Literature thesis

Combining EGFR inhibitors with Chemo- and Radiotherapy in lung cancer treatment

F. M. Katoen
Supervisor: Dr. M. van Vugt

Department of Medical Oncology, University Medical Center Groningen, Groningen, the Netherlands

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Abstract

Lung cancer is the leading cause of cancer death and leads approximately to 1.18 million deaths each year worldwide. Chemotherapy, radiotherapy, antibodies and small molecules are clinically used to treat lung cancer. Chemo- and radiotherapy trigger apoptosis through provoking an enormous amount of DNA damage. Antibodies and small molecules work on the epidermal growth factor receptor (EGFR), a cell surface receptor involved in cancer development and progression. Antibodies, which are used to inhibit epidermal growth factor receptor signaling, work on the extracellular domain of the EGFR. Antibodies bind to the ERF receptor to block the binding of epidermal growth factor (EGF), which inhibits the normal function of the EGFR such as cell proliferation, angiogenesis, invasion, metastasis, blocking of differentiation and apoptosis inhibition. Small molecules, which are also used to target the EGFR, work both on the extracellular and intracellular domain of the EGF receptor. Extracellularly, the small molecules bind to the EGFR to block the activation of the EGF receptor. Intracellularly, small molecules compete with ATP in binding to the tyrosine kinase domain of the EGF receptor. Binding of small molecules to the tyrosine kinase domain leads to inhibition of cell proliferation, angiogenesis, invasion and metastasis; because the kinase domain does not become activated by ATP and can no longer exert its function. Because none of these treatments work efficiently in all lung cancer patients, combined treatments become a substantially interest. The most promising therapy is the Cetuximab antibody combined with chemo- and radiotherapy. It is shown that this combination is effective, although the mechanism behind this improved effect is still uncertain. In this literature thesis I will discuss a number of mechanisms that may explain the improved effect of Cetuximab combined with radio/chemotherapy. First, I will discuss the influence of membrane composition on EGFR and checkpoint signaling. Second, the effect of DNA damage on EGFR levels will be analyzed. Third, I will discuss cell cycle kinetics in response to EGFR inhibition. Finally, the intracellular trafficking of the EGFR will be analyzed. In conclusion, I will discuss potential molecular mechanisms of EGFR inhibition combined with chemo- and radiotherapy.

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Introduction

Lung cancer is the leading cause of cancer death for both men and women. In Europe, 385 300 new cases of lung cancer were diagnosed (12,1 % of all diagnosed cancers) and 334 800 lung cancer patients die (19,7 % of total cancer deaths) in the year 2006 (Ferlay et al., 2006). In 2002, there were 1.35 million cases and approximately 1.18 million deaths world-wide (Parkin et al., 2002). The biggest cause of lung cancer is tobacco smoking, which based on data from 2000, result in estimated 85% of lung cancer in men and 47% in women (Parkin et al., 2002). The percentage in men is much higher, because men started earlier with tobacco smoking than women. As a consequence, almost two and a half times more men than women have lung cancer; 965 241 men and 386 891 women, respectively (Parking et al., 2002). The percentages of lung cancer patients who die from the disease, are 87, 9 % of male patient and 85, 5 % of female patients (Parkin et al., 2002).

Two types of lung cancer are recognized; small cell lung (SCL) cancer and non-small cell lung (NSCL) cancer (e.g. adenocarcinoma, squamous cell carcinoma, large cell carcinoma, Collins et al., 2007). These categories are used for treatment decisions and determining prognosis. Small cell lung carcinomas (SCLC) are highly aggressive and are almost always caused of tobacco smoking. SCLCs are rapidly growing and around 60 % of the patients have widespread metastasis at time of diagnosis (Collins et al., 2007). Although initially there is a good response to chemotherapy, the prognosis for SCLC patients is very poor (Collins et al., 2007). In contrast, NSLC is less aggressive and grows slower. The prognosis for NSCL cancer is much better. Complete surgical resection is currently the best treatment for non small cell lung carcinomas. But only 25 % of all patients are suitable of surgical treatment (Jensen et al., 2006). In about 40 % of the patient there will be metastasis at time of diagnosis (Collins et al., 2007). The most common form of lung cancer is the non-small cell lung cancer (85%, Stinghcombel et al., 2009). In this study I will only focus on NSCL, because of its high incidence.

Currently, non-small cell lung cancer is clinically treated by using surgical resection, radiotherapy, chemotherapy and EGF receptor inhibitors. Because none of these treatments have a sufficient response on all NSCL patients, more and more interest has been expressed in combining several therapies to treat lung cancer optimally. In this study, I will analyze the current scientific literature concerning the treatment using EGFR inhibitors combined with radio- or chemotherapy. Specifically, I will focus on the question whether this is a better or worse treatment for lung cancer than the therapies separately. I will also try to find an explanation why these combined treatments works better or worse.

EGF receptor

The Epidermal Growth Factor receptor (EGFR) is a cell surface tyrosine kinase receptor, involved in cancer development and progression (Imai et al., 2006). The EGF receptor is a family member of the epidermal growth factor receptor family, which contains of four subgroups: EGFR (ErbB-1), an important receptor in lung cancer, HER2/c-neu (ErbB-2), an important receptor in breast and ovarian cancer, Her 3 (ErbB-3) and Her 4 (ErbB-4), both important in endometrial cancer (Slamon et al., 1989; Srinivasan et al., 1999; Imai et al., 2006).

The EGF receptor is a 170 kDa transmembrane glycoprotein that consists of an intracellular domain, with a tyrosine kinase domain and an extracellular domain, a binding place for specific ligands (Ciardiello et al., 2004). Ligand binding (such as Epidermal Growth Factor) to the extracellular domain of the EGF receptor result in the activation of a cascade of intracellular signals, which control (directly or indirectly) cell proliferation, angiogenesis, invasion and metastasis (Figure 1, reviewed in Sebolt-Leopold and English, 2006). After ligand binding to a single chain EGF receptor, the receptor forms a dimer that leads to autophosphorylation of the intracellular domain of the receptor (the protein-tyrosine kinase domain). Docking protein such as GRB2, contain SH2 domains that bind to the phosphorylated residues of the activated receptor. Subsequently GRB2 binds the exchange factor Son-of-Sevenless (SOS) through the SH3 domain of GRB2. When the GRB2-SOS complex binds to the phosphorylated EGF receptor, SOS becomes activated. The exchange factor SOS, with cooperation of GRB2, replaces the GDP into a GTP to activate Ras. Activated Ras leads to the activating of two pathways: The mitogen-activated protein kinase pathway (MAPK pathway) and the phosphatidylinositol-3-OH kinase pathway (PI(3)K pathway).

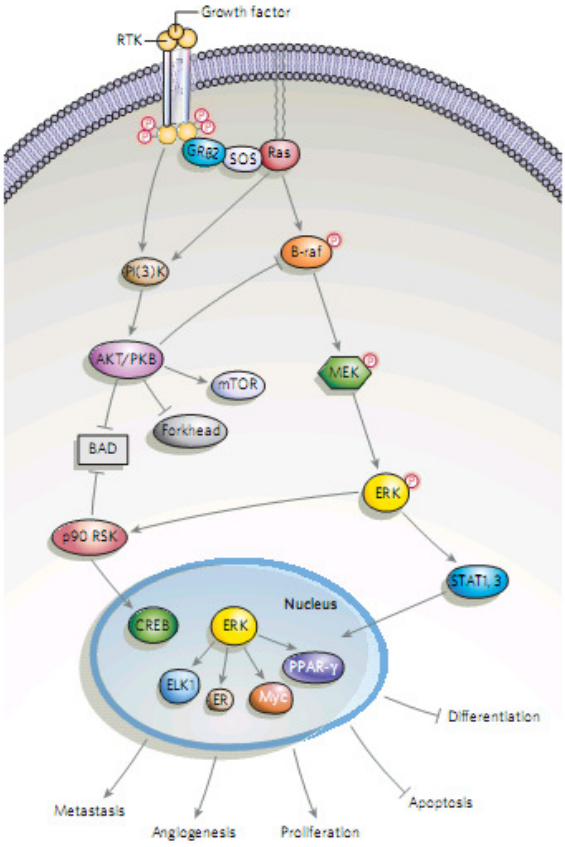

Figure 1: Pathway activation by EGFR. Epidermal Growth Factor binding to the EGFR causes activation of an intracellular cascade that leads to metastasis, angiogenesis, proliferation and an inhibition of apoptosis and differentiation. (Sebolt-Leopold et al., 2006)

In the MAPK pathway, Ras activates B-raf which causes the phosphorylation and the activating of the specific MAP kinases: MEK1 and MEK2 (reviewed in Sebolt-Leopold and English, 2006). Activated MEK phoshorylates ERK1 and ERK 2 (extracellular signal-regulated kinase), which can translocate across the nuclear

membrane, resulting in the activation of numerous transcription factors: ELK1, oestrogen receptor (ER), Myc, peroxisome proliferator-activated receptor-γ (PPAR-γ) and other transcription factors (Davids, 1995; Camp and Tafuri, 1997; Sebolt-Leopold and English, 2006). ELK1, Myc and PPAR-γ are all transcription factors. Furthermore the oestrogen receptor was thought only to play a role in breast cancer, although Giovannini et al. showed that there is an interaction between ER and the EGF receptor (Giovannini et al., 2008). Treatment with oestradiol causes a decrease in EGFR expression, whereas treatment with anti-oestradiol causes an increase in EGFR expression. In addition, Nijkawa et al. showed that 73% of non-small cell lung carcinomas (NSCLC) express higher concentration of estradiol in carcinoma issues than the corresponding non-neoplastic lung tissues from the same patient, and intratumoral oestradiol concentrations were significantly higher than the corresponding non-neoplastic lungs (Nijkawa et al., 2008). The intratumoral concentration of oestradiol was positively correlated with tumor size (Niikawa et al., 2008). Activated ERK also phosphorylates cytoplasmic p90 ribosomal protein S6 kinase (RSK), leading to phosphorylation and inactivation of the pro-apoptotic protein BAD (reviewed in Sebolt-Leopold and English, 2006). RSK activation further promotes cell survival by leading to the phosphorylation of cyclic AMP responsive element binding (CREB), a transcription factor protein. ERK also phosphorylates the Signal Transducers and Activator of Transcription (STAT). STAT is actively transported in the nucleus after phosphorylation. In the nucleus the protein activates transcription of the genes that regulates many aspects of cell growth, survival and differentiation (Sebolt-Leopold and English, 2006).

In the PI(3)K pathway, activated Ras interacts with PI(3)K to generate second-messenger lipids that are critical for activation of numerous target proteins, including the survival signaling kinase AKT/protein kinase B (PKB) (reviewed in Sebolt-Leopold and English, 2006). AKT provides strong anti-apoptotic signals through its negative regulation of Raf, forkhead transcription factors that play important roles in regulating the expression of genes involved in cell growth, proliferation, differentiation, longevity and BAD, an pro-apoptosis protein (Sebolt-Leopold and English, 2006). The PI(3)K–AKT pathway is also important in modulating mammalian target of rapamycin (mTOR), which is a serine/threonine kinase that acts as a central sensor for nutrient/energy availability, thereby regulating cell growth in response to the environment (Sebolt-Leopold and English, 2006).

In conclusion, activating of the EGF receptor by Epidermal Growth Factor leads to a cascade of intercellular signals involving proteins in both the MAPK pathway as well as the PI(3)K pathway, which can both lead to cell proliferation, angiogenesis, invasion, metastasis, and inhibition of differentiation and apoptosis.

EGF receptor in normal tissue and cancer

Between 40 and 80 percent of non small cell lung cancer show an enhanced expression of the EGF receptor (Ciardiello et al., 2004). Overexpression of the EGF receptor can be caused by an increased number of EGFR genes in the DNA (gene amplification) and by mutations in the DNA that lead to an increased number of EGFR gene transcripts and translations. EGFR gene amplification is observed only in approximately 10% of NSCLCs (Ciardiello et al., 2004). Thus, in most cases enhanced transcriptions or related processes are responsible for higher levels of the EGFR. The enhanced expression of the EGF receptor causes, through the earlier explained mechanism, cancer proliferation, angiogenesis, invasion and metastasis. All of these changes are beneficial for the cancer to develop and survive.

The EGF receptor can also be mutated itself. Of the women 16,8 % showed an EGFR mutation and only 2,7 % of the men showed an EGFR mutation (Tapia et al., 2009). The likelihood that a female patient has an EGFR mutation in a lung carcinoma is four times bigger than in male patients (Tapia et al., 2009). The mutations affect mostly the N-lobe (exon18-20) and the C-lobe (exon 21) of the EGFR kinase domain (Kancha et al., 2009). Furthermore, Tapia et al. showed that the majority of mutations are in residues 747—750 of exon 19 (50% of the mutations), followed by a percentage of 12,5 % in the L858R point mutation of exon 21 (Tapia et al., 2009; Costa et al., 2007).

Therapy through EGFR blocking

At the moment there is much research aiming to find a treatment for lung cancer, which works for all patients. It is very difficult to develop an effective treatment, because the mechanisms of cancer are so complex and medicines can target all involved proteins of the pathways. Many studies have investigated the blocking of components in the EGFR pathway, activated by growth factor binding to the EGF receptor. Promising results for example with BAY 43-9006, an inhibitor

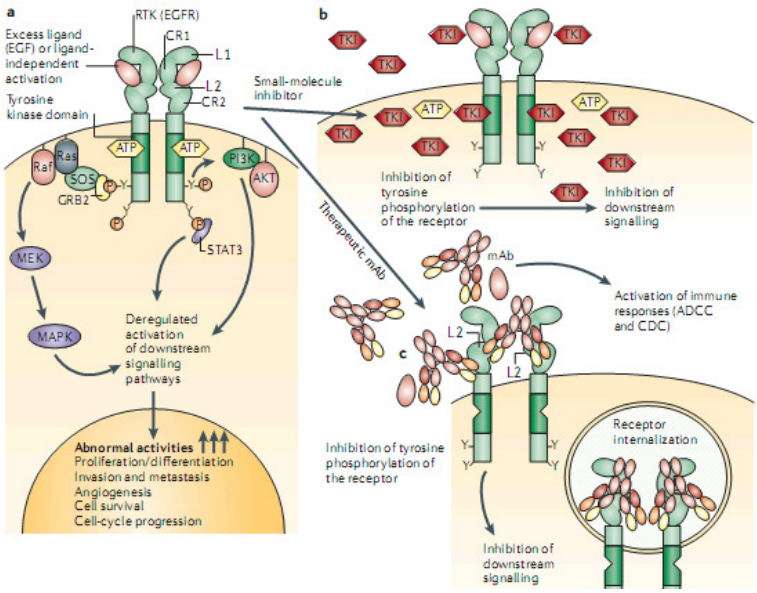

Fig. 2. Mechanism of EGFR blocking. A. Binding of EGF cause activation of signaling pathways leading to abnormal activities as proliferation, differentiation, invasion, metastasis, angiogenesis, cell survival and cell-cycle progression.
B. Blocking of the EGF receptor by binding small molecules (above) and by binding antibodies (below). Both bindings lead to inhibition of tyrosine phosphorylation of the EGF receptor. (Imia et al., 2006)

of Raf Kinase, CCI-779 (Temsirolimus) and RAD001 (Everolimus), both mTOR inhibitors have been reported (Lyons et al., 2001; Katzel et al., 2009). In this study, I shall look at therapies based on the source of these pathways; inhibition of the EGF receptor.

Therapies that block the EGF receptor can be divided in two groups (figure 2). The first group blocks the EGF receptor by using monoclonal antibodies. The second group blocks the intracellular domain of the receptor (the protein-tyrosine kinase domain) by using small molecules to block EGFR-mediated signal transduction which can normally lead to proliferation, metastasis, angiogenesis, and the blocking of apoptosis and differentiation (reviewed in Imia et al., 2006). Below these, different agents are discussed in more detail.

Antibodies

Antibody-mediated therapy is generally designed to block the EGF receptor on its extracellular part. Antibodies bind to the EGF receptor to block the binding of Epidermal Growth Factor, which normally leads to the cascade of intracellular signaling that causes proliferation, metastasis, angiogenesis and the blocking of apoptosis and differentiation. An example of a monoclonal, EGF receptor inhibiting antibody which is already used in treatments is Cetuximab, also known as C225 and Erbitux (Imia et al., 2006). Cetuximab is a human-mouse chimeric monoclonal antibody (IgG1 subtype, Katzel, et al., 2009). Cetuximab alone inhibited the *in vitro* growth of some but not all EGFR-expressing NSCLC cell lines in a dose-dependent manner (Beselga et al., 2000; Raben et al., 2005). Interestingly, EGFR expression did not always correlate with growth inhibition (Raben el al., 2005). Study of Raben et al. showed that Cetuximab reduced EGF-induced phosphorylation of EGFR only in Cetuximab-sentivive cell lines, but not in Cetuximab-resistent cell lines (Raben et al., 2005). Surprisingly, Cetuximab reduces EGF-induced phosphorylation of extracellular regulated kinases 1,2 (pERK) in all EGFR-expressing cell lines (Raben et al., 2005).

At present, also other monoclonal antibodies are tested. Panitumumab and Matuzumab are targeting EGFR at different epitopes. Panitumumab is a fully human antibody (IgGk2 subtype) and binds to domain III of the EGFR, the same domain as Cetuximab (Katzel et al., 2009). Matuzumab is a humanized antibody (IgG1 subtype, 90% human and 10 % murine) and binds to a distinct portion of domain III. Matuzumab sterically blocks the domain rearrangement required for high-affinity binding and receptor dimerization (Katzel et al., 2009).

Small molecules

In contrast to EGFR antibodies that only work on the extracellular part of the EGFR, the small molecules target the EGFR both on the intracellular and the extracellular part (Imia et al., 2006). Extracellularly, the small molecules bind to the EGF receptor to block the activation of the EGF receptor. Intracellularly, the small molecules bind the tyrosine kinase domain which causes the inability for ATP to bind. The tyrosine kinase domain does not become activated, consequently the cascade of intercellular signals can not occur.

It is not new to use small molecules for blocking kinases, many medicines are aimed at the blocking of kinases (De Azevedo et al., 1996; Rabindran et al., 2004). Small molecules are created in a way that they resemble ATP, which normally bind to the ATP binding pocket of a kinase. Kinases convert ATP into ADP and use the free phosphor molecule to modify a substrate. Treatment with small molecules for EGFR inhibition also uses this mechanism. Small molecules bind kinase in the ATP binding pocket and because of its high affinity it is an irreversible reaction. If ATP binds to kinase and becomes ADP the affinity decreases, accordingly this reaction is reversible. ATP is no longer able to bind the kinase and therefore the substrate will not be phosphorylated.

Examples of small molecules that are already used in the clinic are Gefitinib (Iressa) and Erlotinib (Tarceva). Gefitinib and Erlotinib specifically inhibit the EGF receptor (Ready et al., 2005). Small molecules are able to translocate through plasma membrane and interact with the intracellular domain of the receptor and intracellular signaling molecules in competition with ATP. If ATP is not able to bind because of the already bound small molecules, phosorylation will not occur and the cascade of intracellular signals will not be activated.

Clinically, the treatment with Gefitinib and Erlotinib seems partially successful. Yang et al. showed an overall response to Gefitinib treatment of 50.9% (Yang et al., 2008). Although Imia et al. showed responses in only 10 to 20% of previously treated patients, in both Gefitinib and Erlotinib treatment (Imia et al., 2006). Imia et al. also showed that Erlotinib significantly prolonged the survival of patients with NSCLC, whereas Gefitinib did not significantly improved survival (Imia et al. 2006).

Patients with deletions in exon 19 or exon 21 L858R EGFR mutations had statistically higher response rates to Gefitinib compared with patients with other types of EGFR mutations, or wild-type EGFR tumors (Yang et al., 2008). The response rate and median time to treatment failure (TTF) of the patients with exon 19 deletion were respectively 95.0% and 8.9 months and for exon 21 L858R mutation 73.9% and 9.1 month (Yang et al., 2008). In addition, Costa et al. showed that response rates (complete response + partial response) for exon 19 deletion and L858R patients were 80.3% and 81.8% respectively (Costa et al., 2007). Katzel et al. also showed that patients with EGFR mutations have a significant higher response rate on Gefitinib (Katzel et al., 2009). While

the patients without an EGFR mutation have a significant higher response on chemotherapy (Katzel et al., 2009). Patients receiving Gefitinib have an improved quality of life score compared with patients receiving chemotherapy (Katzel et al., 2009). Rosell et al. showed that mutations in the EGFR tyrosine kinase domain also benefit in treatment with Erlotinib. (Rosell et al., 2009) The median survival exceeded 28 months in patients with EGFR mutations treated with Erlotinib, with a median time to progression of 13 months and a two-year survival of 73.3% (Rosell et al., 2009). In addition Ready et al. showed increased likelihood of responding to both Gefitinib and Erlotinib (Ready et al., 2005). Mutations in exon 20 (T790M and A767-V769) are associated with resistance against EGFR inhibitors (such as Gefitinib and Erlotinib). The T790M mutation is responsible for 50 % of the secondary resistance (Tapia el al., 2009). This mutation changes the three-dimensional structure of the ATP bindings place with the consequences that Erlotinib and Gefitinib can not bind anymore (Tapia et al, 2009). Overall, it thus appears that the mutation status of the EGF receptor determines the response rate.

DNA damage in cancer therapy

Chemo- and radiotherapy are often clinically used to treat non small cell lung carcinomas. Both chemotherapy and radiotherapy lead to damage in the DNA, especially double stand breaks (DSB). The goal of both therapies is to activate apoptosis by causing high doses of DNA damage.

Radiotherapy leads to a range of biochemical lesions in the genomic DNA. The lesions that cause the most cell death are the double strand breaks, which can be induced directly through ionization of the DNA and indirectly by the generation of free radials (Ross et al., 1999). Chemotherapy leads especially to double strand breaks in the DNA, although the particular mechanism is still uncertain. As a chemo-therapeutic regimen, Cisplatin (Pt) and other platinating agents are some of the most widely used agents for treating lung cancer (Wagner et al., 2009). In this study, I will therefore only focus on Cisplatin. The majority of (small) lesions made by radio/chemotherapy, are quickly repaired by highly conserved enzymic pathways. Some lesions are not repaired correctly (misrepaired lesions), which may lead to changes of the cell phenotype (Ross et al., 1999). Although cells can adapt to low levels of irreparable damage, it can be sufficient to kill a cell if the DNA has double strand breaks by triggering apoptosis. Cell death occurs when a cell is not able to repair DNA damage completely accurately to protect the cell from the changing of genetic information (Ross et al., 1999).

In response to DNA damage, the DNA damage checkpoint is activated. To activate the DNA damage checkpoint, DSB ends must first undergo extensive 5'-to-3' end resection to generate 3' ssDNA tails (Figure 3). Studies in yeast and other organisms have provided evidence that in dividing cells, such tails are first engaged by the

ssDNA-binding protein replication protein A (RPA), and that the RPA-coated ssDNA plays a key role in DNA damage checkpoint activation by recruiting the PI3 kinase ATR (Mec1 in yeast) through its associated protein ATRIP (Ddc2 in yeast) (Cortez et al., 2001; Zou and Elledge, 2003). The ATR-mediated phosphorylation of downstream effector proteins, such as Chk1, leads to the induction of cell cycle arrest and thus facilitates DNA damage repair (Liu et al. 2000). DSBs are exposing 3' ssDNA tails that are immediately bound by RPA. The RPA-coated ssDNA recruits the ATR–ATRIP complex, leading to the activation of ATR and downstream effectors, such as Chk1, to initiate the checkpoint signalling cascade. Checkpoint activating leads to cell cycle arrest, DNA repair or if it is impossible to repair the cell, to apoptosis. (Raynard et al., 2008).

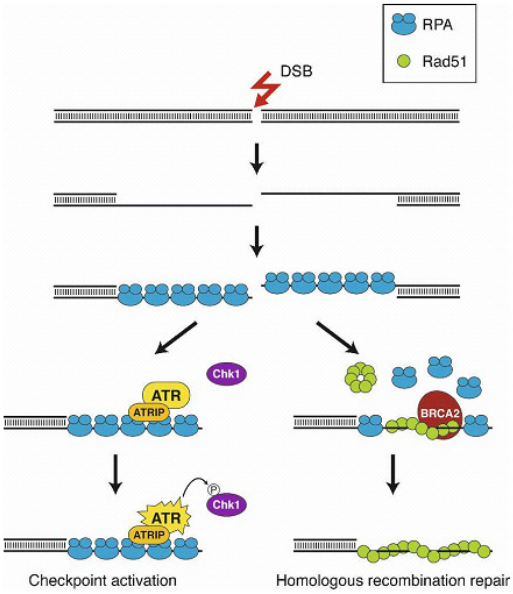

Fig. 3: Activating of the DNA damage Checkpoint. Chemo/radiotherapy leads to double strands breaks. DSBs are processed nucleolytically to expose 3' ssDNA tails that are immediately bound by RPA. The RPA-coated ssDNA recruits the ATR–ATRIP complex, leading to the activation of ATR and downstream effectors such as Chk1 to initiate the checkpoint signaling cascade. For HR repair to occur, RPA is dislodged from the ssDNA and replaced by Rad51 with the assistance of recombination mediator proteins, such as BRCA2, to form the presynaptic filament. (Raynard et al., 2008)

Very important proteins in checkpoint activation are ataxia telangiectasia mutated (ATM) and BRCA1/2. ATM is a Ser/Thr protein kinase and is a member of the phosphoinositide 3-kinase (PI3K)-related protein kinase (PIKK) family, which also includes ATM and Rad3-related protein (ATR), the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs) and SMG1, a protein kinase that is involved in the DNA-damage response (Lavin, 2008). ATM, the product of the AT gene, is emerging as another key participant in the cellular response to ionizing radiation. Sequence comparisons between human mutated in ataxia telangiectasia (ATM) and mouse ATM suggest that the gene is a member of a family of genes involved in cell cycle regulation (TOR1, TOR2, MEC1 of Saccharomyces cerevisiae and rad3 of S. pombe), telomere length monitoring (TEL1 of S. cerevisiae), meiotic recombination (MEC1 of S. cerevisiae and mei41 of Drosophila melanogaster) and DNA repair (DNA-PK), supporting a key role for ATM in DNA DSB repair and cell cycle regulation (Ross et al., 1999). *In vitro*, cells derived from AT heterozygotes demonstrate increased radio sensitivity in comparison with normal individuals, as measured by chromosomal damage (Ross et al., 1999). *In vivo,* defect in the AT gene which is seen by alexia telangiectasia patients, leads to the inability to repair DNA damage (Lavin, 2008). The AT gene, is localized to chromosome 11q22–23 and encodes for ATM, which activates p53 (figure 4, Khanna et

al., 2001; Lavin, 2008). P53 plays a role in activation of the G1- S phase checkpoint and DNA repair (Lavin, 2008). Both processes are damaged in Alexia telangiectasia patients, what often leads to development of cancer.

BRCA1 and BRCA2 are also important proteins in checkpoint activation. Both proteins are co-regulated in the cell cycle and associate with human RAD51, the eukaryotic equivalent of the bacterial recombination protein, recA, which is involved in repair of DSBs and chromosome maintenance (Wang et al., 2001; Ross et al., 1999). This similarity could indicate a role for both genes in the same DNA damage response pathway, either directly involved in repair, or via low level overexpression of p53 induced by failure of repair.

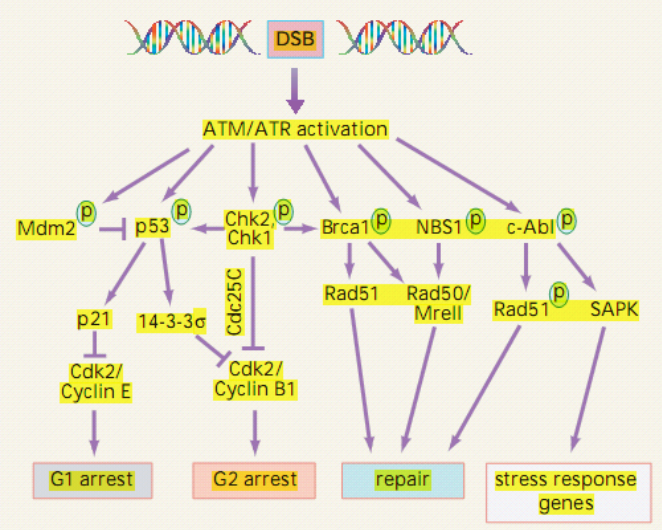

Fig. 4: Mechanism of activating DNA damage repair and DNA damage checkpoints. DSB leads to activation of ATM, which activates p53 (Khanna et al., 2001)

Checkpoint activation gives the cell time to repair the DNA damage. There are two mechanisms for DNA double strand break repair: non-homologous end-joining (NHEJ) and homologous recombination (Hendrikson et al., 1997; Khanna et al., 2001). In the Homologous recombination (HR) extensive homology between the region of DNA with a double strand break and the repair template is required. This pathway involved the RAD52 complex, that acting mainly at late S/G Phase (Hendrikson et al., 1997; Khanna et al., 2001). HR requires Rad52, a DNA-end-binding protein, and Rad51, which forms filaments along the unwound DNA strand to facilitate strand invasion. First the DNA ends resected in the 5' to 3' direction by nucleases (Khanna et al., 2001). The resulting 3'single-stranded tails then invade the DNA double helix of an undamaged, homologous partner molecule. The 3'single-stand is extended by DNA polymerase, which copies the information from the undamaged partner DNA. After branch migration, the resulting DNA crossovers (also called Holliday junctions) are resolved to yield two intact DNA molecules.

In the non-homologous end-joining repair (NHEJ), the DNA-dependent protein kinas (DNA-PK) and the RAD50 (act predominantly during G1/early S phase) complex are involved (Hendrikson et al., 1997; Khanna et al., 2001). NHEJ does not require an undamaged DNA partner and does not rely on extensive homologies between the two recombining end. The two ends are ligated together, sometimes after degradation at the termini (Khanna et al., 2001). NHEJ is often prone to error, and small sequential deletions are usually introduced. DNA

DSBs are considered to be particularly biologically important because their repair is intrinsically more difficult than that of other types of DNA damage (Khanna et al., 2001).

If DNA damage is not fully repaired, apoptosis is trigged. A major pathway of radiation-induced apoptosis involves DNA damage and subsequent induction of a range of genes including ataxia telangiectasia (AT) and p53 (Ross et al., 1999). In the presence of DNA damage, p53-dependent gene transcription is increased and ubiquitin-dependent degradation of the protein is blocked leading to induction of apoptosis and/or cell cycle arrest (Ross et al., 1999).. Activation of p53 is mediated via stress-activated protein kinases. In its latent state p53 can not bind DNA, and it requires phosphorylation to function as a transcription factor. Recent data suggest that DNA-PK is required for the p53 response (Woo et al. 1998). DNA-PK modifies the amino-terminal region of p53, which controls its interaction with the transcriptional apparatus and with MDM2 (Ross et al., 1999).

Radio/chemotherapy also triggers apoptosis. Radiotherapy can lead to apoptosis though the high amount of DNA damage. Chemotherapy can lead to apoptosis though the forming of Platinum adducts on genomic DNA (Pt-DNA), primarily on adjacent guanines. These Pt–DNA adducts inhibit fundamental cellular processes, including replication, transcription, translation and DNA repair by recruiting DNA damage recognition proteins leading to apoptosis. (Ramachandran et al., 2009; Wozniak et al., 2002). Cisplatin exerts its anti-proliferative effects by creating intrastrand and interstrand DNA crosslinks (figure 5), which block DNA replication (Woznaik et al., 2002; Wagner et al., 2009). Intra- and interstrand crosslinks, may cause major local distortions of DNA structure, involving both bending and unwinding of the double helix (Wozniak et al., 2002). In the case of bulky adducts such as intrastrand crosslinks (which comprise the majority of platin-induced lesions), the stalled replication fork triggers the mono-ubiquitylation, a regulatory signal, of Proliferating Cell Nuclear Antigen (PCNA, Wagner et al., 2009). Ubiquitylated PCNA then recruits one or more translesion synthesis (TLS) polymerases, which have active sites that can accommodate bulky lesions, thereby allowing error-prone bypass of the lesion (Wagner et al., 2009). In contrast, interstrand crosslinks, which account for a few percent of cisplatin-induced DNA lesions, are far more cytotoxic and cannot be simply bypassed (Wagner et al., 2009). Instead, their repair involves a complex interplay between a series of DNA repair pathways, including the translesion synthesis and homologous repair (HR) pathways. While the

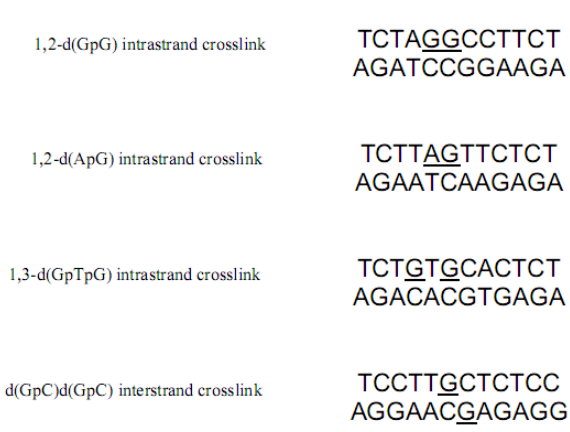

Fig. 5: Schemic representation of Cisplatin adducts. The plastinated nucleosides are underlined. (Wozniak et al., 2002)

complete mechanistic details of how these pathways accomplish this repair remain unknown, it is clear that defects in these pathways dramatically sensitize cells to agents that cause interstrand crosslinks, including the platinating agents (Wagner et al., 2009). A number of cellular proteins, especially those containing the HMG-domain have been shown to bind specifically to PT-GG intrastrand DNA adducts. The binding of structure-specific HMG-domain proteins to Pt–DNA adducts has been shown to inhibit nucleotide excision repair, which may increase the longevity of Pt–DNA adducts. Pt–DNA adducts have also been postulated to sequester some HMG-domain containing transcription factors that are essential for maintaining the uncontrolled cell growth characteristic of tumor cells. Thus, the binding of HMG-domain proteins to Pt–DNA could significantly sensitize cells to Cisplatin.

Taken together, chemo- and radiotherapy leads to double strand breaks and other lesions in the DNA. In response to DNA damage the DNA damage checkpoint is activated, which leads to cell cycle arrest. The most important proteins in checkpoint activation are ATM en BRAC1, 2. DNA damage activates ATM which leads, via BRCA1,2 to cell cycle arrest and double strand break repair. The cell cycle arrest gives the cell more time to repair the DNA. The two mechanisms for DNA double strand break repair are non-homologous end-joining (NHEJ) and homologous recombination (HR). If the cell is not able to repair the DNA damage completely accurately, apoptosis is triggered. Radio/chemotherapy triggers both apoptosis. Radiotherapy leads to apoptosis through the high amount of DNA damage. Cisplatin-based chemotherapy inhibits cellular processes, such as replication, transcription, translation and DNA repair, through formation of platinum adducts on genomic DNA (Pt-DNA), which leads to apoptosis.

Effect of EGFR inhibitors combined with Chemo – and Radiotherapy

The standard treatment of non small cell lung cancer depends on the advance of the disease. In an early stage the best treatment, and accordingly also the standard, is surgical resection (Scott et al., 2009). Radiotherapy remains an important treatment for either cases of early stage NSCLC that are medically inoperable or patients who refuse surgery (Scott el al., 2009). In a later stage surgical resection is not possible because of metastasis. Cisplatin-based adjuvant chemotherapy, an approach that was considered experimental less than two decades ago, is the best and the standard treatment of later staged NSCLC (Chhatwani et al., 2009). The benefits are modest, with 5 to 10% improvement in overall survival at 5 years (Chhatwani et al., 2009). There is still not a good treatment for NSCLC, therefore it is necessary to develop new treatments or, a more efficient and quicker way, to combine existing treatments to improve the performance.

The addition of EGFR inhibition therapy to standard chemo- and radiotherapy regimens for locally advanced NSCLC either with small molecules such as Gefitinib and Erlotinib, or antibody therapy with Cetuximab is promising (Stinchcombel et al., 2009). Ready et al. showed that Cetuximab combined with radiotherapy or chemotherapy is highly effective in locally advanced NSCLC (Ready et al., 2005). In addition, other studies indicated that the combination of Cetuximab with radiotherapy or chemotherapy produced synergistic growth inhibition (Milas et al., 2000; Beselga et al., 2000; Milano et al., 2008; Stinchcombel et al., 2009). In contrast, Raben et al. showed that the cooperative interaction between Cetuximab and radio/chemotherapy only works in Cetuximab-sensitive cells (Raben et al., 2005). Prior studies were done with Cetuximab-sensitive cells, which give the false assumption that Cetuximab combined with radio/chemotherapy works very well in all patients.

There is less success with Gefitinib and Erlotinib in combination with radio/chemotherapy. Several studies showed no significant difference between a combined combination and single treatment. Raben et al. showed combination of chemotherapy and Gefitinib or Erlotinib did not significant improve outcome over chemotherapy alone in NSCLC patients (Raben et al., 2005). In addition, Stinchcombel et al. showed no significant survival benefit in combined treatment with EGFR tyrosine kinase inhibitor therapies with addition to the standard chemotherapy (Stinchcombel et al., 2009).

In conclusion, Cetuximab combined with chemo- or radiotherapy has proved to be successful, despite the fact that it only works in Cetuximab-sensitive cells. For a successful treatment with Gefitinib of Erlotinib combined with radio/chemotherapy more research is necessary.

Why is there an effect of the combined treatment?

Cetuximab, combined with Radio/chemotherapy appears to be a very effective treatment in Cetuximab-sensitive patients. There is less success with kinase inhibitors (Gefitinib and Erlotinib) combined with radio/chemotherapy. The reason that Cetuximab works better in combination with radio/chemotherapy when compared with kinase inhibitors is still unclear. Below a number of potential mechanisms is discussed.

A possible explanation is that chemo/radiotherapy has influence on the cell membrane. Recently it has been recognized that not only the nucleus but also the cell membrane may be important as a target for at least one pathway of radiation-induced apoptosis (Santana et al. 1996). Ionizing radiation activates sphingomyelinase, which catalyses the hydrolysis of sphingomyelin to a lipid second messenger, Ceramide. Ceramide is a lipid molecule which is found in the cell membrane in high concentrations and regulates cell differentiation, proliferation and apoptosis (Ross et al., 1999). It is possible that radiation leads to upregulation of Ceramide in

the Lipid rafts, micro-domains in the cell membrane around receptors involved by membrane protein trafficking and receptor trafficking. The upregulation of Ceramide may cause an interaction with the EGF receptor, which may lead to the blocking of the intercellular signal. So not only Cetuximab, but also the Ceramide in the lipid rafts blocks the intercellular apoptosis signals of the EGFR, which leads to inhibition of proliferation, metastasis, angiogenesis and to inhibitionn of apoptosis and differentiation. But why kinase inhibitors, such as Gefitinib and Erlotinib, combined with chemo/radiotherapy have no significant working advantage could not be explained by this study. It is expected that, if Ceramide interact with the EGFR in that manner, radiotherapy combined with kinase inhibitors would also work efficiently. Maybe it is more difficult for the kinase inhibitors to enter the cell membrane, because of the upgraded Ceramide. Then the kinase inhibitors can only work extracellular. If the extracellular inhibition works better in combination with Chemo/radiotherapy, but the intracellular inhibition is not able to work anymore, it is possible that the defect intracellular inhibition compensate for the better working extracellular inhibition.

Another possible explanation is that DNA damages leads to down regulation of the EGF receptor levels. Garinis et al. showed that long exposure to UV leads to lower expression of the Insulin-like growth factor 1 (IGF1-R) receptor (Garinis et al., 2009). It is possible that a similar reaction occurs in radiotherapy with the EGF receptor. If radiotherapy leads to fewer EGFR, it is logical that EGFR inhibitors, such as Cetuximab, work better in combination with radiotherapy. If there are fewer receptors, it is easier to block the remaining receptors. But why Cetuximab combined with chemotherapy works also better could not be explained by the current study. There is more research necessary to investigate if it also works on the EGFR in stead of the IGF1-R and if it also works with radio/chemotherapy. Maybe long exposure of radiation or accumulation of the chemo-therapeutic regimen Cisplatin, has the same effect as long exposure to UV and will also leads to fewer EGF receptors.

In contrast, Madson et al. showed that the Erbb2 receptor, a family member of EGFR, is activated by UV radiation (Madson et al., 2009). Radiation also leads to checkpoint response causing apoptosis, although this effect is blocked by the Erbb2 receptor. Inhibition of Erbb2 reduced cdc25a levels and increased S-phase arrest in UV-irradiated cells lacking Erbb2 activity. UV-induced Erbb2 activation increases tumorigenesis through inhibitory phosphorylation of Chk1, cdc25a maintenance, and suppression of S-phase arrest via a PI3K/AKT dependent mechanism. It is possible that is mechanism also works in EGFR; radiotherapy activates EGFR and inhibition of EGFR by Cetuximab or kinase inhibitors, leads to checkpoint activation. So blocking of the EGFR before radiation leads to less EGFR activation, causes increased S-phase arrest and suppressing UV-induced tumorigenesis.

Third, Dittmann et al. showed that ionizing radiation triggers EGF receptor import into the nucleus (Dittmann et al., 2003). An increase in nuclear EGFR is also observed after treatment with Cisplatin. During this process, the proteins Ku70/80 and the protein phosphatase 1 are transported into the nucleus. As a consequence, an increase in the nuclear kinase activity of DNA-dependent kinase (DNA-PK) and increased formation of the DNA end-binding protein complexes containing DNA-PK, essential for repair of DNA-strand breaks, occurred. Blockade of EGFR by the anti- EGFR monoclonal antibody Cetuximab abolished EGFR import into the nucleus and radiation-induced activation of DNA-PK, inhibited DNA repair, and increased radio sensitivity of treated cells. More research is necessary to investigate this function of the EGF receptor is a different function or just a subsequent step in the pathway. Or is the EGFR acting different in the nucleus by the changed environment? Or is the mechanism of the sureface EGFR different from the nucleus EGFR.

Fourth, Wang et al showed that nuclear EGFR phosphorylates Tyr211 on PCNA. Moreover, a tyrosine-to-phenylalanine mutant of proliferating cell nuclear antigen (PCNA) stabilizes chromatin-bound PCN protein (Wang et al., 2006). PCNA is an essential protein for DNA replication and damage repair. Increased PCNA Tyr211 phosphorylation coincides with pronounced cell proliferation, and is better correlated with poor survival of breast cancer patients, as well as nuclear EGFR in tumours, than is the total PCNA level. If EGFR inhibitors leads to fewer nuclear EGF receptors, as Dittmann et al. showed, it may cause lower Tyr211 phosphorylation on PCNA (Dittmann et al., 2003). As a consequence there will be less DNA replication, proliferation and DNA damage repair.

Conclusion

It seems almost impossible to create a single treatment that gives good results in all NSCL patients. To treat non-small lung cancer it is very important to analyze individual EGFR status tumor stage, because every carcinoma is different in this respect. If the patient is in a very early stage of NSLC the best treatment is surgical resection. This in only possible if the tumor has not yet metastasized. In case of metastasis NSCL cancer it is very useful to screen the patient for EGFR mutations. There are very good results with Gefitinib or Erlotinib treatment if the patient has a deletion in exon 19 or a L858R EGFR mutation. For the non-mutated patient the best treatment is Cetuximmab combined with chemo/radiotherapy. This combination therapy has showed very good results. The reason why Cetuximab combined with radio/chemotherapy works better than Cetuximab unaccompanied is still uncertain. Probably there is an interaction between Cetuximab and radio/chemotherapy which can act at different levels. It is possible that chemo/radiotherapy leads to an upgrading of Cermide in the lipid rafts, the cell

membrane around the receptor, which inhibits the intercellular signal of the EGFR leading to apoptosis. Another explanation is that DNA damages leads to less expression of EGF receptors. For Cetuximab it is then easier to block the residual receptors. It is not proven that radio- or chemotherapy leads to less expression specifically for EGFR, but maybe it occurs through long radiation or accumulation of Cisplatin. It is also possible that EGFR inhibiting before radiotherapy shows improved results, because radiation activates EGFR. But inhibition of EGFR just leads to S-phase arrest and suppressing of tumorigenesis. So inhibition of EGFR before radiotherapy leads to fewer activated ERG receptors and therefore to increased S-phase arrest and suppressing UV-induced tumorigenesis. A final possible explanation is that radio/chemotherapy triggers EGF receptor import into the nucleus. Blocking of the EGFR decreases the EGFR import activation, which leads to inhibition of DNA repair and increased radiosensitive of treated cells. Fewer nuclear EGFR also leads to decreasing phosporylated Tyr211 level on PCNA, which inhibit replication, proliferation and DNA damage repair.

Although combined treatment seems very efficient, these therapies don't work good enough in all SNCLC patients. Cetuximab combined with radio/chemotherapy is a very promising treatment, but the reason why combined therapies work better than single therapies is still uncertain. There is much more research necessary to investigate the mechanisms of combined treatments. If these mechanisms are better understood, it can be helpful to optimize the current therapies for NSCL cancer.

Reference

Baselga J, Pfister D, Cooper MR, Cohen R, Burtness B, Bos M, D'Andrea G, Seidman A, Norton L, Gunnett K, Falcey J, Anderson V, Waksal H, Mendelsohn J, Phase I Studies of Anti–Epidermal Growth Factor Receptor Chimeric Antibody C225 Alone and in Combination With Cisplatin, Journal of Clinical Oncology, Vol. 18:904-14 (2000)

Camp HS, Tafuri SR. Regulation of peroxisome proliferator-activated receptor gamma activity by mitogen-activated protein kinase, Journal of Biological Chemistry, Vol. 272: 10811-6 (1997)

Chhatwani L, Cabebe E, Wakelee HA, Adjuvant Treatment of Resected Lung Cancer, Proceedings of the American Thoracic Society, Vol. 15:194-200 (2009)

Ciardiello F, De Vita F, Orditura M, Tortora G, The role of EGFR inhibitors in non-small cell lung cancer, Current Opinion in Oncology, Vol. 16: 130-5 (2004)

Collins LG, Haines C, Perkel R, Enck RE, Lung cancer: diagnosis and management, American Family Physicians, Vol. 75: 56-63 (2007)

Cortez, D., Guntuku, S., Qin, J., and Elledge, S.J, ATR and ATRIP: Partners in checkpoint signalling, Science, Vol. 294: 1713–1716 (2001)

Costa DB, Kobayashi S, Tenen DG, Huberman MS, Pooled analysis of the prospective trials of gefitinib monotherapy for EGFR-mutant non-small cell lung cancers, Lung Cancer, Vol. 58: 95-103 (2007)

Davis RJ, Transcriptional regulation by MAP kinases, Mol Reprod Dev., Vol 42: 459-67 (1995)

De Azevedo WF Jr, Mueller-Dieckmann HJ, Schulze-Gahmen U, Worland PJ, Sausville E, Kim SH, basis for specificity and potency of a flavonoid inhibitor of human CDK2, a cell cycle kinase, Proceedings of the National Academy of Sciences, Vol. 93:2735-40 (1996)

Dittmann K, Mayer C, Fehrenbacher B, Schaller M, Raju U, Milas L, Chen DJ, Kehlbach R, Rodemann HP, Radiation-induced epidermal growth factor receptor nuclear import is linked to activation of DNA-dependent protein kinase, The Journal of biological Chemistry, Vol. 280: 31182-9 (2005)

Ferlay J, Autier P, Boniol M, Heanue M, Colombet M, Boyle P, Estimates of the cancer incidence and mortality in Europe in 2006, Annals of Oncology, Vol. 18: 581-92 (2007)

Garinis GA, Uittenboogaard LM, Stachelscheid H, Fousteri M, van Ijcken W, Breit TM, van Steeg H, Mullenders LH, van der Horst GT, Brüning JC, Niessen CM, Hoeijmakers JH, Schumacher B, Persistent transcription-blocking DNA lesions trigger somatic growth attenuation associated with longevity, Nature Cell Biology, Vol. 11: 604-15 (2009)

Giovannini M, Belli C, Villa E, Gregorc B, Estrogen Receptor (ER) and Epidermal growth Factor Receptor (EGFR) as Targets for Dual Lung Cancer Therapy: Not Just a Case?, Journal Thoracic Oncology, Vol. 3:684-685 (2008)

Hendrikson EA, Cell-Cycle Regulation of Mammalian DNA Double-Strand-Break Repair, American Journal for human genetics, Vol. 61: 795-800 (1997)

Imai K, Takaoka A, Comparing antibody and small-molecule therapies for cancer, Nature Reviews Cancer, Vol. 6: 714-27 (2006)

Jensen AD, Münter MW, Bischoff H, Haselmann R, Timke C, Krempien R, Sterzing F, Nill S, Heeger S, Hoess A, Haberkorn U, Huber PE, Steins M, Thomas M, Debus J, Herfarth KK, Treatment of non-small cell lung cancer with intensity-modulated radiation therapy in combination with cetuximab: the NEAR protocol (NCT00115518), BMC Cancer, Vol. 6:122- 29 (2006)

Kancha RK, von Bubnoff N, Peschel C, Duyster J, Functional analysis of epidermal growth factor receptor (EGFR) mutations and potential implications for EGFR targeted therapy, Clinical Cancer Research, Vol. 15: 460-7 (2009)

Katzel JA, Fanucchi MP, Li Z, Recent advances of novel targeted therapy in non-small cell lung cancer, Journal of Hematology & Oncology, Vol. 2: 2-20 (2009)

Khanna KK, Jackson SP, DNA double-strand breaks: signaling, repair and the cancer connection, Nature Genetics, Vol. 27: 247-54 (2001)

Lavin MF, Ataxia-telangiectasia: from a rare disorder to a paradigm for cell signalling and cancer, Nature Revieuws Molecular Cell Biology, Vol. 9: 759-69 (2008)

Liu Q, Guntuku S, Cui XS, Matsuoka S, Cortez D, Tamai K, Luo G, Carattini-Rivera S, DeMayo F, Bradley A, Donehower LA, Elledge SJ, Chk1 is an essential kinase that is regulated by Atr and required for the G(2)/M DNA damage checkpoint, Genes & Development, Vol. 14: 1448–1459 (2000)

Lyons JF, Wilhelm S, Hibner B, Bollag G, Discovery of a novel Raf kinase inhibitor, Endocrine-Related Cancer, Vol. 8: 219-25 (2001)

Madson JG, Lynch DT, Svoboda J, Ophardt R, Yanagida J, Putta SK, Bowles A, Trempus CS, Tennant RW, Hansen LA, Erbb2 Suppresses DNA Damage-Induced Checkpoint Activation and UV-Induced Mouse Skin Tumorigenesis, American Journal of Pathology, 30 April, Epub ahead of print: PMID: 19406993 (2009)

Milano G, Spano JP, Leyland-Jones B, EGFR-targeting drugs in combination with cytotoxic agents: from bench to bedside, a contrasted reality, Britisch Journal of Cancer, Vol. 99: 1-5 (2008)

Milas L, Mason K, Hunter N, Petersen S, Yamakawa M, Ang K, Mendelsohn J, Fan Z., In vivo enhancement of tumor radioresponse by C225 antiepidermal growth factor receptor antibody, Clinical Cancer Research, Vol. 6: 701-8 (2000)

Niikawa H, Suzuki T, Miki Y, Suzuki S, Nagasaki S, Akahira J, Honma S, Evans DB, Hayashi S, Kondo T, Sasano H, Intratumoral Estrogens and Estrogen Receptors in Human Non-Small Cell Lung Carcinoma, Clinical Cancer Research, Vol. 14:4417-26 (2008)

Parkin DM, Bray F, Ferlay J, Pisani P, Global cancer statistics, 2002, CA: Cancer Journal for Clinicians, Vol. 55: 74-108 (2005)

Raben D, Helfrich B, Chan DC, Ciardiello F, Zhao L, Franklin W, Barón AE, Zeng C, Johnson TK, Bunn PA Jr, The effects of cetuximab alone and in combination with radiation and/or chemotherapy in lung cancer, Clinical Cancer Research, Vol. 15:795-805 (2005)

Rosell R, Perez-Roca L, Sanchez JJ, Cobo M, Moran T, Chaib I, Provencio M, Domine M, Sala MA, Jimenez U, Diz P, Barneto I, Macias JA, de Las Peñas R, Catot S, Isla D, Sanchez JM, Ibeas R, Lopez-Vivanco G, Oramas J, Mendez P, Reguart N, Blanco R, Taron M, Customized treatment in non-small-cell lung cancer based on EGFR mutations and BRCA1 mRNA expression, PloS ONE Vol. 4: e5133 (2009)

Ross GM, Induction of cell death by radiotherapy, Endocrine-Related Cancer, Vol. 6:41-4 (1999)

Ready N, Inhibition of the Epidermal Growth Factor Receptor in Combined Modality Treatment for Locally Advanced Non–Small Cell Lung Cancer, Seminars in Oncology, Vol. 32:S35-41 (2005)

Ramachandran S, Temple BR, Chaney SG, Dokholyan NV, Structural basis for the sequence-dependent effects of platinum-DNA adducts, Nucleic Acids Research, Vol. 47:2434-48 (2009)

Raynard S, Niu H, Sung P, DNA double-strand break processing: the beginning of the end, Genes & Development., Vol. 22:2903-7 (2008)

Santana P, Peña LA, Haimovitz-Friedman A, Martin S, Green D, McLoughlin M, Cordon-Cardo C, Schuchman EH, Fuks Z, Kolesnick R, Acid sphingomyelinase-deficient human lymphoblasts and mice are defective in radiation-induced apoptosis, Cell, Vol. 86: 189-199 (1996)

Scott WJ, Howington J, Feigenberg S, Movsas B, Pisters K; American College of Chest Physicians, Treatment of Non-small Cell Lung Cancer Stage I and Stage II: ACCP Evidence-Based Clinical Practice Guidelines (2nd Edition), Chest, Vol. 132: 234S-242S (2007)

Sebolt-Leopold JS and English JM, Mechanisms of drug inhibition of signalling molecules, Nature, Vol. 25: 457-62 (2006)

Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, Levin WJ, Stuart SG, Udove J, Ullrich A, et al., Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer, Science, Vol. 244:707-12 (1989)

Srinivasan R, Benton E, McCormick F, Thomas H, Gullick WJ, Expression of the c-erbB-3/HER-3 and c-erbB-4/HER-4 Growth Factor Receptors and Their Ligands, Neuregulin-1 alpha, Neuregulin-1 beta , and Betacellulin, in Normal Endometrium and Endometrial Cancer, Clinical Cancer Research, Vol. 5:2877-83 (1999)

Stinchcombe TE and Socinski MA, Current Treatments for Advanced Stage Non–Small Cell Lung Cancer, Proceedings of the American Thoracic Society, Vol. 15:233-41 (2009)

Tapia C, Savic S, Bihl M, Rufle A, Zlobec I, Terracciano L, Bubendorf L., EGFR mutation analysis in non-small-cell lung cancer : Experience from routine diagnostics, Der Pathologe, April 10, Epub ahead of print: PMID: 19357847 (2009)

Wagner JM, Karnitz LM, Cisplatin-induced DNA damage activates replication checkpoint signaling components that differentially affect tumor cell survival, Molecular Pharmacology, April 29, Epub ahead of print: PMID: 19403702 (2009)

Wang H, Zeng ZC, Bui TA, DiBiase SJ, Qin W, Xia F, Powell SN, Iliakis G, Nonhomologous end-joining of ionizing radiation-induced DNA double-stranded breaks in human tumor cells deficient in BRCA1 or BRCA2. Cancer research, Vol. 61:270-7 (2001)

Wang SC, Nakajima Y, Yu YL, Xia W, Chen CT, Yang CC, McIntush EW, Li LY, Hawke DH, Kobayashi R, Hung MC, Tyrosine phosphorylation controls PCNA function through protein stability, Nature Cell biology, Vol. 8: 1359-68 (2006)

Woo RA, McLure KG, Lees-Miller SP, Rancourt DE, Lee PW, DNA-dependent protein kinase acts upstream of p53 in response to DNA damage, Nature, Vol. 394:700-704 (1998)

Woźniak K, Błasiak J, Recognition and repair of DNA-cisplatin adducts, Acta Biochimica Polonica, Vol. 49: 583-96 (2002)

Yang CH, Yu CJ, Shih JY, Chang YC, Hu FC, Tsai MC, Chen KY, Lin ZZ, Huang CJ, Shun CT, Huang CL, Bean J, Cheng AL, Pao W, Yang PC, Specific EGFR mutations predict treatment outcome of stage IIIB/IV patients with chemotherapy-naive non-small-cell lung cancer receiving first-line gefitinib monotherapy, Journal of Clinical Oncology. Vol. 26: 2745-53 (2008)

Zou L and Elledge SJ, Sensing DNA damage through ATRIP recognition of RPA–ssDNA complexes, Science, Vol. 300:1542–1548 (2003)