Apoptosis: a relevant tool for anticancer therapy

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Apoptosis is a form of cell death that permits the removal of damaged, senescent or unwanted cells in multicellular organisms, without damage to the cellular microenvironment. Defective apoptosis represents a major causative factor in the development and progression of cancer. The majority of chemotherapeutic agents, as well as radiation, utilize the apoptotic pathway to induce cancer cell death. Resistance to standard chemotherapeutic strategies also seems to be due to alterations in the apoptotic pathway of cancer cells.

Recent knowledge on apoptosis has provided the basis for novel targeted therapies that exploit apoptosis to treat cancer. These new target include those acting in the extrinsic/intrinsic pathway, proteins that control the apoptosis machinery such as the p53 and proteosome pathway. Most of these forms of therapy are still in preclinical development because of their low specificity and susceptibility to drug resistance, but several of them have shown promising results. In particular, this review specifically aims at providing an update of certain molecular players that are already in use in order to target apoptosis (such as bortezomib) or which are still being clinically evaluated (such ONYX-015, survivin and exisulind/aptosyn) or which, following preclinical studies, might have the necessary requirements for becoming part of the anticancer drug programs (such as TRAIL/Apo2L, apoptin/VP3).

Key words: apoptosis, TRAIL/Apo2L, apoptin/VP3, ONYX015, Bortezomib, exisulind, survivin

introduction

An organism uses two main mechanisms for the elimination of cells: necrosis and apoptosis. Necrosi consists of the rupture of the plasmatic membrane and the formation of an inflammatory process that damages the cells and their surrounding tissues. Apoptosis, instead, involves a ‘cleaner’ type of death, where the chromatin is condensed, the DNA becomes fragmented and vesicles, known as ‘apoptotic bodies’, are formed. These are rapidly phagocytized by the macrophages with the result that the cell disappears without any inflammatory phenomena [1].

Apoptosis is, therefore, considered to be the most suitable method of anticancer therapy. Its main aim is, in fact, specifically to bring about tumoral cell death while limiting as far as possible cytotoxic effects on healthy tissues. This might be achieved in several ways, for example, by promoting the expression of pro-apoptotic factors while reducing the expression of anti-apoptotic factors only in the tumor cells, or else by means of the infection of viral particles that act specifically within the transformed cells.

Apoptosis is a fundamental physiological process which maintains cell homeostasis; it is a genetically determined mechanism which is, therefore, regulated by cell factors such as proliferation and differentiation [1]. This means that, like all other molecular events, programmed cell death may be compromised by mutations in genes implicated in this intricate process and, in fact, the events regulating the apoptotic pathway are very often altered in tumor cells [2].

The apoptotic pathway is triggered off by two different signals, one extrinsic, which responds mainly to extracellular stimuli, and the other intrinsic, activated by modulators within the cell itself. Although, at least at the beginning, the two pathways are apparently separate from each other, at the end they converge in a single crucial point, i.e. the conversion of procaspase into caspase, a protease whose activation is the biochemical event that has the strongest influence on the structural modifications of the apoptotic cell [3].

With regard to the extrinsic pathway, the activation of the receptors belonging to the TNF family (Fas/Apo1, TNFR1, DR3/ TRAMP/Apo3, DR4/TRAILR1/Apo2, DR5/TRAILR2 and DR6/TR7), by means of specific ligands (TNF-α, TNF-β, TRAIL, FasL, etc), bring about the recruitment of the TNFR (Fas associated death domain) and TRADD (TNFR associated DD) family members and the chain activation of the caspases 8, 3 e 7 [4].

In the intrinsic pathway, the mitochondria release a series of molecules, including cytochrome c. In cytosol, the association of cytochrome c with the adaptor protein Apaf-1 and several procaspase 9 molecules, gives rise to the formation of apoptosome, which is responsible for bringing several pro-caspase 9 molecules into close contact with one another in order to allow their self-processing. Caspase 9 is thus able to recruit and activate caspase 3 [5], which is the effector of both pathways.

The mitochondrial apoptotic pathway is negatively modulated by anti-apoptotic factors belonging to the Bcl-2
family; these stop the mitochondria from releasing cytochrome c. Furthermore, caspase activity can be blocked by the ‘inhibitor of apoptosis proteins’ (IAPs), which, in their turn, are inhibited by Smac/DIABLO or OMI/HtrA2, the regulator proteins released by the mitochondria.

The aim of this review is to focus attention on the most promising pro-apoptotic drugs involved in present-day preclinical (such as TRAIL/Apo2L, apoptin/VP3) and clinical trials (such as bortezomib ONYX-015, survivin and exisulind) for the treatment of human tumors.

‘death receptors’ and ‘death ligands’

Considerable progress has been made in the last few years with regard to the understanding of the molecular mechanisms behind programmed cell death activated by ‘death receptors’ (DR) and ‘death ligands’ (DL).

DRs are transmembrane proteins which, after binding with a DL, transmit the apoptotic signal to the interior of the cell. The DLs are also transmembrane proteins, which, after being processed by specific proteases, are then transformed into soluble cytochines, which are capable of binding their receptors trimerically. Included in this group are the members of the tumor necrosis factor (TNF) and their receptor superfamily (TNFR) which regulate several biological functions, including cell metabolism, proliferation, cytochine production and apoptosis [6–8].

TNF-α, Fasl and TRAIL/Apo2L have aroused a great deal of interest as possible candidates for anti-cancer therapy, since in the form of trimers, they are able to trigger off apoptosis in many transformed cells but not in normal cells. Taken singly, TNF and Fasl are extremely efficient but not in a large variety of tumor cells but, unfortunately, their in vivo use leads to ischemic phenomena and hemorrhagic lesions [9]. The transfection of the gene TRAIL/Apo2L, unlike TNF and Fasl, results in a very low level of toxicity both in vitro and in vivo, which has led to the hope that it may possibly be useful in the treatment of a large number of human tumors [10].

TRAIL/Apo2L

In spite of the fact that a recombinant form of human TRAIL with a polyhistidine tail kills cultured human hepatocytes [11], a more recent study has shown that the native form of TRAIL, not only is non-toxic for in vitro human hepatocytes, but that it is also well-tolerated in chimeric mice expressing human hepatocytes [12].

The receptor system for TRAIL/Apo2L includes four specific receptors, two of which, DR4 and DR5, are death receptors containing a cytoplasmatic death domain (DD) able to transduct the apoptotic signal; the other two receptors, DcR1 and DcR2, known as decoy receptors, have no intracellular DD and are therefore unable to induce the apoptotic pathway [13] (Figure 1).

The expression levels of the decoy receptors DcR1 and DcR2 appear to play an important role in the specific induction of apoptosis by TRAIL; these levels are higher in normal cells compared with tumor cells [14–16].

preclinical studies. Recent studies have demonstrated that, apart from regulation by the receptors, several other cytosolic factors are able to modulate the apoptosis induced by TRAIL, often giving rise to resistance to the action of this protein [17]. For example, cell lines presenting mutations in FADD (Fas-associated death domain), the molecular adaptor that possesses not only an interaction domain with the death receptor but also an interaction domain with caspase 8/10, prove to be completely resistant to TRAIL-dependent apoptosis [18]. Similarly, cells obtained from infant neuroblastomas, which often have a silent caspase 8 gene, are insensitive to the effects of TRAIL [19].

Apart from the mutations of genes involved in the apoptotic signal, the overexpression of anti-apoptotic proteins such as Bcl-2 and Bcl-xL may also interfere with the action of TRAIL, thus preventing the release of the pro-apoptotic molecules by the mitochondria [20].

The foregoing data suggest that TRAIL alone is not sufficient for the therapy of several forms of cancer. Nevertheless, the treatment of TRAIL-resistant cell lines with chemotherapy agents may convert them into TRAIL-responsive elements [21], although in certain cases the molecular basis of such a synergic action is not clearly understood.

TRAIL-resistant cells from renal, prostate and bladder carcinomas respond to subtoxic concentrations of several chemotherapy agents such as doxorubicin, epirubicin and cisplatin associated with TRAIL [22]. Human U2OS osteosarcoma cells which are resistant to TRAIL-induced apoptosis respond to TRAIL following treatment with doxorubicin and cisplatin, without interfering with either death receptor or decoy receptor expression [23].
Paclitaxel and TRAIL used together bring about a reduction of the tumor in vivo and induce apoptosis by means of the interaction of TNF-family death receptors, the activation of caspases and/or the release of cytochrome c from the mitochondria [24]. The sequential treatment of nude mice with chemotherapeutic drugs (paclitaxel, vincristine, vinblastine, etoposide, camptothecin and Adriamycin) followed by TRAIL induced caspase-3 activity and apoptosis in xenografted breast tumors. Complete eradication of established tumors and survival of mice were achieved without detectable toxicity [25], which leads to high hopes regarding the treatment of patients with TRAIL associated with chemotherapy agents.

Another in vivo study involving nude mice has shown that the use of TRAIL associated with chemotherapy agents not only blocks breast tumor growth, but is also able to cause a reduction in the number of lung metastases, suggesting its possible value in the treatment of metastatic tumors [11].

Clinical studies. Although no clinical studies are in progress at the present time, the results obtained until now from in vivo and in vitro studies lead to the hope that it will soon be possible to produce this new type of anticancer drug therapy for future use.

**Viral protein and viruses to target apoptosis**

In order to complete their life cycle, viruses require actively proliferative cells and for this reason has been considerable interest regarding the use of viral proteins and attenuated viruses in antitumor therapy.

**Apoptin**

Apoptin or VP3 is a virus protein of avian anemia, which induces apoptosis in a large variety of transformed cells but not in primary cells [26].

**Preclinical studies.** It must be borne in mind, however, that in normal cells, although the co-expression of apoptin with the transforming agent, a large T antigen of SV40, is transient, it leads to susceptibility to apoptin-dependent apoptosis [27].

The mechanism behind the induction of apoptosis by apoptin specifically in transformed cells is still not fully understood. The reason for the phenomenon might be the different site; whereas in tumor cells, apoptin accumulates in the nucleus, in normal cells, it is mainly found in the cytoplasm [28]. Apoptin has a particular nuclear localization sequence, between the 70 and 121 residues, which shows a greater affinity with transformed cells [26] (Figure 2).

Nuclear localization, however, is apparently not the only factor determining the apoptotic action of this protein; in several cases, in fact, the forcing of apoptin into the nucleus of normal cells does not cause apoptosis [29]. Moreover, it has also been demonstrated that the induction of apoptosis requires the phosphorylation of the apoptin threonine residue (Thr-108) [30] in spite of the fact that this event has no effect on the protein localization, since the abolition of the apoptin phosphorylation site does not lead to any significant reduction in tumor cell nuclei [30].

Recent studies report that the only requirement for the accumulation within the nucleus and the selectivity of cancer cells by apoptin is the protein expression level. Tumor cells, in fact, are often more easily transfected compared with normal cells, which leads to an accumulation of the protein within the cytosol, indispensable for its translocation to the nucleus [29].

Apoptin over-expression may bring about the death of several normal cells. The discordance of the data regarding different cell types may be due to the different techniques used for the insertion of apoptin within the cell. The main aim, therefore, is to identify the most suitable method, either by proteic transduction or the expression of inducible vectors, for an accurate assay of the cell proteins [31].

It is surprising that, in the last few years, spectroscopic studies have reported that the biologically-active form of a recombinant apoptin (recombinant MBP-apoptin) is a multimer made up of about 30–40 monomers [32]. This complex would appear to be produced by the interaction in the hydrophobic regions of the N-terminal (aa 1–69) of each monomer, which contain the nuclear export sequence (NES) (aa 33–46). If this is so, the formation of the multiproteic complex might complicate the exportation of the nucleus [31]. On the other hand, the C-terminal tip of each monomer which contains the nuclear localization sequence (NLS) and the phosphorylation site still remains available.

The molecular mechanism by which apoptin is able to kill is still not fully understood. It acts independently of the p53 status [33] and it has recently been shown that it binds to the anaphase promotor complex (APC/C) with resulting cell cycle block in G2M and p53-independent cell death. This leads to the hope that this viral protein might be useful for the treatment of those tumors which have lost their p53 and are therefore resistant to many forms of anticancer therapy [34].

It has been demonstrated that apoptin interacts with FADD (Fas-associating protein with death domain) and with Bcl110, which are both involved in apoptosis mediated by Fas and TNFR [29], although the significance of this is still unknown. On the other hand, MCF7 cells, which have neither FADD or caspase 8 functions, are just as responsive to apoptosis, indicating that in these cells at least apoptin probably acts by means of a pathway which is independent of death receptors.

Apoptin is responsible for the release by the mitochondria of cytochrome c and the protein which induces apoptosis (AIP). However, in MCF7 cells, Bcl-2 and Bcl-X, intervene in order to protect the cell from apoptosis [35]. These results seem to disagree with those obtained in the past on other cell lines, for example, in human cells, Saos-2 transfected with plasmids which codify for apoptin and Bcl-2 undergo apoptosis much more frequently than the same cells expressing apoptin on their
Bladder

Table 1. Bortezomib: preclinical studies

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Study type</th>
<th>Combination</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder</td>
<td>In vitro/in vivo</td>
<td>Gemcitabine</td>
<td>Kamat et al. [59]</td>
</tr>
<tr>
<td>Breast</td>
<td>In vitro/in vivo</td>
<td>Radiotherapy, cyclophosphamide, cisplatin</td>
<td>Teicher et al. [60]</td>
</tr>
<tr>
<td>Breast</td>
<td>In vitro/in vivo</td>
<td>Doxorubicin</td>
<td>Thornton et al. [61]</td>
</tr>
<tr>
<td>Colon</td>
<td>In vitro/in vivo</td>
<td>Irinotecan</td>
<td>Casadevall et al. [62]</td>
</tr>
<tr>
<td>Lung</td>
<td>In vivo</td>
<td>Docetaxel</td>
<td>Gumerlock et al. [63]</td>
</tr>
<tr>
<td>Ovarian</td>
<td>In vitro/in vivo</td>
<td>Docetaxel</td>
<td>Pink et al. [64]</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>In vitro/in vivo</td>
<td>Irinotecan</td>
<td>Shah et al. [65]</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>In vivo</td>
<td>Paclitaxel</td>
<td>Sclabas et al. [66]</td>
</tr>
<tr>
<td>Prostate</td>
<td>In vitro/in vivo</td>
<td>Doxorubicin, Etoposide, Gemcitabine</td>
<td>Williams et al. [67]</td>
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</table>

ONYX-015

At present, several preclinical and clinical trials involving strategies for the treatment of tumors with mutated p53 are in progress. One of these, ONYX-015, regarding the use of viruses that specifically replicate in deficient p53 cells, has aroused particular interest.

ONYX-015 is an oncolytic virus, which seems to replicate selectively in p53-defective tumor cells. It lacks the E1B-55K gene product and, therefore, fails to degrade p53 during viral replication [39].

Preclinical studies. The exact role of p53 in determining ONYX-015 selectivity remains controversial. ONYX-015, in fact, is able to replicate in several tumor cell lines retaining wild-type p53 [40]. This apoptotic response appears to be due to the activity of the viral E1A protein, which is not altered in the ONYX-015 virus and which is capable of activating the host cell p53 via p14/ARF [41]. The selectivity of ONYX-015 for tumor cells has therefore been called into question.

Recently a tumor specific replicative adenovirus vector ZD55 (E1B 55KD deleted Adv.) which is similar to ONYX-015 in targeting function but significantly different in construction has been produced and various single therapeutic genes have been into ZD55 (ZD55-Gene). In mice with xenograft tumors, the ZD55-Gene seems to produce better results [42].

Clinical studies. Different phase I and II trials, with intratumoral and peritumoral injections, have been conducted in multiple tumor types with proven safety and evidence of promising clinical activity from several indications [41, 43–45].

A phase II clinical trial reported that ONYX-015 treatment, combined with chemotherapy, was promising in 30 patients with head and neck cancers. Tumors disappeared completely in eight patients and another 19 experienced a dramatic reduction in tumor size [43].

Pilot trials in patients with refractory cancer, have shown that ONYX-015 can be administered safely in combination with CPT11, 5FU or low-dose IL-2 and is able to access malignant tissue following intravenous infusion [46].

Recently, a clinical trial (phase I and II) using ONYX-015 was completed in patients with pancreatic cancer. The phase II trial yielded beneficial results (tumor reduction or stabilization) in about 50% of the patients [47].

Proteosome inhibitors

Because of its importance in cell homeostasis, in the past proteosome was studied with a view to using it in anticancer therapy. Particular interest was focused on proteosome inhibitors, which are molecules able to trigger selective apoptosis in tumor cells [48].

Cells can only function correctly when there is a highly regulated turnover of the proteins, brought about, in eukaryotes, by the proteosome complex. The proteins to be eliminated are first ‘labeled’ by a polyubiquitine tail and then degraded [49]. Proteosome 26S is a multiproteic complex that includes a core (20S) with enzymatic activity and two regulatory complexes (19S), one at each end of the core, responsible for the recognition and for the binding of the polyubiquitine tail [50]. It has been shown that, apart from damaged or mutated proteins, about 80% of the cell proteins are proteosome targets, since they are cell cycle regulators, oncosuppressors and transcription factors [51].

There exist various molecules, both natural and synthetic, able to inhibit protein degradation through the proteosome, for example the original bacterial compound lactacystin, and...
pharmacological inhibitors such as synthetic peptidyl aldehydes. These both act by inhibiting the proteosome through the binding of the 20S catalytic core to the simil-chemotrypsin, thus imitating the substrate binding to the active site [52].

Since the proteosome possesses a large number of substrates, the inhibition of its function leads to the alteration of several pathways. There is, therefore, an increase of important cell cycle regulators, for instance of the oncopsuspressor p53, which acts as a negative transcription regulator and plays an important part in apoptosis induction following DNA damage, and also of cell cycle inhibitors such as p21 and p27, able to induct cell cycle block in G1, thus making the cell more susceptible to apoptosis.

Considerable interest has been aroused by the inhibition of NF-κB, whose constitutive expression is frequently associated with phenomena of resistance to traditional forms of anticancer therapy [53]. Proteosome inhibitors, in fact, act indirectly on NF-κB, thus stabilizing Ik-B levels and blocking its translocation to the nucleus. NF-κB is a transcription factor that activates the transcription of a whole series of molecules implicated in proliferation and in angiogenesis.

VEGF, a cytokine with angiogenic activity, is also a transcriptional target of NFκB [54]. It has recently been reported that in multiple myeloma, the non-phosphorylation of a transcriptional target of NFkB [54]. It has recently been reported that in multiple myeloma, the non-phosphorylation of NFκB was associated with a high-risk cytogenetic profile and with a shorter median survival [55].

NFκB is a transcription factor that activates the transcription of a whole series of molecules implicated in proliferation and in angiogenesis.

bortezomib

It has been seen that if the aldehyde group of the synthetic peptidyl aldehydes is replaced by boronic acid, the selectivity and affinity of these compounds towards proteosome increases [57]. This occurs with bortezomib or PS-341 or velcade, a modified dipeptyl-boronic acid that has proved to be capable of triggering an irreversible and highly selective 385 inhibition of proteosome 26 S activity (Figure 4).

Figure 3. Inhibition of the proteosome by bortezomib results in activations of JNK and stabilization of p53, Bax, Bid, p21, P27, cavelon-1, IkBα. Thus stabilization of Ik-B levels results in inhibition of NF-kB.

Figure 4. Chemical structure of the proteosome inhibitor bortezomib: pyrazylcarbonyl-Phe-Leu-boronate.
A list of additional clinical studies is given in Table 2. However, further studies of this drug are needed to establish its full spectrum of activity, the ideal regimens for various tumor types, and clinically useful prognostic indicators that predict favorable outcomes.

**targeting IAP: survivin**

Survivin is highly expressed during embryo development whereas it is more or less absent in a large number of normal differentiated tissues [78]. The only adults tissues where it is found are the thymocytes, the CD34+ bone marrow stem cells and basal colonic epithelium [78–80].

An important feature of survivin is its different expression in tumor cells compared with that of normal differentiated tissues. It is, in fact, overexpressed in a large number of human neoplasias, including those of the lungs, colon, pancreas, bladder, uterus, ovary, breast and liver. Furthermore, it is re-expressed in numerous pre-neoplastic and/or benign lesions, such as colonic polyps and breast adenomas [78]. In tumor cells it has a cytoprotective role by contrasting apoptosis, thus guaranteeing a correct progression by means of mitosis.

In several solid tumors, such as in colorectal and pancreatic cancer [81, 82], the nuclear survivin overexpression was associated with a more favorable prognosis, while its cytoplasmic overexpression proved to be a negative prognostic factor. All these factors have aroused a great deal of interest in the last few years, and have given rise to hope that it might be possible to use this drug as a diagnostic marker and as a target for anticancer therapy.

In order to have an effect on the survivin pathway in tumoral cells, a complete understanding of its mechanism of action in normal cells is required. It is now well-known that survivin induces apoptosis by inhibiting, both directly and indirectly, the activity of caspases 3, 7 and 9 [83] and that a fundamental event for survivin regulation is the phosphorylation of the Thr34 residue (Thr34) by the cyclic-dependent p34cdc2 chinase [84].

Several methods have been proposed for the reduction of survivin levels in tumor cells, in order to increase their response to agents inducing apoptosis. One of these proposes the use of survivin-antagonist molecules such as antisense oligonucleotides [85] and small interfering RNA (siRNA) [86] and another is the use of negative dominant mutants [87] and cyclin-dependent chinase inhibitors [88].

**preclinical studies**

The results obtained from the various in vivo and in vitro studies have shown that survivin inhibition not only increases the efficiency of traditional chemotherapy drugs, but that it is also able to reduce tumoral angiogenesis [89].

**clinical studies**

Clinical trials are in progress at present on the use of antisense oligonucleotides of survivin [90].

**synthetic activation of caspases: exisulind**

Exisulind (sulindac sulfone, FGN-1, Aptsosyn) is a metabolic product of sulindac, a non-steroid, anti-inflammatory drug, belonging to a new group of pro-apoptotic compounds known as selective apoptotic antineoplastic drugs (SAANDS). The pro-apoptotic effects of exisulind differ from the other sulindac derivatives, OSI-461 (formerly CP461) and OSIP486 821 (formerly CP248) in that they do not affect microtubule polymerization [91, 92].

Exisulid is a specific activator of programmed cell death in cancerous and pre-cancerous cells but not of normal cells. It works by means of a pathway which is independent from p53 and Bcl2 [93]. It is able to induce apoptosis through the inhibition of cyclic guanosine monophosphate (cGMP) phosphodiesterases 2 and 5. This inhibition gives rise to an increase in cGMP levels with the resulting activation of PKG (c-GMP-dependent protein kinase G). PKG activation promotes the degradation by the proteosome of β-catenine and the activation of JNK, leading to caspase activation and thus to apoptosis [94].

**Table 2. Bortezomib: clinical studies**

<table>
<thead>
<tr>
<th>Solid tumor</th>
<th>Treatment</th>
<th>Phase</th>
<th>Results</th>
<th>Investigator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advanced solid tumors</td>
<td>Bortezomib and docetaxel</td>
<td>I</td>
<td>MTD: docetaxel 25 mg/m² days 1,8 plus bortezomib 0.8 mg/m² days 2, 5, 9, and 12 given every 21days</td>
<td>Meserssmith [72]</td>
</tr>
<tr>
<td>Advanced solid tumors and lymphomas</td>
<td>Bortezomib</td>
<td>I</td>
<td>Well tolerated at doses not exceeding 3.0 mg on day 1 and day 4 every other week</td>
<td>Hampton [73]</td>
</tr>
<tr>
<td>Pediatric refractory advanced solid tumors</td>
<td>Bortezomib</td>
<td>I</td>
<td>Recommended phase II dose: 1.2 mg/m²/dose twice weekly for 2 weeks followed by a 1-week break</td>
<td>Blaney [74]</td>
</tr>
<tr>
<td>Metastatic neuroendocrine tumors</td>
<td>Bortezomib</td>
<td>II</td>
<td>Single-agent bortezomib did not induce any objective responses</td>
<td>Shah [75]</td>
</tr>
<tr>
<td>Androgen-independent prostate cancer</td>
<td>Bortezomib</td>
<td>I</td>
<td>Antitumor activity was seen at tolerated doses of bortezomib</td>
<td>Papandreou [76]</td>
</tr>
<tr>
<td>Advanced solid tumors</td>
<td>Bortezomib</td>
<td>I</td>
<td>Dose-limiting toxicities on this schedule were diarrhea and sensory neurotoxicity</td>
<td>Aghajanian [78]</td>
</tr>
</tbody>
</table>
preclinical studies
In the murine model it has been demonstrated that exisulind, like sulindac, is able to inhibit the growth of several tumor cells, for example, of the colon, prostate, bladder and breast [95], prostate [96] and lung [97]. Unlike sulindac, however, exisulind does not inhibit Cox-1 and Cox-2 activity.

Pre-clinical in vitro studies on prostate and lung tumor cells have reported synergic effects when exisulind is used together with docetaxel or paclitaxel [98], probably because both drugs lead to JNK activation and to the promotion of apoptosis.

clinical studies
Several clinical studies, either already concluded or still in progress, have shown that exisulind, because of its tolerability and activity, could be used for the treatment of solid tumors such as for prostate tumors [99].

A recent phase I study has determined the maximal tolerated dose (MTD) of the combination of weekly docetaxel and exisulind in patients with advanced solid tumors. [100]. However, although preclinical data demonstrate increased apoptosis and prolonged survival for the combination of exisulind and docetaxel, multiple clinical trials do not support further clinical development of this combination regimen in patients with advanced NSCLC [101]. Furthermore, in sporadic colon adenomas, exisulind causes significant regression of sporadic adenomatous polyps but is associated with toxicity [102].

conclusions
The last decade has seen an extraordinary increase in our understanding of the complexities of apoptosis and the mechanisms evolved by tumor cells to resist engagement of cell death. The activation of alternative pathways by proapoptotic approaches such as death receptors (e.g. TRAIL) or the introduction of exogenous proapoptotic molecules such as apoptosis inhibitors are capable of inducing apoptosis even in a genetically altered context. Although at present there are still many components of the apoptotic pathways that are still not fully understood, the information collected so far has led to a better knowledge of the mechanisms of resistance to standard chemotherapeutic and radio-therapy, as well as possible strategies aimed at restoring apoptotic sensitivity. Furthermore, the genetic features of each individual tumor and apoptotic response will make it possible to choose a more suitable therapeutic approach with the aim of overcoming treatment resistance and limiting cytotoxic effects in normal tissues.

Based on the present knowledge, the use of these “biological drugs” in synergistic association with the traditional cytotoxic drugs, might represent an important goal in the treatment of malignant cells.

references


89. Nicholson DW. From bench to clinic with apoptosis-based therapeutic agents.

88. Sausville EA. Complexities in the development of cyclin-dependent kinase


86. Kappler M, Bache M, Bartel F et al. Knockdown of survivin expression by small


84. O'Connor DS, Wall NR, Porter ACG, Altieri DC. A p34cdc2 survival checkpoint in

83. Deveraux QL, Reed JC. IAP family proteins-suppressors of apoptosis. Gen

82. Tonini G, Vincenzi B, Santini D et al. Nuclear and cytoplasmic expression of


80. Gianani R, Jarboe E, Orlicky D et al. Expression of survivin in normal,

79. Carter BZ, Milella M, Altieri DC, Andreeff M. Cytokine-regulated expression of

78. Ambrosini G, Adida C, Altieri DC. A novel anti-apoptosis gene, survivin, expressed

77. Aghajanian C, Soignet S, Dizon DS et al. A phase I trial of the novel proteasome

76. Papandreou CN, Dallan IC, Nix D et al. Phase I trial of the proteasome

75. Shah MH, Young D, Kindler HL et al. Phase II study of the proteasome inhibitor

74. Tirosh S, Shtivelman E, Barbash V et al. Survival factor expression in established

73. Raffeld M, Bander NH, Greenson JK et al. Prognostic significance of survivin

72. Martin D, Paulmier G, Tsidemiakos G et al. Expression of survivin in formalin-

71. Droz D, Fraitag S, Probst F et al. New insights into the biology and potential

70. Connecticut Radiation Oncology Group. The randomized trial of tamoxifen,


64. Sausville EA, Birt DF, Begg C et al. The clinical relevance of survivin expression

63. Goldstein LJ, Hairala K, Pajusalu A et al. Survivin expression is associated with

62. Zaffaroni N, Pennati M, Daidone MG. Survivin as a target for new anticancer

61. Zaffaroni N, Pennati M, Daidone MG. New developments in clinical use of


58. Rosselli S, Zaffaroni N, Pennati M et al. Survival implication of survivin

57. Azzoni E, Zaffaroni N, Pennati M et al. Survivin expression in elderly patients

56. Frieri MN, Schelbert ER, Bazan V et al. Prognostic value of survivin expression


54. Zaffaroni N, Pennati M, Daidone MG. Prognostic significance of survivin


52. Zaffaroni N, Pennati M, Daidone MG. Survivin: a novel marker of tumor

51. Zaffaroni N, Pennati M, Daidone MG. Prognostic significance of survivin

50. Zaffaroni N, Pennati M, Daidone MG. Role of survivin as a potential marker

49. Zaffaroni N, Pennati M, Daidone MG. Survivin expression in squamous cell

48. Zaffaroni N, Pennati M, Daidone MG. Prognostic relevance of survivin

47. Zaffaroni N, Pennati M, Daidone MG. Elevated expression of survivin is a

46. Zaffaroni N, Pennati M, Daidone MG. Prognostic significance of survivin

45. Zaffaroni N, Pennati M, Daidone MG. Prognostic significance of survivin

44. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin

43. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin

42. Zaffaroni N, Pennati M, Daidone MG. Prognostic significance of survivin

41. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin

40. Zaffaroni N, Pennati M, Daidone MG. Prognostic significance of survivin

39. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin

38. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin

37. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin

36. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin

35. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin

34. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin

33. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin

32. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin

31. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin

30. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin

29. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin

28. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin

27. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin


25. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin

24. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin

23. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin

22. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin


20. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin


18. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin

17. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin

16. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin

15. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin


13. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin

12. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin

11. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin

10. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin


8. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin

7. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin

6. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin

5. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin

4. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin

3. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin

2. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin

1. Zaffaroni N, Pennati M, Daidone MG. Prognostic value of survivin