Mechanisms determining efficacy of tyrosine kinase-targeting anti-cancer drugs

Maja Bradić Lindh
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You have not lived a perfect day, even though you have earned your money, unless you have done something for someone who will never be able to repay you.

~ *Ruth Smeltzer* ~

The human spirit is stronger than anything that can happen to it.

~ *George C. Scott* ~

Do not follow where the path may lead. Go instead where there is no path, and leave a trail ...
ABSTRACT

The expanding field of cancer research and the introduction of multiple molecular biology techniques have significantly improved cancer drug discovery, presented new drugs, including tyrosine kinase inhibitors, and thereby gave new hope to the cancer patients and their oncologists. Using this knowledge it is now becoming increasingly possible to determine outcome of malignant disease more accurately, to estimate response to specific therapies and to get closer to the ideal of personalized targeted therapy adjusted to each patient, and to the specific cancer type and its unique genetic profile. This thesis tries to explain some mechanisms lying behind the sensitivity or the resistance to new targeted therapies of two cancer types: glioblastoma (GBM) and colorectal cancer (CRC).

The IGF-1- and PDGF- receptor tyrosine kinases are widely expressed in GBMs and involved in their self-sustained proliferation, glioblastoma tissue invasion, resistance to apoptotic stimuli, insensitivity to growth control signals and are thus potential therapeutical targets. The first study of the thesis analyzed the sensitivity of GBM cultures to the IGF-1 receptor inhibitor NVP-AEW541. This small molecule caused variable reduction in growth of GBM cultures, allowing grouping of cultures as “responders” and “non-responders”. Use of the IGF-1 receptor siRNA gave very similar result. The sensitivity to NVP-AEW541 was significantly reduced in cultures with PIK3CA mutations or ligand-independent Akt phosphorylation and in a “responder cell culture” treated with PTEN siRNA as well. Combination treatments with either PI3 kinase- or mTOR-inhibitors together with NVP-AEW541 resulted in prominent reduction in growth of “non-responders”. In the second study, the prognostic or predictive potential of PDGFRs status was evaluated, as a translational part of the randomized CST1571BDE40 trial, which compared hydroxyurea monotherapy and a combination of hydroxyurea and imatinib, a small molecule targeting PDGFRs in recurrent glioblastoma patients. Both PDGFR alpha protein expression and phosphorylation correlated with worse outcome, but did not define a group that showed benefit from the combination therapy with hydroxyurea and imatinib. The third study deals with putative cancer stem cells, associated with radio- and chemo-resistance of GBMs. Gene expression analyses of 11 GBM cell cultures identified two subsets - designated type I and type II cultures. The type I cultures showed high expression of CXCR4, SOX2, EAAT1 and GFAP and low expression of CNP, PDGFRB, CXCL12 and extracellular matrix proteins. Type I cultures had a higher capacity to form xenograft tumors and neurospheres, displayed low or no sensitivity to mono-treatment with PDGF- and IGF-1-receptor inhibitors, but were growth inhibited by combination treatment with low concentrations of the two inhibitors. SOX2 down-regulation conferred sensitivity to PDGF- and IGF-1-receptor inhibitors.

The fourth study describes the influence of one part of the tumor microenvironment, activated fibroblasts, on sensitivity of colorectal cancer cells to cetuximab, a monoclonal EGFR-targeting antibody. LIM1215 colorectal cancer cells were growth-inhibited by cetuximab. However, when co-cultured with PDGF stimulated fibroblasts they become significantly less sensitive to cetuximab with regard to inhibition of growth, migration and invasion. LIM1215 cells co-cultured with activated fibroblasts displayed increased c-met phosphorylation suggesting fibroblast-produced HGF as a mediator of the protective effects of fibroblasts.
LIST OF PUBLICATIONS

I. Daniel Hägerstrand*, Maja Bradić Lindh*, Cristina Peña, Carlos-Garcia Echeverria, Monica Nistér, Francesco Hofmann and Arne Östman
PI3K/PTEN/AKT status affects the sensitivity of high grade glioma cell cultures to the insulin like growth factor-1 receptor inhibitor NVP-AEW 541
Neuro Oncology. 2010 Sep;12(9):967-75.

II. Janna Paulsson, Maja Bradić Lindh, Malin Jarvius, Marjut Puputti, Monica Nistér, Nina N. Nupponen, Werner Paulus, Ola Södeberg, Gregor Dresemann, Andreas von Deimling, Heikki Joensuu, Arne Östman and Martin Hasselblatt
Prognostic but not predictive role of platelet-derived growth factor receptors in patients with recurrent glioblastoma

III. Daniel Hägerstrand*, Xiaobing He*, Maja Bradić Lindh*, Saskia Hoefs, Göran Hesselager, Arne Östman and Monica Nistér
Identification of a SOX2-dependent subset of tumor- and sphere-forming glioblastoma cells with a distinct tyrosine kinase inhibitor sensitivity profile
Under revision in Neuro-Oncology

IV. Maja Bradić Lindh, Cristina Peña, Patrik Andersson, Martin Augsten and Arne Östman
PDGF-dependent effects of cancer associated fibroblasts on sensitivity of colorectal cancer cells to cetuximab
Manuscript

*Authors contributed equally
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<tr>
<td>AKT or PKB</td>
<td>protein kinase B</td>
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<tr>
<td>AML</td>
<td>acute myeloid leukemia</td>
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<td>APC</td>
<td>adenomatous polyposis coli gene</td>
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<td>BRACA 1 and 2</td>
<td>breast cancer type 1 and 2 susceptibility protein</td>
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<td>BRAF</td>
<td>v-Raf murine sarcoma viral oncogene homolog B</td>
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<td>CAF</td>
<td>cancer-associated fibroblast</td>
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<td>CML</td>
<td>chronic myeloid leukemia</td>
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<td>COX-2</td>
<td>cyclooxygenase 2</td>
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<td>CR</td>
<td>complete response</td>
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<td>CRC</td>
<td>colorectal cancer</td>
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<tr>
<td>DFS</td>
<td>disease free survival</td>
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<td>EGF/R</td>
<td>epidermal growth factor/receptor</td>
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<td>FAP</td>
<td>familial adenomatous polyposis</td>
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<td>FDA</td>
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<td>5-FU</td>
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<td>GBM</td>
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<td>GIST</td>
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<td>HGF</td>
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<td>HNPCC</td>
<td>hereditary nonpolyposis colon cancer</td>
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<td>HER2/neu</td>
<td>human epidermal growth factor receptor 2</td>
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<td>IDH1</td>
<td>isocitrate dehydrogenase 1</td>
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INTRODUCTION

Involvement of tyrosine kinases in tumor growth

Protein tyrosine kinases
Tyrosine kinases (TK) and protein tyrosine phosphatases (PTP) play a central role in the regulation of cell signal transduction processes. Their activity is tightly controlled in normal cells. The human genome encodes approximately 90 tyrosine kinases and 40 protein tyrosine phosphatases. TKs are divided into receptor tyrosine kinases (RTK) and cytosolic tyrosine kinases.

RTK are activated by phosphorylation after the ligand binding to the extracellular domain (Schlessinger 2000). Once phosphorylated, they trigger a downstream signaling cascade resulting in activation or suppression of certain genes in the cell nucleus that regulate cell proliferation, differentiation, migration and programmed cell death.

Cytosolic tyrosine kinases are, in most cases, components of intracellular pathways activated by RTKs or other cell surface proteins.

Tyrosine kinases as drivers of tumor growth
Malignant tumor growth is determined by the advantage of malignant cells in survival and growth, and by tumor-associated stroma processes. TKs contribute to oncogenic signaling not only in malignant cells, but also in cells in tumor stroma such are pericytes and endothelial cells in blood and lymph vessels, cancer associated fibroblasts (CAFs), leukocytes and macrophages (Blume-Jensen and Hunter 2001).

Activation of tyrosine kinases in malignant cells
To acquire advantage in growth and survival and ability to invade and migrate, malignant cells often have activating genetic or epigenetic changes in TK encoding genes. Their protein products are known as oncogenic TKs (Klein et al. 2005).

Mutational activation of oncogenic TKs can occur through gene amplifications, chromosomal translocations, point mutations and small deletions.

Gene amplifications. Gene amplification leads to over expression of RTKs leading to constitutive, ligand independent, kinase activation.

One example is HER2/neu (ERBB2) which is amplified in breast cancer. HER2+ breast cancer is characterized by aggressive clinical behavior. Assessment of HER2 expression status on breast cancer biopsies through immunohistochemistry therefore has been incorporated into diagnostic and prognostic routine.

Activating translocations. Genomic re-arrangements, such as chromosomal translocations, can result in oncogenic fusion protein characterized by ligand independent receptor dimerization.
In chronic myeloid leukemia (CML) the t(9; 22) translocation, also known as the Philadelphia chromosome, encodes the constitutively active fusion protein Bcr-Abl (Bernstein 1988; Groffen et al. 1984). In chronic myelomonocytic leukemia (CMML) the t(5;12) translocation creates a gene encoding ligand-independent platelet-derived growth factor receptor-beta (PDGFR-beta) (Golub et al. 1994).

**Point mutations and small deletions.** Gain of function mutations or small deletions lead to RTKs with enhanced enzymatic activity. In gastrointestinal stromal tumors (GIST) more than 80% of tumors have activated mutant c-kit (stem cell factor receptor) (Rubin et al. 2001). Mutant EGF receptor is responsible for malignant behavior and growth of non-small cell lung cancer (NSCLC) in subset of NSCLC patients (see below).

**Target tyrosine kinases in tumor stroma**
Prominent RTKs involved in angiogenesis and recruitment of cancer associated fibroblasts (CAFs) are the vascular endothelial growth factor (VEGF) and PDGF receptor TK subfamilies (Sawyers 2004).

Activated VEGFR induce endothelial cell proliferation and the formation of new blood vessels in tumors. VEGF is a ligand with high affinity binding to the extracellular domain of two homologous endothelial cell membrane receptors: VEGFR-1 and VEGFR-2 (Olsson et al. 2006; Shibuya and Claesson-Welsh 2006). Platelet-derived growth factor (PDGF) receptor signaling participates in different processes in solid tumors, including recruitment of tumor stroma fibroblasts, and stimulation of tumor angiogenesis by pericyte recruitment to tumor vessels (Ostman and Heldin 2007).

**Novel cancer drugs targeting growth factor signaling and their clinical effects**
Tyrosine kinases form a significant share of all oncproteins and have now become important targets for the new generation of “targeted drugs”. Inhibitors of tyrosine kinases can broadly be divided into monoclonal antibodies and low-molecular weight (LMW) inhibitors.

**TK-targeting antibodies**
Antibodies act extracellularly and show high specificity. They, most commonly, block signaling by preventing ligand-receptor interactions by binding either to the ligand or the receptor. Mostly, they are combined with the standard chemotherapy specific for treatment of different cancer types. As macromolecules they require intravenous (i.v.) administration.

*Trastuzumab (Herceptin)* was the first FDA-approved tyrosine kinase targeting drug (February 2000). The antibody is binding to the extracellular part of HER2 receptor which is amplified in approx. 25% of breast cancers. Indication for application are in adjuvant setting of lymph node positive, HER2 expressing breast cancer after surgery (approved 2005) and in recurrent metastatic HER2 positive breast cancer.
In metastatic HER2 positive disease the patients randomized to trastuzumab and chemotherapy compared with patients randomized to chemotherapy alone, experienced a significantly longer median time to disease progression (TTP) (7.4 vs. 4.6 months), a higher overall response rate (ORR) (50% vs. 32%), a longer median duration of response (9.1 vs. 6.1 months). The same group had longer median survival (25.1 vs. 20.3 months) and one year survival (79% vs. 68%) (Slamon et al. 2001).

Next approved indication was use of trastuzumab for the adjuvant treatment of women with node-positive, HER2-overexpressing breast cancer, as part of a standard chemo regimen with doxorubicin, cyclophosphamide, and paclitaxel, or as a single agent following anthracycline chemotherapy treatment. The approval was based on 50% reduction in recurrence rate after 1 year treatment in women receiving trastuzumab and chemotherapy compared to those receiving chemotherapy alone (Piccart-Gebhart et al. 2005; Romond et al. 2005).

Recently FDA granted approval for trastuzumab, in combination with cisplatin and a fluoropyrimidine, for the treatment of patients with HER2-overexpressing metastatic gastric or gastroesophageal junction adenocarcinoma who didn’t receive prior treatment for metastatic disease. The approval is based on results of an international multicenter randomized clinical trial, which enrolled 594 patients with locally advanced or metastatic HER2-overexpressing stomach- or GE junction adenocarcinoma. Median overall survival was 13.8 months in combination group, with trastuzumab plus chemotherapy, compared with 11.1 months in chemotherapy alone group (Bang et al. 2010).

**Bevacizumab (Avastin)** works by targeting VEGF-A ligand in a manner that prevents it from binding to the angiogenic VEGFR in vascular endothelial cells. It was the first tyrosine kinase inhibitor to target tumor associated stroma.

The FDA approved bevacizumab in 2004 as a first-line treatment for metastatic colorectal cancer in combination with intravenous 5-FU-based chemotherapy. In the phase III randomized, double blind study with 800 patients the group receiving irinotecan/5-fu/leucovorin (IFL) plus bevacizumab showed a median survival of 20.3 months vs. 15.6 months for the group given IFL plus placebo. Progression free survival was 10.6 vs. 6.2 months respectively. The corresponding rates of response were 44.8 % and 34.8% (Hurwitz et al. 2004). Bevacizumab is also indicated in combination with intravenous 5-FU-based chemotherapy for second-line treatment of patients with metastatic CRC (Giantonio et al. 2007).

In 2006 bevacicumab was approved in combination with carboplatin and paclitaxel for the first-line treatment of patients with unresectable, locally advanced, recurrent or metastatic non-small cell lung cancer (NSCLC). This recommendation was based on the results from randomized, phase III study. The OS was significantly longer in patients receiving bevacizumab in combination with paclitaxel and carboplatin as compared to those receiving paclitaxel and carboplatin alone (median OS 12.3 vs 10.3 months) (Sandler et al. 2006).

2008, the FDA granted accelerated approval for bevacizumab to be used in combination with paclitaxel for the treatment of patients who have not received chemotherapy for metastatic HER2-negative breast cancer. The approval was based on the demonstration of an improvement
in progression-free survival (PFS) in patients receiving bevacizumab with paclitaxel compared to those receiving paclitaxel alone as a first-line treatment for metastatic breast cancer. The addition of bevacizumab to paclitaxel resulted in an improvement in PFS with no significant improvement in overall survival. The median PFS was 11.3 vs. 5.8 months for the bevacizumab plus paclitaxel arm versus the paclitaxel alone. Partial response rates in patients with measurable disease were higher with bevacizumab plus paclitaxel (48.9% vs. 22.2%). No complete responses were observed (Miller et al. 2007).

The researchers are exploring bevacizumab uses in other cancers, as well. One of them is advanced kidney cancer. In the international phase III, double blind study, with 649 patients with advanced kidney cancer, patients were randomly assigned to receive either bevacizumab plus interferon or a placebo plus interferon. Disease progression was delayed nearly twice as long in patients treated with the bevacizumab combination vs. the placebo group (median TTP 10.2 vs. 5.4 months). Although there was a trend toward longer survival for patients treated with the bevacizumab combination, the follow-up period was too short for the researchers to be certain that the survival difference was not due to chance (Escudier et al. 2007b).

Bevacizumab was FDA approved 2009 for the 2nd and 3rd line treatment of recurrent GBM patients according to the results of an open-label, phase II clinical study where patients received irinotecan in combination with bevacizumab. 35 patients with GBM were included after they relapsed and treatment with radiotherapy and temozolomide failed. The 6-month PFS among all 35 patients was 46%, 6-month OS was 77%. Twenty of the 35 patients had at least a partial response (Vredenburgh et al. 2007).

Bevacizumab have shown activity in several phase II clinical studies involving recurrent ovarian cancer patients, which may be explained by the dual antitumor and anti-angiogenic effects on the VEGF. Bevacizumab may be useful when incorporated into treatment together with first-line platinum/taxane therapy (P.G. Rose 2009; Penson et al. 2010).

**Cetuximab (Erbitux)** is a monoclonal antibody targeting EGFR. Cetuximab is used for the treatment of locally or regionally advanced squamous cell carcinoma of the head and neck (SCCHN), in combination with radiation therapy (RT) or as a single agent for the treatment of patients with recurrent or metastatic SCCHN for whom prior platinum-based therapy has failed.

Between 1999 and 2002, a total of 424 patients with SCCHN stage III or IV were enrolled in phase III trial and randomly assigned to receive radiation therapy alone or radiation plus weekly cetuximab. All patients had tumors in their tonsils, tongue, or voicebox that may have involved lymph nodes. Median survival for patients treated with cetuximab+RT vs. radiotherapy alone was 49 vs. 29.3 months. 55% of the cetuximab-treated patients survived for three years, compared with 45 % of those in the radiation-only group. The median PFS was 24.4 months for combined therapy vs. 14.9 months for radiation alone (Bonner et al. 2006).

Adding cetuximab to platinum-based chemotherapy with fluorouracil (platinum/fluorouracil) in metastatic SCCHN patients significantly prolonged the median overall survival (10.1 vs. 7.4 months), the cetuximab+chemo group had prolonged median progression-free survival time (5.6 vs. 3.3 months) and increased response rate (36% vs. 20%)(Vermorken et al. 2008).
FDA approved cetuximab (2004) for second line treatment of EGFR-expressing metastatic colorectal carcinoma in combination with irinotecan in patients who are refractory to irinotecan-based chemotherapy. In the cetuximab+chemotherapy group, response rate was significantly higher than that in the monotherapy group (22.9% vs. 10.8%). Median time to progression was 4.1 vs. 1.5 month; median survival in the combination group was 8.6 vs. 6.9 months in the control group (Cunningham et al. 2004).

Cetuximab was approved for first line treatment for metastatic CRC in the combination with chemo, according to the results shown in the randomized study with cetuximab plus irinotecan, fluorouracil, and leucovorin (FOLFIRI) vs. FOLFIRI alone. Patients in the combination treated group had reduced the risk of disease progression by 15% and increased the response rate by nearly 10%. After the study was closed it was noted that only patients with wt K-Ras tumors benefited from cetuximab treatment (Van Cutsem et al. 2009).

A study in NSCLC reported positive results with cetuximab and chemotherapy as first-line treatment. Patients with advanced non-small-cell lung cancer (NSCLC) who received cetuximab plus chemotherapy lived on average 5 weeks longer than patients who received chemotherapy alone (Lynch et al. 2010).

Panitumumab was FDA approved for treatment of chemorefractory metastatic CRC patients. The approval was based on the results of a single, open label, randomized, multinational study that enrolled 463 patients with metastatic colorectal cancer. Patients were randomly assigned to either best supportive care (BSC) alone or BSC plus panitumumab. Mean PFS time was 13.8 weeks for panitumumab and 8.5 weeks for BSC, after a 12-month minimum follow-up (Giusti et al. 2008).

The use of cetuximab and panitumumab are in Europe restricted to CRC patients without K-Ras mutations (see below).

Low-molecular weight tyrosine kinase inhibitors (LMW-TKIs)
The small molecules, inhibitors of tyrosine kinases, bind directly to the intracellular kinase domain where they act as competitive inhibitors of ATP-binding. They are orally administered drugs.

FDA approved LMW-TKIs
Imatinib mesylate (Glivec/Gleevec) was the first member of this category of drugs to be approved by FDA based on its activity in chronic myeloid leukemia. Imatinib blocks the kinase activity of the tyrosine kinases abl, c-kit (SCF-1 receptor) and PDGF alpha- and beta-receptors. Imatinib is now standard of care for chronic myelogenous leukemia, where it acts by blocking the Bcr-Abl protein encoded by the fusion gene of the Philadelphia chromosome. This drug is also approved for treatment of patients with gastrointestinal stromal tumors (GISTs). In patients with this tumor-type, 85% have tumor mutations in c-kit.

The pivotal study for FDA approval of imatinib for CML treatment in 2001 was a phase I, dose-escalating trial for 83 patients with CML in the chronic phase in whom treatment with interferon alpha had failed. Complete hematologic responses were observed in 53 of 54 patients treated. Of
the 54 patients treated with doses of 300 mg or more, cytogenetic responses occurred in 29, including 17 with major responses. 7 of these patients had complete cytogenetic remissions (Druker et al. 2001). Survival data from a randomized clinical trial with 5 years of follow-up (the International Randomized Study of Interferon and STI571 (IRIS) study, involving 1000 CML patients, confirmed imatinib superiority to interferon-alpha plus ARA-C, when 5 year OS was achieved in 89% of the patients (Druker et al. 2006)

Imatinib was approved for treatment of recurrent GIST according to the results from the randomized study in 2001, where 147 patients with far-advanced, bulky disease were recruited to receive the imatinib treatment vs. placebo. 79 patients (53.7%) had a partial response, 41 patients (27.9%) had stable disease (Demetri et al. 2002).

Imatinib was approved in 2007 for adjuvant treatment of GIST as a result of phase III double blind randomized study with adjuvant imatinib vs. placebo in GIST patients following the resection of primary tumor. After one year of treatment there was no recurrence in 97% of patients in the imatinib group vs. 83% of patients in the placebo group. No difference in overall survival was reported in the original study. Patients with tumors larger than 10 cm benefited more from imatinib than those with smaller tumors (Dematteo et al. 2009).

Additional indications for imatinib include hypereosinophilic syndrome or chronic eosinophilic leukemia; relapsed or refractory Philadelphia chromosome positive acute lymphoblastic leukemia; dermatofibrosarcoma protuberans; myelodysplastic/myeloproliferative disorders and systemic mastocytosis (Apperley et al. 2002; Gleich et al. 2002; McArthur et al. 2005).

Dasatinib (Sprycel) is a LMW TKI of SRC-family protein-tyrosine kinases, which is structural distinct from imatinib. Apparently because it binds to BCR-ABL kinase with second mutation and some other tyrosine kinases such as c-Kit, PDGFR beta, FYN, SRC and EPH receptor-A2, dasatinib has ability to overcome the imatinib resistance of chronic myeloid leukemia (CML) cells with BCR-ABL kinase domain point mutations (Bradeen et al. 2006; Branford et al. 2002). Another indication is treatment of adults with Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL) with resistance or intolerance to prior therapy.

FDA granted accelerated approval (2006) of dasatinib for use in the treatment of adults with chronic phase (CP), accelerated phase (AP), or myeloid or lymphoid blast (MB or LB) phase chronic myeloid leukemia (CML) with resistance or intolerance to prior therapy, including imatinib. In a phase II study Investigators enrolled 42 patients with lymphoid blast crisis (LBC) and 74 patients with myeloid blast crisis (MBC). All patients were either resistant or intolerant to imatinib. Among patients with MBC, 34% had a major hematologic response at eight months of follow-up and from patients with LBC, 31 % had a major hematologic response at both 6 and 8 months of follow-up. 27% of MBC patients and 43% of LBC patients had a complete cytogenetic response (Cortes et al. 2007; Talpaz et al. 2006).

In a multinational study chronic-phase CML patients were randomly assigned to receive dasatinib or imatinib with the primary end point of complete cytogenetic response at 1 year. Dasatinib induced significantly higher and faster rates of complete cytogenetic response and has been associated with better long-term, progression-free survival as compared to imatinib (Kantarjian et al. 2010).
Nilotinib (Tasigna) is Bcr-Abl kinase inhibitor, approved to treat Ph(+) chronic- and accelerated phase chronic myelogenous leukemia (CML). It is used in adult patients who cannot take imatinib mesylate or whose condition got worse while taking it.

It was approved 2007 after phase II nilotinib study with 280 patients who had also all been previously treated with imatinib. Either that drug had stopped being effective against their disease or they could no longer take it because of side effects. After at least six months of follow-up, nearly half of the patients responded to nilotinib treatment. In 31% the Ph chromosome was eliminated from the bone marrow and in 16 % it was reduced to low levels. Patients whose disease had become resistant to imatinib and those no longer able to take imatinib because of side effects responded equally well to treatment with nilotinib (Kantarjian et al. 2007).

In a phase III study, untreated, chronic-phase, Ph(+) CML patients who received nilotinib had significantly higher complete cytogenetic response at 1 year of 80% compared to 65% for imatinib treated group (Saglio et al. 2010).

Erlotinib hydrochlorid (Tarceva) is a LMW TKI that inhibits the EGFR. Erlotininb was approved for treatment of patients with metastatic chemo-resistant non-small cell lung cancer (NSCLC). A number of recent studies suggest that this drug is particularly active in a subset of patients which have activating mutations and/or amplification of the EGF receptor in their tumors (see below).

Erlotinib was FDA approved (2004) for the second or third line treatment of NSCLC patients according to the results from double-blind, multinational, randomized trial, with 731 patient, comparing erlotinib hydrochloride to placebo. Survival was significantly prolonged in the erlotinib arm with a median overall survival of 6.7 vs. 4.7 months in placebo group. Progression-free survival (PFS) was significantly prolonged for erlotinib group (Shepherd et al. 2005).

Erlotinib is also approved in 2005 to be used together with a gemcitabine as a first-line therapy for metastastatic and/or unresectable pancreatic cancer, as a result of single, multicenter, double-blinded, placebo-controlled, randomized, phase III study of erlotinib hydrochloride plus gemcitabine (EG) versus gemcitabine plus placebo (PG). Erlotinib plus gemcitabine gave a 27% increase in survival and reduced the risk of death by 21% compared with treatment with gemcitabine alone (Moore et al. 2007).

Gefitinib (Iressa), an EGFR LMW TKI, was FDA approved 2003 for patients with NSCLC based on the 10% response rate in patients taking the drug. But after additional two phase III clinical trials which showed no gefitinib survival benefit, the indications were limited to cancer patients who, in the opinion of their treating physician, are currently benefiting, or have previously benefited, from gefitinib treatment.

Recently, new randomized, phase III clinical studies comparing gefitinib with standard chemotherapy, selected previously untreated NSCLC patients with either the exon 19 deletion or L858R point mutation of the EGFR. In one study this subgroup of NSCLC showed better response rate, 73.7% for gefitinib vs. 30.7% for standard chemotherapy group, and better median overall survival, 30.5 months for gefitinib group vs. 23.6 months for the chemotherapy group. In
the second study with the similar design primary endpoint was progression free survival which was 9.2 months in the gefitinib group vs. 6.3 months in the chemotherapy group (Maemondo et al. 2010; Mitsudomi et al. 2010).

**Lapatinib ditosylate (Tykerb)** is LMW-TKIs for use in combination with capecitabine (Xeloda) for the treatment of patients with advanced or metastatic breast cancer whose tumors over express HER2 (ErbB2) and who have received prior therapies including trastuzumab. It inhibits also EGFR (ErbB1).

FDA approval (2007) of lapatinib was based on phase III, randomized trial, where HER2+ breast cancer patients, having relapsed to anthracycline, taxane, and trastuzumab treatment, received either combination lapatinib+capecitabine or capecitabine alone, with median TTP 27.1 and 18.6 weeks respectively (Geyer et al. 2006).

**Sorafenib (Nexavar) and sunitinib (Sutent)** are representatives of a category of small molecule-drugs sometimes referred to as „multi-kinase-inhibitors“. These drugs block both VEGF- and PDGF-receptors. Their inhibitory profile allows them to exert potent anti-angiogenic activity through simultaneous targeting of endothelial cells and pericytes which are dependent on VEGF- and PDGF-receptors, respectively.

Sorafenib was FDA approved (2005) after two studies in patients with advanced renal cell carcinoma. Both studies showed that patients treated with sorafenib had longer time before tumor progression or death. In the larger, phase III, double-blind study, with 900 patients, most of them had previously received treatment with interleukin-2 or interferon. The median time to tumor progression or death in the sorafenib arm was 167 vs. 84 days in the placebo arm (Escudier et al. 2007a).

Sorafenib was also approved in 2007 for the treatment of metastatic hepatocellular carcinoma according to finding in multicenter, phase III, double-blind, placebo-controlled trial. 602 patients with advanced hepatocellular carcinoma were randomized to receive either sorafenib or placebo as a first line treatment. Median overall survival in the sorafenib group was 10.7 vs. 7.9 months for the placebo group. The median time to radiologic progression was 5.5 vs. 2.8 months (Llovet et al. 2008).

Previously untreated elderly patients (≥70 years) with stage IIIIB or IV NSCLC were randomized to receive sorafenib with either erlotinib or gemcitabine in phase II clinical study. The combination of erlotinib and sorafenib was feasible and was associated with a longer 1-year survival rate than the sorafenib and gemcitabine arm (Gridelli et al. 2011).

**Sunitinib** prolong time to progression after first-line treatment of advanced renal cell cancer patients and it’s also active in patients with imatininb-resistant GIST. This activity involves inhibition of imatininb-resistant variants of c-kit.

FDA approved (2006) sunitinib for the treatment of advanced renal cell carcinoma based on results from the multicenter, international randomized trial enrolling 750 patients with treatment-naive metastatic renal cell carcinoma. Patients were randomized to receive either sunitinib or
interferon-alpha. The median progression-free survival was significantly longer in the sunitinib group (11 vs. 5 months in interferon alpha group (Motzer et al. 2007).

Additional approval (2006) for sunitinib was done after a phase III trial, with 312 GIST patients with relapse of GIST after imatinib therapy. Disease progression was delayed in sunitinib treated patients for 27.3 vs. 6.4 weeks, compared with placebo group. This means that patients taking sunitinib were 67% less likely to suffer a return of GIST than those taking a placebo. Overall survival was also prolonged by sunitinib. At six months, in sunitinib group 13.4% patients had died vs. 26.3% from placebo group (Demetri et al. 2006).

Other promising LMW-TKIs

*ARQ 197* is a small molecule, MET inhibitor, which efficacy was tested in a randomized, placebo-controlled, phase II study designed to compare treatment with ARQ 197 plus erlotinib to erlotinib plus placebo in patients with locally advanced or metastatic non-small cell lung cancer. The study included patients who received one prior chemotherapy regimen (other than erlotinib) for NSCLC. The result was promising – PFS was increased 1.5 months for the combination group. The effects were especially good for patients who had tumors without mutations in EGFR or RAS (L.V. Sequist 2010).

Oncogenic fusion genes consisting of EML4 and anaplastic lymphoma kinase (ALK) are present in a subgroup of 2 to 7% non-small-cell lung cancers and are more common in younger, non-smoker patients. *Crizotinib*, a small molecule that targets rearranged ALK gene in patients with non-small cell lung cancer (NSCLC), showed promising therapeutic effects in a phase I clinical study. After 6 months from the beginning of the treatment 33% of the patients had stable disease and the overall response rate was 57% with one confirmed complete response, compared to 10 % response rate after second-line standard chemotherapy treatment (Kwak et al. 2010).

Targeted therapies with other mechanism of action

*Temsirolimus (Torisel) and everolimus (Certican, Afinitor)* are two synthetic analogues of antibiotic rapamycin (sirolimus), which inhibits mTOR. After promising results in preclinical models, these drugs failed to show benefit in 6-months PFS (only 2.5%-7.8%) in phase II clinical studies, as a monotherapy treatment for GBM (Chang et al. 2005; Galanis et al. 2005).

Temsirolimus was FDA approved in 2007 for the treatment of advanced renal cell carcinoma (RCC) after a phase II randomized, 3 arm study, comparing interferon-alpha alone, temsirolimus or the combination of temsirolimus+interferon-alpha. Single-agent temsirolimus was associated with a statistically significant improvement of 10.9 months in OS vs. 7.3 months on the interferon arm. Median PFS was 5.5 months on the temsirolimus arm vs. 3.1 months on the interferon arm. Combination of two agents didn’t give additional benefit compared to interferon alone, on the contrary, it gave more adverse effects (Kwitkowski et al. 2010).

Everolimus was approved in 2009 for the treatment of patients with advanced RCC resistant to sunitinib or sorafenib. The efficacy and safety of everolimus were evaluated in an international, randomized, double-blind study comparing everolimus to placebo. The median PFS was in the everolimus arm 4.9 and 1.9 months in placebo arm (Motzer et al. 2008).
FDA granted in 2010 accelerated approval to everolimus for patients with subependymal giant cell astrocytoma (SEGA) associated with tuberous sclerosis (TS) who require therapy but are not candidates for surgical resection. The efficacy and safety of everolimus was shown in a single-arm, open-label clinical trial involving 28 kids 11-year median age, with SEGA. Nine of 28 patients had more than 50% and rest 21 patients had at least 30% reduction in the volume of their largest SEGA lesion at 6 months. Median response duration for the 9 responding patients was 266 days. No complete responses were observed (Krueger et al. 2010).

The majority of malignant melanomas harbour B-Raf (V600E) activating somatic mutations which gives the therapeutic opportunity to test oncogene-targeted treatment for this disease. A multicenter, phase I, dose-escalation study in advanced malignant melanoma patients treated with PLX4032 (also known as RG7204), small molecule, inhibitor of mutated B-Raf, resulted in around 80% response rate. There is a possibility to apply high doses of PLX4032 because this drug targets the mutant form of the B-Raf protein without affecting wt B-Raf in normal cells (Flaherty et al. 2010).

Several forms of cancer are more dependent on poly (ADP-ribose) polymerase (PARP) than regular cells, making PARP an attractive target for targeted cancer therapy. Olaparib is a PARP inhibitor that induces synthetic lethality in homozygous BRCA-deficient cells. Olaparib is, by inhibiting the DNA repair, causing accumulation of specific DNA lesions in the malignant cells. Some breast and ovarian tumors lack functional BRCA1 and BRCA2 specialized repair mechanisms and that’s why they are 1000-fold more sensitive to PARP inhibition than normal cells (Ashworth 2008). Olaparib’s efficacy and safety was tested in a phase II clinical study involving 70 women with advanced ovarian cancer carrying BRCA1 or BRCA2 mutations. The primary efficacy endpoint, objective response rate (ORR), was 33% with 2% CR and 27% PR for the patients treated with 400 mg olaparib twice daily, with acceptable toxic adverse effects (Audeh et al. 2010).

Mutations in hedgehog pathway genes, commonly genes encoding patched homologue 1 (PTCH1) and smoothened homologue (SMO), occur in basal-cell carcinoma, brain tumors, lung, esophagus, stomach, pancreas, biliary tract, breast and prostate cancer (Scales and de Sauvage 2009). GDC-0449 is a small-molecule inhibitor of SMO, which showed antitumor activity in phase I clinical studies for the locally advanced or metastatic basal-cell carcinoma and medulloblastoma (Rudin et al. 2009; Von Hoff et al. 2009). In another phase I study 68 patients with different solid tumors refractory to standard treatments were treated with GDC-0449. These were the patients diagnosed for advanced basal cell carcinoma, pancreatic cancer, medulloblastoma and 17 other types of cancer. Tumor responses were observed in 20 patients, 14 patients had stable and 28 had progressive disease (Lorusso et al. 2011).

Ipilimumab, a monoclonal antibody binding to the CTLA4 receptor, activates T cells directly by blocking a brake on T-cell activity, has shown promising results in a phase III study (from 2010) on 676 patients diagnosed for unresectable, metastatic melanoma who had previously failed standard melanoma chemotherapy. Ipilimumab nearly doubled the rates of survival at 12 months (46% vs 25%) and 24 months (24% vs 14%) compared with the peptide control treatment. The new phase III study in advanced malignant melanoma patients comparing ipilimumab+dacarabazine vs dacarabazine alone will possibly confirm earlier results (Weber et al. 2009; Wolchok et al. 2010).
**Denosumab** *(Xgeva)* is a monoclonal antibody that binds to RANKL, a ligand for the RANK receptor on the osteoclasts. It was FDA approved in 2010 for the prevention of skeletal-related events (SREs) in patients with bone metastases from solid tumors. By binding to the RANKL denosumab blocks proliferation and survival of the osteoclasts and resorption of the bone in bone cancer metastasis which prevents pathological fractures of the bones. The approval is based on results from three international randomized trials in patients with bone metastases showing superiority of denosumab treatment - significantly less SREs compared to zoledronic acid. First trial enrolled 1,901 patients with hormone-refractory prostate cancer, second enrolled 2,046 patients with breast cancer, and the third enrolled 1,776 patients with advanced multiple myeloma or solid tumors other than breast or prostate cancer (Fizazi et al. 2009; Stopeck et al. 2010; Vij et al. 2009).

**Determinants of resistance and sensitivity**

**Responses to TKIs targeting malignant cells are predominantly occurring in tumors with genetically activated TKs**

Mutated TK receptors are constantly transducing antiapoptotic and proliferative signals making malignant cell dependent - “addicted” - on this signaling. Therefore, blocking of such TKs by TKIs is predicted to give inhibitory tumor effect.

As a good example of the relationship between sensitivity to TK inhibitor and presence of activated mutations in drug target, is **mutated c-kit in gastrointestinal stromal tumors (GIST)**. This tumor-type expresses almost homogenous point mutation of c-kit in 85% of cases. A similar situation is the presence of t(9;22) translocation in more than 90% of cases of CML resulting in constitutively active Bcr-Abl fusion protein.

Another drug supporting this concept is trastuzumab which was, also, from the beginning given to specific preselected subgroup of **breast cancer patients over expressing HER2 receptor** (25% of patients). Detection of expression of HER2 in the tumor tissue is now standard in every hospital in Europe and an important marker for the planning of the treatment (Holden et al. 2008).

In the case of erlotinib use in NSCLC, the initial studies were performed on an unselected patient population. After that, the clinical development led to the identification of a novel subpopulation of NSCLC patients, with specific **somatic mutations**, which was in their case, “driving” mutation. Somatic mutations in EGFR are found in 10% to 15% of Caucasian and in 30% to 40% of Asian NSCLC patients (Sequist et al. 2007). Analysis of tumors from erlotinib studies subsequently showed that benefit from the treatment was much bigger in the patients with EGFR mutations or amplification (Ciardiello and Tortora 2008).

Similar observation has been made with another EGFR inhibitor, gefitinib, used for the treatment of NSCLC patients. With this drug major effects are observed in patients harboring either the exon 19 deletion or L858R point mutation of the EGFR (Maemondo et al. 2010; Mitsudomi et al. 2010).
**Mutational activation of downstream signaling molecules is common in solid tumors**

After the ligand induced activation of the RTK, effector molecules are recruited, such as phospholipase C, PI3-kinase and Ras. These conduct information further on, downstream through the complex network of signaling pathways to the nucleus, resulting in regulation of activity of different genes important for the cell survival, growth or apoptosis. A series of pre-clinical studies have predicted that mutational activation of signaling downstream from RTKs will lead to resistance to drugs targeting RTKs (Lievre et al. 2006; Pao et al. 2005). Clinical data is now being accumulated which support this concept (Figure 1.) (see below).

Deregulation of the PTEN/PI3K/Akt pathway leads to malignant transformation. The gene encoding PI3K catalytic subunit p110a (PIK3CA) has been found mutated in several malignancies: colon, lung, ovaries, liver, brain, stomach and breast (Samuels et al. 2004). **PTEN** is a tumor suppressor, PI (phosphoinositide) 3-phosphatase. It inhibits cellular proliferation and survival through inactivating PI3K -dependent signaling. PTEN is one of the most commonly mutated or lost tumor suppressors in human cancer. PTEN loss has been reported in endometrial cancer (35%) and glioblastoma (54%) as well as in other cancer types such as breast (32%), CRC (30%), prostate (27%) and lung cancer (19%) (Frattini et al. 2007; Nagata et al. 2004).

**Ras** mutations conducting downstream signaling independent of RTK activation status is also very common in different types of cancer. K-Ras mutations are frequently detected in NSCLC (30%), CRC (50%) and pancreatic cancer (90%). N-Ras mutations have been reported in hematological malignancies (25%), melanoma, and hepatocellular carcinoma and H-Ras mutations in kidney, thyroid and bladder carcinomas (Schubbert et al. 2007).

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<th>Sensitivity to RTK inhibitor:</th>
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Figure 1. The type of growth factor pathway activation, used by a particular tumor, is predicted to determine the sensitivity to different targeted therapies. Modified from Östman A, 2010

**B-Raf** serine-threonine kinase is in the pathway between RAS and MAP kinase. It is mutated in malignant melanoma (70%), and at lower frequency in other malignancies (thyroid cancer, CRC, ovarian, lung cancer). ERK is constitutively active in melanoma cells with mutated B-Raf giving them advantage in growth.
Clinical studies on resistance-determinants

**Cetuximab** was FDA approved TKI for treatment of EGFR-expressing metastatic CRC in combination with irinotecan-based chemotherapy. In subsequent studies subgroups of patients were retrospectively analyzed for **K-Ras** mutational status. Response to cetuximab appeared to be restricted to patients without mutations in the K-Ras gene. In another similar phase II study comparing FOLFOX+cetuximab vs. FOLFOX alone, only patients with wt K-Ras status treated with the combination cetuximab+chemo obtained benefit in both RR (61% for combination vs. 37% chemo alone), and PFS (7.7 months vs. 7.2 months) (Van Cutsem et al., 2009). Similar findings were reported in the case of panitumumab, another monoclonal antibody approved for treatment of metastatic CRC patients (Amado et al. 2008). Because of these findings both cetuximab and panitumumab are in Europe approved only for use in CRC without K-Ras mutations.

The loss of expression of **PTEN** protein has been observed in 30% of CRC and may be a useful marker in predicting response to cetuximab (Frattini et al. 2007). The first clinical study supporting this notion included 27 metastatic CRC patients after recurrence. They were cetuximab-treated, evaluated for drug response and investigated for EGFR protein and gene status, K-Ras mutational status and PTEN protein expression. None of the 11 patients with loss of PTEN expression responded to the treatment with cetuximab, while response was observed in 10 out of 16 patients with intact PTEN expression.

Another clinical study analyzed 110 metastatic CRC patients treated either with cetuximab or panitumumab. Mutational analyses were performed for **PIK3CA** and K-Ras together with the evaluation of PTEN expression, to clarify how these genes affect clinical response to anti-EGFR–TKIs. 13.6% of the patients had PIK3CA and 29% K-RAS mutations. PIK3CA mutations were significantly associated with resistance to both MoAbs. None of the patients with mutations achieved objective response. Patients with PIK3CA mutations displayed a worse clinical outcome also in terms of progression-free survival. In summary, 70% of the CRC patients were found to have alterations in either the PIK3CA/PTEN or K-Ras pathways and none of them responded to the MoAb treatment (Sartore-Bianchi et al. 2009).

In the wt K-Ras patients, **B-Raf** mutations could have a predictive/prognostic value since the B-Raf is principal effector of K-Ras. In a single arm clinical study, involving 113 metastatic CRC treated either with cetuximab or panitumumab, mutational analysis were performed for K-Ras and B-Raf. 30% of the patients had K-Ras mutations and were resistant to MoAb treatment; 11% of patients had B-Raf mutations and none of them responded to the treatment. B-Raf-mutated patients had significantly shorter progression-free survival and OS compared to the B-Raf wt. In this study some in vitro experiments were done. Introduction of mutant-B-Raf V600E allele decreased the inhibitory effect of cetuximab or panitumumab in CRC cells, while treatment with sorafenib-B-Raf inhibitor restored sensitivity to both cetuximab and panitumumab in these cells (Di Nicolantonio et al. 2008)

**Trastuzumab** is inhibiting growth of HER2-overexpressing breast cancer cells through the down regulation of HER2 and the subsequent inhibition of its downstream PTEN/ PI3K/Akt signaling pathway. A study on 47 metastatic breast cancer patients who received trastuzumab+taxan vs. a
control group of 37 patients treated with taxane alone, was performed to determine clinical significance of PTEN expression status in predicting response to trastuzumab. Less than 35% of HER2 positive breast cancer patients responded to trastuzumab treatment. PTEN-negative tumors demonstrated a highly significant worse response to trastuzumab plus taxane therapy than patients with PTEN-positive tumors (11.1% vs. 65.8% CR+PR) (Nagata et al. 2004). In addition, in this study, some in vitro experiments were done which support clinical data. SiRNA PTEN downregulation in responsive breast cancer cells decreased sensitivity to trastuzumab, and introduction of PTEN in resistant breast cancer cell line restored sensitivity to the TK inhibitor. Additionally, PI3K inhibitors could overcome PTEN loss-induced trastuzumab resistance, suggesting a therapeutic solution for the future treatment of subgroup of HER2+ PTEN- breast cancer patients.

In another study with a cohort of 55 breast cancer patients, activation of the PI3K pathway, estimated by detection of mutated PI3K or low PTEN expression, was associated with poor prognosis after trastuzumab treatment, and the joined analysis of PTEN and PI3K identified twice as many patients at increased risk for progression compared to PTEN alone. Oncogenic mutants of PI3K also conferred resistance to trastuzumab in breast cell cultures in vitro (Berns et al. 2007).

In the case of treatment with the EGFR-targeting LMW-TKIs erlotinib and gefitinib similar type of data are emerging. Glioblastomas (GBM) have frequent EGFR over expression or mutation, but only 10 to 20% of patients respond to EGFR kinase inhibitors. Tumor material from 49 GBM patients was collected in a clinical study with erlotinib or placebo to analyze determinants of resistance and sensitivity. EGFR mutations and PTEN expression levels were determined and correlated with drug response and outcome. Coexpression of EGFR deletion mutant variant III (EGFRvIII) and PTEN was significantly associated with a clinical response. In vitro, coexpression of EGFRvIII and PTEN sensitized glioblastoma cells to erlotinib (Haas-Kogan et al. 2005; Mellinghoff et al. 2005).

Similar to CRC and cetuximab treatment, active only in wt K-Ras patients, only wt K-Ras lung adenocarcinoma patients will benefit from erlotinib/gefitinib. The study to first prove this relationship was single arm study, with 60 lung adenocarcinoma patients. Retrospectively tumor tissue was analyzed for EGFR and K-Ras mutations. K-Ras mutations were found in 24% of tumors. None of these cases displayed any response to EGFR TKI treatment. All cases with no K-Ras mutations responded to the treatment (Pao et al. 2005). Another mechanism of resistance to erlotinib/gefitinib is secondary mutations EGFR (T790M). Patients whose tumors harbor this secondary mutation develop resistance to these inhibitors (Engelman and Janne 2008).

Secondary resistance can develop in both chronic phase (CP) and advanced phase (AP) CML patients. Point mutations in the kinase domain of the BCR/ABL enzyme are the most frequent mechanism of secondary or acquired resistance. These mutations cause amino acid substitutions inside the kinase domain of the BCR/ABL protein, impairing imatinib binding and resulting in a loss of sensitivity to the drug. Two new small molecules, dasatinib and nilotinib were approved for treatment of CML patients with acquired imatinib resistance (Cortes et al. 2007; Talpaz et al. 2006). Several different mechanisms of imatinib resistance in GIST have been described. Among these are secondary mutations in c-kit which generate c-kit variants which are not blocked by imatinib (Heinrich et al. 2006).
Glioblastoma

Glioblastoma (GBM) is the grade IV, or the most malignant, type of the brain tumor. It is accounting for approximately 60-70% of astrocytic tumors. The incidence is in range 3-4 new cases per 100 000 population per year in most of the European countries with peak incidence between 45 and 75 years of age (Ohgaki and Kleihues 2005). GBM occurs most often in the subcortical white matter of the combined fronto-temporal zone, infiltrating towards adjacent cortex and to the contralateral hemisphere. In approximately 9-11% of patients, GBM is multifocal from the beginning.

Expansive and infiltrative growth of GBM can produce generalized and focal symptoms. Generalized symptoms, as a consequence of raised intracranial pressure, can include headache confusion, lethargy, personality change and somnolence. Focal symptoms can involve seizures, sensory or motorical dysfunction of the limb or half of the body, speech problems, trouble with vision, etc. The clinical history of the disease is usually short, less than 3 months for 50% of the patients. Median survival is 10 months, with less than 5% of patients surviving 5 years. Standard treatment involve surgery followed by radio- and chemotherapy (Gaya et al. 2002)

Primary and secondary glioblastoma

Primary or de novo glioblastoma makes up to 95% of newly diagnosed GBMs. They develop more rapidly without evidence of less malignant precursor lesion and are typically occurring in older patients (mean age 62 years). Secondary GBMs develop more slowly, through progression from diffuse astrocytoma (WHO grade II) or anaplastic astrocytoma (WHO grade III) and they are typical for younger patients (mean age 45 years).

Primary and secondary GMBs are thus relatively distinct disease entities affecting different age group of patients, developing through different genetical pathways, showing different expression profiles and having different response to treatment.

Genetical alterations of primary and secondary glioblastomas

GBMs, like other cancers, display characteristic malignant phenotypes, including self-sustained proliferation, resistance to apoptotic stimuli, insensitivity to growth control signals and immunsurveillance, tissue invasion and ability to form and develop blood vessels.

Malignant transformation which leads to primary or secondary GBM is a multistep process in which new mutations are acquired as the disease progresses (Fig.2 A and B).

The hallmarks of primary glioblastoma are mutation in INK4a (30-40%), EGFR amplification (40%) and loss of heterozygosity of 10q (LOH 10q). The loss of negative regulator of PI3K, PTEN, has been described in 25% of primary GBMs. TP53 mutations were described in approximately 30% of the primary GBMs (2008; Verhaak et al. 2010).

On the other hand majority of secondary GBMs harbor TP53 mutations. The metabolism related isocitrate dehydrogenase 1 (IDH1) gene is mutated in 70% of secondary GBMs with a similar mutation frequency in the grade II and III gliomas that give rise to the secondary GBMs (Yan et
al. 2009). Dysregulation of EGFR, INK4a and PTEN are less frequent in secondary GBMs (Noushmehr et al. 2010; Ohgaki et al. 2004).

LOH 10q is equally common in both primary and secondary GBMs. Other common mutations are activating mutations of PI3CA in about 30% of GBMs.

Beside the amplified, overexpressed or mutated EGFR, oncogenesis in GBMs is driven by several other activated growth factor receptor signaling pathways. These are platelet derived growth factor receptor (PDGFR), insulin growth factor-1 receptor (IGF-1R) and vascular endothelial growth factor receptor (VEGFR), driving angiogenesis in GBMs (Hagerstrand et al. 2006; Hermansson et al. 1988; Sandberg et al. 1988).

![Diagram](image.png)

Fig.2 A) Major signaling pathways involved in pathogenesis of glioblastomas. B) Timing and frequency of genetic alterations during glioblastoma development. LOH 10q is frequent in primary and secondary glioblastomas, and TP53 mutations are early and frequent genetic alterations leading to secondary glioblastomas. Modified from Ohgaki et al, 2004 and 2009.

**Standard treatment of glioblastoma**

Tumors of the CNS are usually diagnosed by magnetic resonance imaging (MRI). The next step in the diagnostic procedure is stereotactic surgical biopsy followed by histological grading and neurosurgical removal of the tumor. MRI, even as independent diagnostic tool, can show correlation with good or bad outcome. Patients with presence of radiographic necrosis and heterogeneity of tumor density have shorter survival. Regarding neurosurgery, patients who undergo more radical surgery have better prognosis (Franco Cavalli 2004a).
Both external beam radiation therapy (conformal and whole brain) and maximum tolerated doses of chemotherapy with temozolomide gave 46% 1 year overall survival compared to 40% with radiotherapy alone (Schonekaes et al. 2002; Stupp et al. 2005). The epigenetically silencing of MGMT gene, in about 45% of GBMs, is good predicative marker for response to temozolomid (Fukushima et al. 2009; Hegi et al. 2005). When this gene is silenced, glioblastoma cells have less capability of repairing their DNA damaged by alkylating agents as temozolamid (Esteller et al. 2000).

Despite existing treatments most of GBMs recur at or within 1-2 cm of their original location. Due to low morbidity and mortality associated with aggressive resection, glioblastoma patients commonly undergo more than one surgical resection. The survival advantage from reoperation can be as good as for standard chemotherapy with acceptable morbidity, but still not better.

Because of the lack of effective chemotherapies, new therapies targeting underlying pathogenesis of glioblastomas are obviously needed.

**Targeted therapy of glioblastoma**

Based on genetical alterations in GBMs described above, and knowing that these alterations are “driving” tumor growth, invasion, migration and survival, there has been several attempts to target these pathways.

**Targeting EGFR**

A monoclonal antibody targeting EGFR, **cetuximab (Erbitux)** has shown preclinical antitumor activity as a single agent and combinatorial effect together with radiation therapy against EGFR-amplified GBM (Combs et al. 2008). Cetuximab underwent clinical evaluation in patients with recurrent GBMs in a two-arm, open-label, phase II study where patients were stratified according to their EGFR gene amplification status. As a single agent, cetuximab was well tolerated but had limited activity in this patient population with progressive glioblastoma (Neyns et al. 2009). Cetuximab has also been tested together with irinotecan and bevacizumab for the treatment of 43 patients with recurrent primary GBM. This phase II clinical study demonstrated that cetuximab, bevacizumab plus irinotecan, had an acceptable safety profile and induced a considerable number of clinically relevant, durable responses. The response rate was 26%, 6-month, PFS was 33% and median PFS was 16 weeks for the combination group (Hasselbalch et al. 2010).

Two reversible, small molecules, tyrosine kinase inhibitors, **gefitinib (Iressa) and erlotinib (Tarceva)**, have been evaluated in GBMs. Several phase I and II studies on recurrent GBM patients have been performed using gefitinib or erlotinib as a single agent. The response rates varied from 0%-14% and the median PFS was approximately 8-12 weeks while 6-months PFS rate varied in the range of 9%-14.3% (Franceschi et al. 2007; Mellinghoff et al. 2005; Rich et al. 2004). Retrospective studies demonstrated that high expression of wild-type EGFR and low levels of phosphorylated AKT or coexpression of EGFRvIII and wild-type PTEN were associated with increase in radiographic response after treatment with erlotinib or gefitinib (Haas-Kogan et al. 2005; Mellinghoff et al. 2007).
Targeting PDGFR

**Imatinib (Gleevec, STI571)**, a small molecule, tyrosine kinase inhibitor of PDGFR, c-kit and Bcr-Abl kinases, showed antiglioma activity in preclinical studies (Hagerstrand et al. 2006; Kilic et al. 2000). Despite that, monotherapy with imatinib failed to show benefit for GBM patients in phase I/II clinical studies (Wen et al. 2006). In the combination with the hydroxyurea (HU), imatinib has demonstrated modest but promising activity in the initial pilot study including 30 patients where 32% 6-months PFS was achieved (Dresemann 2005). This finding was confirmed in phase II study with 6-months PFS of 27% involving 33 GBM patients (Reardon et al. 2005). Based on these two studies a multicenter, two arm, phase III study was performed involving 240 recurrent GBM patients. The objective was to evaluate if a combination of imatinib and HU was superior to HU alone in prolonging PFS. No significant differences were found in median PFS between the two treatment arms (Dresemann et al. 2010).

Targeting VEGF/VEGFR

**Bevacizumab (Avastin)**, monoclonal antibody against VEGF, was FDA approved 2009 for the 2nd and 3rd line treatment of recurrent GBM patients according to the results of an open-label, phase II clinical study where patients received irinotecan in combination with bevacizumab. 35 patients with GBM were included after they relapsed and treatment with radiotherapy and temozolomide failed. The 6-month PFS among all 35 patients was 46%, 6-month OS was 77%. Twenty of the 35 patients had at least a partial response (Vredenburgh et al. 2007).

**Vatalanib (PTK787/ZK222584)** a small molecule, tyrosine kinase inhibitor of VEGFR and PDGFR, has demonstrated modest efficacy in multicenter phase I/II studies either alone or in combination with different cytostatic drugs (Brandes et al. 2010). Another pan-VEGFR inhibitor, **cediranib (AZD2171)**, has shown encouraging antiangiogenic efficacy with 56% radiographic response and 30% 6-months PFS in the phase II clinical study (Batchelor et al. 2007).

Targeting m-TOR

**Temsirolimus (Torisel) and everolimus (Certican)** are two synthetic analogues of antibiotic rapamycin (sirolimus), which inhibits mTOR. After promising results in preclinical models, these drugs failed to show benefit in 6-months PFS (only 2.5%-7.8%) in phase II clinical studies, as a monotherapy treatment for GBM (Chang et al. 2005; Galanis et al. 2005).

Colorectal cancer

Every year 1.2 million cases of colorectal cancer (CRC) are diagnosed globally and 600 000 patients die from this disease. Long-term relative survival for these patients is for the Nordic countries and most other countries in Europe between 50%-60% and slightly above 60% for USA. The difference in the survival can be mainly explained by the higher proportion of adenomatous polyps in the US population, not registered as cancers in e.g. Sweden. CRC is the fourth most common cancer type in the USA and the third leading cause of cancer-related death in the Western world (Franco Cavalli 2004b).

The great majority of CRCs arise from premalignant lesions-adenomatous polyps which develop into advanced adenomas with high-grade dysplasia and then progress to an invasive cancer. CRC is rare under the age of 45. The incidence and the cumulative risk are increasing with age. Thorough and regular screening of people in the risk group will lead to detection of asymptomatic
and earlier stages of disease, when is potentially treatable and curable resulting in reduction of morbidity and mortality of CRC. According to CRC recommendations for average-risk individuals, by American Cancer Society, screening should start at the age of 50 with annual fecal occult blood test and either flexible sigmoidoscopy every 5 years or colonoscopy every 10 years. The advantage of an endoscopic procedure is detection of CRC and removal of adenomas which decreases mortality from rectal and colon cancer up to 80% (Jemal et al. 2010). Screening is also recommended in Europe by EU since 2003.

**Genetic alterations in colorectal cancer**

Malignant transformation which leads to development of invasive CRC is a multistep process in which new mutations are acquired as the disease progresses. Most common are loss of genomic stability, mutational inactivation of tumor suppressor genes, and activation of oncogene- and growth factor pathways (Fig. 3).

![Fig. 3 Colorectal cancer multistep carcinogenesis. Modified from P. Li and E.J. Lin 2009](image)

*The loss of genomic stability* can drive the development of CRC by multiple tumor-associated mutations. The most common type of genomic instability, in around 85% of all CRC, is chromosomal instability (CIN) which results in loss of wild-type copy of a tumor suppressor genes, such as APC, P53 and SMAD 4 family (Lengauer et al. 1997). In a subgroup of CRC patients, around 15% from all CRCs, there is inactivation of genes responsible for repair of base-base mis-matches in DNA called microsatellite instability (MSI) (Boland and Goel 2010). The inactivation can be inherited like in hereditary nonpolyposis colon cancer (HNPCC) also known as Lynch syndrome, or it can be acquired as methylation silencing of a gene responsible for a DNA mis-match repair. In the Lynch syndrome mis-match repair defects are detected in germ line MLH1 and MSH2 genes. Family members carrying that mutation have a lifetime risk of CRC of 80% by the age of 45 (Bronner et al. 1994; Vasen 2005). In mis-match repair deficiency tumor-suppressor genes such as TGF-beta, TGFBR2 and BAX are inactivated (Komarova et al. 2002; Wood et al. 2007). The expression of MLH1 gene can be epigenetically silenced in sporadic CRC with microsatellite instability (Issa 2004).

*Mutational inactivation of tumor-suppressor genes* is one of the key factors in CRC carcinogenesis. CRC commonly starts with a mutation in the Wnt signaling pathway by inactivating mutation of APC gene. In the absence of functional APC protein the Wnt pathway is
constitutively activated (Schneikert and Behrens 2007). Germ-line APC mutations are found in familial adenomatous polyposis, an inherited cancer-predisposition syndrome in which more than 100 adenomatous polyps can develop. Members of these families have a risk of developing CRC of almost 100% by the age of 40 (Lynch et al. 2008). Most of sporadic colorectal adenomas and cancers have somatic mutations and deletions that inactivate both copies of APC gene. In a small subgroup of tumors with wild-type APC, mutations of β-catenin activate Wnt signaling (Goss and Groden 2000).

A second key genetic step in CRC development is inactivation of TP53 pathway. The inactivation of TP53 is often a part of transition of large adenomas into invasive carcinomas. The two TP53 alleles are inactivated, in most tumors, usually by a combination of a missense mutation that inactivates the transcriptional activity of p53 and a 17p chromosomal deletion that eliminates the second TP53 allele (Baker et al. 1990a; Baker et al. 1990b).

Another common step in the progression of CRC is the mutational inactivation of TGF-beta signaling (Meulmeester and Ten Dijke 2011). TGF-beta has important role in inhibiting normal cell proliferation and regulation of the processes such as cell invasion, immune regulation, and microenvironment modification. Mutations in the TGF-beta type II receptor (TGFBR2) occur in approximately 30% of CRCs (Bellam and Pasche 2010). These mutations coincide with the transition from adenoma to high-grade dysplasia or carcinoma. Smad4 and Smad7 mutations are also common in CRCs and correlate with higher risk for recurrence of CRC (Gulubova et al. 2010). Loss of tumor suppressor Smad4 expression in primary CRC correlates with loss of E-cadherin expression, supporting the assumption that Smad4 controls expression of the tumor and invasion suppressor, E-cadherin (Reinacher-Schick et al. 2004).

Activation of oncogene pathways. Components of downstream signaling pathways are frequently mutated in CRC (Fearon 2011). K-RAS is mutated in 40-50% and BRAF in 10-15% of CRCs. BRAF mutations are detectable even in small polyps and they are more common in hyperplastic polyps, serrated adenomas, and proximal colon cancers (Bos et al. 1987; Siena et al. 2009). Around 30% of CRCs have activating somatic mutations in PI3CA. Less common genetic alterations include loss of PTEN, as well as amplification of insulin receptor substrate 2 (IRS2), an upstream activator of PI3K signaling, and co-amplification of AKT and PAK4, which are downstream mediators of PI3K signaling (Parsons et al. 2005).

Staging and prognosis of colorectal cancer

Tumor–node–metastasis - TNM classification is a staging system that includes two parts of CRC evaluation, clinical-before start of the treatment (cTNM) and pathohistological-after tumor (pTNM) excision. cTNM is the basis for the choice of treatment and pTNM for prognostic assessment (Table 1).

Staging procedure consists of several steps. Patient’s history of the disease together with physical examination, laboratory evaluation like blood-, carcinoembryonic antigen (CEA) values, intestinal evaluation (endoscopy), preoperative chest radiograph and liver ultrasonography or CT scan of the chest and abdomen, taken together will give estimation of the stage of CRC. But only after surgery with tumor and lymph nodes excision, exact stage of the disease will be established. Surgical staging includes detection of liver metastases, lymph nodes spread of disease and growth
of tumor through the bowel wall and onto adjacent structures. For proper pN-staging at least 12–14 nodes should be removed. Intraoperative ultrasound is a more accurate assessment for liver metastases: occult liver metastases can be found in 15% of patients. In 5% of cases they are solitary and could easily be resected (Labianca et al. 2010).

<table>
<thead>
<tr>
<th>Primary tumour (T)</th>
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<tbody>
<tr>
<td>TX</td>
<td>Primary tumour cannot be assessed</td>
</tr>
<tr>
<td>T0</td>
<td>No evidence of primary tumour</td>
</tr>
<tr>
<td>Tis</td>
<td>Carcinoma in situ intraepithelial or invasion of the lamina propia</td>
</tr>
<tr>
<td>T1</td>
<td>Tumour invades submucosa</td>
</tr>
<tr>
<td>T2</td>
<td>Tumour invades muscularis propria</td>
</tr>
<tr>
<td>T3</td>
<td>Tumour invades through the muscularis propria into the subserosa, or into the non-peritonealized pericolic tissues</td>
</tr>
<tr>
<td>T4</td>
<td>Tumour directly invades other organs or structures and/or perforates the visceral peritoneum</td>
</tr>
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<table>
<thead>
<tr>
<th>Regional lymph nodes (N)</th>
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<tbody>
<tr>
<td>NX</td>
<td>Regional nodes cannot be assessed</td>
</tr>
<tr>
<td>N0</td>
<td>No regional lymph node metastases</td>
</tr>
<tr>
<td>N1</td>
<td>Metastases in 1–3 regional lymph nodes</td>
</tr>
<tr>
<td>N2</td>
<td>Metastases in ≥4 regional lymph nodes</td>
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</table>

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<tr>
<th>Distant metastases (M)</th>
<th></th>
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<tbody>
<tr>
<td>MX</td>
<td>Presence of distant metastases cannot be assessed</td>
</tr>
<tr>
<td>M0</td>
<td>No distant metastases</td>
</tr>
<tr>
<td>M1</td>
<td>Distant metastases</td>
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<table>
<thead>
<tr>
<th>Stage grouping</th>
<th></th>
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<tbody>
<tr>
<td>Stage 0</td>
<td>Tis N0 M0: (carcinoma in situ)</td>
</tr>
<tr>
<td>Stage I</td>
<td>T1 N0 M0; T2 N0 M0; stage I equivalent to Dukes’ A or MAC A or B1</td>
</tr>
<tr>
<td>Stage IIA</td>
<td>T3 N0 M0</td>
</tr>
<tr>
<td>Stage IIIB</td>
<td>T4 N0 M0; stage II equivalent to Dukes’ B or MAC B2 or B3</td>
</tr>
<tr>
<td>Stage III (A, B, C)</td>
<td>Any T1–2, N1, M0 (IIIA); any T3–4, N1 M0 (IIIB); any T N2 M0 (IIIC); stage III equivalent to Dukes’ C or MAC C1–C3</td>
</tr>
<tr>
<td>Stage IV</td>
<td>any T, any N, M1</td>
</tr>
</tbody>
</table>

Table 1. TNM classification of colorectal cancer; Modified from Labianca R., ESMO clinical practice guidelines, 2011

**Additional Staging:**

<table>
<thead>
<tr>
<th>venous invasion (V)</th>
<th>lymphatic invasion (L)</th>
<th>histologic grade (G)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V0 no venous invasion</td>
<td>L0 no lymphatic vessel invasion</td>
<td>G1 well differentiated</td>
</tr>
<tr>
<td>V1 microscopic venous invasion</td>
<td>L1 lymphatic vessel invasion</td>
<td>G2 moderately differentiated</td>
</tr>
<tr>
<td>V2 macroscopic venous invasion</td>
<td></td>
<td>G3 poorly differentiated</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G4 undiffererentiated</td>
</tr>
</tbody>
</table>
The prognosis of colon cancer is related to the extent of penetration of the tumor through the bowel wall and presence or absence of lymph nodes metastases, in other words stage of the disease. Other important parameters are lymphatic, venous or perineural invasion, lymphoid inflammatory response and positivity of resection margins, all characteristics that TNM classification does not take into account. Many other potentially prognostic factors such as p53, K-Ras and bcl-2 expression, TGF alpha, EGFR, proliferation index and aneuploidy are important as single or combined parameters for high-risk conditions. Bowel obstruction and perforation are clinical signs of a poor prognosis. Elevated serum levels of CEA and/or carbohydrate antigen 19-9 (CA19-9) before treatment have negative prognostic value.

For the localized CRC one of the most important determinants of prognosis is lymph node status. It is estimated that for T1-T3 tumors, at least 40 lymph nodes should be examined to get 85% probability of stating truly node negative CRC. In practice, recommendation for standard diagnostics is 12 lymph nodes to be examined after radical surgery with lymph node excision. In rectal cancer progressive increase in T stage (T1-2 vs. T3-4) has a negative impact on survival and disease relapse. Similar finding is true for nodal status, therefore patients with T3-4 and N2 should be considered for more aggressive treatment (Chau et al. 2003).

Standard treatment strategies for localized and metastatic colorectal cancer before targeted therapy era

Treatment of localized colorectal cancer

Invasive cancers that are restricted to the wall of the colon, TNM stages I and II, are curable by surgical excision. Up to 73% of cases of stage III disease are curable by surgery combined with adjuvant chemotherapy.

About 70%-80% of patients with colon cancer have localized disease at diagnosis. Despite curative surgery, these patients still have significant risk of relapse. The risk is stage dependent, for stage I is 5-10%, stage II 20-30% and for stage III 40-50%. Adjuvant chemotherapy has become standard in some patients with stage II and in all patients with stage III CRCs.

Adjuvant chemotherapy in stage II CRC remains controversial with no international consensus. Results from QUASAR study involving 3187 patients, (90% had stage II CRC) which were randomized to adjuvant bolus 5-FU/LV or observation, showed a small, 3.6%, survival benefit for the chemotherapy group, 5-year mortality without chemotherapy is 20%. Chemotherapy can prevent a proportion of recurrences and deaths. For younger patients, the life-years gained are more substantial (Gray et al. 2007). In contrast some previous studies didn’t support adjuvant therapy with 5-FU/LV because there was no difference in 5-year disease-free survival (DFS) and overall survival (OS) (Chau et al. 2003). In conclusion, adjuvant therapy with 5-FU/LV should be considered in the individual cases of stage II CRC when patients belong to the “high risk group” with intestinal obstruction, perforation, T4 tumors, poorly differentiated tumors, venous- or perineural invasion.
Several studies involving stage III CRC patients have shown an advantage of adjuvant chemotherapy with modulated 5-FU with up to 40% reduction in recurrence and up to 33% reduction in mortality. The European study (MOSAIC) involved 2248 stage II and III CRC patients. They were treated either with a combination of oxaliplatin/5-FU/LV (FOLFOX) or 5-FU/LV. Combination treatment showed significant increase in DFS and this combination can be recommended for the treatment of the stage III CRC patients at least if risk of recurrence is high. Oxaliplatin can result in long lasting disabling neuropathy and should thus be used only when the recurrence risk is high (40-50% or higher) (Andre et al. 2009). In rectal cancer, preoperative radiotherapy or chemoradiotherapy is a standard of care depending upon a risk category (Glimelius et al. 2010).

**Treatment of advanced colorectal cancer**

After potentially curative surgery for their primary tumors approximately 1 out of 5 patients will develop liver metastases and 1 out of 12 pulmonary metastases. With the FOLFOX protocol it is possible to “downsize” unresectable liver metastases in selected group of patients and then surgically remove them. For this group of patients 5 year DFS can be up to 30% (Fornarini G 2005).

The majority of the patients diagnosed for stage IV CRC have non-resectable disease and more than one metastatic site, so surgery is no longer an option. Systemic chemotherapy with 5-FU/LV alone or in the combination with oxaliplatin is the standard treatment for these group patients with median survival in the trials of 18-22 months (Van Cutsem E 2010).

**Targeted therapy of metastatic colorectal cancer**

**Targeting EGFR**

*Cetuximab (Erbitux)* is a human-mouse chimeric IgG1 monoclonal antibody targeting EGFR with 5-10 fold higher affinity then natural ligands. It binds to the extracellular domain of EGFR. Binding of cetuximab to EGFR results in inhibition of the phosphorylation of the receptor as well as inhibition of downstream signaling. It causes internalization and degradation of EGFR and activation of immune response through antibody-dependent cellular cytotoxicity (ADCC). Blockade of EGFR also inhibits angiogenesis by down-regulating VEGF expression (Pfeiffer et al. 2007).

As described above, cetuximab was FDA approved first in 2004 for second or third line treatment of EGFR-expressing metastatic colorectal carcinoma in combination with irinotecan in patients who are refractory to irinotecan-based chemotherapy, based on the result of clinical trial showing 2 months gain in median survival for the patients in the combination therapy group (Cunningham et al. 2004). In 2007 the FDA granted approval for cetuximab as a single-agent for the treatment of patients with EGFR-expressing mCRC after failure of both irinotecan- and oxaliplatin-based chemotherapy regimens (Jonker et al. 2007). After the randomized phase III study, finalized in 2009, where patients in cetuximab + FOLFIRI treatment group showed better RR and reduced risk of disease progression compared to FOLFIRI group alone, cetuximab was FDA approved for the first line treatment of mCRC. The cetuximab+FOLFIRI combination slightly reduced the risk of progression for wt K-Ras CRC (Van Cutsem et al. 2009).

The efficacy of the cetuximab was not correlated with the extent of expression of EGFR on the CRC cells but it was strongly related to the skin rash (Chung et al. 2005). If no rash after
Cetuximab treatment was seen the RR was 6%, if they had mild rush, RR was 20% and 55% if patients had severe skin reaction (Cunningham D et al. 2004, NEJM).

Panitumumab (Vectibix) was FDA approved for treatment of chemo refractory metastatic CRC patients. The approval was based on the results of a single, open label, randomized, multinational study with metastatic colorectal cancer patients, comparing best supportive care (BSC) alone to BSC plus panitumumab. Mean PFS time was approximately two months longer for patients in the panitumumab group (Giusti et al. 2008).

The use of cetuximab and panitumumab are in Europe restricted to CRC patients without K-Ras mutations (Amado et al. 2008; Van Cutsem et al. 2009). Recent clinical study from 2010, involving chemotherapy-refractory colorectal cancer patients, was performed to test hypothesis that K-Ras codon 13 mutations are associated with a better outcome after treatment with cetuximab compared to other K-Ras mutations. The study could confirm that use of cetuximab was associated with longer overall and progression-free survival among patients with p.G13D K-Ras-mutated tumors compared to the patients with other K-Ras-mutated tumors (De Roock et al. 2010).

In two large randomized trials reported during 2010 (Coin and Nordic VII), cetuximab was added to an oxaliplatin-fluoropyrimidine combination showed limited or no extra influence on PFS or OS in the wt K-Ras population.

Despite the fact that there have been several clinical trials in CRC with gefitinib and erlotinib, no significant responses were seen with single-agent therapy. Combination trials with chemotherapy were more promising, but EGFR TKIs with irinotecan-based regimens have proven to be quite toxic (Ng and Zhu 2008).

**Targeting VEGF**

Angiogenesis is a very important event in the process of tumor growth and metastatic dissemination. Clinical studies have shown that angiogenesis plays an important role in the growth and progression of colorectal cancer (Willett et al. 2004).

The FDA approved anti-VEGF antibody bevacizumab (Avastin) 2004 as a first-line treatment for metastatic colorectal cancer in combination with intravenous 5-FU/irinotecan-based chemotherapy, as described above. In the phase III randomized, double blind study, group of patients receiving irinotecan/5-fu/leucovorin (IFL) plus bevacizumab showed almost 5 months longer median survival compared to the group given IFL plus placebo. The corresponding rates of response were 44.8 % and 34.8% (Hurwitz et al. 2004).

Bevacizumab is also indicated in combination with intravenous 5-FU-based chemotherapy for second-line treatment of patients with metastatic CRC (Giantonio et al. 2007). The identification of molecular differences between cancers that benefit from this treatment and those that do not remains a challenge.

In the adjuvant setting, tested in phase III study as a part of a first-line treatment for stage II and III CRC patients together with FOLFOX, bevacizumab failed to show advantage in DFS. Based on that, the use of bevacizumab can’t be recommended for use in the adjuvant treatment of patients with CRC (Allegra et al. 2011).
A randomized phase III clinical study, involving 755 patients with previously untreated metastatic colorectal cancer, compared the effects of capecitabine, oxaliplatin, and bevacizumab with the same combination plus cetuximab. The primary end point was progression-free survival. The addition of cetuximab to capecitabine, oxaliplatin, and bevacizumab resulted in significantly shorter progression-free survival and inferior quality of life. Mutation status of the K-RAS gene was a predictor of outcome in the cetuximab group (Tol et al. 2009).

**Targeting COX-2**

An early event in the development of an adenoma is the activation of prostaglandin signaling. This activation can be induced by inflammation or mitogen-associated up-regulation of COX-2, an enzyme that mediates the synthesis of prostaglandin E2, an agent strongly associated with CRC. Increased levels of COX-2 are found in approximately two thirds of CRC (Chan et al. 2007).

Clinical trials have shown that the inhibition of COX-2 by nonsteroid anti-inflammatory drugs prevents the development of new adenomas and mediates regression of established adenomas (Steinbach et al. 2000). **Celecoxib** is nonsteroid anti-inflammatory, FDA approved, drug for chemoprevention of CRC and only for high-risk patients with familial adenomatous polyposis (FAP) due to cardiovascular toxicity. Aspirin have also demonstrated efficacy in this setting (Giovannucci et al. 1995; Kune et al. 2007).
Aims of the thesis

The aims of the thesis were to explore mechanisms which determine efficacy of tyrosine kinase-targeting anticancer drugs in two different tumor types, glioblastoma and colorectal cancer.

More specifically the thesis aims at:
- investigating glioblastoma cell properties determining resistance or sensitivity to IGF-1R and PDGFR targeting drugs
- investigating role of activated cancer stroma in modulating sensitivity of colorectal cancer cells to new EGFR targeting drugs

Results and discussion

Paper I
PI3K/PTEN/AKT status affects the sensitivity of high grade glioma cell cultures to the insulin like growth factor-1 receptor inhibitor NVP-AEW 541

IGF-1 receptor signaling contributes to the growth of many solid tumors, including glioblastoma. This study analyzed the sensitivity of glioblastoma cultures to the IGF-1 receptor inhibitor NPV-AEW541. As a result of both anti-proliferative and pro-apoptotic effects NPV-AEW541 caused variable growth reduction of 8 GBM cultures, separating them in to “non-responding” and “responding” cultures. Activating mutations in the PIK3CA were found in two “non-responding” cultures, and the other two of “non-responding” cultures displayed ligand-independent Akt phosphorylation. PTEN siRNA experiments supported the notion that status of the PI3K/PTEN/Akt pathway is involved in determining NVP-AEW541 sensitivity. Combination treatments with either PI3 kinase- or mTOR-inhibitors together with NVP-AEW541 resulted in reduction in growth in cultures resistant to mono-treatment with the IGF-1 receptor inhibitor.

Together, the studies support continued clinical development of IGF-1 receptor antagonists for glioblastomas, and identify links between PI3K/PTEN/Akt status and sensitivity to mono-treatment with NVP-AEW541. Furthermore, the studies suggest that NPV-AEW541 is also active together with PI3 kinase- and mTOR-inhibitors in cultures with dysregulated PI3K/PTEN/Akt pathway.

Paper II
Prognostic but not predictive role of platelet-derived growth factor receptors in patients with recurrent glioblastoma

PDGFR signaling has been implicated in the pathogenesis of glioblastomas and is a target for the tyrosine kinase inhibitor imatinib. In this study, the prognostic or predictive role of PDGFR status in 101 GBM tumor samples was evaluated. Samples originate from tumors of the CST1571BDE40 trial which compared hydroxyurea monotherapy and a combination of hydroxyurea and imatinib. In addition to PDGFR alpha expression, PDGFR alpha
phosphorylation was investigated using in situ proximity ligation assay. PDGFR alpha protein was expressed in 33% of tumors and associated with male sex, young age and shorter median survival (142 vs. 187 days, P=0.028). Similarly, PDGFR alpha phosphorylation was associated with shorter survival (P=0.030). The subset of PDGFR alpha positive cases did not display longer survival upon treatment with hydroxyurea and imatinib as compared to hydroxyurea monotherapy.

In conclusion, both PDGFR alpha protein expression and phosphorylation status had a prognostic role in recurrent glioblastomas, but did not define a group that showed benefit from the combination therapy with hydroxyurea and imatinib.

**Paper III**

**Identification of a SOX2-dependent subset of tumor- and sphere-forming glioblastoma cells with a distinct tyrosine kinase inhibitor sensitivity profile**

Putative cancer stem cells have been identified in glioblastomas and are associated with radio- and chemo-resistance. Further knowledge about these cells is thus highly warranted for the development of better glioblastoma therapies. Gene expression analyses of eleven high grade glioma cultures identified two subsets designated type I and type II cultures. The type I cultures displayed high expression of CXCR4, SOX2, EAAT1 and GFAP, and low expression of CNP, PDGFRB, CXCL12 and extracellular matrix proteins. Clinical significance of the two subsets was indicated by the coordinated expression of type I and II "markers" in human glioblastoma samples. Type I cultures possessed a higher capacity to form xenograft tumors and neurospheres, displayed low or no sensitivity to mono-treatment with PDGF-and IGF-1-receptor inhibitors, but were efficiently growth inhibited by combination treatment with low doses of these two inhibitors. Furthermore, siRNA-induced down-regulation of SOX2 reduced sphere formation of type I cultures and decreased expression of type I "marker" genes. SOX2 down-regulation also conferred sensitivity to PDGF-and IGF-1-receptor inhibitors.

This study thus describes a tumor-and neurosphere-forming SOX2-dependent subset of glioblastoma cells, which are resistant to monotherapy with IGF-1R- or PDGFR targeted drugs, but express striking sensitivity to combination treatment with these drugs.

**Paper IV**

**PDGF-dependent effects of cancer associated fibroblasts on sensitivity of colorectal cancer cells to cetuximab**

This study describes experiments that test the hypothesis that paracrine signaling derived from the tumor microenvironment have impact on tumor drug sensitivity. More specifically, the study describes the influence of one part of the tumor microenvironment, activated fibroblasts, on sensitivity of colorectal cancer cells to EGFR targeting monoclonal antibody, cetuximab.

In agreement with previous studies LIM1215 colorectal cancer cells were shown to be growth-inhibited by cetuximab. However, co-culturing of LIM1215 cells with fibroblasts significantly reduced the sensitivity of cancer cells to cetuximab. Furthermore, when co-cultured with PDGF-stimulated fibroblasts they became significantly less sensitive to cetuximab regarding growth, migration and invasion. Co-cultured fibroblasts did not affect the ability of cetuximab
to block EGFR signaling suggesting that the protective effects involved activation of non-
EGFR signaling in cancer cells. LIM1215 cells co-cultured with fibroblasts displayed increased
c-met phosphorylation suggesting fibroblast-derived hepatocyte growth factor (HGF) as a
mediator of the protective effects of fibroblasts. This notion was supported by demonstrations
of PDGF-induced HGF-production by fibroblasts, and by the observation that stimulation of
cancer cells with HGF also induced resistance to cetuximab. Importantly, down-regulation of
HGF production in fibroblasts by siRNA significantly reduced their ability to confer resistance
of LIM1215 cells to cetuximab.
Conclusions and future perspectives

Together, all four studies could explain some of the mechanisms determining resistance of cancer cells to the targeted therapies used in these studies, as well as indicate new directions for extended studies towards improved understanding of the sensitivity properties of malignant tumors.

The first study suggests IGF-1R as a good target for IGF-1R targeted therapies of GBM, and identify links between PI3K/PTEN/Akt status and sensitivity to mono-treatment with NVP-AEW541. Furthermore, the study suggests that NVP-AEW541 is also active together with PI3 kinase- and mTOR-inhibitors in cultures with dysregulated PI3K/PTEN/Akt pathway.

The second study evaluated how PDGFRs status impact on survival of GBM patients and their response to imatinib treatment. Both PDGFR alpha protein expression and phosphorylation correlated with the worse outcome of GBM patients, but did not define a group that benefit from the combination therapy with hydroxyurea and imatinib.

The third study focused on observation that some GBM cell cultures displayed high expression of CXCR4, SOX2, EAAT1 and GFAP, and low expression of CNP, PDGFRB, CXCL12 and extracellular matrix proteins which gave them “stem cell like” properties. These GBM cultures possessed a higher capacity to form xenograft tumors and neurospheres, displayed low or no sensitivity to mono-treatment with PDGF-and IGF-1-receptor inhibitors, but were efficiently growth inhibited by combination treatment with low doses of these two inhibitors. SOX2 down-regulation also conferred sensitivity to PDGF-and IGF-1-receptor inhibitors.

These findings support continued clinical development of IGF-1 receptor antagonists for glioblastomas and other tumors and clinical trials with combination therapy of IGF-1R- and mTOR/PI3K- inhibitors with supporting translational research studies including sampling of the tumors and characterisation of PI3K/PTEN/PAKT status. Because of the negative correlation between PDGFR status and survival of the patients, observed in the second study, further clinical studies with PDGFR inhibitors in combination with other targeted therapies such as VEGF/VEGFR or EGFR inhibitors, deserve evaluation. The third study of this thesis suggested that resistance to PDGF-and IGF-1-receptor inhibitors can be related to, and dependent on, SOX2 expression. This finding deserves further pre-clinical and clinical evaluation. Isolation of new set of glioblastoma cells with “stem cell like” properties from GBM tumor material and confirmation of single/combination treatment effects is highly motivated. If these results are conclusive and reproducible, they could possibly lead to pilot clinical studies with combination treatment.

The fourth study describes how paracrine signaling of PDGF-activated fibroblasts protects colorectal cancer cells from the EGFR targeting monoclonal antibody cetuximab. The paracrine effect involves HGF, produced by activated fibroblasts. When co-cultured with activated fibroblasts, c-met receptor on the colorectal cancer cells is activated by HGF regardless drug inhibition of the EGFR. In conclusion, by activating c-met receptor and its signaling pathway, colorectal cancer cells can overcome growth, migratory and invasive inhibitory effects of cetuximab observed when colorectal cancer cells are treated without presence of activated fibroblasts. These findings support pre-clinical and clinical trials using either c-met inhibitors or PDGFR inhibitors together with cetuximab, followed by translational research studies including
sampling of the tumors and characterization of the PDGFRs status of the tumor stroma and c- 
met receptor status of the colorectal cancer cells in the tumor sections. Furthermore, more 
detailed analyzes of stromal cell types expressing PDGFR alpha and PDGFR beta, their 
properties, relationship to other tumor markers and role in colon cancer biology appear highly 
motivated.
Acknowledgements

As a junior member of the European Society for Medical Oncology (ESMO) I had the opportunity to participate in Translational Research Unite Visit (TRU) “Tumor immunology and targeted tumor therapies” at Cancer Center Karolinska in April 2004. Thanks to this visit, and an ESMO research grant one year later, I was able to start my PhD studies in tyrosine kinase-targeting anti-cancer drugs in Professor Arne Östman’s group. A big thanks to ESMO for organizing TRU visits and giving the opportunity to young oncologist to be involved and learn about basic cancer research.

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CCK is a magic place. It is a great mixture of smart and talented people from all around the world. A wonderful combination of many cultures and religions, resulting in friendship, prosperity and good science! I’m thankful to all of you showing me your cultures and opening your hearts to me! My dear friends Liping, Tao, Walid, Amir, Sasha, Katja, Daiana, Jelena, Slavica, Dali, Naveen, Shahab, Inga, Thomader, Ulrica, Anna, Betzy, Masako, Rona, Nathalie,
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