Cell death pathologies: targeting death pathways and the immune system for cancer therapy

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Abstract
Alterations in the molecular mechanisms of cell death are a common feature of cancer. These alterations enable malignant cells to survive intrinsic death signalling leading to accumulation of genetic aberrations and helping them to cope with adverse conditions. Regulated cell death has historically been exclusively associated with classical apoptosis; however, increasing evidence indicates that several alternative mechanisms orchestrate multiple death pathways, such as ferroptosis, entosis, necroptosis and immunogenic cell death, each with distinct underlying molecular mechanisms. Although pharmacological targeting of cell death pathways has been the subject of intensive efforts in recent decades with a dominant focus on targeting apoptosis, the identification of these novel death pathways has opened additional venues for intervention in cancer cells and the immune system. In this mini-review, we cover some recent progress on major recently emerged cell death modalities, emphasizing their potential clinical and therapeutic implications. We also discuss the interplay between cell death and immune response, highlighting the potential of the combination of traditional anticancer therapy and immunocheckpoint blockade. While attempting to stimulate discussion and draw attention to the possible clinical impact of these more recently emerged cell death modalities, we also cover the major progress achieved in translating strategies for manipulation of apoptotic pathways into the clinic, focusing on the attempts to target the anti-apoptotic protein BCL2 and the tumour suppressor p53.

Targeting cell death in human disease
Over the past 20 years, significant efforts of the biomedical scientific community have been dedicated to the development of therapeutic strategies aimed to target cell death signalling pathways in multiple clinical scenarios in which cytoprotection, in the case of ischaemic disorders, or cellular lethality, in oncological conditions, are the desired outcomes [1, 2] (Fig. 1). This major investment has led to partial success as exemplified by patients with relapsed or refractory chronic lymphocytic leukaemia (CLL) currently receiving clinical benefit from the treatment with venetoclax, an inhibitor of the anti-apoptotic protein BCL2 [3, 4] (Fig. 2b). Given our current knowledge of the molecular mechanisms underlying apoptotic cell death, tilting the balance of this cell death modality today appears possible, and a therapeutic benefit might require a more thorough understanding of the integration of the different cell death modalities adopted or preferred in specific physiological conditions.

Necroptosis, ferroptosis and entosis have recently emerged as regulated cell death modalities that execute their death programme following different molecular pathways [5]. Defining how and whether these mechanisms exert a role in pathological conditions and whether interconnectivity of these signalling and modularity of their execution occur is crucial from a therapeutic standpoint. In this minireview, we provide an overview of the major recently emerged novel cell death modalities emphasizing, where possible, the clinical relevance and therapeutic implications of these molecular signalling pathways. In addition, we discuss the strategies currently in clinical
practise or development employed to target cell death, which are mainly confined to targeting apoptotic signalling.

**Cell death modalities**

The longest studied mechanisms of cell death is skin cornification [6, 7], which involves a p53 family member, namely, p63 [8]. In contrast to accidental cell death associated with catastrophic exposure of cells to severe physical, chemical or mechanical insults, regulated cell death is the result of the activation of defined signal transduction molecular mechanisms, implying that, in theory, such death modes can be pharmacologically or genetically manipulated. All the different modalities of regulated cell death maintain the purpose of responding to microenvironment perturbations to promote cellular and organismal homeostasis in both physiological and pathological conditions, providing obvious advantages to multicellular organisms [9] (Fig. 1).

The best studied and defined mechanism of regulated cell death is apoptosis. Apoptosis is characterized by nuclear fragmentation, chromatin condensation, cytoplasmic shrinkage and plasma membrane blebbing. This process results in the accumulation of apoptotic bodies (intact small vesicles) that are neatly cleared by phagocytic cells through a process known as efferocytosis, which is classically viewed as a non-inflammatory mode of cell disposal. At the molecular level, apoptosis is categorized into two major pathways, the extrinsic and intrinsic

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**Fig. 1** Diagrammatic representation of cell death aspects and therapeutic implications. Mild alterations of cellular homeostasis, produced by exogenous or endogenous factors, induce an adaptive response to restore homeostasis. Failure of such a response leads to the activation of the process of regulated cell death that might (or might not) involve release of danger-associated molecular pattern (DAMP) and trigger an immunoresponse, with the ultimate goal of restoring tissue homeostasis. The adaptive response, executors of the regulated cell death programme and immunoresponse represent or can potentially represent therapeutic targets. Severe homeostatic perturbations lead to accidental cell death that generally involves release of cytotoxic molecules that reiterate the cell death signalling in the tissue. Accidental cell death cannot be therapeutically targeted, but molecules released from the cells succumbing to the primary insult can represent an alternative strategy to pharmacological intervention in these conditions.

**Fig. 2** Interaction between BCL2 and its inhibitor navitoclax (ABT-263). Navitoclax is an oral form of BCL2 inhibitor that showed efficacy in BCL2-overexpressing CLL and in follicular lymphoma. Molecular docking analysis shows navitoclax interaction in the binding site of BCL2. The inhibitor is shown as a ball and stick, while BCL2 is shown in a space filling (a) and ribbon diagram (b) model.
pathways that mainly differ based on the original trigger that initiates the cascade of events. The transcriptional factor p53 and its family members p63 and p73 are major sensors of cellular stress leading to intrinsic apoptosis [10–13] (Fig. 3a). Cellular damage, such as genotoxic stress, can enable stabilization of p53 that subsequently promotes a transcriptional programme aimed to promote repair and fidelity or otherwise kill the damaged cells [14]. In addition, non-cell autonomous mechanisms have also been associated with p53 tumour suppression [15–17] and partially shared by the family members [18–24]. For many years, this mechanism was thought to be responsible for p53 tumour suppression; however, recent evidence highlighted larger complexity in the p53 tumour suppressor network [25–27]. Numerous studies have been dedicated to review different aspects of these cell death modalities, dissecting many different mechanistic details. In this section of the article, we wish to dedicate more attention to the less characterized regulated cell death modalities and discuss their therapeutic implications.
Necroptosis

Necroptosis is a form of regulated cell death generally manifested with a necrotic morphology, which is initiated by signals from the microenvironment that can be detected by specific death receptors, such as TNFR1 and FAS [28–33], or receptors for pathogen recognition, such as DAI, TLR3 and TLR4 [34–37]. Engagement of death receptors triggers the activation of the receptor-interacting protein kinase 1 (RIPK1), which autophosphorylates and recruits RIPK3 through RIP homotypic interaction motif (RHIM) domains present on both kinases [38]. Then, activation of necroptosis critically depends on the sequential activation by RIPK3 of the mixed lineage kinase domain-like pseudokinase (MLKL) (provided that CASP8 is inactive) [39, 40]. Once activated, MLKL translocates onto the plasma membrane, inducing its rupture and subsequent cell death with the release of the intracellular content including pro-inflammatory cytokines [41] and many types of disease-associated molecular patterns (DAMPs) into the microenvironment.

Necroptosis was originally associated with adaptive functions upon failing responses to stress; however, increasing evidence indicates that it also participates in developmental programmes by ensuring the elimination of potentially defective organisms and in T-cell homeostasis [42–44]. Triggering regulated necrosis emerged as a new possible antitumoural strategy given that one of the hallmarks of cancer is the blockade or evasion of apoptosis [45]. Necroptosis can bypass apoptosis resistance and consequentially kill cancer cells. Moreover, the release of DAMPs in combination with cytokines and chemokines make cells dying through this programme immunogenic and able to potentially induce antitumour immunity [46–48]. This regulated cell death modality can become especially important for the design of novel cancer treatments.

Beyond cancer, for which boosting necroptosis could be beneficial, necroptosis is associated with a variety of human diseases, including atherosclerosis, pancreatitis, inflammatory bowel disease and neurodegenerative diseases, such as amyotrophic lateral sclerosis, multiple sclerosis and Alzheimer’s [49]. In these settings of inflammatory and degenerative diseases, targeting key necroptotic players represents a promising strategy. Indeed, the small molecule necrostatin-1 (Nec-1) targets RIPK1 and dramatically inhibits TNFR1-driven necroptosis [50, 51]. Nec-1 is protective in various ischaemic and neurodegenerative diseases [52, 53].

Ferroptosis

Similar to necroptosis, ferroptosis manifests with a necrotic morphotype, but this regulated cell death programme is initiated by specific perturbations of the intracellular redox homeostasis associated with iron availability [54–57]. The major molecular mechanism leading to ferroptotic cell death is severe lipid peroxidation, and it is potentially associated with a consistent release of immunostimulatory DAMPs [58, 59]. Alterations in reduced glutathione (GSH) synthesis and restoration are the main causes associated with the initiation of ferroptosis. Inhibitors of oxidized GSH (GSSG)-GSH turn over, such as RSL3, and de novo synthesis of GSH, such as Erastin, modulate ferroptosis [55, 60, 61]. Accordingly, intracellular imbalances in glutamine and cysteine, which are required for the synthesis of intracellular GSH, also activate this iron-dependent cell death modality [62–65].

Implications for human therapy associated with ferroptosis might exhibit relevance in the glutamine addiction observed in cancer cells. For example, triple-negative breast carcinoma (TNBC) displays a severe glutamine addiction related to its ability to drive cystine uptake via cystine/glutamate antiporter system xc– [66, 67]. The antiporter xc – is a target of Erastin that indeed is thought to trigger ferroptosis by influencing cystine/cysteine intracellular balance and thus indirectly affecting the activity of the GSH-dependent enzyme glutathione peroxidase 4 (GPX4). Xc– may therefore constitute a therapeutic target in this cancer setting. Interestingly, the tumour suppressor function of p53 is partially attributed to its ability to induce ferroptosis through inhibition of the Xc– system [68, 69]; however, the role of p53 in ferroptosis is highly context dependent [70]. Beyond carcinogenesis, ferroptosis is linked to the pathological cell death associated with neurodegenerative diseases, brain haemorrhage or injury, ischaemia-reperfusion injury and kidney degeneration [56]. To successfully target ferroptosis in different clinical settings, its contribution to necroinflammation and immune cell activation must be thoroughly dissected.

Entosis

Entosis is a mechanism involving engulfment of viable cells by non-phagocyte cells of the same or a different cell type defined as homotypic or heterotypic entosis, respectively [71–73]. This form of cellular cannibalism has been observed in healthy and malignant mammalian tissues [71]. The current understanding of the underlying mechanism suggests that the internalization of entotic cells involves a process of cell invasion rather than a canonical mechanism of phagocytosis [71, 74]. Cell-in-cell invasion is promoted by the cellular junctions between the engulfing and entotic cell, involving E-cadherin (also known as cadherin 1, CDH1) and catenin alpha 1 [75]. The Rho-associated coiled-coil containing protein kinase 1 (ROCK1), ROCK2, Ras homologue family member A (RHOA) and
diaphanous-related formin 1 (DIAPH1) promote contraction of the cell cytoskeleton that results in engulfment [74, 76, 77]. Once engulfed, entotic cells are often eliminated by a BCL2/caspase-independent cell death programme that generally requires a specific autophagy-related process commonly known as LC3-associated phagocytosis (LAP) [78].

To date, three main mechanisms triggering entosis have been characterized, including matrix de-adhesion, aberrant mitosis and glucose deprivation, each corresponding to well-known cancer hallmarks (anchorage independence, deregulated proliferation and metabolic stress, respectively), suggesting that different cancer cell features can induce entotic cell killing and cannibalism [79]. Indeed, entotic cell death has been observed in several cancer types [75]. Interestingly, chemical inhibition of ROCK abrogates entosis, favouring the anchorage-independent growth of malignant cells and indicating that entosis can act as an oncosuppressor mechanism [75]. Conversely, entosis can promote tumour progression by inducing a non-genetic route to aneuploidization and polyploidization [80–82].

Considering such a dual role of entosis in cancer, potential therapeutic strategies must carefully consider when and how to act on this delicate balance towards entotic host/inner cell survival.

**Cell death as a therapeutic target: BCL2 and p53**

Targeting cell death pathways has been the subject of intensive efforts in the past decades with most studies focusing on the mechanisms regulating apoptosis [83, 84], the best characterized programme of cell demise, and particularly BCL2 and p53. Approximately 30 years after their initial discovery, the long road to the clinic culminated successfully, as mentioned above, with the recent FDA approval of the orally bioavailable and highly selective BCL2 inhibitor, venetoclax, for relapsed or refractory CLL. These results pave the way for further development of agents, such as small molecules BH3 mimetics, which displace pro-apoptotic BCL2 family proteins from the constraint of pro-survival members [85]. Consistent with the fact that tumour suppressor genes are more difficult to target with drugs, approaches able to reactivate p53 function and provide significant advances in the standard of care for patients have been more difficult to achieve. Here, we briefly review some aspects of BCL2 and p53 targeting, report current approaches using these strategies in interventional clinical trials and highlight the challenges epitomized by these two important regulators of apoptosis for the successful translation of therapeutic approaches targeting cell death.

**BCL2 inhibition**

BCL2 pro- and anti-apoptotic family member interaction establishes the apoptotic threshold regulating the life/death decisions [86]. BCL2 is overexpressed in a variety of human cancers through either chromosomal alteration or other mechanisms, such as deregulation of BCL2-targeting microRNAs [85, 87–90]. Most tumours indeed bear high levels of one or more pro-survival family member or carry mutations impairing the induction of pro-apoptotic members, such as PUMA and NOXA, which are normally activated by p53 [25, 91, 92]. Nonetheless, cancers retain the bulk of the apoptotic machinery and are therefore prone for killing induced by BCL2 homology 3 (BH3) mimetics, agents mimicking the BH3 domains of pro-apoptotic family members, which neutralize their anti-apoptotic siblings by binding their surface hydrophobic grooves. In fact, cancer cells with elevated expression of pro-survival factors such as BCL2 are prone to undergo apoptosis, indicating that their ‘addiction’ to pro-survival factors makes them more susceptible than normal cells. The first BH3 mimetic compound targeting BCL2, ABT-737 (developed by AbbVie), exhibited low solubility and oral bioavailability compared with its orally bioavailable derivative navitoclax (ABT-263) (Fig. 2). However, upon binding, BCL-XL, which regulates platelet lifespan, also caused acute thrombocytopenia, which limited their application. Nonetheless, navitoclax exhibited efficacy in BCL2-overexpressing CLL and in follicular lymphoma [93]. In both diseases, the combined use of rituximab, an antibody recognizing the CD20 antigen expressed on the majority of mature B-cells, increased response rates [94].

Further optimization of the lead compound led to venetoclax (ABT-199), the potent selective BCL2 inhibitor that exerts antitumour activity while sparing platelets. Venetoclax is currently used against some CLL forms as mentioned above [95]. Beyond CLL, venetoclax has achieved favourable responses as monotherapy in mantle cell lymphoma and to a minor extent in follicular lymphoma, myeloma, diffuse large B-cell lymphomas and acute myeloid leukaemia. In the latter, tumours bearing mutations of isocitrate dehydrogenase 1 or 2 were found to be BCL2 dependent, facilitating patient stratification [96]. Interestingly, positive results also emerged from venetoclax used in combination with anti-CD20 antibodies and/or chemotherapy in CLL and B-cell lymphomas; ibrutinib, which inhibits Bruton tyrosine kinase in CLL and mantle cell lymphomas; the proteasome inhibitor bortezomib in multiple myeloma and rituximab in relapsed CLL. Moreover, the drug development pipeline is further enriched with other selective inhibitors for the main pro-survival family members BCL2, BCL-XL and MCL1: S55746 [97] (BCL2 selective); WEHI-53978 [98] and its more potent derivatives.
Moreover, BH3 pro-BH3 mimetics are potentially effective in most cancer types on a universal apoptotic pathway downstream of p53; thus, advantages as anticancer therapeutics. In particular, they act and/or disease. Overall, BH3 mimetics offer various and other trials can be retrieved searching for specific drugs and/or disease. Nonetheless, BH3 mimetics offer various advantages as anticancer therapeutics. In particular, they act on a universal apoptotic pathway downstream of p53; thus, BH3 mimetics are potentially effective in most cancer types [3, 103, 104]. Moreover, BH3 profiling, as proposed by Letai [105], could help to identify tumours sensitive to specific BH3 mimetics, guiding their use in the clinical practise. Interestingly, BCL2 exhibits intriguing connections with autoimmune diseases, suggesting that evaluation of BH3 mimetics for a possible repurposing in autoimmune pathologies is worthy of investigation [106].

**Pharmacological targeting of p53**

Restoring the function of *TP53*, the most frequently altered gene in human cancers, has been an obvious goal for cancer therapy that has been addressed by disparate strategies [107, 108]. Alterations in p53 can result in loss of protein function, leading to development of an unstable genome, evasion of apoptosis and gain of function activities that confer a survival advantage. Despite a variety of underlying mechanisms, both processes foster cancer development and progression [25, 109]. Among the first clinical approaches to reactivate p53, a retrovirus carrying the wt *TP53* gene was directly injected into non-small cell lung cancers in 1996, successfully inducing apoptosis and tumour regression/stabilization in six of the nine treated patients [110]. However, concerns were raised about the use of retroviruses, paving the way for the use of adeno viral vectors, such as the recombinant adeno viral human *TP53* vector gendicine, which was approved in China in 2003 for the treatment of head and neck cancer in combination with radiotherapy [111]. Given the low transduction efficiency of *TP53*-expressing adeno viruses, replicating viruses have been developed and engineered to selectively replicate in *TP53*-deficient tumours, such as dl-1520 (Onyx-015). In addition, its derivative H101 in combination with che motherapy was approved in China for the treatment of late-stage refractory nasopharyngeal cancer [112]. Various phase II/III studies failed to demonstrate efficient activity of Onyx-015 mostly due to inefficient systemic delivery and limited intratumoral dissemination, setting the stage for next-generation approaches, including mesenchymal and neural stem cells as delivery vehicles [113, 114]. Recently, administration of p53 has been attempted through the scL nanocomplex (SGT-53), and results from a first-in-man phase I clinical trial demonstrated that the compound is well tolerated, exhibits anticancer activity and reaches metastatic lesions [115]. Ablation of the p53 negative regulator MDM2 leads to p53-dependent cell death [116]. Strategies to reactivate endogenous wt p53 in tumours in which it is not mutated have been attempted by targeting its MDM2 and MDMX through the use of peptides and small molecules (Fig. 3b, c). Among the first, peptides designed to mimic p53 and block the p53 binding site of MDM2 through steric hindrance have been recently optimized through ‘stapling’ via introducing non-natural amino acids into the peptide that increases affinity, half-life and cellular uptake [112, 117].

The archetype of small molecules acting as MDM2 inhibitors are the nutlin. Nutlin-3a was first developed in 2004 [118] and exhibited efficacy against various cancer types. However, both poor bioavailability and high toxicities hindered its clinical use. Nonetheless, many studies are investigating the use of nutlin and its derivative compounds in combination strategies for a variety of tumours (reviewed in refs. [114, 117] for both pre-clinical and clinical approaches). Among these new drugs, spiro indoles, such as SAR405838, and piperidinones, such as AMG-232, are being tested in clinical trials for their safety (NCT01636479 and NCT01985191 both completed) and efficacy in various solid and haematological tumours, respectively (NCT03107780; NCT03041688; NCT02110355; NCT02016729/completed; NCT01723020/completed). As resistance to MDM2 inhibition might arise from MDMX overexpression, dual MDM2/MDMX or small molecule inhibitors of MDMX have also been pursued. Currently, nine compounds are under clinical trial assessment (recently reviewed in ref. [119]). Other approaches indirectly targeting p53 are directed against p53-regulating microRNAs; p53 upstream regulators, such as agents that block p53 acetylation; p53 vaccination with a mixture of synthetic p53-derived peptides or through its
| Study description                                                                 | Condition                                                                 | Phase | Clinical trial identifier |
|----------------------------------------------------------------------------------|---------------------------------------------------------------------------|-------|---------------------------|
| **Venetoclax (ABT-199) and combination strategies in haematological malignancies and solid tumours** |                                                                           |       |                           |
| Study of venetoclax                                                              | Patients with relapsed or refractory Waldenström macroglobulinemia         | II    | NCT02677324               |
| Venetoclax in combination with standard intensive AML induction/consolidation therapy with FLAG-IDA | Newly diagnosed or relapsed/refractory AML                                | Ib/II | NCT03214562               |
| Venetoclax in combination with the mIDH1 Inhibitor ivosidenib (AG120)            | IDH1-mutated haematologic malignancies                                     | Ib/II | NCT03471260               |
| BCL2 inhibitor venetoclax (ABT-199) in combination with obinutuzumab and ibritinib | Relapsed, refractory or previously untreated CLL                           | I/I   | NCT02427451               |
| Study of ibritinib in combination with venetoclax (ABT-199)                      | Relapsed/refractory mantle cell lymphoma                                   | I/b   | NCT0241956                |
| Study of venetoclax in combination with obinutuzumab and bendamustine as front line therapy | High tumour burden follicular lymphoma                                      | II    | NCT03113422               |
| Study comparing the efficacy of venetoclax + fulvestrant vs. fulvestrant         | Patients with oestrogen receptor-positive, Her2-negative locally advanced or metastatic breast cancer who experienced recurrence or progression during or after CDK4/6 inhibitors | II    | NCT03584009               |
| Study of duvelisib and venetoclax                                                | Relapsed or refractory CLL or SLL                                          | I/II  | NCT03534323               |
| Study of venetoclax (ABT-199) in combination with liposomal vincristine         | Relapsed or refractory T-cell or B-cell ALL                                | Ib/II | NCT03504644               |
| Venetoclax in combination with ibrutinib and umbralisib (TGR-1202)              | Relapsed or refractory CLL/SLL                                              | I/II  | NCT03579051               |
| Study of the combination of venetoclax with chemotherapy as frontline therapy    | Older patients with ALL                                                     | Ib    | NCT0319901                |
| Trial evaluating combination of atezolizumab with venetoclax and obinutuzumab    | Relapsed or refractory lymphomas                                           | II    | NCT03276468               |
| Trial of venetoclax in combination with R-ICE (V+RICE) chemotherapy             | Relapsed or refractory diffuse large B-cell lymphoma                       | I     | NCT03064867               |
| Study of venetoclax in combination with dose-adjusted EPOCH-R                    | Patients with Richter syndrome                                              | II    | NCT03054896               |
| Study of venetoclax in combination with carfilzomib and dexamethasone           | Relapsed or refractory multiple myeloma                                     | II    | NCT02899052               |
| Trial of obinutuzumab in combination with venetoclax                             | Previously, untreated follicular lymphoma                                   | I     | NCT02875550               |
| Study of bortezomib and dexamethasone in combination with either venetoclax or placebo | Relapsed or refractory multiple myeloma sensitive or naïve to proteasome inhibitors | III   | NCT02755597               |
| **Navitoclax (ABT-263) and combination strategies in solid tumours**            |                                                                           |       |                           |
| MEK inhibitor trametinib in combination with navitoclax                          | KRAS or NRAS mutation-positive advanced solid tumours                      | Ib/I  | NCT02079740               |
| Navitoclax in combination with sorafenib tosylate (Nexavar)                      | Relapsed or refractory solid tumours                                        | I     | NCT02143401               |
| Study of AZD9291 in combination with navitoclax                                  | Patients with EGFR-positive previously treated advanced or metastatic non-small cell lung cancer | I/b   | NCT02520778               |
| Study of dabrafenib, trametinib and navitoclax                                    | Patients with BRAF mutant melanoma or metastatic unresectable solid tumours | I/I   | NCT01989585               |
| **Other BCL2 targeting approaches**                                               |                                                                           |       |                           |
| Dose-escalation study of the orally administered selective BCL2 inhibitor S55746 | Refractory or relapsed CLL and B-cell non-Hodgkin lymphoma                 | I     | NCT02920697               |
|                                                                              | AML or high or very high-risk myelodysplastic syndrome                      | I     | NCT02920541               |
expression in dendritic cells; and use of synthetic lethal strategies, i.e., pharmacologically forcing p53-defective cells, which have a faulty G1/S checkpoint in response to DNA damage and thus rely on the G2/M checkpoint, into a lethal G2/M transition (all approaches reviewed in ref. [112]).

Given that p53 gain of function mutants, which have been identified in 42% of cases across 12 cancer types [120–123], have such a high impact on cancer, the development of numerous strategies aimed at reactivating wild-type p53 function in mutated cancers has been stimulated [124]. Small molecules that restore p53 DNA binding include PRIMA and its derivative APR-246, CP31398, Ellipticine analogues and JNJ26854165 [112]. Interestingly, APR-246 exhibited positive results in a phase I/IIa clinical trial including patients with refractory haematological or prostate cancer [125] and is currently under investigation in six clinical trials in combination strategies for oesophageal cancer (NCT02999893), myelodysplasies (NCT03588078/ not yet recruiting and NCT03072043), melanoma (NCT03391050), and ovarian cancer (NCT03268382 and NCT02098343). Remarkably, APR-246 is also able to restore the function of p63 mutations that are associated with several human diseases [117]. Other approaches in this direction are promising, such as the use of Zn2+ chelators and others (all extensively reviewed [117, 126]). Here, a list of selected currently active interventional phase I–III clinical studies targeting p53, including some of the approaches overviewed above, is provided in Table 2. Hopefully, these studies will soon provide information on their potential as new therapeutic avenues for a variety of cancer patients.

Despite such an arsenal of compounds and strategies, reestablishing p53 function in the clinical setting has proven difficult mainly owing to the lack of efficacy, resistance development, side effects and shortfalls in defining first which subset of patients would have more likely benefited specific approaches (Table 3). In particular, many of these compounds exhibited both on-target and off-target effects, and this feature coupled with the difficulty in determining the outcome of p53-activated response further complicated the design of suitable trials. Indeed, although early studies suggested that p53 tumour suppressor function relies on its ability to induce cell cycle arrest, senescence or apoptosis, new studies challenged this paradigm. Beyond the complexity due to the type/amount of stress required to elicit a p53 response, the context dependence, the ability to interact and/or regulate hundreds of genes, the possible overlapping function of family members, and the different gain-of-function-specific mutants, p53 also regulates many autonomous and non-autonomous additional processes, including metabolism, autophagy, stem cell reprogramming, fertility, invasion metastasis and longevity. These findings indicate

| Study description | Condition | Phase | Clinical trial identifier |
|-------------------|-----------|-------|--------------------------|
| Venetoclax (ABT-199) and combination strategies in haematological malignancies and solid tumours | Dose-escalation study of the orally administered selective BCL2 inhibitor | I | NCT03387332; NCT0882111 |
| Study of the safety, pharmacokinetic and pharmacodynamic properties of intravenously administered APO-1352 highly potent BCL2 family inhibitor | Patients with relapsed or refractory diffuse large B-cell lymphoma | II | NCT02266668 |
| Venetoclax, venetoclax and rituximab in patients with relapsed or refractory chronic lymphocytic leukaemia | Dose-escalation study of the orally administered selective BCL2 inhibitor | I | NCT01325846; NCT01870640; NCT01541810; NCT01419071; NCT0188672; NCT01346685; NCT01352063; NCT0137788; NCT0139781; NCT0139371; NCT0138967; NCT0138672; NCT0138672 | II | NCT02226965 |
| Study of PNT2258 Patients with relapsed or refractory diffuse large B-cell lymphoma | Study of the safety, pharmacokinetic and pharmacodynamic properties of intravenously administered APO-1352 highly potent BCL2 family inhibitor | II | NCT02226965 |

The table reports selected studies that used BCL2 as a target for treatment retrieved from a search of clinicaltrials.gov (NIH, US National Library of Medicine). Other terms: BCL2; Status: recruiting/enrolling by invitation; Study type: interventional studies; Phase: I, II, III. We did not report studies using BCL2 as a biomarker for patient selection (NCT03132584; NCT03103971; NCT03038672). Other approaches in this direction are promising, such as the use of Zn2+ chelators and others (all extensively reviewed [117, 126]). Here, a list of selected currently active interventional phase I–III clinical studies targeting p53, including some of the approaches overviewed above, is provided in Table 2. Hopefully, these studies will soon provide information on their potential as new therapeutic avenues for a variety of cancer patients.

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| Study description                                      | Condition                                                                 | Phase | Clinical trial identifier |
|-------------------------------------------------------|---------------------------------------------------------------------------|-------|--------------------------|
| Neoadjuvant AMG-232 concurrent with preoperative radiotherapy | wt p53 Soft tissue sarcoma                                                | Ib    | NCT03217266              |
| Study of MDM2 inhibitor AMG-232                        | Newly diagnosed GBM harbouring unmethylated MGMT promoters and wt TP53 or recurrent GBM | I     | NCT03107780              |
| Study of AMG-232 in combination with decitabine       | Relapsed, refractory or newly diagnosed wt TP53 AML                       | Ib    | NCT03041688              |
| Study evaluating AMG-232 combined with trametinib and dabrafenib or trametinib | Adult patients with metastatic cutaneous melanoma                         | Ib/Ia | NCT0210355               |
| APR-246 in combination with carboplatin/PLD chemotherapy vs. carboplatin/PLD chemotherapy alone (PiSARRO) | Platinum-sensitive recurrent high-grade serous ovarian cancer with mutated p53 | Ib    | NCT02098343              |
| APR-246 in combination with PLD chemotherapy (PiSARRO-R) | Platinum-resistant high-grade serous ovarian cancer (positive for p53 nuclear expression by IHC) | II    | NCT03268382              |
| Dose-escalation study evaluating the efficacy of APR-246, in combination with standard chemotherapy (cisplatin and 5-FU) | Platinum-resistant advanced and metastatic oesophageal or gastrooesophageal junction cancers | Ib/Ii | NCT02999893              |
| Study to investigate the safety and clinical activity of APR-246 in combination with dabrafenib | BRAF V600 mutant unresectable and/or metastatic cutaneous melanoma resistant to dabrafenib/trametinib combination | I/Ii  | NCT03391050              |
| Study to evaluate the safety and efficacy of APR-246 in combination with azacitidine | TP53 mutant myeloid neoplasms                                             | Ib/Ii | NCT03072043              |
| Dose-escalation study of imidazolopyrroldinone analogue p53-MDM2 inhibitor HDM201 | Selected advanced solid and haematological wt TP53 tumours                 | I     | NCT02143635              |
| Dose-escalation study of HDM201                        | Adult patients with advanced solid and haematological wt TP53 tumours      | I     | NCT02143635              |
| Study of oral HDM201 in combination with oral LEE011   | Adult patients with liposarcoma                                           | Ib/Ii | NCT02343172              |
| Study of PKC inhibitor LXS196 antitumour activity as a single agent and in combination with HDM201 | Metastatic uveal melanoma                                                 | I     | NCT02601378              |
| Dose-escalation study of oral CGM097, a p53/MDM2-interaction inhibitor | Selected advanced solid tumours with wt p53                              | Ib/Ii | NCT01760525              |
| Dose-escalation study of oral CGM097, a p53/MDM2-interaction inhibitor | Adult patients with selected advanced solid tumours                       | I     | NCT01760525              |
| Study of the safety, pharmacokinetic and pharmacodynamic properties of orally administered APG-115 | Advanced solid tumours or lymphomas                                       | I     | NCT02935907              |
| Multiple ascending dose study of the oral MDM2 inhibitor DS-3032b | Advanced solid tumours or lymphomas                                       | I     | NCT01877382              |
| Dose-escalation study of DS-3032b                      | AML, ALL, CML in blast phase, or high-risk MDS                            | I     | NCT02319369              |
| Study of DS-3032b                                      | Relapsed and/or refractory multiple myeloma                              | I     | NCT02579824              |
| Trial of anti-PD-L1 atezolizumab with MEK1/2 inhibitor cobimetinib or MDM2 antagonist idasanutlin | Metastatic ER+ breast cancer                                              | Ib/Ii | NCT03566485              |
| Study of idasanutlin with cytarabine vs. cytarabine plus placebo | Relapsed or refractory AML                                                | III   | NCT02545283              |
| Idasanutlin in combination with ixazomib and dexamethasone | 17p Deleted, relapsed multiple myeloma                                   | I/Ii  | NCT02633059              |
| Dose-escalation study of BI 907828                    | Adult patients with wt TP53 enriched advanced solid tumours and expansion in patients with MDM2 amplified advanced solid tumours | Ia/Ib | NCT03449381              |
| Study to determine the safety and tolerability of the stapled peptide ALRN-6924 | Advanced solid tumours or lymphomas expressing wt p53                    | I/Iia | NCT02264613              |
| Study of COTI-2—orally available third-generation thiosemicarbazone and activator of mutant forms of the p53 | Advanced or recurrent gynaecologic malignancies and HNSCC                 | I     | NCT02433626              |
| Study description                                                                 | Condition                                                                 | Phase | Clinical trial identifier |
|----------------------------------------------------------------------------------|---------------------------------------------------------------------------|-------|---------------------------|
| **TP53 vaccination and gene therapy approaches**                                  |                                                                           |       |                           |
| Evaluation of Ad-p53 in combination with capecitabine (Xeloda) or anti-PD1       | Unresectable liver metastases of CRC and other solid tumours, recurrent  | I/II  | NCT02842125               |
|                                                                                  | HNSCC and primary hepatic cancers with known disease progression on        |       |                           |
|                                                                                  | standard therapy                                                          |       |                           |
| Study of Ad-p53 transduced DC vaccine in combination with 1-methyl-D-tryptophan  | Metastatic solid tumours and invasive breast cancer                        | I/II  | NCT01042535               |
| in                                                                               |                                                                           |       |                           |
| Vaccine therapy with Ad-p53-infected autologous DCs in combination with           | Women with p53-overexpressing stage III breast cancer                      | Ib/II | NCT00082641               |
| neoadjuvant or adjuvant chemotherapy and adjuvant radiotherapy                    |                                                                           |       |                           |
| Ad-p53 DCs in combination with chemotherapy with or without all trans RA         | Patients with extensive stage small cell lung cancer                       | II    | NCT00617409               |
| Study to evaluate efficacy and safety of Ad-p53 in combination with nivolumab vs.| Recurrent HNSCC                                                           | II    | NCT03544723               |
| nivolumab alone                                                                  |                                                                           |       |                           |
| Combination immunotherapy with ipilimumab and nivolumab plus a DC-based p53     | Relapsed small cell lung cancer                                            | II    | NCT03406715               |
| vaccine                                                                         |                                                                           |       |                           |
| Study of a p53/MVA vaccine in combination with pembrolizumab                      | Solid tumours (bearing TP53 mutation) that failed prior therapy            | I     | NCT02432963               |
| Study of metastatic cancer that overexpress p53 using lymphodepleting conditioning| Progressive or recurrent metastatic cancer                                 | II    | NCT00704938               |
| followed by infusion of anti-p53 TCR-gene engineered lymphocytes and DC          |                                                                           |       |                           |
| Study of a tumour-targeted IL-2 fusion protein, ALT-801, capable of binding a    | Patients with Bacillus Calmette-Guerin failure non-muscle invasive bladder | Ib/II | NCT01625260               |
| tumour associated p53 peptide presented in the context of HLA-A2                 | cancer                                                                    |       |                           |
| First-in-human clinical study with RNA-immunotherapy combination of               | Triple-negative breast cancer patients                                     | I     | NCT02316457               |
| IVAC_W_bre1_U1D and IVAC_M_u1D for individualized tumour therapy (RNA-based     |                                                                           |       |                           |
| vaccination                                                                      |                                                                           |       |                           |
| Study of SGT-53 in combination with topotecan and cyclophosphamide               | Paediatric patients with recurrent or refractory solid tumours            | I     | NCT02354547               |
| Study of SGT-53 plus temozolomide                                                | Recurrent GBM                                                             | II    | NCT02340156               |
| Study of SGT-53 plus gemcitabine/nab-paclitaxel                                  | Metastatic pancreatic cancer                                               | II    | NCT02340117               |

The table reports selected studies that used p53-based approaches retrieved from a search of clinicaltrials.gov (NIH, US National Library of Medicine). Other terms: p53 or MDM2/HDM2; Status: recruiting/active, not recruiting/enrolling by invitation; Study type: interventional studies; Phase: I, II, III. We did not report studies using p53 as a biomarker for patient selection (such as NCT03149679 ‘The p53 Colorectal Cancer Trial’; NCT02965950 ‘The p53 Breast Cancer Trial’; NCT02042989; NCT03144804; NCT03077243; NCT02734537), tumour classification/biomarker, or readout of treatment.

Ad-p53 adenovirus expressing p53, ALL acute lymphocytic leukaemia, AML acute myelogenous leukaemia, CML chronic myelogenous leukaemia, CRC colorectal carcinoma, DC dendritic cell, GBM glioblastoma multiforme, HLA-A2 major histocompatibility complex, class I, A2, HNSCC head and neck squamous cell carcinoma, MDS myelodysplastic syndrome, MGMT O-6-methylguanine-DNA methyltransferase, MVA modified vaccinia Ankara, PKC protein kinase C, PLD pegylated liposomal doxorubicin hydrochloride, RA retinoic acid, 5-FU 5-fluorouracil.
that we need to achieve p53-mediated tumour suppression without promoting ageing in the clinical setting [25, 107, 127]. Moreover, recent studies indicate that some of the tumour suppressor mechanisms of p53 might be related to its role in other programmed cell death pathways, such as necroptosis [128] and ferroptosis [55], suggesting that a careful dissection of all the aspects connected to the recently identified mechanisms of cell death is needed to establish how and in what clinical setting we can target these pathways.

**Immunogenic cell death and immunotherapy**

Immunogenic cell death refers to all the forms of regulated cell death that stimulate a T cell-dependent immune response specific for dead cell-derived antigens. Immunogenic cell death indeed requires that dying cells activate adaptive responses associated with the expression and secretion of DAMPs in the microenvironment. A number of currently employed and well-established chemotherapeutics can elicit immunogenic cell death, including anthracyclines, mitoxantrone, bleomycin, bortezomib and cyclophosphamide. Apoptosis, necroptosis and in theoretical terms also ferroptosis can stimulate the activation of the immune system. Tumour transplantation experiments in immunocompetent BALB/c mice have demonstrated that doxorubicin treatment stimulated an immune response that was abolished by the presence of the pan-caspase inhibitor z-Vad [129]. Analogous experiments have demonstrated similar immunostimulatory capacities in processes of necroptotic cell death [130].

The relevance of immunogenic cell death in clinical setting lies on the ability of this process of reactivating physiological anticancer immunity. DAMPs released by dying cancer cells favour the recruitment, activation and interaction with T lymphocytes, thus impairing the immuno-evasion that is often at the basis of tumour development and progression. The combination of immunostimulating anticancer therapy and the immunocheckpoint blockade have subsequently become crucial [131].

The table reports selected studies that used p53-based approaches retrieved from a search of clinical trials.gov (NIH, US National Library of Medicine)

| Study description | Condition | Phase | Clinical trial identifier |
|-------------------|-----------|-------|--------------------------|
| Combine TACE and autologous Tcm immunotherapy | Hepatocellular carcinoma | I | NCT03575806 |
| Autologous Tcm cells immunotherapy | Urinary bladder neoplasm | II | NCT03389438 |
| Immunotherapy (nivolumab, atezolizumab) plus radiotherapy | Metastatic renal cell carcinoma | II | NCT03115801 |
| Metastatic urothelial carcinoma | NCT00834093 |
| Epstein–Barr virus-specific immunotherapy | Nasopharyngeal carcinoma | II | NCT01818323 |
| Intra-tumoral T4 immunotherapy | Head and neck cancer | I | NCT01774578 |
| Docetaxel, gemcitabine, pemetrexed | Non-small cell lung cancer | II/III | NCT0393858 |
| Non-small cell lung cancer recurrent | NCT02491697 |
| HyperAcute®-lung immunotherapy drug | Mesothelioma, malignant | I/II | NCT03389438 |
| Anti-PD-1 antibody | NCT03012242 |
| DC-CIK immunotherapy | Breast cancer | II | NCT02843156 |
| Thermotron RF-8EX | Malignant solid tumour | I/II | NCT02843204 |
| DC-CIK immunotherapy | NCT03012242 |
| Capecitabine monotherapy | Non-small cell lung | II | NCT02843204 |
| Atezolizumab | NCT03102242 |
| Nivolumab | NCT03012242 |
| NK immunotherapy | NCT02843165 |
| Anti-PD-1/PD-L1 immunotherapy | Metastatic cancer | II | NCT02843165 |
| Radiation therapy at 9.5 Gy | | |

The table reports selected studies that used p53-based approaches retrieved from a search of clinical trials.gov (NIH, US National Library of Medicine).
tumour responses in patients with a variety of cancers. In chemotherapy-induced immunogenic cell death, secretion of DAMPs by dying cancer cells facilitates recruitment of tumour-infiltrating dendritic cells, resulting in immunogenic phagocytosis [132]. These mechanisms suggest a potential synergism between traditional chemotherapy and immune-checkpoint blockade [133].

The success of immunotherapy and its combinations relies on pre-existing levels of antitumour immune cells. Traditional and more modern anticancer approaches, such as chemotherapy, radiotherapy and oncogene-targeted therapies, might have the potential to reshape the tumour microenvironment, including the immune content, and therefore further promote responses to immune checkpoint blockade [134].

With the recent success of cancer immunotherapy, it is imperative to invest in more research on the mechanisms of immunogenic cell death to identify routes for overcoming unresponsiveness and preventing acquisition of resistance.

Concluding remarks

This recent discovery of novel modalities of regulated cell death opened an entirely new therapeutic perspective for the field. However, the clear contributions of the individual mechanisms for human disease are not clear, and a general consensus has not always been reached in the field. Two major approaches should be adopted to potentially succeed in therapeutically targeting cell death. The first approach should aim to develop strategies designed to switch cell death modalities rather than enhancing or abrogating the execution of a specific cell death programme [135–138]. A second approach should aim to develop agents that intercept DAMPs or regulate DAMP-dependent signalling pathways [139, 140].

It is reasonable to optimistically envisage the targeting of cell death as a promising approach for human cancer and generally for several human disorders. On the other hand, considerable effort has been made to develop strategies to target cell death for clinical purposes; however, it appears clear that additional studies are still required to devise the most efficient strategies.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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