Cancer stem cells and epithelial-to-mesenchymal transition in lung cancer

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Chapter 7

Targeting apoptosis pathways in lung cancer


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Abstract

Lung cancer is a devastating disease with a poor prognosis. Non-small cell lung cancer (NSCLC) and small-cell lung cancer (SCLC) represent different forms of lung cancer that are associated with distinct genetic causes and display different responses to therapy in the clinic. Whereas SCLC is often sensitive to chemotherapy at start of treatment NSCLC are less chemo-sensitive. In NSCLC different histological subtypes are distinguished and increasing efforts are made to identify subtypes that respond to specific therapies, such as those harbouring epidermal growth factor receptor (EGFR) mutations that have benefit from treatment with EGFR inhibitors. Targeting of the apoptotic machinery represents another approach that aims to selectively kill cancer cells while sparing normal ones. Here we describe different ways that are currently explored to induce apoptosis in lung cancer cells, specifically pathways controlled by TNF-related apoptosis-inducing ligand (TRAIL), BCL-2 family members and apoptosis-inhibitory proteins (IAPs). Preclinical studies are discussed and for some agents results from early clinical studies and future perspectives are considered.
## List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ALK</td>
<td>Anaplastic Lymphoma Kinase</td>
</tr>
<tr>
<td>BAX</td>
<td>B-cell lymphoma associated X protein</td>
</tr>
<tr>
<td>BAK</td>
<td>B-cell lymphoma antagonist/killer 1</td>
</tr>
<tr>
<td>BCL-2</td>
<td>B-cell lymphoma 2</td>
</tr>
<tr>
<td>BCL-XL</td>
<td>B-cell lymphoma-extra large</td>
</tr>
<tr>
<td>BH3</td>
<td>B-cell lymphoma-2 homology 3</td>
</tr>
<tr>
<td>BID</td>
<td>BCL-2 interacting domain</td>
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<tr>
<td>BIM</td>
<td>B-cell lymphoma 2 interacting mediator protein</td>
</tr>
<tr>
<td>BIR</td>
<td>Baculovirus Apoptosis Inhibitory Protein Repeat</td>
</tr>
<tr>
<td>BRAF</td>
<td>B-Raf proto-oncogene serine/threonine-protein kinase</td>
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<tr>
<td>cFLIP</td>
<td>Cellular flle-like inhibitory protein</td>
</tr>
<tr>
<td>DcR</td>
<td>Decoy Receptor</td>
</tr>
<tr>
<td>DISC</td>
<td>Death Inducing Signaling Complex</td>
</tr>
<tr>
<td>DLT</td>
<td>Dose Limiting Toxicity</td>
</tr>
<tr>
<td>DR</td>
<td>Death Receptor</td>
</tr>
<tr>
<td>EGFR</td>
<td>Epidermal Growth Factor Receptor</td>
</tr>
<tr>
<td>EML</td>
<td>Echinoderm Microtubule-associated like protein</td>
</tr>
<tr>
<td>ERBB2</td>
<td>Erythroblastic Leukemia Viral Oncogene homolog 2</td>
</tr>
<tr>
<td>ERK</td>
<td>Extracellular signal Regulated Kinase</td>
</tr>
<tr>
<td>FADD</td>
<td>Fas-associated protein with Death Domain</td>
</tr>
<tr>
<td>HDAC</td>
<td>Histone Deacetylases</td>
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<tr>
<td>IAP</td>
<td>Apoptosis Inhibitory Protein</td>
</tr>
<tr>
<td>KRAS</td>
<td>Kirsten rat sarcoma viral oncogene</td>
</tr>
<tr>
<td>MCL-1</td>
<td>myeloid cell leukemia factor 1</td>
</tr>
<tr>
<td>MEK</td>
<td>Mitogen activated ERK activating Kinase</td>
</tr>
<tr>
<td>MET</td>
<td>Mesenchymal epithelial transition factor</td>
</tr>
<tr>
<td>MOMP</td>
<td>Mitochondrial Outer Membrane Permeabilization</td>
</tr>
<tr>
<td>NSCLC</td>
<td>Non small cell Lung Cancer</td>
</tr>
<tr>
<td>OPG</td>
<td>Osteoprotegerin</td>
</tr>
<tr>
<td>PARK2</td>
<td>Parkinson disease 2</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>Phosphoinositide-3-kinase, catalytic, alpha polypeptide</td>
</tr>
<tr>
<td>PUMA</td>
<td>p53 Upregulated Modulator of Apoptosis</td>
</tr>
<tr>
<td>rhTRAIL</td>
<td>Recombinant human soluble TRAIL</td>
</tr>
<tr>
<td>SCLC</td>
<td>Small cell Lung Cancer</td>
</tr>
<tr>
<td>SMAC</td>
<td>Second Mitochondria-derived Activator of Caspases</td>
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<tr>
<td>STK11</td>
<td>Serine/threonine kinase 11</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor Necrosis Factor</td>
</tr>
<tr>
<td>TRAIL</td>
<td>TNF Related Apoptosis-inducing Ligand</td>
</tr>
<tr>
<td>TRAIL-R</td>
<td>TRAIL Receptor</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular Endothelial Growth Factor</td>
</tr>
<tr>
<td>XIAP</td>
<td>X-linked Apoptosis Inhibitory Protein</td>
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</table>
Chapter 7

1. Lung Cancer

Lung cancer is the leading cause of cancer related deaths worldwide. It has been estimated that more than 1 million people die with it annually and approximately 1.4 million are diagnosed per year, 12% of which are new cases [1]. Lung cancers are divided into small cell lung cancer (SCLC), which comprises of 15-20% of total lung cancer cases and remaining 80-85% are attributed to the non-small cell lung cancers (NSCLC). On the basis of histological characteristics NSCLC is further divided into adenocarcinoma, squamous cell carcinoma and large cell lung cancer [2]. Smoking is the main causative agent in all types of lung cancers; it is strongest associated with squamous cell carcinoma and SCLC. The main histology in never smokers is adenocarcinoma. Although several attempts have been made to develop effective treatment strategies to combat lung cancer, still overall 5 years prognosis is less than 15% in NSCLC and for SCLC it is even lower [3]. SCLC’s are neuroendocrine tumors and they differ from NSCLC in several aspects like biology, prognosis and response to therapy. SCLC is one of the most aggressive tumor types in man. At presentation patients most often have metastasized disease. SCLC’s are initially sensitive to the chemotherapy, but even in limited disease with concurrent chemoradiotherapy and prophylactic cranial irradiation to prevent brain metastasis the 5- year survival is less than 15%. In most cases of lung cancer no symptoms or only a few are reported, and therefore most patients with lung cancer have advanced disease on diagnosis. The median survival in this group without therapy is 4 month [4].

New therapies are greatly needed for improving lung cancer treatment. Genetic analyses of NSCLC has identified both genetic and somatic mutations in EGFR and p53 genes, and somatic mutations in KRAS, BRAF, ERBB2, MET, STK11, PIK3CA and PARK2 genes. These mutations in genes have led to new strategies, aiming at these targets, such as for example mutated version of EGFR [5,6]. In this review we describe the progress made in the field of apoptosis targeted therapy for lung cancer.

2. Apoptotic cell death

Apoptosis or programmed cell death is a physiological process that provides an effective, non inflammatory way to remove redundant or damaged cells from tissues thereby securing tissue homeostasis [7]. Inhibition of apoptosis is considered as an essential step in tumourigenesis and is one of the hallmarks of cancer, allowing the survival of cells that accumulate oncogenic events that otherwise would have been
removed by apoptosis [8]. A multitude of signals activated by variable triggers, such as growth factors, cell-cell interactions, changing nutrient conditions, hypoxic conditions, and cytotoxic damage affect the status of the apoptotic machinery [7]. The caspases, specific cysteine proteases, are instrumental in the initiation and execution of apoptosis. Two main caspase activation pathways have been identified: the intrinsic or mitochondrial pathway and the extrinsic or death receptor pathway.

The intrinsic pathway is triggered upon disruption of mitochondria, for example as a result of DNA damage inflicted by cytotoxic agents, resulting in the release of cytochrome c into the cytoplasm [9]. Cytochrome c and dATP are required for the assembly of the apoptosome consisting of Apaf-1 and procaspase-9 and the subsequent cleavage and activation of caspase-9. Mitochondrial disruption is regulated by the BCL-2 family proteins [10,11], comprising of antiapoptotic members, such as BCL-2, BCL-XL, and MCL-1, and proapoptotic members, such as BAX and BAK. Together with BH3-only proteins, including BID, PUMA, and NOXA, which appear to be sensors for particular types of stress, interactions amongst the BCL-2 family members determine whether apoptotic thresholds are exceeded [12]. BAX and BAK then translocate from the cytoplasm to the mitochondrial membrane where they make pore-like structures resulting in mitochondrial outer membrane permeabilization (MOMP) and subsequently the release of cytochrome c, second mitochondria-derived activator of caspases (SMAC) and caspase activation.

The extrinsic or death receptor pathway is triggered via specific cell membrane receptors, such as Fas/CD95 TRAIL receptors, which after ligand binding can recruit FADD (Fas-associated protein with death domain) and procaspase-8 causing caspase-8 activation in a complex named the death-inducing signaling complex (DISC) [13]. Active initiator caspases-8 and -9 on their turn cleave and activate the effector caspases-3, -6, and -7 that result in the proteolytic disassembly of cells. Cellular flice-like inhibitory protein (cFLIP), a non-functional procaspase-8 homologue, can compete with procaspase 8 for FADD binding leading to suppression of apoptosis. The full activation of extrinsic apoptosis often requires the cross activation of intrinsic apoptosis that is mediated by caspase-8-dependent cleavage of BID and subsequent mitochondrial disruption [14]. The inhibitor of apoptosis protein (IAPs) family comprises proteins that can bind and inactivate caspases via one or more baculovirus IAP repeat (BIR) domains [15]. For example, X-linked IAP (XIAP) is known to inhibit caspases-3 and -9 and its antiapoptotic activity is neutralized by the release of SMAC following MOMP [16].
3. Apoptosis as target for therapy

Standard cancer therapies such as chemotherapy and radiation eradicate tumor cells at least in part by indirectly activating the apoptotic machinery [17]. In this process the tumor suppressor p53 is an important effector that predominantly induces transcription-dependent mechanisms of apoptosis by upregulating proapoptotic genes such as PUMA, BAX and death receptors and subsequent activation of intrinsic and/or extrinsic apoptosis [18]. It is observed that around 50% of all types of lung cancers are associated with mutated p53 [19]. In NSCLC and SCLC defects in the p53 gene are observed generally caused by complete loss of one allele and point mutation in the other allele. Interestingly, G → T transversion is frequently observed that have been associated with the mutagenic activity of polycyclic aromatic hydrocarbons from cigarette smoke [19]. Non-functional p53 leads to less efficient apoptosis activation by conventional treatments. Therefore, the possibility to develop agents that directly target apoptotic mechanisms has generated a lot of excitement and could lead to more effective therapies with less toxic side effects. Currently, different apoptosis targeted therapies are being evaluated in preclinical and clinical studies in different tumor types. One may distinguish proapoptotic and apoptosis sensitizing strategies. Proapoptotic approaches aim to selectively trigger apoptosis in tumor cells such as for example by targeting tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) receptors. Sensitizing strategies, for example by neutralizing antiapoptotic proteins like the inhibitor of apoptosis protein (IAPs) or BCL-2, lower the threshold for apoptosis that in some cases is sufficient to cause apoptosis but mostly sensitize tumor cells for eradication by other apoptotic triggers such as chemo- and radiotherapy. Below we describe the current status on the use of apoptosis modulating approaches in lung cancer. First, examples of different apoptosis targeted strategies in laboratory models are presented, followed by an overview of completed and ongoing clinical trials.

4. TRAIL receptor targeted therapy

Targeting of the TRAIL receptor signaling pathway is an attractive approach since it provides a direct apoptosis trigger that selectively kills tumor cells without harming normal cells [20]. Two membrane receptors mediate apoptosis, designated TRAIL-R1 (DR4) and TRAIL-R2 (DR5), whereas TRAIL-R3 (DcR1), -R4 (DcR2) and circulating osteoprotegerin (OPG) are nonfunctional decoy receptors that are able to sequester TRAIL causing suppression of apoptosis [21]. The mechanisms underlying the selective sensitivity of tumor cells to TRAIL are not completely
understood, but may be related to high TRAIL receptor expression levels in tumor cells, increased expression of decoy TRAIL receptors in normal cells, and likely also involve oncogene-dependent activation of the TRAIL apoptotic pathway in tumor cells [22]. Moreover, TRAIL-induced apoptosis is independent of p53 gene status implicating efficacy in tumors with nonfunctional p53. A number of TRAIL receptor targeting agents have been developed, including preparations of recombinant human soluble TRAIL (rhTRAIL) and derived variants, and agonistic monoclonal antibodies [23]. An advantage of agonistic monoclonal antibodies is their long half-life (18-21 days) compared with rhTRAIL (20-30 minutes). In addition, high affinity and selective binding of either TRAIL-R1 or TRAIL-R2 by monoclonal antibodies and the reduced sequestering by decoy receptors may lead to increased antitumor effects.

SCLC appears not to be a suitable tumor type for TRAIL-based therapy because it frequently lacks CASP8 expression amongst other DISC compounds in contrast to NSCLC [24]. DNA methylation of genes, including CASP8, appears to be a major cause of its absence [25] and the demethylation agent 5-aza-2-deoxycytidine together with INFg was shown to partially revert TRAIL resistance [26]. Moreover, TRAIL exposure was found to even promote cell survival and proliferation in SCLC cells involving TRAIL-R2-dependent ERK1/2 activation [27]. Combined treatment with IFN-g could alter the methylation status of CASP8 and restore its expression resulting in partially restored caspase activation. An alternative approach for altering the epigenetic status of tumor cells in order to sensitize for TRAIL is provided by histone deacetylases (HDAC) inhibitors. HDACIs are known to de-repress apoptotic pathways for example by elevating death receptor levels, stimulation of DISC formation and down-regulation of apoptosis inhibitors [28]. In NSCLC cells both the HDACIs valproic acid and trichostatin A could increase TRAIL induced caspase activation [29, 30]. HDACI activity in SCLC remains to be examined. Recently, in a number of TRAIL-resistant SCLC cell lines that do express caspase-8 apoptosis activation could be established by combined treatment with doxorubicin or etoposide. This was associated with elevated TRAIL-R2 expression and decreased cFLIP and it was suggested that in a subset of caspase-8 expressing SCLC tumors such a treatment may have benefit [31].

In contrary, NSCLC appears to be an interesting tumor type for TRAIL therapy for the following reasons: i. the p53 pathway is inactive in a large portion of NSCLC patients as mentioned earlier; ii. DNA damage-induced mitochondrial-dependent
caspase-9 activation may be disrupted in NSCLC [32] whereas the caspase-8-BID-mitochondrial pathway is intact [33]; iii. Both TRAIL-R1 and –R2 are expressed in the large majority of tested NSCLC samples [34]. In preclinical NSCLC models the efficacy of TRAIL based therapy alone appeared to be limited. The largest proportion of NSCLC cell lines show moderate sensitivity or high resistance towards different TRAIL receptor targeting agents that could be enhanced or restored by combined treatment with various standard or experimental agents, which was extensively reviewed recently [35]. Intrinsic TRAIL resistance in NSCLC involves blocks at different levels in the pathway, such as high levels of decoy receptors, limitations in DISC formation due to cFLIP- or PED-mediated inhibition, and high expression of anti-apoptotic BCL-2 proteins. Combined therapy with standard chemo- and radio therapy can overcome these blocks generally involving p53-dependent or independent effects, including the upregulation of TRAIL receptors and enhancement of mitochondria dependent apoptosis by affecting BCL-2 family protein expression [35]. Furthermore, the availability of other targeted agents allows the rationalized testing of combinations. For example, the apoptosis suppressing activity of NF-kB by transcriptional activation of cFLIP, BCL-XL and IAP proteins can be blocked by preventing the proteasomal degradation of IkB and may therefore potentiate TRAIL-induced apoptosis. Indeed, the proteasome inhibitor bortezomib enhanced TRAIL-induced apoptosis in NSCLC cells, although inhibition of NF-kB appeared partially instrumental for sensitization and upregulation of TRAIL receptors and proapoptotic BCL-2 proteins were also involved [36,37].

5. BCL-2 targeted therapy
BCL-2 is frequently overexpressed in tumors and in lung cancer a meta-analysis has reported 76% and 35% in SCLC and NSCLC, respectively, of patients samples positive for BCL-2 [38]. Overexpression of antiapoptotic BCL-2 family members is known to cause apoptosis and therapy resistance in a wide range of tumors and the development of therapies that target these apoptosis modulators appears to be a promising approach [39,40]. Two main strategies to target BCL-2 and related family members are currently exploited, in which one aims to inhibit the expression of these proteins and the other to interfere with BH-3 domain-mediated protein interactions. Based on initial observations that anti-sense oligonucleotides that inhibit BCL-2 and BCL-XL expression could induce apoptosis in lung cancer cell lines [41,42,43], an 18-mer phosphorothioate antisense oligonucleotide directed against BCL-2 (Genasense, Oblimersen, G3139, Genta Inc.Berkeley Heights, NJ) has been explored as a therapeutic approach in NSCLC and SCLC. In preclinical
models, in particular SCLC cells appeared to be sensitive for down-regulation of BCL-2 [41], although combined use with the chemotherapeutic agents etoposide, doxorubicin or cisplatin displayed strong synergistic activity [44]. In cell culture models also NSCLC cells showed sensitivity for antisense mediated BCL-2 inhibition [42,43], and in xenograft mouse models the combined use with the antimitotic drug vinorelbine further enhanced antitumor activity [45].

Gossypol, one of the first known BH3 mimetics, is a natural polyphenol derived from cotton plants that was found to bind the BH3 pocket of BCL-2, BCL-XL and MLC-1 to cause inhibition of their activities [46]. Molecular modeling and structure-based analyses has led to the development of an improved variant, named apogossypol, which also inhibits BLF-1 [47]. Further improvements have been recently reported leading to derivatives with EC50 concentrations in the nanomolar range in NSCLC cells [48]. Also a racemic mixture of gossypol has been produced, named AT-101 (Ascenta Therapeutics, Michigan), which has demonstrated potent anti-tumor activity in B-cell lymphoma when combined with chemotherapy [49]. Clinical studies have been rapidly initiated with AT-101 in lung cancer patients (see below) and only recently it was tested in preclinical studies causing apoptosis activation and sensitization for radiotherapy in NSCLC cell lines [50].

Non-peptide small molecule inhibitors of BCL-2 and BCL-XL have been developed based on the resolved structure of BCL-2 and BCL-XL/ BAK peptide complexes [51] and these BH3 mimetics are showing promising anti-tumor effects in lung cancer cells. ABT-737 (Abbott Oncology, IL, USA), a high affinity inhibitor of BCL-2, BCL-XL and BCL-W, displayed potent antitumor activity as single agent in SCLC cell culture and mice models and also enhanced chemotherapy-induced cell death in other tumor types including NSCLC [52]. In a SCLC xenograft mice model ABT-737 demonstrated potent single agent anti-tumor activity that was associated with elevated caspase-3 activation and Cytokeratin 18 levels in plasma [53]. In another study, the presence and level of BCL-2 as well as levels of proapoptotic BAX and BIM were shown to determine sensitivity to ABT-737 [54]. Importantly, resistance to ABT-737 in SCLC was found to correlate strongly with MCL-1 expression [55,56]. Also NOXA was identified as a critical factor for ABT-737 sensitivity [57]. NOXA binds predominantly MCL-1 causing the release of proapoptotic BAX or BAK that trigger MOMP and low levels of NOXA in SCLC cells were associated with ABT-737 resistance.
In NSCLC cell lines ABT-737 resistance also could be related to MCL-1 expression levels and vector-dependent overexpression of NOXA sensitized cells for ABT-737 as well as combined treatment with chemotherapeutic agents [58]. In the same study MCL-1 expression was determined in tumor samples from 84 chemo-naive NSCLC patients and in 56% of the cases MCL-1 expression was detected suggestive of relevance as resistance factor in the clinical setting. Interestingly, in NSCLC synergistic interactions have been reported between ABT-737 and the EGFR inhibitor gefitinib, which has activity in a subpopulation of patients with mutated EGFR [59]. Gefitinib was found to induce a rapid increase in BIM that was mediated by MEK–ERK1/2 (mitogen-activated protein kinase kinase–extracellular) signalling. Elevated BIM was essential for gefitinib-dependent cell killing that could be further enhanced by neutralizing BCL-2 with ABT-737. Also synergistic interactions between TRAIL and ABT-737 have been reported in cell lines representing different tumor types, including A549 NSCLC cells [60]. Sensitisation by ABT-737 involved an enhancing effect on TRAIL-dependent mitochondrial apoptosis but also upregulation of TRAIL-R2 via a NF-kB-dependent mechanism was observed. An oral derivative of ABT-737, named ABT-263, has been generated with improved pharmokinetic and pharmacodynamic properties [61]. Potent dose-dependent antitumor activity has been reported in SCLC cell culture and xenograft mouse models [62].

Another promising small molecule BH3 mimetic is GX15-070 (Obatoclax, GeminX Inc., Malvern, PA, USA) that inhibits BCL2/BCL-XL and MCL-1 [63]. Its ability to inhibit MCL-1 provides an alternative for MCL-1-based mechanism of resistance encountered with ABT-737 [64]. In NSCLC cell lines GX15-070 demonstrated cell killing potential that was strongly enhanced by chemotherapeutic agents such as cisplatin, gemcitabine or paclitaxel and also enhanced cytotoxic effects of gefitinib in EGFR mutant NSCLC cells [63].

6. Targeting the IAP family

The IAP family comprises a group of eight structurally related proteins that share the presence of one or three zinc-binding motifs named the baculovirus IAP repeat (BIR) domain [65]. They constitute a functionally heterogeneous family and two members of the family, XIAP and survivin, are particularly involved in the inhibition of apoptosis. XIAP has three BIR domains that can interact and interfere with caspases-9, -3, and -7 activity. The BIR3 domain is involved in caspase-9 inhibition and the BIR2 domain in caspase-3 and -7 inhibition [65]. Upon cellular commitment
to undergo apoptosis XIAP is relieved from caspases by release of antagonistic SMAC from mitochondria [16]. XIAP is expressed in NSCLC and SCLC cells and its abundant presence in clinical samples derived from NSCLC may be associated with treatment resistance [66, 67]. Small molecules that interfere with the apoptotic inhibitory activity of XIAP and antisense oligonucleotide-based approaches that down-regulate the expression of XIAP are currently being explored as therapies. For example, a SMAC mimetic (compound 3) has been developed that sensitizes tumor cells to treatment with chemotherapeutic agents or TRAIL [68]. In NSCLC cells this compound also enhanced cisplatin-induced apoptosis involving increased caspase-3 activation [69]. More recently, a XIAP antagonistic phenylurea-based compound showed weak cytotoxic activity when administered alone and strongly enhanced apoptosis in combination with chemotherapeutic agents in NSCLC cell lines [70]. Antisense oligonucleotide mediated inhibition of XIAP displayed potent radiosensitizing properties in H460 NSCLC cell culture and xenograft mice models [71]. Another antisense oligonucleotide, named AEG35156 (Aegera Therapeutics, Montreal, Canada) exhibited strong antitumor activity in xenograft mice models of prostate, pancreatic and NSCLC cancer when combined with chemotherapy (cisplatin, docetaxel) and was able to synergistically enhance TRAIL-induced apoptosis in NSCLC cells in vitro [72]. AEG35156 is the only anti-XIAP agent to have advanced into clinical testing to date (see below).

Survivin has an important role in regulating cell survival and cell division [73]. Survivin can inhibit apoptosis by BIR-dependent binding and inhibition of caspases 3 and 7 and through direct interactions with SMAC. In addition, survivin can interact with microtubules and a role for survivin as a chromosomal passenger protein that regulates mitotic progression has been established [73]. Survivin is hardly detectable in most normal adult tissues whereas high levels of survivin are present in tumor cells and its expression has been found to be an indicator of poor patient prognosis, including in NSCLC in which also nuclear localization appeared to have prognostic value [74, 75]. However, contradicting reports have been published on nuclear survivin as a prognostic predictor for survival and further studies are warranted [75-77]. Together these properties have suggested that targeting survivin may be a promising therapeutic approach, and in different tumor types evidence for this has been obtained [73]. In NSCLC cells antisense-mediated downregulation of survivin was found to potently sensitize for radiotherapy [71, 78]. A small molecule inhibitor that suppresses activity of the survivin promoter, named YM155 (Astellas Pharma, Japan), has been developed showing potent antitumor activity in a p53-
independent fashion [79]. Furthermore, YM155 sensitized NSCLC cell lines and xenografts in nude mice for radiotherapy [80]. In SCLC Survivin-directed therapy has hardly been examined but some evidence for Survivin being a regulator of cisplatin-mediated cytotoxicity has been reported [81].

7. Apoptosis targeting strategies in clinical studies

Based on the promising preclinical results a number of therapeutic strategies that target the extrinsic or intrinsic pathway of apoptosis are being explored in early clinical studies in lung cancer. Agents that are most likely to produce approved products in the near future are TRAIL receptor targeted agents in NSCLC, antagonising inhibitors of XIAP or Survivin and inhibition of the BCL-2 family of proteins both in NSCLC and SCLC (see Figure 1). A summary of ongoing and completed clinical studies with these agents is provided below and in Table 1.

**Figure 1. Schematic representation of apoptotic targeted agents that have or are being tested in lung cancer.**

Pro-apoptotic TRAIL receptor targeting agents can trigger apoptosis selectively in cancer cells. Inactivation of anti-apoptotic proteins belonging to the BCL-2 or IAP family spontaneously cause apoptosis or result in apoptosis sensitization in tumor cells. In SCLC DISC formation is frequently impaired making it less suitable for single agent treatment with TRAIL receptor agonists. Combined treatments with standard therapy or other targeted agents are most effective (see text for more details).

7.1 TRAIL receptor targeting agents

Both agonistic antibodies against TRAIL-R1 (anti-DR4) or TRAIL-R2 (anti-DR5) and recombinant Apo2L/TRAIL agents are being studied in phase I and phase II setting.
Recently, an open label phase Ib study of rhTRAIL (AMG951 also known as dulanermin) in combination with paclitaxel, carboplatin and bevacizumab has been published as first line treatment in patients with advanced NSCLC, with acceptable toxicity and the suggestion of an additive effect (response rate 58%) [82]. This drug is now being tested in a randomized phase II trial in NSCLC, which started in 2007 and is ongoing.

Four studies have been published on the TRAIL-R1 agonist mapatumumab. Three of these were phase I studies in solid tumors with either mapatumumab alone [83, 84] or in combination with cisplatin and gemcitabin [85] or paclitaxel and carboplatin [86]. All of these studies demonstrated acceptable toxicity either alone or in combination with cytotoxic drugs. The most striking side effects related to the combination of cytotoxic drugs and mapatumumab appear to be peripheral sensory neuropathy, diarrhea and anorexia. A randomized phase II study on mapatumumab and paclitaxel and carboplatin started in 2007 and is ongoing. A monotherapy phase II study of mapatumumab was performed in relapsed NSCLC [84]. No objective single agent activity of mapatumumab was demonstrated but the drug was safe and well tolerated.

Several TRAIL-R2 agonists are being studied [87-92] of which three have been published as phase I trials in solid tumors as monotherapy [88-90]. Dose limiting toxicity (DLTs) were elevations of serum amylase, transaminases and bilirubin (liver toxicity), but lexatumumab can be safely administered every 14 days at 10 mg/kg. Three randomized phase II trials in patients with advanced NSCLC are ongoing, a trial with CS-1008, paclitaxel and carboplatin started in 2007, a trial with AMG-655 and paclitaxel and carboplatin started in 2008, and finally a trial with PRO95780, paclitaxel, carboplatin and bevacizumab started in 2009 (Table 1, clinicaltrials.gov).

7.2. XIAP and Survivin
A number of studies have evaluated Survivin and XIAP as targets for therapy. Two phase I trials have been performed in solid tumors with YM155, a small molecule inhibitor of Survivin. The first study found a maximal tolerated dose (MTD) of 4.8 mg/m^2 by 168 hour continuous i.v. infusion (CIVI) [93]. The second study found a MTD at 8.0 mg/m^2 by 168-hour, CIVI every 3 weeks. In both studies DLT was based on elevations in blood creatinine (kidney toxicity).
Table 1. Overview completed or ongoing clinical studies with apoptosis targeting agents in lung cancer.

<table>
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<tr>
<th>Targeting drug</th>
<th>Combination drugs</th>
<th>Phase</th>
<th>Patients</th>
<th>Remarks</th>
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<tr>
<td><strong>TRAIL receptor targeting drug</strong></td>
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<tr>
<td>Dulanermin (rh Apo2L/TRAIl)</td>
<td>Paclitaxel, Carboplatin, Bevacizumab</td>
<td>Ib</td>
<td>n=24 NSCLC IIb/IV or recurrent</td>
<td>no DLT</td>
<td>[82]</td>
</tr>
<tr>
<td>AMG 951</td>
<td>Paclitaxel, Carboplatin, Bevacizumab</td>
<td>II</td>
<td>n=200 NSCLC IIb/IV</td>
<td>started 2007 ongoing, not recruiting</td>
<td>§</td>
</tr>
<tr>
<td>Mapatumumab (TRAIL-R1)</td>
<td>-</td>
<td>I</td>
<td>Advanced solid tumors (5/49 NSCLC)</td>
<td>no DLT</td>
<td>[83]</td>
</tr>
<tr>
<td>Mapatumumab</td>
<td>-</td>
<td>II</td>
<td>n=32 NSCLC IIb/IV or recurrent</td>
<td>no DLT, no clinical effect</td>
<td>[84]</td>
</tr>
<tr>
<td>Mapatumumab</td>
<td>Cisplatin, Gemcitabine</td>
<td>I</td>
<td>Advanced solid tumors (3/49 NSCLC)</td>
<td>MTD not reached</td>
<td>[85]</td>
</tr>
<tr>
<td>Mapatumumab</td>
<td>Paclitaxel, Carboplatin</td>
<td>I</td>
<td>Advanced solid tumors (6/27 NSCLC)</td>
<td>MTD not reached</td>
<td>[81]</td>
</tr>
<tr>
<td>AMG 655 (TRAIL-R2)</td>
<td>Paclitaxel, Carboplatin</td>
<td>Ib/II</td>
<td>n=172 NSCLC IIb/IV or recurrent</td>
<td>started 2008 ongoing, not recruiting</td>
<td>[87]§</td>
</tr>
<tr>
<td>AMG 655</td>
<td>AMG 479 (IGF-1R antagonist)</td>
<td>Ib/II</td>
<td>n=108 advanced solid tumors</td>
<td>started 2009 ongoing, recruiting</td>
<td>§</td>
</tr>
<tr>
<td>PRO95780</td>
<td>-</td>
<td>I</td>
<td>Advanced solid tumors (3/50 NSCLC)</td>
<td>MTD not reached</td>
<td>[88]</td>
</tr>
<tr>
<td>CS-1008 (IAP-targeting drugs)</td>
<td>Paclitaxel, Carboplatin, Bevacizumab</td>
<td>II</td>
<td>n=120 NSCLC IIb/IV or recurrent</td>
<td>started 2007 ongoing, not recruiting</td>
<td>§</td>
</tr>
<tr>
<td>Lexatumumab</td>
<td>-</td>
<td>I</td>
<td>Advanced solid tumors (0/37 NSCLC)</td>
<td>10 mg/kg iv/3 wks MTD</td>
<td>[89]</td>
</tr>
<tr>
<td>Lexatumumab</td>
<td>-</td>
<td>I</td>
<td>Advanced solid tumors (8/31 NSCLC)</td>
<td>10 mg/kg iv/2 wks MTD</td>
<td>[90]</td>
</tr>
<tr>
<td>CS-1008</td>
<td>-</td>
<td>I</td>
<td>n=40 Solid tumors/lymphoma</td>
<td>2006-2007 completed, not published</td>
<td>§</td>
</tr>
<tr>
<td>CS-1008</td>
<td>Paclitaxel, Carboplatin</td>
<td>II</td>
<td>n=100 NSCLC IIb/IV</td>
<td>started 2009 ongoing, recruiting</td>
<td>§</td>
</tr>
<tr>
<td>LBY135</td>
<td>-</td>
<td>I</td>
<td>n=56 advanced solid tumors</td>
<td>discontinued(11)</td>
<td>[91]</td>
</tr>
<tr>
<td>HGS-TR2J</td>
<td>-</td>
<td>I</td>
<td>Advanced solid tumors</td>
<td>Discontinued</td>
<td>[92]</td>
</tr>
<tr>
<td><strong>IAP-targeting drugs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YM-155 (survivin supressor)</td>
<td>-</td>
<td>I</td>
<td>n=6 Solid tumors/lymphoma</td>
<td>started 2009 ongoing, recruiting</td>
<td>§</td>
</tr>
<tr>
<td>YM-155</td>
<td>-</td>
<td>I</td>
<td>Advanced solid tumors (2/41NSCLC)</td>
<td>4.8 mg/m2/d 168 h iv/3wks, MTD</td>
<td>[93]</td>
</tr>
<tr>
<td>YM-155</td>
<td>-</td>
<td>I</td>
<td>Advanced solid tumors (7/33 NSCLC)</td>
<td>8.0 mg/m2/d 168h iv/3wks, MTD</td>
<td>[94]</td>
</tr>
<tr>
<td>YM-155</td>
<td>-</td>
<td>II</td>
<td>n=37 NSCLC IIb/IV</td>
<td>PFS 1.7 (1.3-2.8) ; OS 6,6 (4-12,2) months 1 year survival 35,1%</td>
<td>[95]</td>
</tr>
<tr>
<td>AEG35156 (XIAP-antisense)</td>
<td>-</td>
<td>I</td>
<td>Advanced solid tumors (2/38 NSCLC)</td>
<td>125mg/m2/d 7d iv/3wks, MTD; 213 mg/m2/d 3d iv/3wks</td>
<td>[96]</td>
</tr>
<tr>
<td>AEG35156</td>
<td>Docetaxel</td>
<td>I</td>
<td>n=30 advanced solid tumors</td>
<td>started 2006 ongoing, not recruiting</td>
<td>§</td>
</tr>
<tr>
<td>AEG35156</td>
<td>Paclitaxel, Carboplatin</td>
<td>I/II</td>
<td>n=54 NSCLC IIb/IV</td>
<td>terminated, unacceptable neurotoxicity</td>
<td>§</td>
</tr>
<tr>
<td>TL32711 (SMAC mimetic)</td>
<td>-</td>
<td>I</td>
<td>n=56 advanced solid tumor/lymphoma</td>
<td>started 2009 ongoing, recruiting</td>
<td>§</td>
</tr>
<tr>
<td><strong>BCL-2 targeting drugs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oblimersen</td>
<td>Docetaxel</td>
<td>I/II</td>
<td>Advanced solid tumors (2/22 NSCLC; 1/22 SCLC)</td>
<td>MTD 9 mg/kg/d d1-5/4wks</td>
<td>[97]</td>
</tr>
<tr>
<td>Oblimersen</td>
<td>Paclitaxel, Carboplatin</td>
<td>I</td>
<td>Advanced solid tumors (3/46 NSCLC)</td>
<td>MTD not reached</td>
<td>[98]</td>
</tr>
</tbody>
</table>
A nonrandomised phase II trial in 37 patients with recurrent NSCLC treated with 4.8mg/m2/d by 168-h CIVI was well tolerated and suggested a modest single agent activity for YM155.

One phase I trial have been performed with the antisense to X-linked inhibitor of apoptosis (XIAP) protein AEG35156 [96]. DLT compromised elevated hepatic enzymes, hypophosphatemia and thrombocytopenia. A phase I/II study in patients with recurrent NSCLC of AEG3516 in combination with paclitaxel and carboplatin was terminated prematurely due to unacceptable neurotoxicity (clinicaltrials.gov). A phase I trial with the SMAC mimetic TL32711 recently started in adults with refractory solid tumors (Table 1, clinicaltrials.gov).
7.3. Targeting BCL-2 family proteins
For the BCL-2 targeting agents thus far only trials with the BCL-2 antisense agent oblimersen have been published. In the first phase I trial in solid tumors oblimersen combined with docetaxel was well tolerated [97]. Next the combination of oblimersen with paclitaxel and carboplatin appeared to be safe as well [98]. However, the results of a randomized phase II trial combining oblimersen with carboplatin and etoposide in SCLC were negative [99]. The authors concluded that additional evaluation of this agent in SCLC is not warranted. A randomized phase III study in recurrent NSCLC started in 2002 to determine the effectiveness of docetaxel with or without oblimersen and is still ongoing (Table 1, clinicaltrial.gov).

The first results on obatoclax, a small molecule antagonist of the BCL-2 prosurvival proteins, in combination with carboplatin and etoposide in SCLC ED [100] seem to be promising and a randomized phase II study is ongoing. In addition, obatoclax is being tested in a phase II trial with topotecan in SCLC [101]. AT-101, an oral pan BCL-2 family protein inhibitor has been tested in a small phase I/II study where it was safely combined with topotecan in relapsed SCLC; however, the response rates observed did not meet the criteria for additional enrolment [102]. A randomized phase II trial in 106 patients with recurrent NSCLC showed favorable overall survival for AT-101 when combined with docetaxel as compared to placebo [103]. Several phase I and II trials of combination therapies with AT-101 are ongoing (see table 1). Finally the oral BH3 mimetic (ABT-263) is being tested as monotherapy as well as combination therapy in both SCLC and solid tumors. The first results on these phase I studies are awaited.

8. Conclusions and future perspectives
Apoptosis targeting agents have shown promising antitumor activity in preclinical lung cancer models. Some of the agents appear to be particularly effective in either NSCLC or SCLC. For example, TRAIL receptor targeted agents are only tested in NSCLC patients, since DISC formation in SCLC is generally impaired. On the other hand, BCL-2 targeting agents as monotherapy display preferential activity for SCLC. The apoptotic drugs seem to be promising especially in combination with traditional cytotoxic chemotherapy. For all promising apoptotic drugs Phase III studies remain to be performed to validate possible therapeutic benefit.

It will be interesting to also test combinations of targeted agents, such as BCL-2 targeting drugs with TRAIL receptor targeting agents because antiapoptotic BCL-2
family proteins frequently are main resistance factors in laboratory models. It also seems logical to study combinations of BCL-2 inhibitors with EGFR inhibitors considering their enhancing effects in cell lines [59] and clinical studies are under way (see Table 1).

The identification of subgroups within lung cancer patients that qualify for specific targeted therapies appears to be of utmost importance. As mentioned earlier, EGFR inhibitors are active in NSCLC patients with EGFR mutations. A Biomarker-Integrated Approach of Targeted Therapy for Lung Cancer Elimination (so called BATTLE trial) program has started that bases its treatment on four molecular pathways in NSCLC: EGFR, Kras, Braf and VEGF [6] Recently, the presence of EML/ALK fusion gene in about 4% of lung adenocarcinomas leading to high ALK expression demonstrated high response rates to the ALK tyrosine kinase inhibitor PF-02341066 in a phase I trial of this agent [clinicaltrials.gov]. Similarly, different patient populations may be identified that will most likely benefit from specific apoptosis targeted agents. Thus far, no correlations have been found between responses to apoptosis targeted agents and histological subtypes within NSCLC. The presence of the apoptotic targets, such as TRAIL receptors and DISC compounds, anti-apoptotic BCL-2 and IAP family members is important but does not predict response to the corresponding targeted treatments. Biomarkers that better predict responses to apoptosis targeted agents remain to be identified.

**Acknowledgements**

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**Conflict of interest**

The authors have nothing to disclose.
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