CDDpress

www.nature.com/cdd

REVIEW ARTICLE

Check for updates

What can we learn from mice lacking pro-survival BCL-2 proteins to advance BH3 mimetic drugs for cancer therapy?

Kerstin Brinkmann[1,2]✉, Ashley P. Ng[1,2], Carolyn A. de Graaf[1,2] and Andreas Strasser[1,2]✉

In many human cancers the control of apoptosis is dysregulated, for instance as a result of the overexpression of pro-survival BCL-2 proteins. This promotes tumorigenesis by protecting nascent neoplastic cells from stress and renders malignant cells resistant to anti-cancer agents. Therefore, several BH3 mimetic drugs targeting distinct pro-survival proteins have been developed. The BCL-2 inhibitor Venetoclax/ABT-199, has been approved for treatment of certain blood cancers and tens of thousands of patients have already been treated effectively with this drug. To advance the clinical development of MCL-1 and BCL-XL inhibitors, a more detailed understanding of their distinct and overlapping roles in the survival of malignant as well as non-transformed cells in healthy tissues is required. Here, we discuss similarities and differences in pro-survival BCL-2 protein structure, subcellular localisation and binding affinities to the pro-apoptotic BCL-2 family members. We summarise the findings from gene-targeting studies in mice to discuss the specific roles of distinct pro-survival BCL-2 family members during embryogenesis and the survival of non-transformed cells in healthy tissues in adults. Finally, we elaborate how these findings align with or differ from the observations from the clinical development and use of BH3 mimetic drugs targeting different pro-survival BCL-2 proteins.

Cell Death & Differentiation (2022) 29:1079–1093; https://doi.org/10.1038/s41418-022-00987-0

FACTS

- Pro-survival BCL-2 proteins have overlapping roles in securing the survival of certain cell types, whereas other cell populations rely on the expression of a specific pro-survival BCL-2 family member.
- Many cell types during embryogenesis as well as in the adult cannot tolerate the loss of the pro-survival protein MCL-1.
- On-target toxic effects of BH3 mimetic drugs targeting specific pro-survival BCL-2 proteins on healthy cells are generally less severe than the defects seen in gene-targeted mice lacking these proteins.

OPEN QUESTIONS

- Why can the survival dependence of distinct cell types not be explained exclusively by the expression profile of the different pro-survival BCL-2 proteins?
- Why is MCL-1 critical for early embryogenesis and the survival of so many cell types?
- Are there apoptosis-unrelated functions of pro-survival BCL-2 proteins, in particular MCL-1, that are critical for the survival of distinct cell types?
- Do the reported apoptosis unrelated functions of pro-survival proteins play a role in the efficacy and/or on-target toxic effects of BH3 mimetic drugs?

PRO-SURVIVAL BCL-2 PROTEINS—SAME, SAME BUT DIFFERENT

Mammals have five pro-survival BCL-2 family members: BCL-2, BCL-XL, MCL-1, BCL-W and A1(murine)/BFL-1(human). They regulate mitochondrial apoptosis through interactions with two sub-groups of pro-apoptotic BCL-2 family members [1–3]; the BH3-only proteins (PUMA, BIM, tBID, NOXA, BMF, BIK, HRK, BAD) that are critical for apoptosis initiation as well as the multi-BH (BCL-2 homology) domain effectors of apoptosis, BAX and BAK [2]. The BAX/BAK-related multi-BH-domain protein BOK is not regulated by pro-survival BCL-2 proteins [4, 5]. Apoptosis is initiated in response to diverse stresses, such as cytokine deprivation or oncogene activation, resulting in the transcriptional and/or post-transcriptional upregulation of pro-apoptotic BH3-only proteins [6]. BH3-only proteins bind the pro-survival BCL-2 proteins with high affinity and thereby neutralise them. This unleashes BAX and BAK from their restraint by the pro-survival BCL-2 proteins, allowing them to cause mitochondrial outer membrane permeabilisation (MOMP) [2, 3]. Some BH3-only proteins can reportedly also directly activate BAX and BAK [7–9]. MOMP results in the release of apoptogenic factors (e.g.,

[1]The Walter and Eliza Hall Institute of Medical Research, Melbourne, Vic, Australia. [2]The Department of Medical Biology, University of Melbourne, Melbourne, Vic, Australia. ✉email: brinkmann.k@wehi.edu.au; strasser@wehi.edu.au

Edited by G. Melino

Received: 28 September 2021 Revised: 4 March 2022 Accepted: 15 March 2022
Published online: 6 April 2022

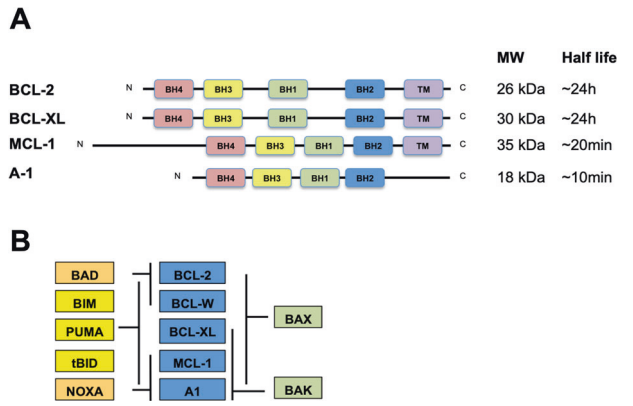

Fig. 1 The regulation of the mitochondrial apoptotic pathway by pro-survival BCL-2 proteins. A Schematic presentation of the structure of the different pro-survival BCL-2 proteins (not to scale). **B** Binding of the different pro-survival BCL-2 proteins to the different pro-apoptotic BH3-only proteins as well as the apoptosis effectors, BAX and BAK.

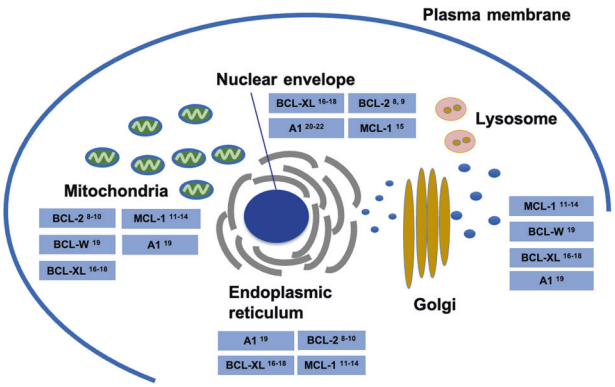

Fig. 2 Subcellular localisation of the pro-survival BCL-2 proteins. Schematic presentation of the subcellular localisation of BCL-2, BCL-XL, MCL-1, A1 and BCL-W. Superscript numbers relate to the literature reference.

Cytochrome c) from the mitochondrial inter-membrane space leading to the activation of the caspase cascade that causes the ordered dismantling of dying cells (Graphical Abstract) [3].

All pro-survival proteins contain four BH domains and interact with both sub-groups of pro-apoptotic BCL-2 family members through their hydrophobic BH3 binding groove (Fig. 1A, B). Even though highly related to each other, different pro-survival BCL-2 proteins have some distinct features. BCL-2, BCL-XL, MCL-1 and BCL-W have a C-terminal transmembrane (TM) domain that allows them to anchor to different intracellular membranes (Fig. 1A). BCL-2 is found at the endoplasmic reticulum (ER), the mitochondrial outer membrane (MOM) and the nuclear envelope (NE) [10–12]. MCL-1 is found at the ER, the MOM as well as the mitochondrial inter-membrane space, the NE and in the cytosol [13–17]. BCL-XL is located in the cytosol and at the MOM but has also been detected at the NE and the ER [18–20]. BCL-W is cytosolic but can insert into the MOM in response to intracellular stress [21]. A1 lacks a TM domain and is therefore mainly found in the cytosol but it is also detected at the MOM, the ER and the NE [22–24] (Fig. 2).

MCL-1 has a long N-terminal stretch harbouring 4 putative PEST sequences, and this is in part responsible for its short (20–30 min) half-life [25, 26]. In contrast, BCL-2, BCL-XL and BCL-W have half-lives of >20 h [27, 28]. A1/BFL-1 protein has a half-life of only 10 min [29] (Fig. 1A).

The pro-survival BCL-2 proteins differ in their binding to the apoptosis effectors BAX and BAK. All pro-survival BCL-2 proteins can restrain BAX, but only BCL-XL, MCL-1 and A1 also bind BAK [30, 31]. Furthermore, whereas all pro-survival BCL-2 proteins can bind to the BH3-only proteins BIM, PUMA and tBID, NOXA interacts only with MCL-1 and A1, while BAD selectively binds BCL-2, BCL-XL and BCL-W [9, 30, 32] (Fig. 1B).

THE ROLES OF THE DIFFERENT PRO-SURVIVAL BCL-2 PROTEINS IN EMBRYOGENESIS

During the initial stages of embryogenesis (days 0–2) the expression of proteins in the zygote is driven from maternal mRNA. Only after several cell divisions (6–8 cell state) around embryonic day 3 (E3) the embryonic genome is activated to allow mRNA and protein synthesis [33].

Among the pro-survival BCL-2 proteins, the poorly studied BCL-B (human)/DIVA(murine) is the most prominently expressed at the mRNA level in oocytes and early embryos [34]. However, loss of BCL-B/DIVA has no impact on oocytes or early embryos [35]. Of note, some reports indicate that BCL-B/DIVA lacks a BH3 domain and its function in regulating cell death is therefore unclear [36–38]. Neither A1 nor BCL-W are expressed during early embryogenesis. MCL-1 and BCL-XL are highly expressed from both maternally inherited mRNA as well as embryonic transcripts [39]. BCL-2 expression levels are low during early embryogenesis but increase after the late blastocyst stage (~E4.5). Here, we focus on the most relevant pro-survival BCL-2 family members during embryogenesis, MCL-1, BCL-XL and BCL-2.

BCL-2 (Table 1**):** BCL-2-deficient mice are born but die soon after weaning due to polycystic kidney disease, a disorder that commences during embryogenesis [40]. This demonstrates an essential role of BCL-2 for renal epithelial cell survival during embryogenesis. BCL-2 levels are high in the central nervous system during embryogenesis but decline after neuronal tube formation [39]. In contrast, high levels of BCL-2 are maintained in the peripheral neuronal system [10, 41–43]. BCL-2-deficient mice show normal neuronal development during embryogenesis but exhibit a significant loss of sympathetic, motor and sensory neurons during the early postnatal period [44].

BCL-XL (Table 2**):** Constitutive absence of BCL-XL (encoded by the *Bcl2l1* gene) results in embryonic lethality ~E13.5 due to erythroid and neuronal defects [45, 46]. To further study the role of BCL-XL during development and in adulthood, chimaeric mice were generated through injection of *Bcl2l1*$^{-/-}$ (in the following referred to as *Bcl-x*$^{-/-}$) or control embryonic stem (ES) cells into wild-type blastocysts. No significant differences in the contribution of *Bcl-x*$^{-/-}$ *vs* control cells were observed in the heart, kidney or muscle [46], but BCL-XL was indispensable for the survival of embryonic erythroid progenitors (EryP) and definitive erythrocytes (EryD) in the adult [45]. Furthermore, *Bcl-x*$^{-/-}$ ES cells contributed less to the spleen and thymus compared to control ES cells [45].

Bcl-x$^{-/-}$ embryos present with massive apoptotic death of post-mitotic immature neurons in the developing brain, spinal cord, and dorsal root ganglia [46]. Accordingly, extensive cell death was detected in cultures of telencephalic neurons derived from *Bcl-x*$^{-/-}$ embryos, and this was rescued by loss of BAX [47, 48]. BCL-XL deficiency specifically in catecholaminergic neurons (tyrosine-hydroxylaseCre-*Bcl-x*$^{fl/fl}$ mice) resulted in viable offspring, although this neuronal population was reduced by one-third [49]. However, some catecholaminergic neurons without BCL-XL were present [49], indicating that their survival can be safeguarded by additional pro-survival BCL-2 proteins.

MCL-1 (Table 3**):** Amongst the pro-survival BCL-2 proteins, only MCL-1 is essential for early embryogenesis. Constitutive absence of MCL-1 in mice results in peri-implantation lethality (~E3.5) [50]. The authors of this study failed to detect an increase in apoptotic cells in the *Mcl-1*$^{-/-}$ blastocysts and therefore hypothesised that the

Table 1. *Bcl-2* gene targeting in mice.

Mouse model	Targeted tissue/cell population	Findings	Reference
constitutive *Bcl-2*$^{-/-}$	whole body	viable offspring but mice succumb to polycystic kidney disease between 4-10 weeks post-birth (lifespan influenced by genetic background); premature greying, lymphocytopenia BCL-2 is essential for the survival of renal epithelial progenitor cells in the embryo, mature B and T lymphocytes BCL-2 is essential for the survival of melanocyte stem and progenitor cells BCL-2 is not important for prenatal neuronal survival but crucial for the maintenance of motoneurons, sensory, and sympathetic neurons during early postnatal period BCL-2 is critical for the survival of endothelial cells and pericytes and its loss results in decreased retinal vascular density BCL-2 is critical in osteoclasts but not osteoblasts	[40] [88] [56] [60] [57, 58] [44] [62] [154]
Bcl-2$^{-/-}$ haematopoietic chimaeras	haematopoietic system	BCL-2 is essential for the survival of B and T lymphoid cells	[86]
constitutive *Bcl-2*$^{-/-}$*Bim*$^{-/-}$	whole body	Viable offspring that is rescued from polycystic kidney disease, premature greying and lymphocytopenia BCL-2 is required for the peripheral survival of naive CD8+ but not CD4+ T cells	[60] [59]
Bcl-2 YFP reporter mouse		BCL-2 is important for the development of effector and memory T lymphocytes	[89, 90]
Pf4-Cre-Bcl-2$^{fl/fl}$	megakaryocyte lineages	viable BCL-2 is dispensable for thrombopoiesis and platelet survival	[92]
NestinCre-Bcl-2 $^{fl/fl}$	central and peripheral nervous system	viable BCL-2 is essential for the survival of doublecortin-expressing immature neurons	[61]
Ncr1Cre-Bcl-2 $^{fl/fl}$	NK cells	viable BCL-2 is required for optimal NK cell survival	[91]

essential role of MCL-1 during implantation is unrelated to its role in inhibiting apoptosis. Further investigations are needed to clarify whether the peri-implantation lethality caused by the absence of MCL-1 is triggered by excess cell death or defects in apoptosis-unrelated functions of MCL-1.

Tissue-restricted gene deletion revealed an essential role for MCL-1 in neuronal and cardiac tissues in embryos. *NestinCre-Mcl-1*$^{fl/fl}$ mice, with neuronal-restricted loss of MCL-1, developed only until ~E12.5 [42, 51]. A wave of apoptosis was detected at E9.5 in the brainstem and cervical spinal cord and at E10.5 in the forebrain. Interestingly, while loss of BAX was sufficient to protect the majority of neuronal precursor cells from apoptosis, this rescued only ~50% of cells in the dorso-medial brainstem and ventral-thoracic spinal cord [51]. Thus, MCL-1 ensures NPC survival mainly by restraining BAX in the brain stem, while the survival of other brain cells must also rely on the inhibition of BAK. The critical role of MCL-1 in neurogenesis in the forebrain was further demonstrated in *Foxg1Cre-Mcl-1*$^{fl/fl}$ mice, which all died before E15 [42].

CkmmCre-Mcl-1$^{fl/fl}$ mice, in which MCL-1 is removed from cardiomyocytes and skeletal muscle cells die around post-natal day 10 due to myocardial degeneration, demonstrating that MCL-1 is essential for cardiomyocyte survival [52]. The loss of MCL-1 had only minor impact on skeletal muscle cells, at least within the short lifetime of the pups [52], suggesting that their survival is safeguarded by additional pro-survival BCL-2 proteins.

THE ROLES OF PRO-SURVIVAL BCL-2 PROTEINS IN ADULT MICE

Some healthy cells in the adult are protected from apoptosis by mostly one pro-survival BCL-2 family member, whereas others are safeguarded by two or more (Fig. 3). Moderate to high expression of MCL-1 is found in most adult tissues, including the brain, digestive system, liver, kidney, reproductive organs (male and female), smooth and skeletal muscle and cardiomyocytes [53–55]. BCL-2 expression is reported as low in the adult heart, liver, skeletal muscle but moderate to high in the colon, male and female reproductive organs, skin, colon and kidneys [53–55]. Moderate to high levels of BCL-XL have been observed in many adult tissues, however, others such as cardiac and smooth muscles as well as the skin lack BCL-XL [53–55]. BCL-W is expressed in the brain, spinal cord, colon, testes, pancreas, liver, heart, spleen and mammary glands of pregnant mice [21]. A1 is only found in haematopoietic cells [53–55] (Fig. 3).

BCL-2 (Table 1**):** BCL-2-deficient mice die ~30 days after birth (C57BL/6 background) due to polycystic kidney disease, a disorder that starts during embryogenesis [40, 56]. BCL-2-deficient mice also present with substantial reductions in mature B and T lymphocytes and melanocytes, the latter causing premature greying [40, 56–58]. All defects caused by the absence of BCL-2 could be prevented by concomitant loss of the pro-apoptotic BH3-only protein BIM [59, 60]. This demonstrates that BCL-2 safeguards the survival of several cell types by preventing BIM-induced apoptosis.

BCL-2-deficient mice also exhibit an abnormal reduction of sympathetic, motor and sensory neurons during the early postnatal period [44]. *NestinCreERT2-Bcl-2*$^{fl/fl}$ mice were generated to facilitate inducible deletion of *Bcl-2* specifically in NPCs in adult mice. This revealed that BCL-2 is essential for the survival of doublecortin-expressing immature neurons in the central and peripheral nervous systems [61].

BCL-2 is also critical for endothelial cell survival. Abnormally decreased numbers of endothelial cells and pericytes were observed in retinas from BCL-2-deficient mice, resulting in decreased retinal vascular density [62].

BCL-XL (Table 2**):** The loss of only one allele of *Bcl2l1* (encoding *Bcl-x*) impairs male fertility, although with incomplete penetrance [63]. A critical role of BCL-XL in male and female reproductive organs was confirmed in a *Bcl-x* hypomorphic mouse strain which

Table 2. *Bcl2l1 (Bcl-x)* gene targeting in mice.

Mouse model	Targeted tissue/cell population	Findings	Reference
constitutive *Bcl-x*$^{-/-}$	whole body	embryonic lethal (E13.5) BCL-XL is essential for the survival of erythroid progenitor cells and catecholaminergic neuronal cells	[46]
haematopoietic chimaeras that were produced by microinjection of *Bcl-x*$^{-/-}$ ES cells into *Rag2*$^{-/-}$ blastocysts	T cells (DN stage) B cells (pro B stage)	viable BCL-XL is dispensable for the survival of lymphocyte progenitors but the survival of DP thymocytes is compromised upon loss of BCL-XL	[93]
chimaeras (microinjection of *Bcl-x*$^{-/-}$ ES cells in wt blastocysts)	whole body	viable offspring with >80% chimaerism BCL-XL is essential for the survival of primitive and definitive erythroid progenitors	[45]
Bcl-x hypomorphic mouse (introduction of a loxP flanked neomycin (neo) cassette into the promoter of *Bcl-x*)	whole body	viable BCL-XL is essential for the survival of mouse germ cells during gonadogenesis in males and females	[63]
MMTV-Cre-Bcl-x$^{fl/fl}$	erythroid cells	mice die ~3 months after birth due to severe haemolytic anaemia and splenomegaly BCL-XL is essential for the survival of erythroid cells at the end of their maturation	[99]
MMTV-Cre-Bcl-x$^{fl/fl}$ *WAP-Cre- Bcl-x*$^{fl/fl}$	mammary epithelium	viable BCL-XL is not essential during mammopoiesis, but critical for controlled apoptosis during the first phase of involution	[155]
AlbCre-Bcl-x	hepatocytes	viable but mice develop severe liver fibrosis 5-7 months after birth BCL-XL is essential for hepatocyte survival	[65]
pCMV-Cre via gene gun delivery to the abdomen of Bcl-x$^{fl/fl}$ *mice*	dendritic cells	viable BCL-XL is essential for the survival of certain dendritic cell populations	[73, 156]
LckCre- Bcl-x$^{fl/fl}$	T lymphoid cells	viable BCL-XL is not essential for the survival of effector and memory T lymphocytes	[94]
tyrosine hydroxylaseCre-*Bcl-x*$^{fl/fl}$	a subset of CNS neurons catecholaminergic neurons	viable BCL-XL is required for proper development of the mouse substantia nigra (catecholaminergic neuronal population reduced by one-third upon induced *Bcl-x* deletion)	[49]
Bcl-x$^{Plt16/Plt16}$ *Bcl-x*$^{Plt20/Plt20}$ (destabilising point mutations)	whole body	Only few *Bcl-x*$^{Plt16/Plt16}$ mice found at birth *Bcl-x*$^{Plt20/Plt20}$ mice are viable anaemia/hyperplasia of erythroid progenitors BCL-XL is essential for normal platelet lifespan	[100]
RIP-Cre-Bcl-x$^{fl/fl}$	pancreatic β-cells	viable BCL-XL is dispensable during islet development in the mouse but *Bcl-x*$^{-/-}$ β-cells are hypersensitive to apoptotic stimuli	[66]
Sftpc-Cre-Bcl-x$^{fl/fl}$	epithelial cell	perinatally lethal in approximately 50% of the expected offspring BCL-XL is not essential for proper lung development in viable offspring but respiratory epithelial cells are hypersensitive to apoptotic stimuli	[67]
Pf4Cre-Bcl-x$^{fl/fl}$	megakaryocyte lineages	mice present with severe thrombocytopenia ~7 weeks after birth BCL-XL is essential for megakaryocyte function to produce platelets but dispensable for their growth and survival	[101]
Pf4Cre-Bcl-x$^{fl/}$*fl-Mcl-1*$^{fl/fl}$	megakaryocyte lineages	BCL-XL is essential for megakaryocyte survival in combination with MCL-1	[102, 103]
RosaCreERT2-Bcl-x$^{fl/fl}$	CreERT2-induced whole body deletion	Mice die ~25 days after CreERT2-induced *Bcl-x* deletion due to severe anaemia and thrombocytopenia BCL-XL is critical for the survival of red blood cells and platelets	[64]
wt BM chimaera; RosaCreERT2-Bcl-x$^{fl/fl}$ *host*	CreERT2-induced deletion only in the non-haematopoietic cells	Mice die ~30 days after CreERT2-induced *Bcl-x* deletion due to severe kidney damage and secondary anaemia and thrombocytopenia BCL-XL is critical for the survival of renal tubular epithelial cells	[64]

Table 3. *Mcl-1* gene targeting in mice.

Mouse model	Targeted tissue/cell population	Findings	Reference
constitutive *Mcl-1*$^{-/-}$	whole body	embryonic lethal peri-implantation (E3.5)	[50]
LckCre-Mcl-1$^{fl/fl}$	T lymphoid cells	viable MCL-1 is essential for the survival of T lymphoid cells; reduced cell numbers from DN2 stage	[32]
CD19Cre-Mcl-1$^{fl/fl}$	B lymphoid cells	viable MCL-1 is essential for the survival of B lymphoid cells; reduced cell numbers from pro B cell stage	[32]
MxCre-Mcl-1$^{fl/fl}$ (pIC/INF-alpha induced ex vivo deletion)	B and T cells in tissue culture	viable MCL-1 is essential for the survival of mature B cells, mature T cells	[32]
MxCre-Mcl-1$^{fl/fl}$	liver lymphocytes	mice die after 12-21 days upon MxCre-induced *Mcl-1* deletion MCL-1 is essential for the survival of haematopoietic stem and progenitor cell populations no liver abnormalities observed	[105]
MxCre-Mcl-1$^{fl/fl}$ *BM chimaeras (with congenic wild-type carrier BM to overcome lethality); wt host*	MxCre-induced deletion in the haematopoietic cells only in the chimaeras	viable MCL-1 is essential for the survival of haematopoietic stem and progenitor cell populations	[105]
wt BM chimaera; MxCre-Mcl-1$^{fl/fl}$ *host*	liver (MxCre-induced deletion only in the non-haematopoietic cells expressing Mx-Cre in the chimaeras)	mice survive more than 14 weeks after MxCre-induced deletion MCL-1 deletion had no impact on the liver	[105]
LysMCre-Mcl-1$^{fl/fl}$	myeloid cells	viable MCL-1 is essential for the survival of neutrophils but dispensable for the survival of monocytes and macrophages	[110] [111]
Foxg1Cre-Mcl-1$^{fl/fl}$	neuronal cells	embryonic lethal (~E16-17) MCL-1 is essential for the survival of neuronal progenitor cells (NPCs)	[42]
NestinCre-Mcl-1$^{fl/fl}$	central and peripheral nervous system	embryonic lethal (before E15) MCL-1 is essential for adult NPCs	[42] [19]
AlbCre-Mcl-1$^{fl/fl}$	hepatocytes	viable offspring MCL-1 is essential for the survival of hepatocytes	[72]
AicdaCre-Mcl-1$^{fl/fl}$	B cells initiating somatic hypermutation (SHM) or class switch recombination (CSR)	MCL-1 is required for the survival of activated B cells, for the formation of the germinal centre and for the persistence of the germinal centre once established	[106]
Pf4Cre-Mcl-1$^{fl/fl}$	megakaryocyte lineages	viable MCL-1 is dispensable for normal megakaryopoiesis and platelet lifespan	[102] [103]
CkmmCre-Mcl-1$^{fl/fl}$	cardiomyocytes skeletal muscle	mice die ~10 d after birth due to myocardial degeneration MCL-1 is essential for the survival of cardiomyocytes but its loss has only minor impact on skeletal muscle cell survival	[52]
MyhCreER-Mcl-1$^{fl/fl}$	CreERT2-induced deletion in cardiac cells	mice die ~3 weeks upon CreERT2-induced *Mcl-1* gene deletion due to heart failure MCL-1 is essential for the survival of cardiomyocytes	[52]
MerCreMer-Mcl-1$^{fl/fl}$	CreERT2-induced deletion in cardiac cells	mice die within 29 days after the initiation of CreERT2-induced *Mcl-1* gene deletion (median survival 16 d) MCL-1 is essential for the survival of cardiomyocytes	[68]
RosaCreERT2-Mcl-1$^{fl/fl}$ *BM chimaeras; wt host*	CreERT2-induced deletion in haematopoietic cells	experiment was terminated 28 days after CreERT2-induced *Mcl-1* gene deletion MCL-1 is essential for plasma cell survival	[157]
Ncr1Cre-Mcl-1$^{fl/fl}$	NK cells	viable MCL-1 is essential for the survival of NK cells	[107]
CD11cCre-Mcl-1$^{fl/fl}$	dendritic cells	viable MCL-1 is essential for conventional DCs (cDCs) and plasmacytoid DCs (pDCs)	[108]
K5Cre-Mcl-1$^{fl/fl}$ *MMTVCre-Mcl-1*$^{fl/fl}$ *WAPiCre-Mcl-1*$^{fl/fl}$	widespread expression, including the female germline basal epithelial cells mammary gland	viable but mothers could not feed their offspring MCL-1 is essential for the survival of mammary epithelium cells	[76]
Tie2Cre-Mcl-1$^{fl/fl}$	endothelial cells	mice die within 5-21 days after birth MCL-1 is essential for the survival of endothelial cells	[77]

Table 3. continued

Mouse model	Targeted tissue/cell population	Findings	Reference
Foxn1Cre-Mcl-1$^{fl/fl}$	thymic epithelial cells	viable MCL-1 is essential for the survival of thymic epithelial cells	[75]
VillinCre-Mcl-1$^{fl/fl}$	intestinal epithelial cells	~40% mortality within the first year MCL-1 is essential for the survival of intestinal epithelial cells	[74]

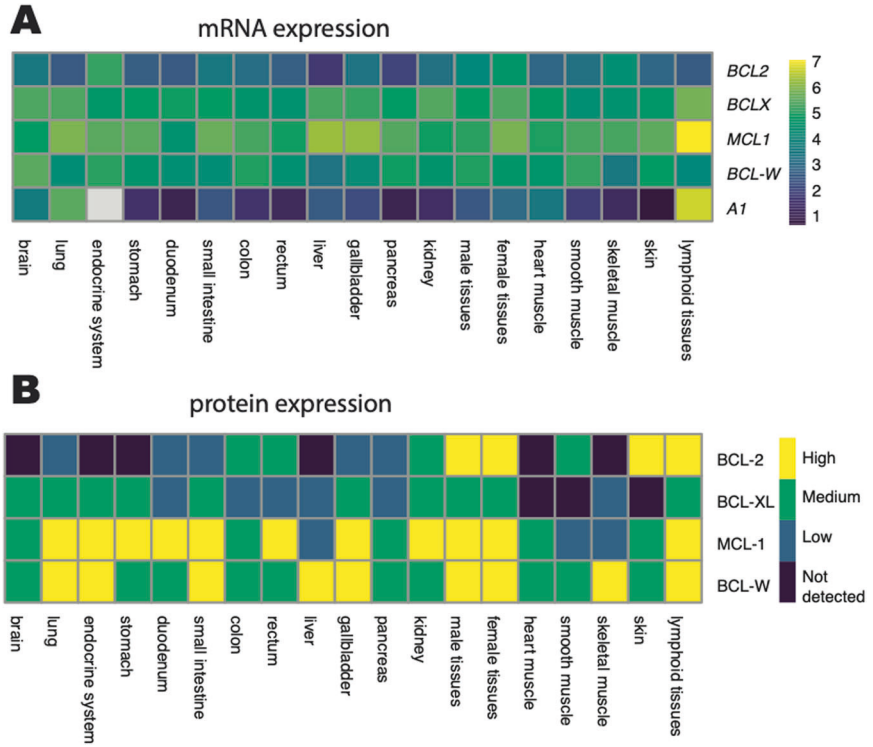

Fig. 3 Expression profile of pro-survival BCL-2 family members in solid tissues. A mRNA expression of the pro-survival BCL-2 family members *BCL2* (encoding BCL-2), *BCL2L1* (encoding BCL-XL), *MCL1* (encoding MCL-1), *BCL2L2* (encoding BCL-W), *BCL2A1* (encoding BFL-1) in human tissues. Data shown are from the Human Protein Atlas representing pooled Consensus Normalized eXpression (NX) levels created by combining the data from the three transcriptomics datasets (HPA, GTEx and FANTOM5) using an internal normalization pipeline [54]. **B** Protein expression profile of pro-survival BCL-2 family members in human tissues, quantified in a range from not detected to high expression. Data are from the Human Protein Atlas [54].

exhibited reduced germ cell survival during gonadogenesis. All *Bcl-x* hypomorphic males were sterile and had abnormally small testes. Interestingly, 25% of the *Bcl-x* hypomorphic females were fertile but had abnormally small litters [63].

CreERT2-induced whole-body deletion of *Bcl-x* in *Bcl-x*$^{fl/fl}$; *RosaCreERT2* mice was fatal at ~25 days due to severe anaemia and thrombocytopenia caused by the loss of erythroid progenitors and platelets [64]. Unexpectedly, deletion of BCL-XL in all cells other than haematopoietic ones also caused fatal anaemia and thrombocytopenia, in this case due to severe kidney damage resulting in the loss of the red blood cell-stimulating and megakaryocyte-stimulating hormones erythropoietin (EPO) and thrombopoietin (TPO), respectively [64]. This identifies an essential role of BCL-XL for the survival of mitochondria-rich renal tubular cells in the kidneys [64].

Hepatocyte-specific loss of BCL-XL in *AlbCre-Bcl-x*$^{fl/fl}$ mice caused hepatocyte apoptosis and liver fibrosis [65]. *RIPCre-Bcl-x*$^{fl/fl}$ mice, with specific loss of BCL-XL in pancreatic β-cells, are viable and show normal development of the pancreatic islets but these cells are hypersensitive to apoptosis-inducing agents [66]. Approximately 50% of mice in which BCL-XL is absent in lung epithelial cells (*SftpcCre-Bcl-x*$^{fl/fl}$) die soon after birth. Interestingly, the analysis of the viable offspring revealed that BCL-XL expression is not essential for lung development, but the pulmonary epithelial cells are hypersensitive to apoptotic stimuli [67].

MCL-1 (Table 3)**:** Loss of MCL-1 in cardiomyocytes is fatal in adults [19, 52, 68]. *MyhCreER-Mcl-1*$^{fl/fl}$ as well as *MerCreMer-Mcl-1*$^{fl/fl}$ mice died around 3–4 weeks after induced deletion of *Mcl-1* [52, 68, 69]. Interestingly, while *CkmmCre-Mcl-1*$^{fl/fl}$ as well as tamoxifen-treated (to activate CreERT2) *MyhCreERT2-Mcl-1*$^{fl/fl}$ mice developed until late adulthood in the absence of BAX and BAK (*CkmmCre-Mcl-1*$^{fl/fl}$;*Bax*$^{fl/fl}$;*Bak*$^{-/-}$ and *MyhCreERT2-Mcl-1*$^{fl/fl}$;*Bax*$^{fl/fl}$;*Bak*$^{-/-}$ mice), defects in mitochondrial dynamics and oxygen consumption were observed in these triple knockout mice [52]. It was therefore proposed that loss of an apoptosis-unrelated function of MCL-1 in mitochondrial dynamics and energy production is at least partially responsible for the cardiac defects observed [52, 68]. Specifically, it was reported that a proteolytically cleaved form of MCL-1 is imported into the mitochondrial intra-membrane space where it regulates mitochondrial fusion and mitochondrial respiratory complex assembly [70]. However, the CRE-mediated deletion of the floxed *Mcl-1* and *Bax* alleles in *CkmmCre-Mcl-1*$^{fl/fl}$;*Bax*$^{fl/fl}$;*Bak*$^{-/-}$ mice as well as tamoxifen-treated *MyhCreERT2-Mcl-1*$^{fl/fl}$;*Bax*$^{fl/fl}$;*Bak*$^{-/-}$ mice was achieved simultaneously [52]. Given the large difference

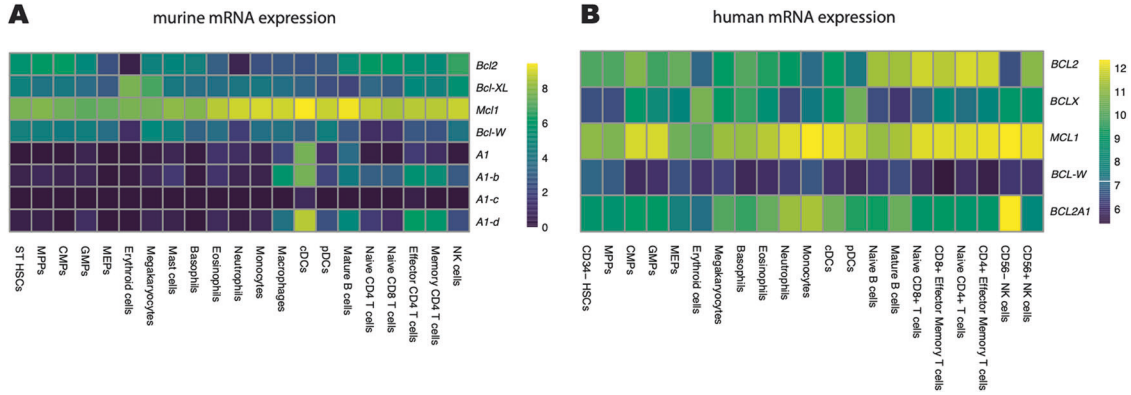

Fig. 4 mRNA expression profile of pro-survival BCL-2 family members in haematopoietic cell populations. A Expression profile of the different pro-survival BCL-2 family members *Bcl2* (encoding BCL-2), *Bcl2l1* (encoding BCL-XL), *Mcl1* (encoding MCL-1), *Bcl2l2* (encoding BCL-W), *Bcl2a1a-d* (encoding A1 proteins) in different murine haematopoietic cell populations. Data are derived from the Haemopedia Mouse RNA-Seq atlas and shown in log2 transcripts per million [84]. **B** Expression profile of the different pro-survival BCL-2 family members *BCL2* (encoding BCL-2), *BCL2L1* (encoding BCL-XL), *MCL1* (encoding MCL-1), *BCL2L2* (encoding BCL-W), *BCL2A1* (encoding BFL-1) in different human haematopoietic cell populations. Data are derived from Novershtern et al. [85] and shown as log2 normalised expression. Data for both heatmaps were sourced from www.haemosphere.org [84]. MPP – multi potential progenitor; ST-HSC - short term haematopoietic stem cell; CMP – common myeloid progenitor; GMP – granulocyte macrophage progenitor; MEP – megakaryocyte erythroid progenitor; cDC – conventional dendritic cell; pDC – plasmacytoid dendritic cell; NK cell – natural killer cell.

in the half-life of MCL-1 (~20 min) [25, 26] *vs* BAX (24 h) [71], it cannot be excluded that the observed defects in mitochondrial ultrastructure and oxygen consumption were a consequence of BAX-mediated induction of MOMP, because these cells would experience several hours when MCL-1 is no longer present but BAX is still there. In order to avoid this complication and be able to better investigate the importance of the postulated apoptosis-unrelated function of MCL-1, a mouse model that allows the deletion of *Mcl-1* and *Bax* independently is needed (e.g., first deletion of *Bax* using the FRT/*flp* system and then deletion of *Mcl-1* using the CreERT2/*loxP* system).

The deletion of MCL-1 in neuronal cells of adult mice was achieved by stereotaxic injection of *NestinCre*-expressing plasmids into the lateral ventricles of adult *Mcl-1*$^{fl/fl}$ mice. This demonstrated a critical role of MCL-1 in adult NPCs of the subventricular zone [19]. MCL-1 is also needed for hepatocyte survival [72]. *AlbCre-Mcl-1*$^{fl/fl}$ mice are born with abnormally small livers due to high rates of hepatocyte apoptosis, resulting in chronic liver damage and hepatic pericellular fibrosis [72]. Other studies revealed that MCL-1 and BCL-XL cooperatively maintain the integrity of hepatocytes in developing and adult mice [73]. Accordingly, *AlbCre-Bcl-x*$^{fl/fl}$*Mcl-1*$^{fl/fl}$ mice display a massive reduction in hepatocytes and abnormally small livers and die within 1 day after birth [73].

A critical role for MCL-1 was reported for epithelial cells, including thymic epithelial (TECs) as well as intestinal epithelial cells (IECs) [74, 75]. In contrast, loss of BCL-2 or BCL-XL had no impact on the survival of TECs or IECs. *Foxn1Cre-Mcl-1*$^{fl/fl}$ mice, in which MCL-1 is absent in TECs produce viable offspring. However, these mice are severely immunocompromised due to early thymic atrophy and T-cell lymphopenia, with near complete loss of thymic tissue by 2 months of age [75]. The loss of MCL-1 in IECs caused increased apoptosis leading to severe entero-colopathy. The increased IEC apoptosis was associated with hyperproliferative crypts, driven by compensatory proliferation of cells that had not recombined *Mcl-1*^{fl}, and this led to epithelial barrier dysfunction, chronic inflammation and accumulation of DNA damage in hyperproliferating IECs. This caused the development of intestinal tumours with morphological and genetic features of human adenomas and carcinomas [74]. MCL-1 is also essential for mammary epithelial cell survival and lactation using *K5Cre-Mcl-1*$^{fl/fl}$, *MMTVCre-Mcl-1*$^{fl/fl}$ and *WAPiCre-Mcl-1*$^{fl/fl}$ mice [76] as well as the survival of endothelial cells. Even though some endothelial cell specific *Mcl-1*-deleted mice develop until adulthood, most *Tie2Cre-Mcl-1*$^{fl/fl}$ pups are lost before weaning. They exhibit abnormally high rates of apoptosis in the angiogenic vasculature and a decline in vessel density [77].

BCL-W: BCL-W *(*encoded by the *Bcl2l2* gene*)*-deficient mice develop normally and most tissues from BCL-W-deficient mice are indistinguishable from those of wild-type controls [21, 78]. However, BCL-W-deficient males are infertile, demonstrating a critical role for BCL-W in spermatogenesis [78, 79].

A1: BFL-1 is expressed from a single gene in humans (*BCL2A1*) but there are four *A1* genes in the mouse genome [80]. *Bcl2a1a*, *Bcl2a1b* and *Bcl2a1d* are expressed and almost identical in sequence, whereas *Bcl2a1c* is a pseudogene. Mice lacking all three functional *A1* genes develop and age normally and only exhibit minor abnormalities in certain haematopoietic cell subsets [80, 81]. This is not surprising, given that A1 expression is largely restricted to haematopoietic cell lineages (Fig. 4) [80].

THE ROLES OF THE DIFFERENT PRO-SURVIVAL BCL-2 PROTEINS IN HAEMATOPOIESIS

Haematopoiesis describes the production and differentiation of all mature blood cell subsets from haematopoietic stem and progenitor cells (HSPCs). HSPCs have self-renewal capability and give rise to multipotent progenitor cells (MPPs) which in turn give rise to the lineage-committed progenitor cells, including the common lymphoid progenitors (CLPs) and common myeloid progenitors (CMPs). The latter further differentiate into megakaryocyte-erythroid progenitor (MEPs) or granulocyte-macrophage progenitors (GMPs). CLPs are the origin of all lymphoid cells, including B as well as T lymphocytes and natural killer (NK) cells. MEPs give rise to erythroid cells and megakaryocytes, which shed platelets, while granulocytes (e.g., neutrophils, eosinophils, monocytes) originate from GMPs.

The survival of immature and mature haematopoietic cells is safeguarded by pro-survival BCL-2 proteins with distinct family members being critical in different cell subsets (Fig. 4). MCL-1 is highly expressed in HSPCs and many mature haematopoietic cell subsets in both mice and humans [82–85]. In contrast, the expression of BCL-XL is moderate throughout haematopoiesis, but with relatively high levels found in erythroid cells and megakaryocytes [82–85]. BCL-2 is most prominently expressed in certain

B and T lymphoid populations as well as NK cells and moderate expression is also found in stem and progenitor populations, including HSCs, MPPs, CMPs and CLPs, but only low expression is seen in erythroid cells [82–85]. A1 is found in antigen receptor stimulated T and B lymphocytes, dendritic cells, neutrophils, eosinophils and monocytes [82–85]. BCL-W is expressed at comparatively low levels in the haematopoietic compartment (Fig. 4) [82–85].

Conditional gene deletion studies in mice have identified the essential roles of distinct pro-survival BCL-2 proteins in the survival of different haematopoietic cell types.

BCL-2 (Table 1**):** Examination of chimaeric mice with a BCL-2-deficient haematopoietic compartment revealed that BCL-2-deficient mature T cells developed normally in vivo but had abnormally short lifespan in vitro and increased sensitivity to glucocorticoids and γ-irradiation compared to control T cells [86]. Antigen receptor stimulation enhanced the in vitro survival of T cells lacking BCL-2 [86], presumably because this causes NF-kB-driven up-regulation of BCL-XL [87]. In these chimaeric mice BCL-2-deficient T and B cells disappeared from the bone marrow, thymus, and peripheral lymphoid organs by 4 weeks of age [86, 88]. Characterisation of a *Bcl-2* YFP reporter mouse strain revealed that BCL-2 is critical for the survival of effector and memory T lymphocytes [89, 90]. Notably, the survival defects of all lymphoid subsets caused by the absence of BCL-2 could be rescued by the concomitant loss of only a single allele of pro-apoptotic *Bim* [60]. This demonstrates that the function of BCL-2 in mature B and T lymphocytes is mainly to oppose BIM-induced apoptosis.

The absence of BCL-2 had no impact on the numbers of myeloid as well as erythroid cells and platelets. Notably, even cells that express moderate to high levels of BCL-2 (e.g., pro-myelocytes, normoblasts, megakaryocytes) are present at normal numbers in BCL-2-deficient mice [88]. Tissue-restricted deletion of floxed *Bcl-2* revealed that BCL-2 contributes to NK cell survival (*Ncr1Cre-Bcl-2*$^{fl/fl}$ mice) [91] but is dispensable for the survival of megakaryocytes and platelets (*Pf4-Cre-Bcl-2*$^{fl/fl}$ mice) [92].

BCL-XL (Table 2**):** Even though moderate to high expression of BCL-XL has been reported for most haematopoietic cell populations (Fig. 4), the absence of BCL-XL only impacts the survival of some. The role of BCL-XL in haematopoiesis was studied in chimaeric mice that were generated by injection of *Bcl-x*$^{-/-}$ ES cells into wild-type or *Rag1*$^{-/-}$ blastocysts, the latter unable to give rise to mature T or B cells [46]. This revealed impaired survival of immature lymphocytes, such as CD4^{+}CD8^{+} thymocytes, but not mature lymphocytes [46, 93]. Conditional deletion of *Bcl-x* specifically in T lymphoid cells from an early stage of differentiation (*LckCre-Bcl-x*$^{fl/fl}$ mice) revealed that the development of effector and memory T lymphocytes was not impacted by the loss of BCL-XL [94].

Analysis of the aforementioned chimaeric mice revealed that *Bcl-x*$^{-/-}$ ES cells did not contribute to circulating EryD cells in the peripheral blood, demonstrating that BCL-XL plays an important role in erythroid progenitor survival. In vitro differentiation analysis confirmed that BCL-XL is critical for the survival of both primitive (EryP) and definite erythroid (EryD) progenitor cells [45]. Interestingly, the differentiation of *Bcl-x*$^{-/-}$ and wild-type ES cells in vitro yielded similar numbers of EryP and EryD cells, however, prominent apoptosis of *Bcl-x*$^{-/-}$ EryP and EryD occurred upon further maturation. This demonstrates that BCL-XL is critical at later stages of erythropoiesis. Accordingly, *MMTVCre-Bcl-x*$^{fl/fl}$ transgenic mice that express the CRE recombinase in various secretory tissues but also the haematopoietic system [95–98] develop fatal anaemia [99]. Notably, the loss of only a single allele of *Bcl-x* or point mutations that reduce BCL-XL protein half-life (*Bcl-x*$^{Plt16/Plt16}$ and *Bcl-x*$^{Plt20/Plt20}$ mice) cause a significant reduction in platelets [100]. Platelets specifically depend on BCL-XL to inhibit BAK-mediated apoptosis. When platelets are shed from megakaryocytes, they no longer produce much protein. Therefore, the relative levels of BCL-XL *vs* BAK set up a timer of platelet lifespan and consequently a reduction in BCL-XL (e.g., in platelets from *Bcl-x*$^{+/-}$ mice) reduces platelet survival [100]. Conditional deletion of *Bcl-x* in megakaryocytes (*Pf4-Cre-Bcl-x*$^{fl/fl}$ mice) demonstrated that BCL-XL is dispensable for the development and survival of these cells [101]. In fact, megakaryocyte survival is safeguarded by the combination of BCL-XL and MCL-1, with only the absence of both causing their depletion [102, 103].

MCL-1 (Table 3**):** A reduction in lymphocytes, red blood cells (RBCs) but not platelets was observed in *Mcl-1*$^{+/-}$ mice in which MCL-1 protein levels are reduced by ~40% compared to wild-type cells [104]. Conditional *Mcl-1* gene-targeted mice were generated to identify the role of MCL-1 in the survival of different haematopoietic cell populations.

LckCre-Mcl-1$^{fl/fl}$ mice with MCL-1 loss from early stages of T lymphocyte development lack all immature and mature T cell populations, with their development arrested at the progenitor (CD3^{-}4^{-}8^{-} triple-negative (TN)) stage [32]. *CD19Cre-Mcl-1*$^{fl/fl}$ mice with loss of MCL-1 in B cells present with an arrest of B lymphocyte differentiation at the pro-B cell stage [32]. Although these studies identify a critical role for MCL-1 in the survival of early T and B cell progenitors, they do not provide insight into possible roles of MCL-1 at later stages of T and B cell differentiation. Inducible deletion of MCL-1 using *MxCre-Mcl-1*$^{fl/fl}$ mice was able to reveal a critical role of MCL-1 for the survival of mature B and T cells in culture [105]. Moreover, MCL-1 is required for the survival of activated B cells and the formation of the germinal centre [106].

Ncr1Cre-Mcl-1$^{fl/fl}$ mice that lack MCL-1 in NK cells are completely deficient in this cell population [107], identifying an essential role of MCL-1 for NK cell survival. The characterisation of *CD11c-Cre-Mcl-1*$^{fl/fl}$ revealed a critical role of MCL-1 in the survival of conventional as well as plasmacytoid dendritic cells [108]. A prominent role for MCL-1 was also reported for erythroid progenitors. *EpoR-Cre-Mcl-1*$^{fl/fl}$ embryos die ~E13.5 due to severe anaemia [109]. Interestingly, MCL-1 is only required during early stages of definitive erythropoiesis but is dispensable for the survival of later stage erythroid progenitor cells that instead depend on BCL-XL [45, 99, 109].

MCL-1 has a less prominent role in myeloid cells and megakaryocytes. *LysMCre-Mcl-1*$^{fl/fl}$ mice have normal numbers of monocytes and macrophages but display an abnormal reduction in neutrophils [110, 111]. It is, however, noteworthy that the *LysMCre* transgene is not as effective at recombining floxed genes as other *Cre* transgenes; therefore the importance of MCL-1 in myeloid cell survival may have been underestimated. Examination of *Pf4-Cre-Mcl-1*$^{fl/fl}$ mice revealed that MCL-1 is dispensable for megakaryocyte development and survival as well as platelet lifespan [102, 103] with megakaryocyte survival safeguarded by both BCL-XL and MCL-1.

BCL-W: Moderate BCL-W expression was detected in most haematopoietic cell populations [21, 112] (Fig. 4). Nevertheless, BCL-W-deficient mice had normal distributions of all immature as well as mature haematopoietic cell types [78]. Enforced expression of BCL-W renders lymphoid and myeloid cells refractory to diverse cytotoxic conditions [112]. Therefore, it was proposed that BCL-W may play a role in the development of haematological cancers. However, initial reports [113, 114] that BCL-W is a driver of lymphomagenesis in various B cell lymphomas could not be reproduced [115].

A1: A1 is expressed in certain haematopoietic cell types but only minor defects are observed in A1-deficient mice, including a small reduction in TCRγ/δ T cells, antigen-experienced conventional as well as regulatory CD4^{+} T cells in vivo and impaired survival of conventional dendritic cells (cDCs) in culture [80]. The absence of A1 did not impair T cell responses during viral infection in mice [81]. A1 expression is induced by inflammatory cytokines, suggesting a role in the survival of inflammatory cells [116–119].

Table 4. Impact of pro-survival gene targeting versus pro-survival protein inhibition using BH3 mimetic drugs.

	Deletion of BCL-XL*	Pharmacological Inhibition of BCL-XL**	Deletion of BCL-2§	Pharmacological Inhibition of BCL-2	Deletion of MCL-1 #	Pharmacological Inhibition of MCL-1##
Heart	*no impact reported* *	*no human data reported/ available***	*no impact reported*§	No adverse events reported from Venetoclax/ABT-199 phase I dose escalation [138]	Loss of cardiomyocyte survival and function [52, 68]	AMG-397: phase 1 dose escalation clinical paused due to cardiotoxicity safety signal (NCT 03465540) [147, 148] AZD5991: phase 1 dose escalation clinical paused due to cardiotoxicity safety signal (NCT03218683) [149, 150]
Skeletal muscle/Bones	*no impact reported**	*no human data reported/ available***	Reduction of osteoclasts [154]	No adverse events reported from Venetoclax/ABT-199 phase I dose escalation [138]	minor impact [52]	*no human data reported/ available* ##
Liver	Loss of hepatocyte survival (in conjunction with deletion of *Mcl-1*) [65, 73]	*no human data reported/ available*** **Pre-clinical:** No liver toxicity of Navitoclax/ABT-263 on human hepatocytes in vitro [135] Liver toxicity of S63845 when combined with A-1331852 [153]	*no impact reported*§	No adverse events reported from Venetoclax/ABT-199 phase I dose escalation [138]	Loss of hepatocyte survival in conjunction with deletion of *Bcl-x* [72, 73]	*no human data reported/ available* ## **Pre-clinical:** Liver toxicity of S63845 when combined with the BCL-XL inhibitor A-1331852 [153]
Kidney	Loss of renal epithelial cells [64]	*no human data reported/ available***	Polycystic kidney disease starting during embryogenesis [40, 60, 86, 88]	Acute renal failure in 2/56 patients who have also suffered from tumour lysis syndrome and 1/56 had transient elevated creatinine kinase levels in a Venetoclax/ABT-199 phase I dose escalation trial (NCT01328626)	*no data available* #	*no human data reported/ available* ##
Gastrointestinal	*no impact reported**	Navitoclax/ABT-263 (BCL2/ BCL-XL/BCL-W inhibitor) phase I trial reported diarrhoea, nausea [122]	*no impact reported*§	Venetoclax/ABT-199 phase I dose escalation trial (NCT01328626) reported diarrhoea [138]	Loss of intestinal epithelial cells [74]	AMG 176 phase I dose escalation trial (NCT02675452) reported nausea and diarrhoea [151]
Pancreas/endocrine system	β-cells are hypersensitive to apoptotic insults [66]	*no human data reported/ available*** **Pre-clinical:** Modest effect of the BCL-XL inhibitor A-1155463 on pancreatic acinar cells Ca2+ response induced by taurolithocholic acid 3-sulfate [158]	*no impact reported*§	**Pre-clinical:** No effect of Venetoclax/ ABT-199 on pancreatic acinar cells Ca2+ response induced by taurolithocholic acid 3-sulfate [158]	*no data available*#	*no human data reported/ available*##
Brain/neuronal cells	Loss of catecholaminergic neuronal cells [46, 49]	*no human data reported/ available*** **Pre-clinical:** Navitoclax/ABT-263 (BCL2/ BCL-XL/BCL-W inhibitor) induced cell death in murine non-dopaminergic neuron cell lines [159]	Loss of motoneurons, sensory and sympathetic neurons during early postnatal phase [44], loss of doublecortin-expressing immature neurons [61]	No adverse events reported from Venetoclax/ABT-199 phase I dose escalation [138] **Pre-clinical:** Venetoclax/ABT-199 induced cell death in a	Loss of neuronal progenitor cells [19, 42]	*no human data reported/ available*## **Pre-clinical:** The MCL-1 inhibitor UMI-77 induced cell death in murine dopaminergic neuron cell line [159]

Table 4. continued

	Deletion of BCL-XL*	Pharmacological Inhibition of BCL-XL**	Deletion of BCL-2§	Pharmacological Inhibition of BCL-2	Deletion of MCL-1 #	Pharmacological Inhibition of MCL-1##
				murine non-dopaminergic neuron cell line in vitro [159]		
Lung	Respiratory epithelial cells are hypersensitive to apoptotic insults [67]	*no human data reported/ available***	*no impact reported*§	No adverse events reported from Venetoclax/ABT-199 phase I dose escalation [138]	*no data available*#	*no human data reported/ available*##
Haematopoietic cells/ Immune system	Loss of erythroid cells [45, 99], dendritic cells [152], megakaryocyte function and survival (in conjunction with deletion of *Mcl-1*) [101–103], decreased platelet lifespan [100], impaired survival of CD4+CD8+ thymocytes [93]	Navitoclax/ABT-263 (BCL2/ BCL-XL/BCL-W) inhibitor) induced thrombocytopenia (dose limiting toxicity) [122]	Loss of B and T lymphocytes [40, 60, 86, 88] including effector and memory T lymphocytes [89, 90,153], dendritic cells [154], NK cells [91]	Venetoclax/ABT-199 phase I dose escalation trial (NCT01328626) reported neutropenia [138]	Loss of HSPCs [105], immature and mature B and T cells [32, 154], neutrophils [110, 111], germinal centre formation and memory B cells [156], NK cells [107], plasma cells [157], dendritic cells [108], thymic epithelial cells [75], erythroid progenitor cells [109]	AMG 176 phase I dose escalation trial (NCT02675452) reported anaemia and neutropenia (≥Grade 3 treatment emergent adverse events) [151] **Pre-clinical:** S63845 induces complete depletion of human CD34 + HSPCs [152]
Vasculature	*no impact reported**	*no human data reported/ available***	Loss of endothelial cells and pericytes [62]	No adverse events reported from Venetoclax/ABT-199 phase I dose escalation [138]	Loss of endothelial cells [77]	*no human data reported/ available*##
Skin	*no impact reported**	*no human data reported/ available***	Loss of melanocytes and melanocyte stem cells [57, 58]	No adverse events reported from Venetoclax/ABT-199 phase I dose escalation [138]	*no data available*#	*no human data reported/ available*##
Female reproductive tissues	Loss of murine germ cells [63], critical role for mammary epithelium during involution [160]	*no human data reported/ available***	*no impact reported*§	*no human data reported/ available**	Loss of mammary epithelial cells [76]	*no human data reported/ available*##
Male reproductive tissue	Loss of murine germ cells [63]	*no human data reported/ available***	*no impact reported* §	*no human data reported/ available**	*Mcl-1*$^{fl/fl}$ males are infertile	*no human data reported/ available*##

**Whole body BCL-XL deletion is lethal during embryogenesis, induced whole body BCL-XL deletion allows the survival of mice for up to 30 days in intact mice or up 105 days in haematopoietic chimaeras, no impact has been reported within this time frame.

**Selective BCL-XL inhibitors (e.g., WEHI-539, A-1155463, A-1331852) have not entered clinical trials at this time. Clinical data that are listed here are from the BCL2/BCL-XL/BCL-W inhibitors Navitoclax/ABT-263 or ABT-737.

§Whole body BCL-2 deletion allows the survival of mice for up to 40 days, no impact has been reported within this time frame.

#Whole-body MCL-1 deletion is lethal during early (E3.5) embryogenesis as well as within 24 h after induced deletion in the adult, no tissue-restricted targeting has been reported yet.

##The MCL-1 inhibitors AMG-176 & 395 (Amgen), AZD5991 (AstraZeneca) and S63845/MIK-665 (Servier/Novarties) have recently entered phase 1 clinical trials. No data from long-term studies have been reported.

However, no defects in neutrophil survival during infection were observed in A1-deficient mice [120].

TARGETING PRO-SURVIVAL BCL-2 FAMILY PROTEINS WITH BH3 MIMETIC DRUGS FOR CANCER THERAPY

In many human cancers apoptosis is dysregulated, often as a result of the overexpression of BCL-2, BCL-XL or MCL-1, and many cancers rely on their aberrant expression for sustained growth [121]. Accordingly, BH3-mimetic drugs that target distinct pro-survival proteins have been developed and the BCL-2 inhibitor Venetoclax is approved for CLL and AML. Given the essential roles of pro-survival BCL-2 proteins for the survival of many non-malignant cells, these compounds may cause undesirable on-target side effects to healthy tissues that may be predicted from the analysis of gene knock-out studies in mice (Table 4). All on-target toxicities observed in patients and mice treated with BH3 mimetic drugs targeting BCL-2, BCL-XL (e.g., thrombocytopenia) or MCL-1 (cardiac, intestinal and haematopoietic toxicity) were also seen in mice deficient for these proteins. Obversely, some of the damages to tissues caused by constitutive or cell type-restricted knockout of pro-survival BCL-2 proteins were not seen in patients or mice treated with BH3 mimetic drugs targeting these proteins. This is not surprising since in contrast to genetic deletion, drug-mediated inhibition is transient and likely does not result in a complete loss of function of a pro-survival BCL-2 protein. Therefore, it is expected that the on-target drug-induced toxicities are milder than the defects caused by constitutive loss of a pro-survival BCL-2 protein upon its genetic deletion. Here, we compare and discuss the consequences of genetic deletion *vs* drug-mediated inhibition of pro-survival BCL-2 proteins in major tissues.

ABT-737 is the first BH3-mimetic compound described, followed by its orally available derivative ABT-263/Navitoclax that was tested in clinical trials [122, 123]. Both compounds are potent inhibitors of BCL-2, BCL-XL and BCL-W [124–127]. Despite high potency in killing several types of malignant cells in pre-clinical tests, clinical trials of Navitoclax in chronic lymphocytic leukaemia were halted because of platelet toxicity [123]. The thrombocytopenia and anaemia observed in Navitoclax-treated patients [122, 123] aligns with the essential role of BCL-XL in platelets and erythroid progenitor cells identified in gene-targeted mice [99, 100]. Accordingly, toxic effects on platelets were also observed in mice treated with BCL-XL-specific inhibitors [128–131]. This will likely complicate clinical development of such agents unless they can be appropriately scheduled to minimise dose limiting toxicity or modified to preferentially target cancerous cells, for example by coupling to an antibody that binds to malignant cells. Another promising approach is the PROTAC-based degradation of BCL-XL, targeting E3-ubiquitin ligases that are not present in platelets but highly expressed in cancerous cells [132–134]. Prolonged treatment with Navitoclax did not cause liver or kidney toxicities that are caused by genetic deletion of BCL-XL [135]. Of note, Navitoclax and ABT-737 were both shown to preferentially inhibit BCL-2 rather than BCL-XL in cells in vivo [136]. This may limit conclusions regarding the spectrum of toxicities related to specific pro-survival BCL-2 proteins from clinical studies of Navitoclax. As platelets express only low levels of BCL-2 and BCL-W, BCL-XL can be inferred to be the primary target of Navitoclax in these cells. In contrast, cells in the kidney express significant levels of BCL-2, BCL-XL and MCL-1 (Fig. 3), and thus BCL-XL might not be efficiently targeted by Navitoclax in this tissue. MCL-1 was shown to safeguard hepatocyte survival in the absence of BCL-XL in genetic knock-out studies [73], perhaps explaining why Navitoclax did not cause liver toxicity. Since no BCL-XL-specific inhibitor has entered clinical trials, it cannot be excluded that consequences of genetic loss of BCL-XL might actually occur in patients treated with such a drug.

To prevent dose-limiting thrombocytopenia in cancer patients, the BCL-2-specific inhibitor Venetoclax/ABT-199 was developed [137]. Treatment with Venetoclax is well-tolerated in patients, with no toxicity in major organs, such as the kidney or liver [138]. This agrees with observations from BCL-2-deficient mice, showing that even though BCL-2 is essential for kidney development in the embryo, it is dispensable for the function of major adult organs [40, 60]. In line with what was seen in BCL-2-deficient mice, many patients develop transient neutropenia (~40%) [139, 140]. While investigators have attributed thrombocytopenia and anaemia as adverse events to single agent Venetoclax, this occurred with a large proportion of enroled patients with relapsed/refractory CLL having cytopenia at baseline [139, 140]. As most haematologic adverse events occurred early in treatment and decreased or resolved over time, these observations are most likely secondary to the high burden of disease rather than a direct effect of Venetoclax. Importantly, the neutropenia in Venetoclax-treated patients could be managed with growth factor support [139, 140]. In contrast to the genetic deletion of BCL-2, treatment with Venetoclax does not cause neuronal toxicity in patients [138]. This may be explained by the poor blood-brain barrier penetration of Venetoclax, with drug concentration in the central nervous system only reaching 0.1% of that observed in the plasma [141].

Despite the essential role of MCL-1 for the survival of many critical cell types, pre-clinical studies in mice have identified a therapeutic window for MCL-1-specific inhibitors [142–145]. This is in line with observations that loss of even a single allele of *Mcl-1* obliterates the expansion of MYC-driven lymphomas in mice, unless they carry a mutation in p53 [146], while loss of one allele of *Mcl-1* is well-tolerated in mice with only minor reductions in mature B lymphocytes and erythroid cells [104]. Conclusions from pre-clinical observations prompted the initiation of phase 1 clinical trials with several MCL-1 inhibitors. Trials of AMG-397 (Amgen) and AZD5991 (AstraZeneca) were paused due to cardiac toxicity signals [147–150]. In contrast, phase 1 clinical dose escalation studies for S64315/MIK665 (Servier/Novartis) have been completed without major adverse events being reported. The cardiotoxicity of AMG-397 and AZD5991 is in line with the findings from genetic studies that MCL-1 is essential for cardiomyocyte survival in mice [52, 68]. MCL-1 is also the major survival factor for HSPCs, and accordingly, AMG-176 induced neutropenia and severe anaemia in patients, which may be caused by a reduction in HSPCs [151]. Consistent with this idea, human HSPCs were depleted during in vitro treatment with S64315/MIK665 [151]. MCL-1 loss results in intestinal epithelial cell apoptosis [74] and, accordingly, treatment with AMG-176 caused gastro-intestinal toxicity in patients. Pre-clinical data demonstrate that the combined inhibition of MCL-1 and BCL-XL causes liver toxicity [152], a scenario that could be predicted from gene-targeting studies, showing that MCL-1 and BCL-XL collectively ensure hepatocellular survival [65, 72, 73].

In conclusion, mouse models have been invaluable to identify the critical roles of the different pro-survival BCL-2 proteins for the survival of a broad range of non-transformed cell types during embryogenesis and in the adult. This highlights the importance of using genetic mouse models to form the foundation for developing effective and safe new treatments for cancer patients.

DATA AVAILABILITY

There are no primary data presented in this review article.

REFERENCES

1. Adams JM, Cory S. The Bcl-2 apoptotic switch in cancer development and therapy. Oncogene. 2007;26:1324–37.

2. Czabotar PE, Lessene G, Strasser A, Adams JM. Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy. Nat Rev Mol Cell Biol. 2014;15:49–63.
3. Green DR. The coming decade of cell death research: five riddles. Cell. 2019;177:1094–107.
4. Ke FFS, Vanyai HK, Cowan AD, Delbridge ARD, Whitehead L, Grabow S, et al. Embryogenesis and adult life in the absence of intrinsic apoptosis effectors BAX, BAK, and BOK. Cell. 2018;173:1217–30 e1217.
5. Llambi F, Wang YM, Victor B, Yang M, Schneider DM, Gingras S, et al. BOK is a non-canonical BCL-2 family effector of apoptosis regulated by ER-associated degradation. Cell. 2016;165:421–33.
6. Puthalakath H, Strasser A. Keeping killers on a tight leash: transcriptional and post-translational control of the pro-apoptotic activity of BH3-only proteins. Cell Death Differ. 2002;9:505–12.
7. Cartron PF, Gallenne T, Bougras G, Gautier F, Manero F, Vusio P, et al. The first alpha helix of Bax plays a necessary role in its ligand-induced activation by the BH3-only proteins Bid and PUMA. Mol Cell. 2004;16:807–18.
8. Marani M, Tenev T, Hancock D, Downward J, Lemoine NR. Identification of novel isoforms of the BH3 domain protein Bim which directly activate Bax to trigger apoptosis. Mol Cell Biol. 2002;22:3577–89.
9. Willis SN, Fletcher JI, Kaufmann T, van Delft MF, Chen L, Czabotar PE, et al. Apoptosis initiated when BH3 ligands engage multiple Bcl-2 homologs, not Bax or Bak. Science. 2007;315:856–9.
10. Krajewski S, Tanaka S, Takayama S, Schibler MJ, Fenton W, Reed JC. Investigation of the subcellular distribution of the bcl-2 oncoprotein: residence in the nuclear envelope, endoplasmic reticulum, and outer mitochondrial membranes. Cancer Res. 1993;53:4701–14.
11. Lithgow T, van Driel R, Bertram JF, Strasser A. The protein product of the oncogene Bcl-2 is a component of the nuclear envelope, the endoplasmic reticulum, and the outer mitochondrial membrane. Cell Growth Differ. 1994;5:411–7.
12. Janiak F, Leber B, Andrews DW. Assembly of Bcl-2 into microsomal and outer mitochondrial membranes. J Biol Chem. 1994;269:9842–9.
13. Liu H, Peng HW, Cheng YS, Yuan HS, Yang-Yen HF. Stabilization and enhancement of the antiapoptotic activity of mcl-1 by TCTP. Mol Cell Biol. 2005;25:3117–26.
14. Nakajima W, Hicks MA, Tanaka N, Krystal GW, Harada H. Noxa determines localization and stability of MCL-1 and consequently ABT-737 sensitivity in small cell lung cancer. Cell Death Dis. 2014;5:e1052.
15. Weng SY, Yang CY, Li CC, Sun TP, Tung SY, Yen JJ, et al. Synergism between p53 and Mcl-1 in protecting from hepatic injury, fibrosis and cancer. J Hepatol. 2011;54:685–94.
16. Yang T, Kozopas KM, Craig RW. The intracellular distribution and pattern of expression of Mcl-1 overlap with, but are not identical to, those of Bcl-2. J Cell Biol. 1995;128:1173–84.
17. Jamil S, Sobouti R, Hojabrpour P, Raj M, Kast J, Duronio V. A proteolytic fragment of Mcl-1 exhibits nuclear localization and regulates cell growth by interaction with Cdk1. Biochem J. 2005;387:659–67.
18. Hsu YT, Youle RJ. Nonionic detergents induce dimerization among members of the Bcl-2 family. J Biol Chem. 1997;272:13829–34.
19. Malone CD, Hasan SM, Roome RB, Xiong J, Furlong M, Opferman JT, et al. Mcl-1 regulates the survival of adult neural precursor cells. Mol Cell Neurosci. 2012;49:439–47.
20. Nijhawan D, Fang M, Traer E, Zhong Q, Gao W, Du F, et al. Elimination of Mcl-1 is required for the initiation of apoptosis following ultraviolet irradiation. Genes Dev. 2003;17:1475–86.
21. O'Reilly LA, Print C, Hausmann G, Moriishi K, Cory S, Huang DC, et al. Tissue expression and subcellular localization of the pro-survival molecule Bcl-w. Cell Death Differ. 2001;8:486–94.
22. Werner AB, de Vries E, Tait SW, Bontjer I, Borst J. Bcl-2 family member Bfl-1/A1 sequesters truncated bid to inhibit is collaboration with pro-apoptotic Bak or Bax. J Biol Chem. 2002;277:22781–8.
23. Guedes RP, Rocha E, Mahiou J, Moll HP, Arvelo MB, Taube JM, et al. The C-terminal domain of A1/Bfl-1 regulates its anti-inflammatory function in human endothelial cells. Biochim Biophys Acta. 2013;1833:1553–61.
24. Kucharczak JF, Simmons MJ, Duckett CS, Gelinas C. Constitutive proteasome-mediated turnover of Bfl-1/A1 and its processing in response to TNF receptor activation in FL5.12 pro-B cells convert it into a prodeath factor. Cell Death Differ. 2005;12:1225–39.
25. Adams KW, Cooper GM. Rapid turnover of mcl-1 couples translation to cell survival and apoptosis. J Biol Chem. 2007;282:6192–200.
26. Maurer U, Charvet C, Wagman AS, Dejardin E, Green DR. Glycogen synthase kinase-3 regulates mitochondrial outer membrane permeabilization and apoptosis by destabilization of MCL-1. Mol Cell. 2006;21:749–60.
27. Blagosklonny MV, Alvarez M, Fojo A, Neckers LM. bcl-2 protein downregulation is not required for differentiation of multidrug resistant HL60 leukemia cells. Leuk Res. 1996;20:101–7.
28. Rooswinkel RW, van de Kooij B, de Vries E, Paauwe M, Braster R, Verheij M, et al. Antiapoptotic potency of Bcl-2 proteins primarily relies on their stability, not binding selectivity. Blood. 2014;123:2806–15.
29. Herold MJ, Zeitz J, Pelzer C, Kraus C, Peters A, Wohlleben G, et al. The stability and anti-apoptotic function of A1 are controlled by its C terminus. J Biol Chem. 2006;281:13663–71.
30. Chen L, Willis SN, Wei A, Smith BJ, Fletcher JI, Hinds MG, et al. Differential targeting of prosurvival Bcl-2 proteins by their BH3-only ligands allows complementary apoptotic function. Mol Cell. 2005;17:393–403.
31. Kuwana T, Bouchier-Hayes L, Chipuk JE, Bonzon C, Sullivan BA, Green DR, et al. BH3 domains of BH3-only proteins differentially regulate Bax-mediated mitochondrial membrane permeabilization both directly and indirectly. Mol Cell. 2005;17:525–35.
32. Opferman JT, Letai A, Beard C, Sorcinelli MD, Ong CC, Korsmeyer SJ. Development and maintenance of B and T lymphocytes requires antiapoptotic MCL-1. Nature. 2003;426:671–6.
33. Flach G, Johnson MH, Braude PR, Taylor RA, Bolton VN. The transition from maternal to embryonic control in the 2-cell mouse embryo. EMBO J. 1982;1:681–6.
34. Boumela I, Assou S, Haouzi D, Dechaud H, Ait-Ahmed O, Hamamah S. Developmental regulated expression of anti- and pro-apoptotic BCL-2 family genes during human early embryonic development. Curr Med Chem. 2014;21:1361–9.
35. Russell HR, Lee Y, Miller HL, Zhao J, McKinnon PJ. Murine ovarian development is not affected by inactivation of the bcl-2 family member diva. Mol Cell Biol. 2002;22:6866–70.
36. Ke N, Godzik A, Reed JC. Bcl-B, a novel Bcl-2 family member that differentially binds and regulates Bax and Bak. J Biol Chem. 2001;276:12481–4.
37. Lee R, Chen J, Matthews CP, McDougall JK, Neiman PE. Characterization of NR13-related human cell death regulator, Boo/Diva, in normal and cancer tissues. Biochim Biophys Acta. 2001;1520:187–94.
38. Song Q, Kuang Y, Dixit VM. Vincenz C. Boo, a novel negative regulator of cell death, interacts with Apaf-1. EMBO J. 1999;18:167–78.
39. Opferman JT, Kothari A. Anti-apoptotic BCL-2 family members in development. Cell Death Differ. 2018;25:37–45.
40. Veis DJ, Sorenson CM, Shutter JR, Korsmeyer SJ. Bcl-2-deficient mice demonstrate fulminant lymphoid apoptosis, polycystic kidneys, and hypopigmented hair. Cell. 1993;75:229–40.
41. Abe-Dohmae S, Harada N, Yamada K, Tanaka R. Bcl-2 gene is highly expressed during neurogenesis in the central nervous system. Biochem Biophys Res Commun. 1993;191:915–21.
42. Arbour N, Vanderluit JL, Le Grand JN, Jahani-Asl A, Ruzhynsky VA, Cheung EC, et al. Mcl-1 is a key regulator of apoptosis during CNS development and after DNA damage. J Neurosci. 2008;28:6068–78.
43. Merry DE, Veis DJ, Hickey WF, Korsmeyer SJ. bcl-2 protein expression is widespread in the developing nervous system and retained in the adult PNS. Development. 1994;120:301–11.
44. Michaelidis TM, Sendtner M, Cooper JD, Airaksinen MS, Holtmann B, Meyer M, et al. Inactivation of bcl-2 results in progressive degeneration of motoneurons, sympathetic and sensory neurons during early postnatal development. Neuron. 1996;17:75–89.
45. Motoyama N, Kimura T, Takahashi T, Watanabe T, Nakano T. bcl-x prevents apoptotic cell death of both primitive and definitive erythrocytes at the end of maturation. J Exp Med. 1999;189:1691–8.
46. Motoyama N, Wang F, Roth KA, Sawa H, Nakayama K, Nakayama K, et al. Massive cell death of immature hematopoietic cells and neurons in Bcl-x-deficient mice. Science. 1995;267:1506–10.
47. Roth KA, Motoyama N, Loh DY. Apoptosis of bcl-x-deficient telencephalic cells in vitro. J Neurosci. 1996;16:1753–8.
48. Shindler KS, Latham CB, Roth KA. Bax deficiency prevents the increased cell death of immature neurons in bcl-x-deficient mice. J Neurosci. 1997;17:3112–9.
49. Savitt JM, Jang SS, Mu W, Dawson VL, Dawson TM. Bcl-x is required for proper development of the mouse substantia nigra. J Neurosci. 2005;25:6721–8.
50. Rinkenberger JL, Horning S, Klocke B, Roth K, Korsmeyer SJ. Mcl-1 deficiency results in peri-implantation embryonic lethality. Genes Dev. 2000;14:23–7.
51. Flemmer RT, Connolly SP, Geizer BA, Opferman JT, Vanderluit JL. The role of Mcl-1 in embryonic neural precursor cell apoptosis. Front Cell Dev Biol. 2021;9:659531.
52. Wang X, Bathina M, Lynch J, Koss B, Calabrese C, Frase S, et al. Deletion of MCL-1 causes lethal cardiac failure and mitochondrial dysfunction. Genes Dev. 2013;27:1351–64.

53. Sjostedt E, Zhong W, Fagerberg L, Karlsson M, Mitsios N, Adori C, et al. An atlas of the protein-coding genes in the human, pig, and mouse brain. Science 2020, 367.
54. Uhlen M, Bjorling E, Agaton C, Szigyarto CA, Amini B, Andersen E, et al. A human protein atlas for normal and cancer tissues based on antibody proteomics. Mol Cell Proteom. 2005;4:1920–32.
55. Uhlen M, Fagerberg L, Hallstrom BM, Lindskog C, Oksvold P, Mardinoglu A, et al. Proteomics. Tissue-based map of the human proteome. Science. 2015;347:1260419.
56. Kamada S, Shimono A, Shinto Y, Tsujimura T, Takahashi T, Noda T, et al. bcl-2 deficiency in mice leads to pleiotropic abnormalities: accelerated lymphoid cell death in thymus and spleen, polycystic kidney, hair hypopigmentation, and distorted small intestine. Cancer Res. 1995;55:354–9.
57. Mak S-S, Moriyama M, Nishioka E, Osawa M, Nishikawa S-I. Indispensable role of Bcl2 in the development of the melanocyte stem cell. Dev Biol. 2006;291:144–53.
58. Yamamura K, Kamada S, Ito S, Nakagawa K, Ichihashi M, Tsujimoto Y. Accelerated disappearance of melanocytes in bcl-2-deficient mice. Cancer Res. 1996;56:3546–50.
59. Wojciechowski S, Tripathi P, Bourdeau T, Acero L, Grimes HL, Katz JD, et al. Bim/Bcl-2 balance is critical for maintaining naive and memory T cell homeostasis. J Exp Med. 2007;204:1665–75.
60. Bouillet P, Cory S, Zhang LC, Strasser A, Adams JM. Degenerative disorders caused by Bcl-2 deficiency prevented by loss of its BH3-only antagonist Bim. Dev Cell. 2001;1:645–53.
61. Ceizar M, Dhaliwal J, Xi Y, Smallwood M, Kumar KL, Lagace DC. Bcl-2 is required for the survival of doublecortin-expressing immature neurons. Hippocampus. 2016;26:211–9.
62. Wang S, Sorenson CM, Sheibani N. Attenuation of retinal vascular development and neovascularization during oxygen-induced ischemic retinopathy in Bcl-$2^{-/-}$ mice. Dev Biol. 2005;279:205–19.
63. Rucker EB 3rd, Dierisseau P, Wagner KU, Garrett L, Wynshaw-Boris A, Flaws JA, et al. Bcl-x and Bax regulate mouse primordial germ cell survival and apoptosis during embryogenesis. Mol Endocrinol. 2000;14:1038–52.
64. Brinkmann K, Waring P, Glaser SP, Wimmer V, Cottle DL, Tham MS, et al. BCL-XL exerts a protective role against anemia caused by radiation-induced kidney damage. EMBO J. 2020;39:e105561.
65. Takehara T, Tatsumi T, Suzuki T, Rucker EB 3rd, Hennighausen L, Jinushi M, et al. Hepatocyte-specific disruption of Bcl-xL leads to continuous hepatocyte apoptosis and liver fibrotic responses. Gastroenterology. 2004;127:1189–97.
66. Carrington EM, McKenzie MD, Jansen E, Myers M, Fynch S, Kos C, et al. Islet beta-cells deficient in Bcl-xL develop but are abnormally sensitive to apoptotic stimuli. Diabetes. 2009;58:2316–23.
67. Staversky RJ, Vitiello PF, Yee M, Callahan LM, Dean DA, O'Reilly MA. Epithelial ablation of Bcl-XL increases sensitivity to oxygen without disrupting lung development. Am J Respir Cell Mol Biol. 2010;43:376–85.
68. Thomas RL, Roberts DJ, Kubli DA, Lee Y, Quinsay MN, Owens JB, et al. Loss of MCL-1 leads to impaired autophagy and rapid development of heart failure. Genes Dev. 2013;27:1365–77.
69. Seibler J, Zevnik B, Kuter-Luks B, Andreas S, Kern H, Hennek T, et al. Rapid generation of inducible mouse mutants. Nucleic Acids Res. 2003;31:e12.
70. Perciavalle RM, Opferman JT. Delving deeper: MCL-1's contributions to normal and cancer biology. Trends Cell Biol. 2013;23:22–9.
71. Fu NY, Sukumaran SK, Kerk SY, Yu VC. Baxbeta: a constitutively active human Bax isoform that is under tight regulatory control by the proteasomal degradation mechanism. Mol Cell. 2009;33:15–29.
72. Vick B, Weber A, Urbanik T, Maass T, Teufel A, Krammer PH, et al. Knockout of myeloid cell leukemia-1 induces liver damage and increases apoptosis susceptibility of murine hepatocytes. Hepatology. 2009;49:627–36.
73. Hikita H, Takehara T, Shimizu S, Kodama T, Li W, Miyagi T, et al. Mcl-1 and Bcl-xL cooperatively maintain integrity of hepatocytes in developing and adult murine liver. Hepatology. 2009;50:1217–26.
74. Healy ME, Boege Y, Hodder MC, Bohm F, Malehmir M, Scherr AL, et al. MCL1 is Required for Maintenance of Intestinal Homeostasis and Prevention of Carcinogenesis in Mice. Gastroenterology. 2020;159:183–99.
75. Jain R, Sheridan JM, Policheni A, Heinlein M, Gandolfo LC, Dewson G, et al. A critical epithelial survival axis regulated by MCL-1 maintains thymic function in mice. Blood. 2017;130:2504–15.
76. Fu NY, Rios AC, Pal B, Soetanto R, Lun AT, Liu K, et al. EGF-mediated induction of Mcl-1 at the switch to lactation is essential for alveolar cell survival. Nat Cell Biol. 2015;17:365–75.
77. Watson EC, Whitehead L, Adams RH, Dewson G, Coultas L. Endothelial cell survival during angiogenesis requires the pro-survival protein MCL1. Cell Death Differ. 2016;23:1371–9.
78. Print CG, Loveland KL, Gibson L, Meehan T, Stylianou A, Wreford N, et al. Apoptosis regulator bcl-w is essential for spermatogenesis but appears otherwise redundant. Proc Natl Acad Sci USA. 1998;95:12424–31.
79. Ross AJ, Waymire KG, Moss JE, Parlow AF, Skinner MK, Russell LD, et al. Testicular degeneration in Bclw-deficient mice. Nat Genet. 1998;18:251–6.
80. Schenk RL, Tuzlak S, Carrington EM, Zhan Y, Heinzel S, Teh CE, et al. Characterisation of mice lacking all functional isoforms of the pro-survival BCL-2 family member A1 reveals minor defects in the haematopoietic compartment. Cell Death Differ. 2017;24:534–45.
81. Tuzlak S, Schenk RL, Vasanthakumar A, Preston SP, Haschka MD, Zotos D, et al. The BCL-2 pro-survival protein A1 is dispensable for T cell homeostasis on viral infection. Cell Death Differ. 2017;24:523–33.
82. de Graaf CA, Choi J, Baldwin TM, Bolden JE, Fairfax KA, Robinson AJ, et al. Haemopedia: an expression atlas of murine hematopoietic cells. Stem Cell Rep. 2016;7:571–82.
83. Uhlen M, Karlsson MJ, Zhong W, Tebani A, Pou C, Mikes J, et al. A genome-wide transcriptomic analysis of protein-coding genes in human blood cells. Science. 2019;366:6472.
84. Choi J, Baldwin TM, Wong M, Bolden JE, Fairfax KA, Lucas EC, et al. Haemopedia RNA-seq: a database of gene expression during haematopoiesis in mice and humans. Nucleic Acids Res. 2019;47:D780–5.
85. Novershtern N, Subramanian A, Lawton LN, Mak RH, Haining WN, McConkey ME, et al. Densely interconnected transcriptional circuits control cell states in human hematopoiesis. Cell. 2011;144:296–309.
86. Nakayama K, Nakayama K, Negishi I, Kuida K, Shinkai Y, Louie MC, et al. Disappearance of the lymphoid system in Bcl-2 homozygous mutant chimeric mice. Science. 1993;261:1584–8.
87. Grossmann M, O'Reilly LA, Gugasyan R, Strasser A, Adams JM, Gerondakis S. The anti-apoptotic activities of Rel and RelA required during B-cell maturation involve the regulation of Bcl-2 expression. EMBO J. 2000;19:6351–60.
88. Nakayama K, Nakayama K, Negishi I, Kuida K, Sawa H, Loh DY. Targeted disruption of Bcl-2 alpha beta in mice: occurrence of gray hair, polycystic kidney disease, and lymphocytopenia. Proc Natl Acad Sci USA. 1994;91:3700–4.
89. Dunkle A, Dzhagalov I, Gordy C, He YW. Transfer of CD8+ T cell memory using Bcl-2 as a marker. J Immunol. 2013;190:940–7.
90. Kurtulus S, Tripathi P, Moreno-Fernandez ME, Sholl A, Katz JD, Grimes HL, et al. Bcl-2 allows effector and memory CD8+ T cells to tolerate higher expression of Bim. J Immunol. 2011;186:5729–37.
91. Viant C, Guia S, Hennessy RJ, Rautela J, Pham K, Bernat C, et al. Cell cycle progression dictates the requirement for BCL2 in natural killer cell survival. J Exp Med. 2017;214:491–510.
92. Debrincat MA, Pleines I, Lebois M, Lane RM, Holmes ML, Corbin J, et al. BCL-2 is dispensable for thrombopoiesis and platelet survival. Cell Death Dis. 2015;6:e1721.
93. Ma A, Pena JC, Chang B, Margosian E, Davidson L, Alt FW, et al. Bclx regulates the survival of double-positive thymocytes. Proc Natl Acad Sci USA. 1995;92:4763–7.
94. Zhang N, He YW. The antiapoptotic protein Bcl-xL is dispensable for the development of effector and memory T lymphocytes. J Immunol. 2005;174:6967–73.
95. Wagner KU, Wall RJ, St-Onge L, Gruss P, Wynshaw-Boris A, Garrett L, et al. Cre-mediated gene deletion in the mammary gland. Nucleic Acids Res. 1997;25:4323–30.
96. Choi YW, Henrard D, Lee I, Ross SR. The mouse mammary tumor virus long terminal repeat directs expression in epithelial and lymphoid cells of different tissues in transgenic mice. J Virol. 1987;61:3013–9.
97. Ross SR, Hsu CL, Choi Y, Mok E, Dudley JP. Negative regulation in correct tissue-specific expression of mouse mammary tumor virus in transgenic mice. Mol Cell Biol. 1990;10:5822–9.
98. Stewart TA, Hollingshead PG, Pitts SL. Multiple regulatory domains in the mouse mammary tumor virus long terminal repeat revealed by analysis of fusion genes in transgenic mice. Mol Cell Biol. 1988;8:473–9.
99. Wagner KU, Claudio E, Rucker EB 3rd, Riedlinger G, Broussard C, Schwartzberg PL, et al. Conditional deletion of the Bcl-x gene from erythroid cells results in hemolytic anemia and profound splenomegaly. Development. 2000;127:4949–58.
100. Mason KD, Carpinelli MR, Fletcher JI, Collinge JE, Hilton AA, Ellis S, et al. Programmed anuclear cell death delimits platelet life span. Cell. 2007;128:1173–86.
101. Josefsson EC, James C, Henley KJ, Debrincat MA, Rogers KL, Dowling MR, et al. Megakaryocytes possess a functional intrinsic apoptosis pathway that must be restrained to survive and produce platelets. J Exp Med. 2011;208:2017–31.
102. Debrincat MA, Josefsson EC, James C, Henley KJ, Ellis S, Lebois M, et al. Mcl-1 and Bcl-x(L) coordinately regulate megakaryocyte survival. Blood. 2012;119:5850–8.

103. Kodama T, Hikita H, Kawaguchi T, Shigekawa M, Shimizu S, Hayashi Y, et al. Mcl-1 and Bcl-xL regulate Bak/Bax-dependent apoptosis of the megakaryocytic lineage at multistages. Cell Death Differ. 2012;19:1856–69.
104. Brinkmann K, Grabow S, Hyland CD, Teh CE, Alexander WS, Herold MJ, et al. The combination of reduced MCL-1 and standard chemotherapeutics is tolerable in mice. Cell Death Differ. 2017;24:2032–43.
105. Opferman JT, Iwasaki H, Ong CC, Suh H, Mizuno S, Akashi K, et al. Obligate role of anti-apoptotic MCL-1 in the survival of hematopoietic stem cells. Science. 2005;307:1101–4.
106. Vikstrom I, Carotta S, Lüthje K, Peperzak V, Jost PJ, Glaser S, et al. Mcl-1 is essential for germinal center formation and B cell memory. Science. 2010;330:1095–9.
107. Sathe P, Delconte RB, Souza-Fonseca-Guimaraes F, Seillet C, Chopin M, Vandenberg CJ, et al. Innate immunodeficiency following genetic ablation of Mcl1 in natural killer cells. Nat Commun. 2014;5:4539.
108. Carrington EM, Zhang JG, Sutherland RM, Vikstrom IB, Brady JL, Soo P, et al. Prosurvival Bcl-2 family members reveal a distinct apoptotic identity between conventional and plasmacytoid dendritic cells. Proc Natl Acad Sci USA. 2015;112:4044–9.
109. Turnis ME, Kaminska E, Smith KH, Kartchner BJ, Vogel P, Laxton JD, et al. Requirement for antiapoptotic MCL-1 during early erythropoiesis. Blood. 2021;137:1945–58.
110. Dzhagalov I, St John A, He YW. The antiapoptotic protein Mcl-1 is essential for the survival of neutrophils but not macrophages. Blood. 2007;109:1620–6.
111. Steimer DA, Boyd K, Takeuchi O, Fisher JK, Zambetti GP, Opferman JT. Selective roles for antiapoptotic MCL-1 during granulocyte development and macrophage effector function. Blood. 2009;113:2805–15.
112. Gibson L, Holmgreen SP, Huang DC, Bernard O, Copeland NG, Jenkins NA, et al. bcl-w, a novel member of the bcl-2 family, promotes cell survival. Oncogene. 1996;13:665–75.
113. Adams CM, Kim AS, Mitra R, Choi JK, Gong JZ, Eischen CM. BCL-W has a fundamental role in B cell survival and lymphomagenesis. J Clin Invest. 2017;127:635–50.
114. Adams CM, Mitra R, Gong JZ, Eischen CM. Non-Hodgkin and Hodgkin lymphomas select for overexpression of BCLW. Clin Cancer Res. 2017;23:7119–29.
115. Diepstraten ST, Chang C, Tai L, Gong JN, Lan P, Dowell AC, et al. BCL-W is dispensable for the sustained survival of select Burkitt lymphoma and diffuse large B-cell lymphoma cell lines. Blood Adv. 2020;4:356–66.
116. Karsan A, Yee E, Harlan JM. Endothelial cell death induced by tumor necrosis factor-alpha is inhibited by the Bcl-2 family member, A1. J Biol Chem. 1996;271:27201–4.
117. Karsan A, Yee E, Kaushansky K, Harlan JM. Cloning of human Bcl-2 homologue: inflammatory cytokines induce human A1 in cultured endothelial cells. Blood. 1996;87:3089–96.
118. Lin EY, Orlofsky A, Berger MS, Prystowsky MB. Characterization of A1, a novel hemopoietic-specific early-response gene with sequence similarity to bcl-2. J Immunol. 1993;151:1979–88.
119. Zong WX, Edelstein LC, Chen C, Bash J, Gelinas C. The prosurvival Bcl-2 homolog Bfl-1/A1 is a direct transcriptional target of NF-kappaB that blocks TNFalpha-induced apoptosis. Genes Dev. 1999;13:382–7.
120. Gangoda L, Schenk RL, Best SA, Nedeva C, Louis C, D'Silva DB, et al. Absence of pro-survival A1 has no impact on inflammatory cell survival in vivo during acute lung inflammation and peritonitis. Cell Death Differ. 2022;29:96–104.
121. Adams JM, Cory S. The BCL-2 arbiters of apoptosis and their growing role as cancer targets. Cell Death Differ. 2018;25:27–36.
122. Roberts AW, Seymour JF, Brown JR, Wierda WG, Kipps TJ, Khaw SL, et al. Substantial susceptibility of chronic lymphocytic leukemia to BCL2 inhibition: results of a phase I study of navitoclax in patients with relapsed or refractory disease. J Clin Oncol. 2012;30:488–96.
123. Wilson WH, O'Connor OA, Czuczman MS, LaCasce LS, Gerecitano JF, Leonard JP, et al. Navitoclax, a targeted high-affinity inhibitor of BCL-2, in lymphoid malignancies: a phase 1 dose-escalation study of safety, pharmacokinetics, pharmacodynamics, and antitumour activity. Lancet Oncol. 2010;11:1149–59.
124. Ackler S, Mitten MJ, Foster K, Oleksijew A, Refici M, Tahir SK, et al. The Bcl-2 inhibitor ABT-263 enhances the response of multiple chemotherapeutic regimens in hematologic tumors in vivo. Cancer Chemother Pharm. 2010;66:869–80.
125. Oltersdorf T, Elmore SW, Shoemaker AR, Armstrong RC, Augeri DJ, Belli BA, et al. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. Nature. 2005;435:677–81.
126. Shoemaker AR, Mitten MJ, Adickes J, Ackler S, Refici M, Ferguson D, et al. Activity of the Bcl-2 family inhibitor ABT-263 in a panel of small cell lung cancer xenograft models. Clin Cancer Res. 2008;14:3268–77.
127. Tse C, Shoemaker AR, Adickes J, Anderson MG, Chen J, Jin S, et al. ABT-263: a potent and orally bioavailable Bcl-2 family inhibitor. Cancer Res. 2008;68:3421–8.
128. Koehler MF, Bergeron P, Choo EF, Lau K, Ndubaku C, Dudley D, et al. Structure-guided rescaffolding of selective antagonists of BCL-XL. ACS Med Chem Lett. 2014;5:662–7.
129. Lessene G, Czabotar PE, Sleebs BE, Zobel K, Lowes KN, Adams JM, et al. Structure-guided design of a selective BCL-X(L) inhibitor. Nat Chem Biol. 2013;9:390–7.
130. Sleebs BE, Kersten WJ, Kulasegaram S, Nikolakopoulos G, Hatzis E, Moss RM, et al. Discovery of potent and selective benzothiazole hydrazone inhibitors of Bcl-XL. J Med Chem. 2013;56:5514–40.
131. Tao ZF, Hasvold L, Wang L, Wang X, Petros AM, Park CH, et al. Discovery of a potent and selective BCL-XL inhibitor with in vivo activity. ACS Med Chem Lett. 2014;5:1088–93.
132. Khan S, Zhang X, Lv D, Zhang Q, He Y, Zhang P, et al. A selective BCL-XL PROTAC degrader achieves safe and potent antitumor activity. Nat Med. 2019;25:1938–47.
133. Zhang X, He Y, Zhang P, Budamagunta V, Lv D, Thummuri D, et al. Discovery of IAP-recruiting BCL-XL PROTACs as potent degraders across multiple cancer cell lines. Eur J Med Chem. 2020;199:112397.
134. Zhang X, Thummuri D, Liu X, Hu W, Zhang P, Khan S, et al. Discovery of PROTAC BCL-XL degraders as potent anticancer agents with low on-target platelet toxicity. Eur J Med Chem. 2020;192:112186.
135. Zhao X, Ogunwobi OO, Liu C. Survivin inhibition is critical for Bcl-2 inhibitor-induced apoptosis in hepatocellular carcinoma cells. PLoS One. 2011;6:e21980.
136. Merino D, Khaw SL, Glaser SP, Anderson DJ, Belmont LD, Wong C, et al. Bcl-2, Bcl-x(L), and Bcl-w are not equivalent targets of ABT-737 and navitoclax (ABT-263) in lymphoid and leukemic cells. Blood. 2012;119:5807–16.
137. Souers AJ, Leverson JD, Boghaert ER, Ackler SL, Catron ND, Chen J, et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. Nat Med. 2013;19:202–8.
138. Roberts AW, Davids MS, Pagel JM, Kahl BS, Puvvada SD, Gerecitano JF, et al. Targeting BCL2 with Venetoclax in Relapsed Chronic Lymphocytic Leukemia. N Engl J Med. 2016;374:311–22.
139. Davids MS, Hallek M, Wierda W, Roberts AW, Stilgenbauer S, Jones JA, et al. Comprehensive safety analysis of venetoclax monotherapy for patients with relapsed/refractory chronic lymphocytic leukemia. Clin Cancer Res. 2018;24:4371–9.
140. Davids MS, von Keudell G, Portell CA, Cohen JB, Fisher DC, Foss F, et al. Revised dose ramp-up to mitigate the risk of tumor lysis syndrome when initiating venetoclax in patients with mantle cell lymphoma. J Clin Oncol. 2018;36:3525–7.
141. Reda G, Cassin R, Dovrtelova G, Matteo C, Giannotta J, D'Incalci M, et al. Venetoclax penetrates in cerebrospinal fluid and may be effective in chronic lymphocytic leukemia with central nervous system involvement. Haematologica. 2019;104:e222–3.
142. Strasser GLKAA. Toward targeting antiapoptotic MCL-1 for cancer therapy. Annu Rev Cancer Biol. 2020;4:299–313.
143. Caenepeel S, Brown SP, Belmontes B, Moody G, Keegan KS, Chui D, et al. AMG 176, a selective MCL1 inhibitor, is effective in hematologic cancer models alone and in combination with established therapies. Cancer Disco. 2018;8:1582–97.
144. Szlavik Z, Csekei M, Paczal A, Szabo ZB, Sipos S, Radics G, et al. Discovery of 04315, a potent and selective Mcl-1 inhibitor. J Med Chem. 2020;63:13762–95.
145. Tron AE, Belmonte MA, Adam A, Aquila BM, Boise LH, Chiarparin E, et al. Discovery of Mcl-1-specific inhibitor AZD5991 and preclinical activity in multiple myeloma and acute myeloid leukemia. Nat Commun. 2018;9:5341.
146. Kelly GL, Grabow S, Glaser SP, Fitzsimmons L, Aubrey BJ, Okamoto T, et al. Targeting of MCL-1 kills MYC-driven mouse and human lymphomas even when they bear mutations in p53. Genes Dev. 2014;28:58–70.
147. Safety, tolerability, pharmacokinetics and efficacy of AMG 397 in subjects with selected relapsed or refractory hematological malignancies. *Clinicaltrials.gov.au* 2018–2019.
148. FDA places trials of MCL-1 inhibitor on clinical hold. Ash Clinical News; 2019.
149. FDA places clinical hold on trials of AZD5991 following cardiac complications. Ash Clinical News; 2021.
150. Study of AZD5991 alone or in combination with venetoclax in relapsed or refractory haematologic malignancies. *Clinicaltrialsgov* 2022.
151. Spencer A, Rosenberg AS, Jakubowiak A, Raje N, Chatterjee M, Trudel S, et al. A Phase 1, first-in-human study of AMG 176, a selective MCL-1 inhibitor, in patients with relapsed or refractory multiple myeloma. Clin Lymphoma, Myeloma Leuk. 2019;19:E53–4.
152. Bohler S, Afreen S, Fernandez-Orth J, Demmerath EM, Molnar C, Wu Y, et al. Inhibition of the anti-apoptotic protein MCL-1 severely suppresses human hematopoiesis. Haematologica. 2021;106:3136–48.
153. Weeden CE, Ah-Cann C, Holik AZ, Pasquet J, Garnier JM, Merino D, et al. Dual inhibition of BCL-XL and MCL-1 is required to induce tumour regression in lung squamous cell carcinomas sensitive to FGFR inhibition. Oncogene. 2018;37:4475–88.
154. Yamashita J, Datta NS, Chun YH, Yang D-Y, Carey AA, Kreider JM, et al. Role of Bcl2 in osteoclastogenesis and PTH anabolic actions in bone. J Bone Miner Res. 2008;23:621–32.

155. Walton KD, Wagner KU, Rucker EB 3rd, Shillingford JM, Miyoshi K, Hennighausen L. Conditional deletion of the bcl-x gene from mouse mammary epithelium results in accelerated apoptosis during involution but does not compromise cell function during lactation. Mech Dev. 2001;109:281–93.
156. Hon H, Rucker EB 3rd, Hennighausen L, Jacob J. bcl-xL is critical for dendritic cell survival in vivo. J Immunol. 2004;173:4425–32.
157. Peperzak V, Vikström I, Walker J, Glaser SP, LePage M, Coquery CM, et al. Mcl-1 is essential for the survival of plasma cells [published correction appears in Nat Immunol. 2013 Aug;14(8):877]. Nat Immunol. 2013;14:290–7.
158. Jakubowska MA, Kerkhofs M, Martines C, Efremov DG, Gerasimenko JV, Gerasimenko OV, et al. ABT-199 (Venetoclax), a BH3-mimetic Bcl-2 inhibitor, does not cause Ca^{2+} -signalling dysregulation or toxicity in pancreatic acinar cells. Br J Pharmacol. 2019;176:4402–15.
159. Robinson EJ, Aguiar SP, Kouwenhoven WM, Starmans DS, von Oerthel L, Smidt MP, van der Heide LP. Survival of midbrain dopamine neurons depends on the Bcl2 factor Mcl1. Cell Death Discov. 2018;4:107.

ACKNOWLEDGEMENTS

We thank Dr Marco Herold for discussion of the data that are reviewed here. We thank Dr Andrew Roberts for sharing his experience from clinical treatment of patients with Venetoclax and Dr David Huang for sharing his insights into the stability of BAX protein.

AUTHOR CONTRIBUTIONS

KB and AS discussed and interpreted all primary research papers mentioned in this review. KB wrote the manuscript with the help of AS. KB designed Figs. 1 and 2. CdG analysed publicly available gene expression data and designed Figs. 3 and 4. AN and KB produced Table 4 and AN described the findings about on-target toxicities of BH3 mimetic drugs in patients and pre-clinical tests in mice.

FUNDING

The work by the authors was supported by grants and fellowships from the Deutsche Krebshilfe (Dr Mildred Scheel post-doctoral fellowship to KB), the National Health and Medical Research Council (Program Grant #1113133, Fellowship 1116937, Investigator Grant #2007887, Synergy Grant #2010275; all to AS) and the Leukaemia and Lymphoma Society (SCOR Grant #7015-18 to AS).

COMPETING INTERESTS

All authors are employees of The Walter and Eliza Hall Institute (WEHI). WEHI receives royalties and milestone payments from the sale of Venetoclax. AS has received funding for his research from Servier and has served on a strategic advisory board from Servier.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41418-022-00987-0.

Correspondence and requests for materials should be addressed to Kerstin Brinkmann or Andreas Strasser.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.