148088;Lemmers, 2007, 17213198;Zhang, 2005, 16043517;Chau, 2005, 16043518;Zhang, 2009, 19109151}. A role for caspases in T cell proliferation has also emerged by the antiproliferative effects of pharmacological caspase inhibitors {Kennedy, 1999, 10601363}. Moreover, the T cell-specific deletion of *Casp8* attenuates autoimmunity and improves the survival of BH3-only protein BCL2 like 11 $(Bcl2l11)^{-/-}$ mice by limiting T cell proliferation and survival {Bohgaki, 2011, 22006951}. Apparently at odds with these findings, the conditional deletion of *Casp8* in T cells has also been associated with an age-dependent, lethal lymphoproliferative and lymphoinfiltrative immune disorder resembling the condition of patients with *CASP8* mutations {Salmena, 2005, 16157684}. Whether mouse strain or other contextual variables (*e.g.*, the mouse microbiota) underlie such apparent discrepancy remains to be elucidated.

The conditional deletion of *Fadd* or *Casp8* has also revealed a role for these proteins in early hematopoiesis, which appears to relate to their ability to promote the proliferation and differentiation of hematopoietic stem and progenitor cells {Rosenberg, 2011, 21115735;Pellegrini, 2005, 15905188;Kang, 2004, 15322156}. Along similar lines, mice expressing a dominant negative mutant of FADD (FADD-DN) exhibit impaired accumulation of intra-epithelial lymphocytes in the intestine {Zhang, 2018, 30250469}, an effect that has recently been attributed to c-FLIP {Bank, 2020, 32103006}. As for other immune cell subtypes, conditional deletion of *Fadd* in myeloid cells results in increased myeloid and B cell populations coupled to activation of inflammatory responses {Schock, 2015, 25874713}. Along similar lines, the macrophage-restricted deletion of *Casp8* induces a mild systemic inflammatory disease potentially linked to altered macrophage polarization {Cuda, 2015, 26471282; Vitale, 2019, 31269428}, while the DC-specific deletion of *Cflar* or *Casp8* leads to splenomegaly, inflammatory responses and/or autoimmune disorders {Cuda, 2014, 24808358;Huang, 2015, 25963626;Wu, 2015, 26238491}. Of note, these effects seem all to be unrelated to pro-apoptotic functions of FADD and CASP8 but to reflect their ability to limit necroptosis {Osborn, 2010, 20615958;Schock, 2015, 25874713;Ch'en, 2011, 21402742;Oberst, 2011, 21368763;Kaiser, 2011, 21368762;Bell, 2008, 18946037;Ch'en, 2008,

18981423;Cuda, 2015, 26471282}. Corroborating these findings, loss-of-function mutations in *FADD* {Bolze, 2010, 21109225;Kuehn, 2011, 21490157;Kohn, 2020, 32350755;Savic, 2015, 25794656}, *CASP8* or *CASP10* {Chun, 2002, 12353035;Wang, 1999, 10412980;Martinez-Feito, 2016, 26323380} and *TRADD* {Dechant, 2008, 18661484} have been associated with ALPS-like syndromes and other hematological diseases in humans. Of note, patients with ALPS bearing *FADD* or *CASP8* (but not ALPS *FAS* or *FASLG*) mutations also exhibit immunodeficiency coupled with lymphocytic infiltrations in multiple organs, granulomas and/or inflammatory bowel disease {Lehle, 2019, 30267714;Niemela, 2015, 25814141;Chun, 2002, 12353035;Kanderova, 2019, 30337362;Bolze, 2010, 21109225}.

Tissue-specific deletion of *Fadd*, *Casp8* and *Cflar* has also revealed a role for these proteins in the homeostasis of the liver, skin and intestine, although phenotype severity varies quite considerably, ranging from mild/severe inflammatory response to embryonic or postnatal lethality. Thus, conditional deletion of *Cflar* in intestinal epithelial cells (IECs), hepatocytes or keratinocytes results in embryonic or perinatal lethality due to unbalanced cell death activation {Piao, 2012, 23250397;Panayotova-Dimitrova, 2013, 24209745;Feoktistova, 2020, 33238518;Wittkopf, 2013, 24036366}. The inducible deletion of *Cflar* from the intestinal epithelium of adult mice also results in lethality following severe inflammation {Wittkopf, 2013, 24036366}. These results are in line with the crucial role of c-FLIP as an inhibitor or necroptosis {Dillon, 2012, 22675671; Gehrke, 2015, 25342470}. Along similar lines, *Fadd* deletion in epidermal keratinocytes or IECs drives severe chronic inflammation because of illicit necroptosis activation {Bonnet, 2011, 22000287;Welz, 2011, 21804564;Kovalenko, 2009, 19720838;Gunther, 2011, 21921917;Li, 2010, 21135236;Kaden-Volynets, 2019, 31411503;Weinlich, 2013, 24095739}. Indeed, abrogation of FADD (or CASP8) in IECs results in chronic inflammatory colitis and ileitis, which are abolished by concomitant deletion of *Ripk3* or *Mklk* {Welz, 2011, 21804564;Gunther, 2011, 21921917;Stolzer, 2020, 31276162;Weinlich, 2013, 24095739;Fritsch, 2019, 31748744;Newton, 2019, 31723262}. In one of these studies, acute deletion of *Casp8* in the gut of adult mice resulted in enterocyte death, culminating in disruption of tissue homeostasis, sepsis and death {Weinlich, 2013, 24095739}. In this context, enterocyte deficient for CASP8 displayed decreased *in vivo* survival and migration potential {Kaemmerer, 2015, 25914458}. Of note, expression of a catalytically inactive variant of CASP8 in IECs induces intestinal inflammation similar to that observed in *Casp8*$^{-/-}$ mice, a phenotype that is aggravated *Mlkl* deletion, resulting in premature death dependent on the induction of the inflammatory responses and pyroptosis {Fritsch, 2019, 31748744}. As an added layer of complexity, deletion of tumor necrosis factor (*Tnf*) or TNF receptor superfamily member 1A (*Tnfrsf1a*) attenuates colitis (but not ileitis) in mice with an IEC-specific deletion of *Fadd* or *Casp8* {Welz, 2011, 21804564;Wittkopf, 2013, 24036366}. A recent study suggests that this effect may also involve the activation of pyroptosis. Indeed, the CASP8-dependent activation of gasdermin D (GSDMD) appears to promote ileitis in mice with FADD-deficient IECs {Schwarzer, 2020, 32362323}. These results are in line with the crucial involvement of CASP8 and FADD in inflammation activation {Galluzzi, 2016, 26885855; Karki, 2019, 30842595}, ultimately suggesting that the FADD-CASP8 axis regulates the organismal development and homeostasis by balancing apoptosis, necroptosis, pyroptosis and inflammation.