An introduction to molecular targeted therapy of cancer

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ABSTRACT

The rapidly advancing elucidation of molecular targets in human cancers during the last decade has provided an excellent basis for the development of novel therapeutics. A huge variety of potential target structures have been identified, many of which are already being exploited for therapeutic purposes. This review introduces the reader into the concept of molecular targeted therapies, and provides some prototypic examples.

Key words: anti-cancer therapy, tyrosine kinase, apoptosis, epidermal growth factor (EGF), death receptors

INTRODUCTION

During the recent years, there has been an increasing and rapid development of molecular markers as targets for innovative therapeutic concepts ("Targeted Therapy"). A high number of molecules have become therapeutic targets already, especially growth factors and growth factor receptors, molecules of signal transduction, tumor-associated antigens, molecules of intracellular protein metabolism (proteasome inhibitors), factors regulating cell survival, cell cycle and cell death, and molecules associated with invasion, metastasis and angiogenesis. An overview on major examples for targets that already entered clinical trials is given in Fig. 1. Some of these molecular targeted compounds are not only efficient as tumor therapeutics, but also improve the patient's quality of life by, for example, reducing pain associated with the reduction of bone which is true, e.g., for compounds as zoledronic acid [1-6]. The list of targets for molecular therapy is growing daily. In the following, we will select representative examples to illustrate the potential, but also open problems to solve of targeted therapy.

REVIEW

Example for success: targeting tyrosine kinase receptors, for example EGF-R

There has been an impressive development of compounds targeted against tyrosine kinase receptors [7]. Here, targeting concepts directed against c-erb-B2 (HER2), such as Herceptin especially in breast cancers, c-Kit-targeted therapy (Gleevec) in Bcr/Abl-positive leukemias and GIST-tumors, VEGF/ VEGF-R-targeted compounds [8,9], and a number of therapeutic concepts targeting EGF-receptor (EGF-R), are standing out as major examples that already have led, or will most probably lead, to paradigm shifts in the treatment of major tumor diseases. With increasing numbers of clinical studies, a part of them having been accompanied by molecular translational studies, however, it becomes clear that most certainly the therapeutic response towards suchlike compounds to a considerable extent will be defined by the individual molecular conditions of the individual patient, the genetic- or population-based background of a patient, and either acquired or inherited peculiar characteristics and changes within the gene encoding the target, such as amplifications, mutations, or polymorphisms.

This can be illustrated, for example, by first experiences from clinical studies on compounds targeting the EGFreceptor. The EGF-receptor is being overexpressed in a Figure 1. Overview on major examples of molecules that have become therapeutic targets, for compounds that already have entered early clinical trials.



number of solid carcinomas, such as colorectal or certain types of lung cancers [10], and its prognostic impact has already been shown for these tumor entities for certain patient subgroups [10-12]. Binding of the ligand EGF leads to a dimerization either with another EGF-R-molecule, or with a molecule out of the Erb-B-receptor tyrosine-kinase family. This is followed by the phosphorylation of the intracellular domain, activating a number of, for example, Ras-associated signalling cascades that can initiate phenomena such as tumor cell proliferation, invasion, metastasis, or anti-apoptosis [5,6]. During the recent years, diverse therapeutic strategies targeting EGF-R have been developed. Small molecular compounds targeting EGF-R are directed against the tyrosine kinase domain of EGF-R and inhibit its activation, thereby inhibiting EGF-R initiated signalling [13-15]. Other EGF-R-targeted strategies are based on antibodies [16-24], e.g., Cetuximab [25,26]. A number of studies already have been conducted especially concerning the tyrosine-kinase inhibitors in colorectal [10] and also lung cancer [27-30]. Especially, large studies in non-small cell lung cancer such as the ISEL- or BR21-study on 1692 or 731 patients have shown that the best survival and best response to therapy was observed in patient subgroups with Asian population background, female gender, adenocarcinoma, and no history of smoking. Furthermore, in different studies, the level of EGF-R protein expression or amplification of the EGF-R gene was associated with response to EGF-R-based therapy [31-38]. Certain studies show an association of certain mutations within the EGF-R gene with the clinical response towards small molecular EGF-R targeted compounds [33]. Most of these mutations have been found within exons 18 to 21 within the EGF-R-gene [32]. In

addition, it has been shown that certain mutations within the EGF-R-gene can be associated with the development of a secondary resistance to therapy [32,33]. In addition, patients harbouring activating k-ras-mutations in non-small cell lung cancers most often show resistance towards EGF-R-based tyrosine kinase inhibitors [32]. In such cases, a combination with, for example-Ras-targeted compounds may be necessary for an individual patient. For antibodies such as Cetuximab[®], preliminary data suggest that response might be independent of EGF-R-mutations, in contrast to the tyrosine kinase inhibitors. Therefore, an antibody-based therapy in case of secondary mutations could be an option. In general, an antibody-based therapy direct against EGF-R (Cetuximab) is already accepted in colorectal cancer for patients with metastasis, this especially in case of an irinotecan resistance [25,26]. At present, ongoing phase III-studies are investigating the potential of Cetuximab in the situation of lung cancer (e.g. Manegold C et al., the ongoing Gemtax IV-Study), and the FLEX-Study recently has shown superiority of Cetuximab with chemotherapy as compared to chemotherapy alone in first-line treatment of NSCLC (ASCO 2008). In parallel to these ongoing studies, first molecular translational research results, in part of them by our own group, implicate first potential molecular indictors of therapy response, and also implicate that this antibody is able to inhibit different steps of metastatic cascade [36]. Taken together, the initial results of the particular example of EGF-R-targeted therapy illustrate that particular molecular, and also potentially genetic, conditions can modify and affect the response to targeted therapy concepts. It emphasizes the notion that detailed molecular analysis of the individual tumor in the individual patient need to accompany further studies

Figure 2. Apoptosis pathways.



Apoptosis pathways can be initiated by ligation of death receptors (DR) such as CD95 or TRAIL receptors (TRAIL-Rs) by their respective ligands, e.g. CD95 ligand (CD95L) or TRAIL, followed by receptor trimerization, recruitment of adaptor molecules (FADD) and activation of caspase-8 (receptor pathway). The mitochondrial pathway is initiated by the release of apoptogenic factors such as cytochrome c, Smac or AIF from mitochondria in the cytosol. Apoptosis can be inhibited by Bcl-2 or by "Inhibitor of Apoptosis Proteins" (IAPs). Smac promotes apoptosis by neutralizing IAP-mediated inhibition of caspase-3 and -9. See text for more details.

on molecular targeted compounds, to precisely classify the subgroups of patients that best respond to novel targeted compounds.

Example: targeting apoptosis pathways for cancer therapy

Moreover, a number of strategies have been developed that target the apoptotic machinery in cancer cells. Apoptosis or programmed cell death is the cell's intrinsic death program that plays an important role in various physiological and pathological situations and is highly conserved throughout evolution [39]. Tissue homeostasis is maintained by a subtle balance between proliferation on one side and cell death on the other side [40]. As a consequence, too little apoptosis can contribute to tumor formation, progression and treatment resistance [41]. Moreover, one of the most important advances in cancer research in recent years is the recognition that killing of tumor cells by anticancer therapies commonly used in the treatment of human cancer, e.g. chemotherapy, γ -irradiation, immunotherapy or suicide gene therapy, is predominantly

mediated by initiating programmed cell death, i.e. apoptosis, in cancer cells [42,43]. The elucidation of signaling pathways involved in the regulation of apoptosis in cancer cells over the last decade has led to the identification of key apoptosis regulatory molecules that may serve as molecular targets for cancer therapy. In principle, apoptosis-based cancer therapeutics may aim at directly activating apoptosis pathways in cancer cells, at restoring defects in the apoptotic machinery or at disabling the antiapoptotic function of molecules involved in treatment resistance. Such strategies may open new perspectives to overcome apoptosis resistance in a variety of human cancers. Some examples how apoptosis pathways could be targeted for cancer therapy will be discussed in the following sections.

APOPTOSIS SIGNALING PATHWAYS

There are two principle pathways of apoptosis, the receptor or extrinsic and the mitochondrial or intrinsic pathway (*Fig. 2*) [43]. Stimulation of either pathway eventually fuels into activation of caspases, a family of cysteine proteases that act

as common effector molecules in various forms of cell death [44]. Caspases are synthesized as inactive proenzymes. Once activated, they cleave various substrates in the cytoplasm or nucleus causing characteristic morphological features of apoptotic cell death [44]. In the extrinsinc apoptosis pathway, stimulation of death receptors of the tumor necrosis factor (TNF) receptor superfamily, e.g. CD95 (APO-1/Fas) or TRAIL receptors, results in activation of the initiator caspase-8, which in turn can directly cleave downstream effector caspases such as caspase-3 [45]. Also, activation of caspase-8 may link the receptor to the mitochondrial pathway by cleavaging Bid, a Bcl-2 family protein with a BH3 domain only that translocates to mitochondria upon cleavage to initiate a mitochondrial amplification loop [46]. In the mitochondrial pathway, the release of apoptogenic factors such as cytochrome c, apoptosis inducing factor (AIF), second mitochondria-derived activator of caspase (Smac)/direct IAP Binding protein with Low PI (DIABLO) or Omi/high temperature requirement protein A (HtrA2) from the mitochondrial intermembrane space into the cytosol initiates caspase-3 activation [47]. Cytochrome c promotes caspase-3 activation through formation of the cytochrome c/Apaf-1/caspase-9-containing apoptosome complex, while Smac/DIABLO promote caspase activation through neutralizing the inhibitory effects of inhibitor of apoptosis proteins (IAPs) [47]. Because of the potential detrimental effects on cell survival in case of inappropriate caspase activation, activation of caspases has to be tightly controlled. The anti-apoptotic mechanisms regulating cell death have also been implicated in conferring drug resistance to tumor cells.

APOPTOTIC SIGNALING MOLECULES AS TARGETS FOR CANCER THERAPY

Most anticancer therapies primarily act by inducing apoptosis in cancer cells [48]. Accordingly, defects in apoptosis programs may lead to resistance of cancers to current treatment approaches. Since evasion of apoptosis is a characteristic feature of human cancers, strategies designed to restore defective apoptosis programs in cancer cells may overcome intrinsic or acquired resistance of tumor cells to current regimens [49]. Also, apoptosis targeted therapies may increase the responsive rate of tumors towards conventional treatments that are currently used in the clinic, e.g. chemo- or radiotherapy [43].

Targeting death receptors for cancer therapy

The idea to trigger death receptors in order to induce apoptosis in cancer cells is attractive for cancer therapy, since death receptors are directly linked to the cell's intrinsic death machinery [50]. Death receptors are cell surface receptors that belong to the tumor necrosis factor (TNF) receptor gene superfamily [45,50,51]. These receptors exert a wide range of biological functions in addition to signal to cell death. For example, death receptors have also been implicated in the regulation of survival, differentiation and immune responses [45,50,51]. Death receptors share an intracellular domain called ,,death domain", which transmits the death signal from the cell's surface to intracellular signaling pathways. The best-characterized death receptors include CD95 (APO-1/Fas), TNF receptor 1 (TNFRI), TNF-related apoptosis inducing ligand (TRAIL) receptor 1 (TRAIL-R1) and TRAIL-R2. There exists also a family of corresponding ligands of the TNF superfamily that comprises CD95 ligand, TNF α or TRAIL. Binding of death receptors by their cognate ligands or by agonistic antibodies leads to oligomerization and activation of death receptors.

The death receptor ligand TRAIL is considered as a promising candidate for clinical development, since TRAIL preferentially kills cancer cells [52]. Recombinant soluble TRAIL or monoclonal antibodies targeting TRAIL receptors TRAIL-R1 or TRAIL-R2 were reported to induce apoptosis in a wide range of cancer cell lines and also *in vivo* in several xenograft models of human cancers [52-54]. Interestingly, TRAIL-R2 antibody-based therapy was recently reported as an efficient strategy not only to eliminate TRAIL-sensitive tumor cells, but also to induce tumor-specific T cell memory that afforded long-term protection from tumor recurrence [55].

Since a large proportion of human cancer turned out to be partially or completely resistant towards monotherapy with TRAIL despite the expression of both agonistic TRAIL receptors, TRAIL-based combination therapies were developed. To this end, TRAIL was reported to synergistically interact with chemotherapy or γ -irradiation in a variety of cancers [56,57].

Targeting the mitochondrial pathway for cancer therapy

Another approach to target apoptosis pathways for cancer therapy is to antagonize antiapoptotic Bcl-2 family members. The Bcl-2 family of proteins consists of both antiapoptotic members, e.g. Bcl-2, Bcl-X, Mcl-1, as well as proapoptotic molecules [46]. The later comprise on one side multidomain proteins such as Bax, Bak and Bad and on the other side BH3domain only molecules, e.g. Bim, Bid, Bmf, Noxa or Puma [46]. Bcl-2 family proteins play an important role in the regulation of the mitochondrial pathway of apoptosis, since they are involved in the control of mitochondrial outer membrane permeabilization [46]. There are currently two models how BH3-only proteins activate Bax and Bak during the course of apoptosis. According to the direct activation model [58], putative activators such as Bim and cleaved Bid (tBid) bind directly to Bax and Bak to trigger their activation, while BH3only proteins that act as sensitizers, e.g. Bad, bind to the prosurvival Bcl-2 proteins. By comparison, the indirect activation model holds that BH3-only proteins activate Bax and Bak by binding and thus inactivating the various antiapoptotic Bcl-2 proteins that in turn inhibit Bax and Bak [59]. Imbalances in the ratio of anti- versus pro-apoptotic Bcl-2 proteins may tipp the balance towards tumor cell survival and thus may contribute to tumor formation and progression. Since high expression

of anti-apoptotic Bcl-2 family proteins may confer resistance to chemo- or radiotherapy by blocking the mitochondrial pathway of apoptosis, there has been much interest to develop strategies to overcome the cytoprotective effect of Bcl-2 and related molecules. A prominent example of these efforts is the development of the small molecule antagonist ABT-737, which binds to the surface groove of Bcl-2, Bcl-X, and Bcl-w that normally interacts with the BH3 domain of Bax or Bak [60]. By preventing the binding of antiapoptotic Bcl-2 proteins to Bax or Bak, ABT-737 frees Bax and Bak to oligomerize and to form pores in the outer mitochondrial membrane, promoting the release of cytochrome c from mitochondria into the cytosol. Studies in cancer cell lines and preclinical models demonstrate that ABT-737 as single agent can trigger apoptosis in some susceptible cancer types, e.g. those that critically depend on Bcl-2 for survival [60]. In addition, ABT-737 sensitized cancer cells for apoptosis when combined with conventional chemotherapeutics [61]. Since ABT-737 targets Bcl-2/Bcl-xL but not Mcl-1, high expression of Mcl-1 may confer resistance to this novel agent. Indeed, several recent reports indicate that Mcl-1 represents a key determinant of ABT-737 sensitivity and resistance in cancer cells [62,63]. Collectively, these findings suggest that small molecule inhibitors of antiapoptotic Bcl-2 family proteins may open new perspectives to reactivate the mitochondrial pathway of apoptosis in cancer cells.

Targeting "Inhibitor of Apoptosis Proteins" (IAPs) for cancer therapy

Another promising therapeutic strategy directed at apoptosis regulators is the neutralization of "Inhibitor of Apoptosis Proteins" (IAPs). The family of endogenous caspase inhibitors "Inhibitor of Apoptosis Proteins" (IAPs) comprise eight human analogues, i.e. XIAP, c-IAP1, c-IAP2, survivin, apollon, livin/ melanoma-IAP (ML-IAP), NAIP and ILP-2 [64]. IAPs have been reported to directly inhibit active caspase-3 and -7 and to block caspase-9 activation [64]. The role of survivin in the regulation of apoptosis and proliferation is more complex compared to other IAP family proteins, since in addition to regulation of apoptosis, survivin is involved in regulation of mitosis [65]. There is mounting evidence that cancer cells have an intrinsic drive to apoptosis that is held in check by IAPs. To this end, high basal levels of caspase-3 and caspase-8 activities and active caspase-3 fragments in the absence of apoptosis were detected in various tumor cell lines and cancer tissues, but not in normal cells [66]. Tumor cells in contrast to normal cells also expressed high levels of IAPs suggesting that upregulated IAP expression counteracts the high basal caspase activity selectively in tumor cells [66].

Since IAPs are expressed at high levels in the majority of human cancers, they present an attractive molecular target. Consequently, several strategies have been developed to target enhanced expression of IAPs in human malignancies. For the design of therapeutic small molecules directed against X-linked inhibitor of apoptosis protein (XIAP), the binding groove of the BIR3 domain of XIAP, to which Smac binds to after its release from mitochondria, has attracted most attention [67]. Smac peptides that neutralize XIAP through binding to its BIR2 and BIR3 domains were able to promote caspase activation and enhanced TRAIL- or chemotherapy-induced apoptosis. In addition, Smac peptides even substantially increased the antitumor activity of TRAIL in vivo in an intracranial malignant glioma xenograft model, resulting in complete eradication of established tumors [68]. Also, XIAP antisense oligonucleotides exhibited potent antitumor activity as single agent and in combination with clinically relevant chemotherapeutic drugs [69,70]. Recently, IAP antagonists were reported to kill cancer cells by inducing autoubiquitination of c-IAPs, NF-KB activation, and TNFalpha-dependent apoptosis [71-73]. Currently, XIAP antisense oligonucleotides are evaluated in phase I/II clinical trials either as single agent or in combination with chemotherapy in advanced tumors. Thus, Smac agonists, low molecular weight XIAP antagonists or XIAP antisense oligonucleotides are promising new approaches to either directly engage apoptosis or to lower the threshold for apoptosis induction in cancer cells.

The challenge of today: defining the right patients for the right therapeutic concept

The examples given above illustrate the high and promising potential of molecular targeted therapy. However, they also illustrate the increasing importance of including molecular diagnosis to achieve an appropriate patient selection for therapy. An increasing attention is begin given to the field of pharmacogenomics, which investigates the genetic conditions of patients defining a particular type of response to certain therapeutics [71]. For example, there is increasing evidence that genetic polymorphisms which, under normal conditions, are not relevant for a disease or a phenotype, can significantly modify the response to certain types of therapies, for example cytochrome p450-dependent substances [74]. Such polymorphisms can also influence the response not only to novel molecular targeted therapies, but also classical chemoor radiation therapy. Prominent examples for this notion are certain enzymes involved in DNA-repair mechanisms. For example, certain polymorphisms within the XRCC3-gene (X-ray repair cross complementing group 3) have been shown to be associated with a significantly longer survival following Cisplatinum/Gemcitabine-based therapy in non-small cell lung cancer, as compared to Cisplatinum/Docetaxel-based therapy. The survival benefit resulting from these polymorphisms was observed especially in young patients with non-small cell lung cancer [75]. The consequence out of such a study would be that younger patients with non-small cell lung cancer harbouring particular polymorphisms of the XRCC3-gene would be treated with Cisplatinum/Gemcitabine rather than Cisplatinum/Docetaxel. In another study [76], it was shown that a particular polymorphism of the ERCC1-gene (excision repair cross complementing group 1), ERCC1-8092A/A, defines a particularly poor survival following treatment with Cisplatinum/Docetaxel. ERCC1 is an important enzyme

conducting nucleotide-excision DNA-repair that is known to remove DNA-adducts following Cisplatinum-based therapy. Certain ERCC1-polymorphisms affect ERCC1-expression, and it has been shown that NSCLC-patients with low ERCC1expression respond better to Cisplatinum-based therapy than patients with high ERCC1 [77].

These are only two out of many recent examples illustrating that genetic polymorphisms within DNA-repair relevant for metabolizing DNA-changes following particular types of chemotherapy can significantly modify the therapeutic response of tumor patients towards classical therapy concepts. They illustrate that pharmacogenomics will be of increasing importance for optimizing therapeutic compounds towards the individual genetic and molecular conditions of an individual tumor patient in the future. Certainly, novel generations of targeted therapy strategies also will increasingly have to consider particular molecular or genetic variations and changes within patients for a further significant improvement of therapy response and survival of cancer patients. Therefore, individual genetic or inherited conditions that by themselves might not be causative for a disease, will become increasingly important even for sporadic types of cancers, and for the therapy of tumors with a non-familiar background.

CONCLUSION

Over the last two decades, the elucidation of molecular conditions, among them being signal transduction pathways involved in the regulation of tumor growth, cell death in human cancers, or molecular markers of cancer progression, have provided the fundamental basis for the development of molecular targeted therapies. Since such strategies are specifically directed against key components that are crucial for the cancer cell's survival and function, they may be more selective and effective in killing malignant over nonmalignant cells. While several approaches have already been translated into medical application, many concepts have still to be evaluated in (pre)clinical trials. Another main goal ahead with molecular targeted therapies will be considering the appropriate patient selection to enrich for a responsive population. Eventually, these efforts are expected to yield more effective yet less toxic treatment options for the sake of patients suffering from cancer.

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REFERENCES

 Green JR. Bisphosphonates: preclinical review. Oncologist. 2004;9 Suppl 4:3-13.

2. Green JR. Antitumor effects of bisphosphonates. Cancer. 2003 Feb 1;97(3 Suppl):840-7.

3. Jonathan R, Green JR. Pharmacologic profile of zoledronic acid: a highly potent inhibitor of bone resorption. Drug Dev Res. 2002;55(4):210-24.

4. Rogers MJ, Gordon S, Benford HL, Coxon FP, Luckman SP, Monkkonen J, Frith JC. Cellular and molecular mechanisms of action of bisphosphonates. Cancer. 2000 Jun 15;88(12 Suppl):2961-78.

5. Liu D, Aguirre Ghiso J, Estrada Y, Ossowski L. EGFR is a transducer of the urokinase receptor initiated signal that is required for in vivo growth of a human carcinoma. Cancer Cell. 2002 Jun;1(5):445-57.

6. Festuccia C, Angelucci A, Gravina GL, Biordi L, Millimaggi D, Muzi P, Vicentini C, Bologna M. Epidermal growth factor modulates prostate cancer cell invasiveness regulating urokinase-type plasminogen activator activity. EGF-receptor inhibition may prevent tumor cell dissemination. Thromb Haemost. 2005 May;93(5):964-75.

7. Jain RK, Duda DG, Clark JW, Loeffler JS. Lessons from phase III clinical trials on anti-VEGF therapy for cancer. Nat Clin Pract Oncol. 2006 Jan;3(1):24-40.

8. Ranieri G, Patruno R, Ruggieri E, Montemurro S, Valerio P, Ribatti D. Vascular endothelial growth factor (VEGF) as a target of bevacizumab in cancer: from the biology to the clinic. Curr Med Chem. 2006;13(16):1845-57.

9. Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E, Ferrara N, Fyfe G, Rogers B, Ross R, Kabbinavar F. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med. 2004 Jun 3;350(23):2335-42.

10. Lund LR, Romer J, Ronne E, Ellis V, Blasi F, Dano K. Urokinase-receptor biosynthesis, mRNA level and gene transcription are increased by transforming growth factor beta 1 in human A549 lung carcinoma cells. Embo J. 1991 Nov;10(11):3399-407.

11. Dumler I, Petri T, Schleuning WD. Induction of c-fos gene expression by urokinase-type plasminogen activator in human ovarian cancer cells. FEBS Lett. 1994 Apr 25;343(2):103-6.

12. Wang Y, Kristensen GsB, Helland A, Nesland JM, Borresen-Dale AL, Holm R. Protein expression and prognostic value of genes in the erb-b signaling pathway in advanced ovarian carcinomas. Am J Clin Pathol. 2005 Sep;124(3):392-401.

13. Pollack VA, Savage DM, Baker DA, Tsaparikos KE,

Sloan DE, Moyer JD, Barbacci EG, Pustilnik LR, Smolarek TA, Davis JA, Vaidya MP, Arnold LD, Doty JL, Iwata KK, Morin MJ. Inhibition of epidermal growth factor receptorassociated tyrosine phosphorylation in human carcinomas with CP-358,774: dynamics of receptor inhibition in situ and antitumor effects in athymic mice. J Pharmacol Exp Ther. 1999 Nov:291(2):739-48.

14. Akita RW, Sliwkowski MX. Preclinical studies with Erlotinib (Tarceva). Semin Oncol. 2003 Jun;30(3 Suppl 7):15-24.

15. Moyer JD, Barbacci EG, Iwata KK, Arnold L, Boman B, Cunningham A, DiOrio C, Doty J, Morin MJ, Moyer MP, Neveu M, Pollack VA, Pustilnik LR, Reynolds MM, Sloan D, Theleman A, Miller P. Induction of apoptosis and cell cycle arrest by CP-358,774, an inhibitor of epidermal growth factor receptor tyrosine kinase. Cancer Res. 1997 Nov 1;57(21):4838-48.

16. Mendelsohn J. Targeting the EGF receptor: experience and lessons. Eur J Cancer Suppl. 2006;4(6):25-6.

17. Schlessinger J. Cell signaling by receptor tyrosine kinases: From basic concepts to clinical applications. Eur J Cancer Suppl. 2006;4(6):3.

18. Schlessinger J. Common and distinct elements in cellular signaling via EGF and FGF receptors. Science. 2004 Nov 26;306(5701):1506-7.

19. Fleishman SJ, Schlessinger J, Ben-Tal N. A putative molecular-activation switch in the transmembrane domain of erbB2. Proc Natl Acad Sci USA. 2002 Dec 10;99(25):15937-40.

20. Schlessinger J. Signal transduction. Autoinhibition control. Science. 2003 May 2;300(5620):750-2.

21. Klein P, Mattoon D, Lemmon MA, Schlessinger J. A structure-based model for ligand binding and dimerization of EGF receptors. Proc Natl Acad Sci USA. 2004 Jan 27;101(4):929-34.

22. Schlessinger J. Ligand-induced, receptor-mediated dimerization and activation of EGF receptor. Cell. 2002 Sep 20;110(6):669-72.

23. Lax I, Wong A, Lamothe B, Lee A, Frost A, Hawes J, Schlessinger J. The docking protein FRS2alpha controls a MAP kinase-mediated negative feedback mechanism for signaling by FGF receptors. Mol Cell. 2002 Oct;10(4):709-19.

24. Reinmuth N, Meister M, Muley T, Steins M, Kreuter M, Herth FJF, Hoffmann H, Dienemann H, Thomas M. Molecular determinants of response to RTK-targeting agents in non small cell lung cancer. Int J Cancer. 2006 Aug 15;119(4):727-34.

25. Cunningham D, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, Bets D, Mueser M, Harstrick A, Verslype C, Chau I, Van Cutsem E. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med. 2004 Jul 22;351(4):337-45.

26. Moosmann N, Heinemann V. Cetuximab in the treatment of metastatic colorectal cancer. Expert Opin Biol Ther. 2007 Feb;7(2):243-56.

27. Thatcher N. The ISEL and BR21 trials - Outcomes similar or different? Eur J Cancer Suppl. 2006;4(6):23-4.

28. Thatcher N, Chang A, Parikh P, Rodrigues Pereira J, Ciuleanu T, von Pawel J, Thongprasert S, Tan EH, Pemberton K, Archer V, Carroll K. Gefitinib plus best supportive care in previously treated patients with refractory advanced non-smallcell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). Lancet. 2005 Oct 29-Nov 4;366(9496):1527-37.

29. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, Tan EH, Hirsh V, Thongprasert S, Campos D, Maoleekoonpiroj S, Smylie M, Martins R, van Kooten M, Dediu M, Findlay B, Tu D, Johnston D, Bezjak A, Clark G, Santabarbara P, Seymour L, National Cancer Institute of Canada Clinical Trials G. Erlotinib in previously treated non-small-cell lung cancer. N Engl J Med. 2005 Jul 14;353(2):123-32.

30. Blackhall F, Ranson M, Thatcher N. Where next for gefitinib in patients with lung cancer? Lancet Oncol. 2006 Jun;7(6):499-507.

31. Hirsch FR. The role of EGFR family in preneoplasia and lung cancer; Perspectives for targeted therapies and selection of patients. Eur J Cancer Suppl. 2006;4(6):13-4.

32. Van Zandwijk N, Mathy A, De Jong D, Baas P, Burgers S, Nederlof P. Impact of epidermal growth factor receptor (EGFR) mutations on responsiveness of non-small cell lung cancer (NSCLC) to tyrosine kinase inhibitors (TKIs): Prospective observations. Eur J Cancer Suppl. 2006;4(6):14-5.

33. Nikolova DA, Asangani IA, Rasheed SAK, Nelson L et al. Cetuximab attenuates EGF induced u-PAR expression: study on the antimetastatic potential of cetuximab in NSCLC. Proceedings of the AACR Congress 2008, San Diego, April 12-16.

34. Pao W, Miller V, Zakowski M, Doherty J, Politi K, Sarkaria I, Singh B, Heelan R, Rusch V, Fulton L, Mardis E, Kupfer D, Wilson R, Kris M, Varmus H. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. Proc Natl Acad Sci USA. 2004 Sep 7;101(36):13306-11.

35. Cappuzzo F, Hirsch FR, Rossi E, Bartolini S, Ceresoli GL, Bemis L, Haney J, Witta S, Danenberg K, Domenichini I, Ludovini V, Magrini E, Gregorc V, Doglioni C, Sidoni A, Tonato M, Franklin WA, Crino L, Bunn PA Jr, Varella-Garcia M. Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. J Natl Cancer Inst. 2005 May 4;97(9):643-55.

36. Hirsch FR, Varella-Garcia M, McCoy J, West H, Xavier AC, Gumerlock P, Bunn PA Jr, Franklin WA, Crowley J, Gandara DR, Southwest Oncology G. Increased epidermal growth factor receptor gene copy number detected by fluorescence in situ hybridization associates with increased sensitivity to gefitinib in patients with bronchioloalveolar carcinoma subtypes: a Southwest Oncology Group Study. J Clin Oncol. 2005 Oct 1;23(28):6838-45.

37. Tsao MS, Sakurada A, Cutz JC, Zhu CQ, Kamel-Reid S, Squire J, Lorimer I, Zhang T, Liu N, Daneshmand M, Marrano P, da Cunha Santos G, Lagarde A, Richardson F, Seymour L, Whitehead M, Ding K, Pater J, Shepherd FA. Erlotinib in lung cancer - molecular and clinical predictors of outcome. N Engl J Med. 2005 Jul 14;353(2):133-44.

38. Hirsch FR, Varella-Garcia M, Bunn PA Jr, Franklin WA, Dziadziuszko R, Thatcher N, Chang A, Parikh P, Pereira JR, Ciuleanu T, von Pawel J, Watkins C, Flannery A, Ellison G, Donald E, Knight L, Parums D, Botwood N, Holloway B. Molecular predictors of outcome with gefitinib in a phase III placebo-controlled study in advanced non-small-cell lung cancer. J Clin Oncol. 2006 Nov 1;24(31):5034-42.

39. Hengartner MO. The biochemistry of apoptosis. Nature. 2000 Oct 12;407(6805):770-6.

40. Evan GI, Vousden KH. Proliferation, cell cycle and apoptosis in cancer. Nature. 2001 May 17;411(6835):342-8.

41. Lowe SW, Lin AW. Apoptosis in cancer. Carcinogenesis. 2000 Mar;21(3):485-95.

42. Makin G, Dive C. Apoptosis and cancer chemotherapy. Trends Cell Biol. 2001 Nov;11(11):S22-6.

43. Fulda S, Debatin KM. Extrinsic versus intrinsic apoptosis pathways in anticancer chemotherapy. Oncogene. 2006 Aug 7;25(34):4798-811.

44. Degterev A, Boyce M, Yuan J. A decade of caspases. Oncogene. 2003 Nov 24;22(53):8543-67.

45. Walczak H, Krammer PH. The CD95 (APO-1/Fas) and the TRAIL (APO-2L) apoptosis systems. Exp Cell Res. 2000 Apr 10;256(1):58-66.

46. Adams JM, Cory S. The Bcl-2 apoptotic switch in cancer development and therapy. Oncogene. 2007 Feb 26;26(9):1324-37.

47. Saelens X, Festjens N, Vande Walle L, van Gurp M, van Loo G, Vandenabeele P. Toxic proteins released from mitochondria in cell death. Oncogene. 2004 Apr 12:23(16):2861-74.

48. Fulda S, Debatin KM. Targeting apoptosis pathways in cancer therapy. Curr Cancer Drug Targets. 2004 Nov;4(7):569-76.

49. Johnstone RW, Ruefli AA, Lowe SW. Apoptosis: a link between cancer genetics and chemotherapy. Cell. 2002 Jan 25;108(2):153-64.

50. Ashkenazi A. Targeting death and decoy receptors of the tumour-necrosis factor superfamily. Nat Rev Cancer. 2002 Jun;2(6):420-30.

51. Krammer PH. CD95's deadly mission in the immune system. Nature. 2000 Oct 12;407(6805):789-95.

52. LeBlanc HN, Ashkenazi A. Apo2L/TRAIL and its death and decoy receptors. Cell Death Differ. 2003 Jan;10(1):66-75.

53. Chuntharapai A, Dodge K, Grimmer K, Schroeder K, Marsters SA, Koeppen H, Ashkenazi A, Kim KJ. Isotypedependent inhibition of tumor growth in vivo by monoclonal antibodies to death receptor 4. J Immunol. 2001 Apr 15;166(8):4891-8. 54. Ichikawa K, Liu W, Zhao L, Wang Z, Liu D, Ohtsuka T, Zhang H, Mountz JD, Koopman WJ, Kimberly RP, Zhou T. Tumoricidal activity of a novel anti-human DR5 monoclonal antibody without hepatocyte cytotoxicity. Nat Med. 2001 Aug;7(8):954-60.

55. Takeda K, Yamaguchi N, Akiba H, Kojima Y, Hayakawa Y, Tanner JE, Sayers TJ, Seki N, Okumura K, Yagita H, Smyth MJ. Induction of tumor-specific T cell immunity by anti-DR5 antibody therapy. J Exp Med. 2004 Feb 16;199(4):437-48.

56. Gliniak B, Le T. Tumor necrosis factor-related apoptosis-inducing ligand's antitumor activity in vivo is enhanced by the chemotherapeutic agent CPT-11. Cancer Res. 1999 Dec 15;59(24):6153-8.

57. Chinnaiyan AM, Prasad U, Shankar S, Hamstra DA, Shanaiah M, Chenevert TL, Ross BD, Rehemtulla A. Combined effect of tumor necrosis factor-related apoptosisinducing ligand and ionizing radiation in breast cancer therapy. Proc Natl Acad Sci USA. 2000 Feb 15;97(4):1754-9.

58. Letai A, Bassik MC, Walensky LD, Sorcinelli MD, Weiler S, Korsmeyer SJ. Distinct BH3 domains either sensitize or activate mitochondrial apoptosis, serving as prototype cancer therapeutics. Cancer Cell. 2002 Sep;2(3):183-92.

59. Willis SN, Fletcher JI, Kaufmann T, van Delft MF, Chen L, Czabotar PE, Ierino H, Lee EF, Fairlie WD, Bouillet P, Strasser A, Kluck RM, Adams JM, Huang DC. Apoptosis initiated when BH3 ligands engage multiple Bcl-2 homologs, not Bax or Bak. Science. 2007 Feb 9;315(5813):856-9.

60. Oltersdorf T, Elmore SW, Shoemaker AR, Armstrong RC, Augeri DJ, Belli BA, Bruncko M, Deckwerth TL, Dinges J, Hajduk PJ, Joseph MK, Kitada S, Korsmeyer SJ, Kunzer AR, Letai A, Li C, Mitten MJ, Nettesheim DG, Ng S, Nimmer PM, O'Connor JM, Oleksijew A, Petros AM, Reed JC, Shen W, Tahir SK, Thompson CB, Tomaselli KJ, Wang B, Wendt MD, Zhang H, Fesik SW, Rosenberg SH. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. Nature. 2005 Jun 2;435(7042):677-81.

61. Shoemaker AR, Oleksijew A, Bauch J, Belli BA, Borre T, Bruncko M, Deckwirth T, Frost DJ, Jarvis K, Joseph MK, Marsh K, McClellan W, Nellans H, Ng S, Nimmer P, O'Connor JM, Oltersdorf T, Qing W, Shen W, Stavropoulos J, Tahir SK, Wang B, Warner R, Zhang H, Fesik SW, Rosenberg SH, Elmore SW. A small-molecule inhibitor of Bcl-XL potentiates the activity of cytotoxic drugs in vitro and in vivo. Cancer Res. 2006 Sep 1;66(17):8731-9.

62. Konopleva M, Contractor R, Tsao T, Samudio I, Ruvolo PP, Kitada S, Deng X, Zhai D, Shi Y-X, Sneed T, Verhaegen M, Soengas M, Ruvolo VR, McQueen T, Schober WD, Watt JC, Jiffar T, Ling X, Marini FC, Harris D, Dietrich M, Estrov Z, McCubrey J, May WS, Reed JC, Andreeff M. Mechanisms of apoptosis sensitivity and resistance to the BH3 mimetic ABT-737 in acute myeloid leukemia. Cancer Cell. 2006 Nov;10(5):375-88.

63. Van Delft MF, Wei AH, Mason KD, Vandenberg CJ, Chen L, Czabotar PE, Willis SN, Scott CL, Day CL, Cory

64. Salvesen GS, Duckett CS. IAP proteins: blocking the road to death's door. Nat Rev Mol Cell Biol. 2002 Jun;3(6):401-10.

65. Altieri DC. Validating survivin as a cancer therapeutic target. Nat Rev Cancer. 2003 Jan;3(1):46-54.

66. Yang L, Cao Z, Yan H, Wood WC. Coexistence of high levels of apoptotic signaling and inhibitor of apoptosis proteins in human tumor cells: implication for cancer specific therapy. Cancer Res. 2003 Oct 15;63(20):6815-24.

67. Shiozaki EN, Shi Y. Caspases, IAPs and Smac/ DIABLO: mechanisms from structural biology. Trends Biochem Sci. 2004 Sep;29(9):486-94.

68. Fulda S, Wick W, Weller M, Debatin KM. Smac agonists sensitize for Apo2L/TRAIL- or anticancer drug-induced apoptosis and induce regression of malignant glioma in vivo. Nat Med. 2002 Aug;8(8):808-15.

69. LaCasse EC, Kandimalla ER, Winocour P, Sullivan T, Agrawal S, Gillard JW, Durkin J. Application of XIAP antisense to cancer and other proliferative disorders: development of AEG35156/ GEM640. Ann N Y Acad Sci. 2005 Nov;1058:215-34.

70. LaCasse EC, Cherton-Horvat GG, Hewitt KE, Jerome LJ, Morris SJ, Kandimalla ER, Yu D, Wang H, Wang W, Zhang R, Agrawal S, Gillard JW, Durkin JP. Preclinical characterization of AEG35156/GEM 640, a second-generation antisense oligonucleotide targeting X-linked inhibitor of apoptosis. Clin Cancer Res. 2006 Sep 1;12(17):5231-41.

71. Varfolomeev E, Blankenship JW, Wayson SM, Fedorova AV, Kayagaki N, Garg P, Zobel K, Dynek JN, Elliott LO, Wallweber HJ, Flygare JA, Fairbrother WJ, Deshayes K, Dixit VM, Vucic D. IAP antagonists induce autoubiquitination of c-IAPs, NF-kappaB activation, and TNFalpha-dependent apoptosis. Cell. 2007 Nov 16;131(4):669-81.

72. Vince JE, Wong WW, Khan N, Feltham R, Chau D, Ahmed AU, Benetatos CA, Chunduru SK, Condon SM,

McKinlay M, Brink R, Leverkus M, Tergaonkar V, Schneider P, Callus BA, Koentgen F, Vaux DL, Silke J. IAP antagonists target cIAP1 to induce TNFalpha-dependent apoptosis. Cell. 2007 Nov 16;131(4):682-93.

73. Li L, Thomas RM, Suzuki H, De Brabander JK, Wang X, Harran PG. A small molecule Smac mimic potentiates TRAIL- and TNFalpha-mediated cell death. Science. 2004 Sep 3;305(5689):1471-4.

74. Tribut O, Lessard Y, Reymann JM, Allain H, Bentue-Ferrer D. Pharmacogenomics. Med Sci Monit. 2002 Jul;8(7):RA152-63.

75. Rosell Costa R, Alberola V, Camps C, Lopez-Vivanco G, Moran T, Etxaniz O, De Las Peñas R, Gupta J, Taron M, Sanchez J. Clinical outcome of gemcitabine (gem)/ cisplatin (cis)-vs docetaxel (doc)/cis-treated stage IV nonsmall cell lung cancer (NSCLC) patients (p) according to X-ray repair cross-complementing group 3 (XRCC3) polymorphism and age. J Clin Oncol. 2006;24(18 Suppl):7055.

76. Taron M, Alberola V, Lopez Vivanco G, Camps C, De Las Penas R, Alonso G, Provencio M, Salvatierra A, Sanchez J, Rosell R. Excision cross-complementing group 1 (ERCC1) single nucleotide polymorphisms (SNPs) and survival in cisplatin (cis)/docetaxel (doc)-treated stage IV non-small cell lung cancer (NSCLC) patients (p): A Spanish Lung Cancer Group study. J Clin Oncol. 2006 Jun 20;24(18 Suppl):7053.

77. Olaussen KA, Dunant A, Fouret P, Brambilla E, Andre F, Haddad V, Taranchon E, Filipits M, Pirker R, Popper HH, Stahel R, Sabatier L, Pignon JP, Tursz T, Le Chevalier T, Soria JC, Investigators IB. DNA repair by ERCC1 in non-smallcell lung cancer and cisplatin-based adjuvant chemotherapy. N Engl J Med. 2006 Sep 7;355(10):983-91.