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Biomarkers in Gastrointestinal Cancer: Focus on Colon, Pancreatic and Gastric Cancer

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1. Introduction

Personalized cancer medicine based on genetic profiling of individual tumors is regarded as the treatment strategy of the future. The targeted drugs for the treatment of cancer have rapidly developed. However, our understanding (at the molecular level) of the precise role that potential targets have in tumorigenesis, and the survival dependence of tumors on these components, has not progressed at the same rate (De Roock et al., 2011). Since patient selection for therapy remains problematic, there has been an increasing interest in biomarkers of cancer risk in predicting future patterns of disease. In the broadest sense, a biomarker is any biological, chemical, or biophysical indicator of an underlying biological process. From a medical perspective, a biomarker is a physiological characteristic that is indicative of health and disease. A cancer biomarker has been defined as "a molecular, cellular, tissue, or process-based alteration that provides indication of current, or more importantly, future behavior of cancer" (Hayes et al., 1996). Cancer biomarkers are employed across the entire healthcare spectrum from the cancer biological research laboratory to patient monitoring in the clinic. Clinical applications include disease risk stratification, chemoprevention, disease screening, diagnosis and prognosis/prediction, treatment planning and monitoring, and posttreatment surveillance. Cancer biomarkers have contributed greatly to our current understanding of the heterogeneous nature of specific cancers and have led to improvements in treatment outcomes. However, full adoption of cancer biomarkers in the clinic has been slow to date, and only a limited number of cancer biomarker products are currently in routine use (http://www.insightpharmareports.com/reports_report.aspx?r=559&id=78452). Two primary challenges in developing cancer biomarkers are the discovery of candidate markers and the validation of those candidates for specific uses. The discovery process depends on the technologies available, and their sensitivity and specificity, to investigate the complex biochemistry of health and disease in order to identify differences that can be detected consistently in diverse populations. The validation process is also arduous and costly, often

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requiring collection of or access to many patient samples with extensive clinical annotation and long-term follow-up. In addition, a biomarker must be validated for each specific application for which it will be used. There must be convincing evidence that a surrogate endpoint accurately predicts the clinical endpoint of interest or in the case of screening, a test must have sufficient sensitivity, specificity, and positive predictive value to accurately identify a disease in the general population (US National Academy Press, Institute of Medicine (U.S.). Committee on Developing Biomarker-Based Tools for Cancer Screening, Diagnosis, and Treatment, 2007). Rapidly growing insights in the molecular biology of cancer and recent developments in gene sequencing, global gene expression profiling or genome wide analysis have led to high expectations for the identification, validation and assessment of cancer biomarkers alongside the established "standards of care" for cancer diagnosis and treatment.

In this review, the most promising biomarkers in gastrointestinal cancer are discussed, focusing on the epidermal growth factor receptor (EGFR)-pathway in colon cancer, the serum biomarkers, the glucose transporter (GLUT) receptors, and human equilibrative nucleoside transporter 1 in pancreatic cancer and HER2 in gastric tumors.

2. Colon cancer

2.1 Introduction

Colorectal cancer (CRC) is a major public health problem. CRC results from the cumulative effects of sequential genetic alterations, leading to a progressive and irreversible loss of normal control of cell growth and differentiation. Treatment of CRC consists of complete surgical removal of the primary tumor and the regional lymph nodes. Despite improvements in surgical techniques, dosing and scheduling of adjuvant and neo-adjuvant systemic therapy, five year survival for early stage colorectal cancer, i.e. without invasion or lymph node metastases, is about 90%, but this falls of to 65% for tumors with regional spread and to 10% for late stage disease in which the cancer has metastasized to distant sites (Deschoolmeester et al., 2010). Currently, the tumor-node-metastasis (TNM) stage is the only proven prognostic marker to aid in the identification of patients with aggressive disease (Tejpar et al., 2010). However, its predictive value is limited because even the outcome within each stage group is not homogeneous (Deschoolmeester et al., 2010). CRC should be regarded as a heterogeneous disease defined by different activating mutations in receptor tyrosine kinases (RTKs), or activating or loss of function mutations in downstream components of the RTK-activated intracellular pathways, some of which could occur in the same tumor. The efficacy of targeted drugs is therefore linked to the specific molecular alterations in the tumor (De Roock et al., 2011). The availability and application of various treatment modalities in CRC has resulted in the elucidation of prognostic and predictive biomarkers that will improve outcome through patient classification and selection for specific therapies. A prognostic biomarker provides information about the patient's overall outcome, regardless of therapy, whereas a predictive marker gives information about the effect of a particular therapeutic intervention (Tejpar et al., 2010). Consequently, in recent years a huge amount of research has been devoted to the study of new biological prognostic/predictive markers as recently reviewed by our group (Deschoolmeester et al., 2010). Several criteria must be met to ensure a biomarker is clinically useful. In addition, the biomarker needs to be tested and validated in a large cohort of randomized patients.

Although hundreds of these markers have been proposed in the last 2 to 3 decades, the current reality is that no molecular marker, other than the *KRAS* gene in the case of epidermal growth factor receptor (EGFR)-targeted therapy for metastatic disease, has made it into clinical practice (Duffy & Crown, 2008)(De Roock et al., 2009).

EGFR is a receptor tyrosine kinase belonging to the HER-family. When activated, EGFR phosphorylates and activates other intracellular proteins that affect cell signaling pathways, (Harding & Burtness, 2005) cellular proliferation, and control of apoptosis and angiogenesis (Figure 1) (Tedesco et al., 2004)(Harding & Burtness, 2005). EGFR has been implicated in colorectal tumorigenesis, tumor progression, and metastasis, as reviewed in Lockhart and Berlin (Lockhart et al., 2005)(Ng & Zhu, 2008). Overexpression of EGFR has been described in up to 65%–70% of human colon tumors and has been associated with the progression of CRC to a more advanced stage (Ng & Zhu, 2008). Therefore, EGFR not only represents a possible prognostic marker in the adjuvant setting of primary tumors but primarily a rational molecular target for a new class of anticancer agents, especially in the setting of metastatic CRC (mCRC) (Scartozzi et al., 2006a)(Scartozzi et al., 2006b)(Overman & Hoff, 2007).

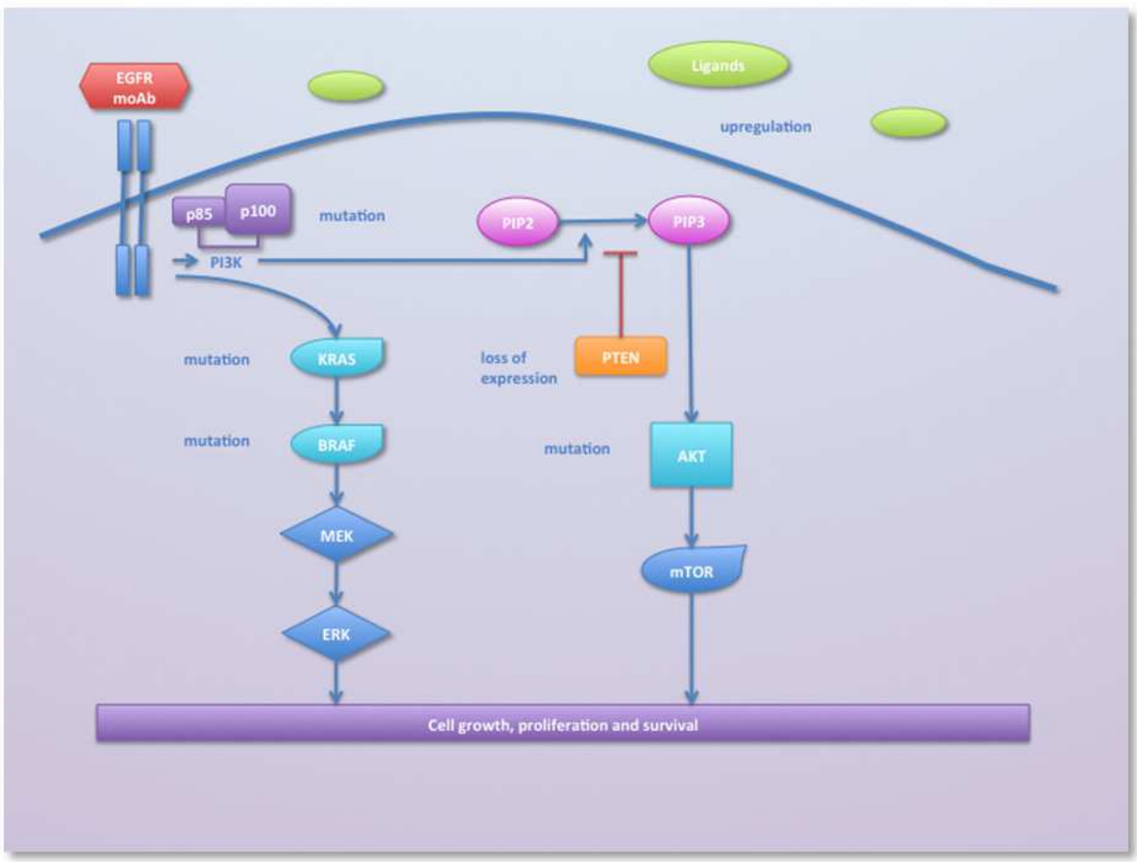

Fig. 1. EGFR signaling pathways and its main transduction pathways.

In preclinical studies, it was found that the inhibition of EGFRs had antitumor activity, and available data suggests synergy with both chemotherapy and radiotherapy (Rivera et al.,

2008). EGFR signaling can be targeted by either monoclonal antibodies (moAb) (cetuximab and panitumumab) or tyrosine kinase inhibitors (TKIs). Cetuximab (a mouse chimeric IgG1) and panitumumab (a fully human IgG2) block ligand induced EGFR tyrosine kinase activation, thereby probably preventing downstream activation of phosphatidylinositol 3-kinase (PI3K)/AKT and RAS/MAPK (mitogen activated protein) signaling pathways, resulting in inhibition of cellular proliferation and induction of apoptosis (Deschoolmeester et al., 2010). Nowadays, anti-EGFR targeted therapy is undergoing extensive clinical evaluation as single agents and in combination with chemotherapy for the treatment of recurrent or first-line mCRC (as reviewed by (Deschoolmeester et al., 2010)). Results of these studies have demonstrated a manageable and acceptable toxicity profile and a promising level of activity. Initially, these therapies were given to unselected populations, but novel insights based on the independent reanalysis of eight randomized trials suggested that these therapies would be effective only in wild type *KRAS* populations (Allegra et al., 2009). Based on these results, the recommended use of these drugs was amended by both the European Medicine Agency (EMEA) and the U.S. Food and Drug Administration (FDA), with important differences, however. The FDA issued a recommendation in 2009 against the use of these drugs in patients with tumors mutated in codon 12 or 13 of *KRAS*, but a label change of the drugs will require additional validation of a single mutation detection assay and reassessment of all randomized trials using this assay. In Europe, the EMEA changed the approval of these drugs for use in wild-type *KRAS* populations only. This has important implications because the exact mutations to be tested are not specified nor is the methodology (see further below) (Bellon et al., 2011).

2.2 KRAS

KRAS belongs to the *RAS* family of genes (*KRAS*, *NRAS* and *HRAS*) that encode guanosine-5′-triphosphate (GTP)-binding proteins. *KRAS* is an important effector of ligand-bound EGFR, mainly, but not exclusively through *BRAF* and *MAPK* axis. KRAS can also activate PI3K through direct interaction with its catalytic subunit (Figure 1) (De Roock et al., 2011). Mutations in the *KRAS* gene are found in 30-40% of CRC and these mutations disable the GTPase activity, causing tumor-associated KRAS to accumulate in the active GTP-bound conformation. About 85-90% of these mutations occur in codons 12 and 13 while the remaining mutations occur in codon 61 (5%) and 146 (5%). The most frequent types of mutations detected are glycine to aspartate on codon 12 (p.G12D, 36.0%), glycine to valine on codon 12 (pG12V, 21.8%), and glycine to aspartate on codon 13 (p.G13D, 18.8%) (Neumann et al., 2009). Several retrospective studies (single-group and randomized clinical trials, summarized by Allegra and colleagues (Allegra et al., 2009)) confirmed the finding by Lievre and colleagues (Lièvre et al., 2006) that mutant *KRAS* is a predictor of resistance to EGFR moAb. This discovery led to the first practical implication of personalized medicine in mCRC. All patients with mCRC are now profiled for seven mutations in *KRAS* codons 12 and 13 before receiving cetuximab or panitumumab (De Roock et al., 2011). However, the picture is not that simple. There is growing evidence for the existence of a whole orchestra of variables and mutations that influence the responsiveness to an anti-EFGR treatment and their role is not fully understood. A European consortium study showed that codon 61 mutations had an adverse effect similar to codon 12 mutations, whereas codon 146 mutations did not affect cetuximab efficacy. Codon 146 mutations co-occurred with other *KRAS* mutations, an additional indication that this might not be an important oncogenic

codon (De Roock et al., 2010b). In vitro data also suggest that *KRAS* codon 13 mutations have a weaker transforming activity than codon 12 mutations and some reports also suggest that some of these patients do respond to cetuximab (Koch et al., 2011). Based on these findings, de Roock and colleagues performed a thorough retrospective subgroup analysis in a pooled data set of 579 patients with chemotherapy-refractory CRC. Their data puzzles the picture of the negative predictive value of a *KRAS* mutation, because patients with the p.G13D mutation seem to respond to cetuximab therapy, in contrast to other *KRAS* mutated tumors, albeit with a lower response rate than those with *KRAS* wild type tumors. The prolonged progression-free and overall survival of patients with p.G13D-mutated tumors in comparison with those with other *KRAS*- mutated tumors may not be due to a real reduction in tumor burden but to a delay in progression. A possible explanation of this clinical observation is that p.G13D mutant tumors do not undergo apoptosis (cytotoxic effect) on EGFR inhibition, but proliferation is inhibited (cytostatic effect). However, prospective randomized trials are needed before conclusions about potential beneficial effects of cetuximab in p.G13D-mutated chemotherapy refractory metastatic colorectal cancer should be inferred (De Roock et al., 2010a).

Furthermore, mutations in the *KRAS* gene can be detected by several different molecular methods and no gold standard methodology is currently available. Because the correctness of the *KRAS* test results is of utmost importance for good patient care, a quality control scheme was set up to (a) assess the performance of *KRAS* testing in Europe, (b) provide remedial measures if necessary, and (c) ensure uniform performance over time by repeated testing rounds. In total, 59 laboratories from eight different European countries participated in the regional *KRAS* external quality assessment (EQA) scheme in 2009. Only 70% of laboratories correctly identified the *KRAS* mutational status in all 10 samples. Genotyping mistakes can be the result of several reasons. A very important issue is the starting material and the type of fixative used. Another important issue in *KRAS* genotyping is the method used for testing. The TheraScreen®DxS kit is considered to be the gold standard for *KRAS* testing in Europe for diagnostic use. However, in this EQA scheme, several mistakes were made using this kit. In addition, the kit is designed to detect only one mutation in a sample, and therefore the mutation scoring ignores possible double mutations, interpreting it as crosstalk. Furthermore, there was a very high variability among laboratories in the estimation of the percentage of tumor cells in H&E stained paraffin sections and the general quality of the reports received in the context of this EQA scheme were very poor. Incomplete or inaccurate exams lead to incorrect diagnoses and can have important consequences for a patient. Therefore, further development of the *KRAS* EQA scheme aims to provide a baseline picture of the accuracy and reliability of the analysis of the *KRAS* test, to identify areas of particular difficulty in testing procedures and to provide a mechanism for improvement for the participating laboratories (Bellon et al., 2011).

In addition, up to 50-65% of patients with *KRAS* wild-type tumors are resistant to EGFR moAb therapies. Therefore the quest for predictive markers continues. Genetic alterations in other EGFR effectors, acting downstream of *KRAS* together with alternative *KRAS* mutations (in codon 61 and 146) could drive primary resistance to anti-EGFR therapy and are currently investigated (Sartore-Bianchi et al., 2009a)(Molinari et al., 2009)(Souglakos et al., 2009)(Laurent-Puig et al., 2009)(Meriggi et al., 2009)(Prenen et al., 2009)(Loupakis et al., 2009a)(Loupakis et al., 2009b)(Perrone et al., 2009)(De Roock et al., 2011). Moreover, Sartore-

Bianchi et al., described that when expression of PTEN and mutation of *KRAS, BRAF* and *PIK3CA* are concomitantly ascertained, up to 70% of mCRC patients unlikely to respond to anti-EGFR therapies can be identified (Sartore-Bianchi et al., 2009a).

It is unclear to what extent the effects of mutant *KRAS* are the same for other RTK-targeted therapies. It is possible that *KRAS*-mutant tumors are not dependent on any RTK upstream component, and therefore will not respond to drugs targeting these RTKs. Alternatively, it might be that *KRAS* mutations confer only part of the survival advantage needed for tumor cells, and therefore will still benefit from RTK inhibition. Moreover, to define CRC as *KRAS* mutant versus *KRAS* wild-type probably underestimates additional heterogeneity found within both populations (De Roock et al., 2011).

2.3 BRAF

BRAF, a member of the *RAF* gene family (*ARAF*, *BRAF* and *CRAF*), encodes a serine-threonine protein kinase, downstream of activated KRAS, and initiates a mitogenic kinase cascade leading to cell proliferation (Figure 1). Activating mutations of *BRAF* have been reported in 5–15% of CRC and >95% of all known mutations involve a thymine to adenine transversion in nucleotide 1799, which leads to a substitution of valine by glutamic acid at amino acid residue 600 (V600E), which results in an upregulation of the ERK signaling pathway independently of *KRAS* mutation (Nash et al., 2010)(Barault et al., 2008)(Oliveira et al., 2004). In addition, the V600E mutation could have additional functions, since *KRAS* and *BRAF* mutations seems to be mutually exclusive in CRC, with very rare exceptions, suggesting they occur in different tumor types and have different outcomes. Moreover, *BRAF* mutations are associated with sporadic microsatellite instability (MSI), CpG island methylator phenotype (CIMP) and right sided tumors, whereas mutant *KRAS* are not (De Roock et al., 2011)(Dasari & Messersmith, 2010).

BRAF mutation status appears to be a valid negative prognostic marker for CRC in the adjuvant and metastatic setting, as demonstrate in the PETACC-3 (Roth et al., 2010), the CRYSTAL (Van Cutsem et al., 2011) and other studies (Yokota et al., 2011)(Park et al., 2011). The presence of CIMP-high appears to eliminate, at least in part, the adverse effect of *BRAF* mutations, whereas the good prognosis associated with MSI-high was abrogated in the presence of a *BRAF* mutation (Ogino et al., 2009a). In contrast, Samowitz et al. (Samowitz et al., 2005) and Roth et al. (Roth et al., 2010) found that *BRAF* mutations were associated with a significantly poorer survival in MSS tumors, but had no effect on the excellent prognosis of MSI-high tumors. Therefore, it has been postulated that it is not the *BRAF* mutation itself which confers a poor prognosis but rather that the mutation has different effects depending on the type of genetic pathway in which it is produced (Barault et al., 2008).

In addition, the currently available data suggest that the *BRAF* V600E mutation confers resistance to EGFR moAb in patients with chemotherapy-refractory *KRAS* wild-type mCRC and might be used as an additional predictive factor in this setting (Siena et al., 2009)(Laurent-Puig et al., 2009)(Sartore-Bianchi et al., 2009a)(Di Nicolantonio et al., 2008)(Tol et al., 2009).

Furthermore, the treatment of *KRAS*-mutated CRC with a selective *BRAF* inhibitor could be an interesting approach since *BRAF* is an important effector downstream of *KRAS* in the ERK signaling pathway. Phase II clinical trials are currently ongoing with the combination of sorafinib (*BRAF* inhibitor) with either FOLFOX, FOLFIRI or cetuximab.

2.4 PIK3CA

The PI3Ks are a family of lipid kinases grouped into three classes with different structure and substrate preferences. Class 1 phosphatidylinositol 3-kinases (PIK3) are heterodimeric proteins composed of a p85 regulatory subunit and one of several p110 catalytic subunits. Among several isoforms of the catalytic subunits, only the α-type, PIK3CA, has been shown to harbor oncogenic mutations or amplifications in its gene in human malignancies (Ogino et al., 2009b)(Jang et al., 2010). Activation of class I PI3K is initiated when a growth factor binds to its cognate RTK, which includes members of the ERBB-family, platelet-derived growth-factor receptor (PDGFR) and the insulin and the insulin-like growth-factor 1 receptors (IGF1R) (De Roock et al., 2011). Activated PIK3CA will phosphorylate phosphatidyl-inositol-4,5-biphospate (PIP2) to produce phosphatidyl-inositol-3,4,5-triphospate (PIP3) which localizes the serine threonine kinase Akt to the cell membrane where it becomes activated (Figure 1). Activated Akt phosphorylates downstream protein effectors and amplifies the signaling cascade, enhancing cell proliferation and survival (Ogino et al., 2009b). Based on the current data, it seems that *PIK3CA* mutation frequency in CRC is probably between 15 and 25% (Dasari & Messersmith, 2010). More than 80% of *PIK3CA* mutations in CRC occur in exon 9 (60-65%) or exon 20 (20-25%). Mutation in *PIK3CA* can co-occur with *KRAS* and *BRAF* mutations. A European consortium recently suggested that only *PIK3CA* exon-20 mutations are associated with a lack of cetuximab activity in *KRAS* wild-type tumors (De Roock et al., 2010b). However, because of the low frequency of this mutation, these data require confirmation in large patient population studies. In contrast, *PIK3CA* exon-9 mutations are associated with *KRAS* mutations and do not have an independent effect on cetuximab efficacy (De Roock et al., 2010b). The apparent difference between exon-9 and exon-20 mutations could explain the conflicting data regarding *PIK3CA* mutations reported by Sartore-Bianchi and colleagues (Sartore-Bianchi et al., 2009b) (lack of response to cetuximab and more exon-20 mutations) and Prenen and colleagues (Prenen et al., 2009) (no correlation). *PIK3CA* mutations as a whole were associated with shorter cancer specific survival in a series of surgically resectable CRC, but exon-9 and exon-20 were not studied separately (Ogino et al., 2009b)(Kato et al., 2007). More studies on large patient populations are needed to establish the prognostic role of *PIK3CA* exon-9 and exon-20 mutations.

2.5 PTEN

PI3K-initiated signaling is inhibited by phosphatase tensin homologue (PTEN). The PTEN protein acts as a phospholipid phosphatase with PIP3 as a substrate. PIP3 is an important lipid second messenger that provides docking sites for multiple downstream components, including AKT, which is activated by phosphorylation and inhibited by PTEN (Figure 1). Since PTEN protein is a negative regulator of the AKT signaling pathway, inactivation of PTEN, which is a common event in human malignancies, facilitates cell proliferation and apoptosis (Sawai et al., 2008)(Goel et al., 2004). PTEN activity may be lost trough various mechanisms, including mutations, deletions, silencing, allelic losses at chromosome 10q23 or hypermethylation of the *PTEN* promoter region (especially in MSI-high CRC). Therefore, ascertainment of PTEN status is usually done on protein level and the recorded frequency of loss of PTEN expression varies from 19% to 36% in CRC. Data on the loss of PTEN are not concordant between primary and metastatic tumors (De Roock et al., 2011)(Dasari & Messersmith, 2010). In addition, PTEN loss in metastatic tumors predicted lack of response

to cetuximab and PTEN null metastasis had shorter progression free survival, which was even more significant in *KRAS* wild-type patients. In sharp contrast, the PTEN analysis on the primary tumor did not reveal any predictive or prognostic information. Although the relative low concordance rate between the primary and metastatic tumors for PTEN expression could be secondary to selection of clonal populations during metastasis, it could be the subjective nature of immunohistochemistry testing with significant method and observer variability. This consideration and the possible need to analyze PTEN from metastatic tumors may limit the role of PTEN as biomarker in CRC (Dasari & Messersmith, 2010).

2.6 Conclusion

In summary, both MAPK and PI3K pathways are stimulated by EGFR, with important implication for EGFR targeted therapy and future drug development. Current American Society of Clinical Oncology (ASCO) guidelines recommend testing only for *KRAS* mutations in codon 12 and codon 13, in patients being considered for EGFR moAb therapy (Dasari & Messersmith, 2010). However, evidence shows that other molecular alterations, such as *BRAF*, *PIK3CA* (exon-20) mutations or loss of PTEN expression, could preclude response to EGFR moAb. The subjective nature of PTEN assessment, however, is a significant challenge. In addition, new drugs are being developed against numerous targets in these pathways, and many are in early clinical stages. Finally, a better understanding of the functional interactions within RTK-activated intracellular pathways is essential to target the individual tumor and to deliver more effective medical treatment to patients with mCRC. Furthermore, the ability of the cancer cell to develop drug resistance via new mutations or alternative signaling pathways also needs to be addressed by combination therapy, and, if possible, analysis of tumor tissue upon progression (Dasari & Messersmith, 2010)(De Roock et al., 2011).

3. Pancreatic cancer

3.1 Introduction

Pancreatic cancer has the worst prognosis of all gastrointestinal malignancies with the mortality approaching the incidence (Buxbaum & Eloubeidi, 2010)(Bünger et al., 2011). Late clinical presentation, intrinsic biological aggressiveness, and resistance to conventional chemotherapy and radiotherapy represent the predominant reasons for its poor prognosis (Pizzi et al., 2009). This demonstrates an urgent demand for improved screening tools for early detection (Buxbaum & Eloubeidi, 2010)(Bünger et al., 2011). While surveillance is performed in individuals with genetic syndromes, hereditary pancreatitis, and a strong family history there are no clear guidelines for those with clinical risk factors like diabetes mellitus, tobacco use, and chronic pancreatitis (Buxbaum & Eloubeidi, 2010). Pancreatic ductal adenocarcinoma is the most commonly diagnosed pancreatic neoplasm, and reported to be the forth or fifth leading cause of cancer death in Western countries. Diagnosis of pancreatic cancer at early stages is crucial because successful surgical resection remains the only possibility of cure (Ansari et al., 2011). Only 10-30% of pancreatic tumor patients are operated on with curative intent. The expected 5-year survival rate of R0 resected patients with additional adjuvant chemotherapy is about 4-26%. In contrast, for the remaining patients who present with unresectable UICC stage III and IV carcinomas, no curative

therapy is available. These patients have median survival times of 8-12 months (stage III) and 5-8 months (stage IV), respectively (Bünger et al., 2011). In addition, early-stage pancreatic cancer is usually clinically silent, and symptoms only become apparent after the tumor invades surrounding tissues or metastasis to distant organs. Therefore, most persons who present with symptoms attributable to pancreatic cancer have advanced disease (Vincent et al., 2011).

The Holy Grail for pancreatic cancer investigators is to identify early markers, which predict the development of pancreatic cancer, uncover early resectable disease, and guide therapy (Buxbaum & Eloubeidi, 2010).

Potential molecular markers are sought in the pancreatic tissue, juice as well as other body fluids including serum and urine. An important consideration is that pancreatic tumor cells and secreted molecules are found in markedly higher concentrations in the pancreas and pancreatic juice compared to serum. Additionally, molecules and proteins in the serum are overwhelmed by high concentrations of albumin, transferrin, and immunoglobulins (Buxbaum & Eloubeidi, 2010).

Both hypothesis driven and high throughput searches for molecular markers to predict disease, early diagnosis, and treatment response are underway. Challenges include differentiation of cancer from chronic inflammatory disease of the pancreas and achieving reproducible results among diverse patients. Minimally invasive methods including endoscopic ultrasound guided fine needle aspiration (EUS-FNA) to acquire tissue may facilitate these important efforts (Buxbaum & Eloubeidi, 2010). This method enabled not only accurate diagnosis, but also the collection of cancer tissue before surgery or chemotherapy even in inoperable cases. Evaluation of the expression status of multiple molecules within the FNA specimen will lead to the establishment of individualized therapeutic strategies based on the prediction of prognosis or response to chemotherapy (Hamada & Shimosegawa, 2011).

3.2 Serum biomarkers

Improved screening for early diagnosis is essential in order to increase the rate of curatively resectable carcinomas, thereby ameliorating patient's prognosis. In present clinical practice, screening for pancreatic cancer is based on state-of-the-art imaging or even invasive diagnostics. A relatively non-invasive, cost efficient possibility could be provided by the measurement of disease-specific markers in peripheral blood. A wide range of serum markers has been reported to be elevated in pancreatic cancer patients since the eighties. Despite these many markers or their combinations with high diagnostic potential for pancreatic cancer screening, none of them have achieved the levels of sensitivity and specificity necessary to be recommended as a screening tool for asymptomatic patients in the general population (Bünger et al., 2011)(Xu et al., 2011). Only a few markers have shown promising results in recent studies with CA19-9 being the most widely investigated and evaluated single marker (Bünger et al., 2011).

3.2.1 CA19-9

The best-established marker is CA19-9, which is a sialylated Lewis antigen of the MUC1 protein with an overall sensitivity ranging from 41 to 86% and specificity from 33 to 100%

(Bünger et al., 2011)(Buxbaum & Eloubeidi, 2010). As a marker for early pancreatic cancer, there are some important weaknesses. Approximately 10% of the population with the Lewis-negative genotype is not able to produce CA19-9, secondary to a lack of the enzyme involved in its synthesis, even if they have advanced pancreatic cancer. Recently it has been reported that patients with undetectable CA19-9 have a better prognosis than those with elevated levels. Patients with small pancreatic cancers often show false negative CA19-9 values, thus eliminating its value in early diagnosis. In addition, patients with certain blood types are incapable of expressing the antigen recognized by CA19-9. Moreover, CA19-9 elevation is common in patients with obstructive jaundice even without malignancy because of the reduction in clearance by the cholestatic liver. Furthermore, false positive CA19-9 elevation is also frequently observed in patients with cancers of the upper gastrointestinal tract, ovarian cancer, hepatocellular cancer, benign conditions of the hepatobiliary system and chronic pancreatitis (Xu et al., 2011). Nevertheless, continuous evaluation of this marker strongly suggests progressive disease during chemotherapy or recurrence after operation (Hamada & Shimosegawa, 2011). Thus CA19-9 is considered the standard for monitoring response to chemotherapy and recurrence following surgical resection in patients with pancreatic cancer but not for the initial diagnosis of the disease in the asymptomatic population (Xu et al., 2011)(Buxbaum & Eloubeidi, 2010)(Vincent et al., 2011).

In addition to serum it has been shown that pancreatic juice might also be a source of pancreatic cancer tumor markers. Several groups evaluated the diagnostic value of CA19-9 in pancreatic juice. Some groups found that CA19-9 concentrations were significantly higher in patients with cancer than in patients with chronic pancreatitis and other non-neoplastic patients (Malesci et al., 1987) showing a diagnostic value approximately similar to that of serum CA19-9 (Nishida et al., 1988)(Chen et al., 1989). Other studies could not confirm the diagnostic value of CA19-9 in pancreatic juice (Matsumoto et al., 1994). Further investigation into the exact role of CA19-9 in pancreatic juice is required. However, other potentially interesting biomarkers, like *KRAS* mutations, 90K, CEA were identified in this pancreatic juice and need further investigation (Nakaizumi et al., 1999)(Gentiloni et al., 1995).

3.2.2 Micro-RNA

Small non-coding RNAs are now attracting increased attention as robust regulators of various biological processes, including cancer progression. The micro-RNAs (miRs) are a class of conserved small non-coding RNA's of 17-25 nucleotides in length that regulate gene expression by either repressing the translation or causing degradation of multiple target mRNAs. *MiR* genes represent about 1% of the genome in different species and it is estimated that about 30% of the protein-coding genes in the human genome are regulated by miRs (Wang & Sen, 2011). These miRs play a central role in the regulation of cellular functions, such as migration, invasion and stem cell functions (Vincent et al., 2011). Extensive mapping of the known *miR* genes revealed that these are often located in the genomic intervals rearranged in cancers including those displaying amplification, loss of heterozygosity, common breakpoints and fragile sites. Furthermore, functional analyses suggest that miRs play roles in cancer initiation, invasion and progression processes and, therefore, may prove to be informative biomarkers of detection, diagnosis and prognosis besides being potential targets of therapy (Wang & Sen, 2011).

Over 300 miRs have been identified, and widespread alterations in these miRs have been recognized in various types of cancer, including pancreatic cancer, and seem to contribute to

their development and progression (see table 1, modified from (Wang & Sen, 2011)). MiR signatures specific for normal pancreas, chronic pancreatitis and cancer tissues have been identified and have been proposed to represent helpful markers for differential diagnosis of pancreatic cancer from chronic inflammatory disease of the pancreas and even other tumors (Hamada & Shimosegawa, 2011)(Vincent et al., 2011)(Wang & Sen, 2011). In addition, these differential-expressing miRs can also be profiled in blood as a minimally invasive biomarker assay for pancreatic cancer. This finding is extremely promising since there is no reliable biomarker assay, much less of minimally invasive nature, currently available for early detection, diagnosis and predicting prognosis of pancreatic cancer patients.

microRNA	Expression profile	References
Let-7f-1	Up	(Lee et al., 2007)
Let-7d	Up	(Lee et al., 2007)
miR-10	Up	(Bloomston et al., 2007)(Zhang et al., 2009b)
miR-15b	Up	(Lee et al., 2007)(Zhang et al., 2009b)
miR-16-1	Up	(Lee et al., 2007)
miR-21	Up	(Lee et al., 2007)(Bloomston et al., 2007)(Zhang et al., 2009b)(Mees et al., 2010)
miR-23	Up	(Bloomston et al., 2007)
miR-24	Up	(Lee et al., 2007)
miR-31	Up	(Szafranska et al., 2007) (Szafranska et al., 2008)
miR-92	Up	(Lee et al., 2007)
miR-95	Up	(Zhang et al., 2009b)
miR-96	Down	(Szafranska et al., 2007) (Szafranska et al., 2008)
miR-99	Up	(Bloomston et al., 2007)
miR-100	Up	(Lee et al., 2007)(Bloomston et al., 2007)
miR-103	Up	(Bloomston et al., 2007)(Zhang et al., 2009b)
miR-107	Up	(Lee et al., 2007)(Bloomston et al., 2007)(Zhang et al., 2009b)
miR-125	Up	(Lee et al., 2007)(Bloomston et al., 2007)
miR-130b	Down	(Szafranska et al., 2007)(Bloomston et al., 2007)(Szafranska et al., 2008)
miR-139	Down	(Lee et al., 2007)
miR-142-P	Down	(Lee et al., 2007)
miR-143	Up	(Szafranska et al., 2007)(Bloomston et al., 2007)(Szafranska et al., 2008) (Zhang et al., 2009b)
miR-145	Up	(Szafranska et al., 2008)(Zhang et al., 2009b)
miR-146	Up	(Szafranska et al., 2007) (Bloomston et al., 2007)
miR-148a	Down	(Szafranska et al., 2007) (Bloomston et al., 2007) (Szafranska et al., 2008)
miR-148b	Down	(Szafranska et al., 2007) (Bloomston et al., 2007)

miR-155	Up	(Lee et al., 2007)(Szafranska et al., 2007)(Szafranska et al., 2008) (Bloomston et al., 2007)(Zhang et al., 2009b)
miR-181a	Up	(Lee et al., 2007)(Bloomston et al., 2007)
miR-181b	Up	(Bloomston et al., 2007)
miR-181c	Up	(Lee et al., 2007)(Bloomston et al., 2007)
miR-181d	Up	(Bloomston et al., 2007)
miR-186	Up	(Zhang et al., 2009b)
miR-190	Up	(Zhang et al., 2009b)
miR-194	Up	{Mees:2010fr}
miR-196a	Up	(Szafranska et al., 2007) (Szafranska et al., 2008)(Zhang et al., 2009b)
miR-196b	Up	(Szafranska et al., 2007)
miR-199a	Up	(Bloomston et al., 2007)
miR-200b	Up	(Zhang et al., 2009b){Mees:2010fr}
miR-200c	Up	{Mees:2010fr}
miR-203	Up	(Ikenaga et al., 2010)
miR-205	Up	(Szafranska et al., 2007) (Bloomston et al., 2007) (Szafranska et al., 2008)
miR-210	Up	(Szafranska et al., 2007) (Bloomston et al., 2007)(Szafranska et al., 2008)(Zhang et al., 2009b)
miR-212	Up	(Lee et al., 2007)
miR-213	Up	(Bloomston et al., 2007)
miR-217	Down	(Szafranska et al., 2007) (Szafranska et al., 2008)
miR-220	Up	(Bloomston et al., 2007) (Lee et al., 2007)
miR-221	Up	(Szafranska et al., 2007)(Bloomston et al., 2007)(Zhang et al., 2009b){Mees:2010fr}
miR-222	Up	(Szafranska et al., 2007) (Bloomston et al., 2007)(Zhang et al., 2009b)
miR-223	Up	(Szafranska et al., 2007)(Bloomston et al., 2007)(Szafranska et al., 2008) (Zhang et al., 2009b)
miR-301	Up	(Lee et al., 2007)
miR-345	Down	(Lee et al., 2007)
miR-375	Down	(Szafranska et al., 2007) (Bloomston et al., 2007) (Szafranska et al., 2008)
miR-376a	Up	(Lee et al., 2007)
miR-424	Up	(Lee et al., 2007)
miR-429	Up	{Mees:2010fr}

Table 1. Deregulated microRNAs in pancreatic ductal adenocarcinoma (modified from (Wang & Sen, 2011)).

In addition, successful therapeutic targeting of miRs (silencing, antisense blocking and miR modification of oncogenic miRs) also holds significant promise towards improved clinical management of patients with cancer, especially those with pancreatic carcinomas, since these patients have very limited treatment options available at this time (Wang & Sen, 2011)(Rachagani et al., 2010).

3.3 Glucose transporter isoforms (GLUT)

Malignant cells have high constitutive glucose uptake and metabolism compared with normal cells (Pizzi et al., 2009). A family of glucose transporter isoforms (GLUT), which is currently composed of 13 members, facilitates the entry of glucose into cells. These are passive carriers and function as an energy-independent system that transports glucose down a concentration gradient. GLUT-1, a member of this family, is considered to be the predominantly upregulated glucose transporter in malignant epithelial tissue and mesothelium, and has been found to correlate with biological behavior in various malignancies (Basturk et al., 2011).

Various studies have shown a close relationship between GLUT-1 expression and tumor aggressiveness and poor prognosis in squamous cell carcinoma of the head and neck and in carcinomas of the lung, stomach, gallbladder, colorectum, kidney, bladder, ovary and cervix. An increased GLUT-1 expression has also proved to be associated with pancreatic cancer invasiveness both *in vitro* and *in vivo* (Basturk et al., 2011). However, literature data regarding the prognostic significance of immunohistochemical GLUT-1 expression in pancreatic ductal adenocarcinoma are limited and non consistent, as a prognostic significance of GLUT-1 expression has been found by some research groups (Sun et al., 2007)(Pizzi et al., 2009) and not by the other (Lyshchik et al., 2007). Differences can be ascribed to heterogeneity of histological types of pancreatic cancer and to the different scoring systems. Overall, GLUT-1 overexpression is regarded as a relative early event in pancreatic carcinogenesis and may be ascribed to local hypoxia. Furthermore, GLUT-1 expression seems to correlate to a higher glucose uptake in undifferentiated and highly proliferative pancreatic cancer cells (Pizzi et al., 2009). Moreover, GLUT-1 promotes cellular invasiveness in pancreatic cancer, which is matrix metalloproteinase 2 (MMP2)-dependent, with MMP-2 being transcriptionally activated by increased GLUT-1 levels (Ito et al., 2004). In addition, an increased expression of GLUT-1 molecules in pancreatic tumors has been suggested to contribute to the higher rate of fluorine 18 fluorodeoxyglucose (^{18}F-FDG) uptake into tumor cells compared with normal pancreatic tissue, as determined by standardized uptake value (SUV). Also, SUV has been found to be a predictor of survival in patients with ductal adenocarcinomas. Therefore, in addition to being of diagnostic value imaging-wise, GLUT-1 may also be a potential therapeutic target to limit glucose uptake and metabolism, thereby limiting the proliferative potential of malignant cells (Basturk et al., 2011). Apigenin, a flavonoid with significant anti-proliferative properties that inhibit pancreatic cancer cell proliferation, has been shown to inhibit glucose uptake as well as both GLUT-1 mRNA and protein expression in human pancreatic cancer cell lines. In addition, the PI3K/Akt pathway may be involved in mediating apigenin's effects on downstream targets such as GLUT-1 (Melstrom et al., 2008). However, literature regarding the biological significance of GLUT-1 expression in pancreatic neoplasia has been limited and controversial (Basturk et al., 2011).

3.4 Human equilibrative nucleoside transporter 1 (hENT1)

Gemcitabine, a pyrimidine nucleoside analogue, has clinically important activity in advanced and metastatic pancreatic adenocarcinoma and for which it is now the standard of care (Maréchal et al., 2009)(Ansari et al., 2011). Gemcitabine is a prodrug that is phosphorylated by deoxycitidine kinase to its mononucleotide in the rate-limiting step of its cellular anabolism. Subsequent nucleotide kinases convert gemcitabine monophosphate to its active metabolites, gemcitabine diphosphate and triphosphate. Permeation of gemcitabine through the plasma membrane requires specialized integral membrane nucleoside transporter proteins. Among these transporters, the major mediators of gemcitabine uptake into human cells appear to be the human equilibrative nucleoside transporter 1 (hENT1) and to a lesser extent the human conservative nucleoside transporter 3 (hCNT3) (Maréchal et al., 2009). Recently, it was reported that tissue mRNA levels of the hENT1, which mediates the cellular entry of gemcitabine, correlated with survival (Ansari et al., 2011). Several subsequent immunohistochemically based studies demonstrated that hENT-1 holds promise as an independent predictive marker to identify those likely to benefit from gemcitabine based monotherapy (Morinaga et al., 2011)(Spratlin et al., 2004)(Farrell et al., 2009) and gemcitabine based chemoradiotherapy (Maréchal et al., 2009)(Murata et al., 2011). In addition, the expression of hENT1 provides independent prognostic information in untreated pancreatic carcinoma patients as well as those treated with adjuvant gemcitabine-based therapy (Kim et al., 2011b)(Maréchal et al., 2009). Whether these assays provide sufficient predictive information to guide treatment decision requires prospective evaluation in randomized clinical trials. However, the consistency and strength of the accumulating preclinical and translational data suggest that nucleoside transporters play an important role in clinical outcomes after gemcitabine adjuvant chemotherapy for pancreatic cancer (Maréchal et al., 2009).

3.5 Conclusion

Although the tumor node metastasis classification provides important prognostic information, it permits only crude stratification of clinical outcome for patients with pancreatic cancer. Although some potential markers were identified, a high degree of inconsistency still exists between reports. Validation through large multicenter prospective studies using standardized protocols is still needed. Considering the complexity of the disease, it seems reasonable to hypothesize that panels of markers, rather than single proteins, might become useful (Ansari et al., 2011).

4. Gastric cancer

4.1 Introduction

Gastric cancer is one of the most common tumors and remains the second leading cause of cancer death worldwide (Gravalos & Jimeno, 2008)(Wagner & Moehler, 2009). Gastric cancer is a heterogeneous disease divided in at least two different tumor entities, the intestinal and the diffuse form, with difference in epidemiology, cause, pathogenesis and disorder. The development of the intestinal form, usually in older patients, is related to *Helicobacter pylori* and usually located in the corpus and the antrum and related to preexisting corpus predominant atrophic gastritis, followed by intestinal metaplasia. In contrast, the diffuse

form is usually poorly differentiated, located most frequently in the proximal stomach and its incidence is rising at an alarming rate in overweight young men suffering from gastroesophageal reflux. Diffuse type cancers have usually a worse prognosis (Wagner & Moehler, 2009).

Surgical resection remains the mainstay of treatment and cure in localized, non-metastatic gastric cancer while no globally accepted consensus exists on the best treatment regimen to be used in advanced gastric cancer (De Vita et al., 2010)(Lorenzen & Lordick, 2011). At present, the combination of a fluoropyrimidine and a platinum analogue either alone or in combination with a third drug such as an antracycline or taxanes are the most effective combinations resulting in a median survival of 8-10 months (Lorenzen & Lordick, 2011). These observations suggest the need for new therapeutic approaches, based on the implementation of predictive biomarkers, to further improve the outcome of patients with advanced gastric cancer (De Vita et al., 2010)(Wagner & Moehler, 2009)(Lorenzen & Lordick, 2011). A better understanding of the molecular basis of cancer has contributed to the development of rationally designed molecular targeted therapies, which interfere with the signaling cascades involved in cell differentiation, proliferation and survival (Gravalos & Jimeno, 2008). Recently, the evidence that upregulation of signaling pathways of EGFR-family plays a central role in cell differentiation, proliferation, and survival has supported the development of antitumor strategies against these targets (De Vita et al., 2010). One of the most considerable innovative targets in human cancer is the HER family.

4.2 HER2

The epidermal growth factor receptor (EGFR) family is composed of four members: HER1 also known as EGFR1, HER2, HER3 and HER4, amongst which the EGFR1 and HER2 represents targets for drugs currently under development for gastric cancer (Wagner & Moehler, 2009). The HER2 protein is a 185 kDa transmembrane tyrosine kinase (TK) receptor encoded by a gene located on chromosome 17q21, with an extracellular ligand-binding domain, a short transmembrane domain and an intracellular domain with TK activity. Up to now, no ligands have been identified for its extracellular domain, but it seems to be the preferred heterodimerization partner for other members of the HER family (De Vita et al., 2010). HER2 functions as an oncogene and its amplification or overexpression plays a central role in the initiation, progression and metastasis of some common cancers. Aberrant HER2 expression or function has been implicated in about 10-34% of invasive breast cancers. In addition, HER 2 also appears to be overexpressed in colon, bladder, ovarian, endometrium, lung, uterine cervix, head and neck, and esophageal carcinomas. The first description of HER2 overexpression in gastric cancer, using IHC, was reported in 1986. Since then, a number of studies have confirmed these findings, reporting a HER2 positivity rate in a wide range (6-35%) of gastric carcinomas. Moreover, HER2 expression varies depending on histology and on primary tumor location (Lorenzen & Lordick, 2011)(De Vita et al., 2010). The randomized open-label, multinational phase III ToGA (Trastuzumb for Gastric Cancer) trial, in which by now the largest population of 3807 gastric cancers were centrally screened for *HER2* gene amplification (Fluorescent in situ hybridization (FISH)) and HER2 protein overexpression (IHC 3+), reported a HER2 positivity of 22.1%, with a high degree of concordance between IHC and FISH (87,2%). Furthermore, HER2 positivity rates were found to be higher in esophagogastric junction cancer than in gastric cancer and

in intestinal cancer than diffuse or mixed type (Lorenzen & Lordick, 2011)(Croxtall & McKeage, 2010).

Trastuzumab, a recombinant humanized IgG1 monoclonal antibody directed against the extracellular domain of HER2, in combination with chemotherapy agents, has recently received approval in the EU and USA for treatment of metastatic HER2-positive gastric cancer without prior anti-cancer treatment for metastatic disease (Croxtall & McKeage, 2010).

4.3 HER2 as prognostic factor in gastric cancer

The TNM stage is the most important prognostic factor for gastric cancer. Prognosis, however, varies among patients in the same stage. Therefore, additional classification parameters, like HER2 need to be defined in addition to the TNM and the classical pathological characteristics of the tumor in order to better identify the biological subset of this disease (Gravalos & Jimeno, 2008). The role of HER2 as prognostic marker in gastric cancer has been controversial because some of the initial studies failed to find an association with outcome (Zhang et al., 2009a)(De Vita et al., 2010)(Gravalos & Jimeno, 2008)(Lorenzen & Lordick, 2011). Other authors, however, reported a direct correlation between HER2 overexpression and poor outcome (Ananiev et al., 2011)(Kim et al., 2011a)(Im et al., 2005). However, the largest study to date investigating the prognostic significance of HER2 expression in 924 gastric cancer patients showed that HER2 expression is not related to gastric cancer patients outcome (Grabsch et al., 2010). Chua et al. (Chua & Merrett, 2011) performed a systematic examination of the literature to identify translational studies that correlated HER2 with clinicopathologic markers and/or survival. This review included 49 studies totaling 11,337 patients. IHC was most commonly used to assess HER2 expression, identifying a median rate of 18% of gastric cancer demonstrating HER2 overexpression. In patients with and without HER2 overexpression, the median 3-year disease-free survival rate was 58% and 86%, respectively. Of the 35 studies reporting the impact of HER2 overexpression on survival, 20 studies (57%) reported no difference in overall survival, two studies (6%) reported significantly longer overall survival in patients with HER2 overexpression and 13 studies (37%) reported significantly poorer overall survival in patients with HER2 overexpression. HER2 overexpression appears to be associated with poorer survival and with intestinal-type gastric cancer in this group of patients for whom majority undergone curative gastrectomy. Results of the ToGA trial, which are discussed below, seem to refute this suggestion as demonstrated by the longer than expected survival of patients in the control arm, who received chemotherapy alone. However possible confounding factors, such as the wide use of second line treatment and the better prognosis associated with the intestinal histology, should be kept in mind when interpreting these results (Fornaro et al., 2011). In addition, these conflicting results could be due to the lack of a standardized definition of HER2 positivity in gastric cancer (De Vita et al., 2010)(Lorenzen & Lordick, 2011).

Hence, definitive answers about the prognostic role of HER2 in gastric cancer and gastric-esophageal cancer cannot be derived from the available data, which thus emphasizes the need for further research in the field (Fornaro et al., 2011).

4.4 Trastuzumab and the predictive role of HER2 in gastric cancer

Trastuzumab, a recombinant humanized IgG1 monoclonal antibody directed against the extracellular domain of HER2, induces antibody-dependent cellular cytotoxicity, inhibits HER2-mediated signaling and prevents cleavage of the extracellular domain of HER2. In HER2 positive breast cancer, trastuzumab has demonstrated survival benefits for patients with early and metastatic disease and is now the standard of care (Bang et al., 2010)(Croxtall & McKeage, 2010). Several studies indicate antitumor activity of trastuzumab in overexpressing HER2 human gastric cancer cell lines or xenograft models (Matsui et al., 2005)(Tanner et al., 2005)(Fujimoto-Ouchi et al., 2007). Most of these studies used the NCI-N87 and or 4-1ST gastric cell lines, which show HER2 expression in IHC and gene amplification on FISH. These studies showed that trastuzumab suppressed the growth of human gastric cancer with HER2 overexpression *in vitro* and *in vivo* and improved the survival of mice with peritoneal dissemination and ascites of gastric cancer. In addition, trastuzumab administered in combination with chemotherapy agents for gastric cancer showed potent antitumor activity, which was significantly greater than did trastuzumab or the chemotherapy agents as single treatments. A three-drug combination of capacetabine, cisplatin, and trastuzumab achieved remarkable tumor growth inhibition in the N87 model (Fujimoto-Ouchi et al., 2007)(Kim et al., 2008)(Gravalos & Jimeno, 2008). In addition, there are currently no data regarding resistance to trastuzumab in gastric cancer cells and there are no *in vitro* tests available to enable the prediction of resistance (Croxtall & McKeage, 2010).

Based on these results there was a strong rationale to investigate the clinical potential of trastuzumab in gastric cancer patients. Several preliminary single-arm phase II trials paved the way for the registration of the large, randomized controlled, open label, multicenter, international phase III trial which was undertaken in 24 centers in Aisa, Central and South America and Europe. The objective of this "Trastuzumab for Gastric Cancer" (ToGA) study was to assess the clinical efficacy and safety of trastuzumab added to chemotherapy for first-line treatment of advanced gastric or gastro-esophageal junction cancer with overexpression of HER2. Tumors were centrally tested for HER2 status with IHC (Hercep test) and FISH. Because of the inherent difference between breast and gastric tumors, notably tumor heterogeneity and the occurrence of baso(lateral) membrane staining, a new set of IHC scoring criteria were developed that are specific for gastric cancer. Patients were eligible if their tumor samples were scored as 3+ on IHC or if they were FISH positive (Bang et al., 2010) (Bang et al., 2010). As mentioned above, 22,1% of all gastric cancer screened in the ToGA trial were HER2-positive, which is broadly comparable with the incidence in breast cancer. Moreover, there was a high degree of concordance between IHC and FISH. Therefore, IHC is suitable for primary testing of HER2 positivity in gastric cancer with a score of IHC3+ indicating eligibility for treatment with trastuzumab, IHC2+ should be retested by FISH to confirm HER2 positivity and a score of IHC0 or 1+ should be considered as HER2-negative. The incidence of HER2 positivity differed according to tumor location and histological subtype. There was a significantly greater incidence of HER2-positive cancers of the oesophagogastric junction than the stomach and in intestinal than diffuse or mixed cancers. In addition, the incidence of HER2 positive gastric cancers was similar between Europe and Asia, but varied between countries (Croxtall & McKeage, 2010). Patients who satisfied all eligibility criteria (594 patients) were randomized in a 1:1 ratio and

584 patients received treatment with trastuzumab plus chemotherapy (5FU or capecitabine and cisplatin) for six cycles or chemotherapy alone (Lorenzen & Lordick, 2011). The primary objective was to compare overall survival in both treatment arms, and the secondary objectives were to compare progression-free survival, time to progression, overall response rate, control disease, duration of response, and quality of life (Gravalos & Jimeno, 2008). The primary endpoint of the study was met: trastuzumab significantly improved overall survival by nearly 3 months (median 11.1 vs 13.8 months) (Lorenzen & Lordick, 2011). In addition, an exploratory post-hoc analysis showed that trastuzumab plus chemotherapy substantially improved overall survival in patients with high expression of HER2 protein (IHC2+/FISH+ or IHC3+, 16 months) compared with patients with low expression of HER2 protein (IHC0 or 1+ and FISH+) (Bang et al., 2010). The secondary endpoints also showed significant improvements when trastuzumab was added to chemotherapy. The addition of trastuzumab to chemotherapy did not increase toxic effects associated with standard fluoropyrimidine-based and platinum-based chemotherapy and therefore trastuzumab can be combined with standard chemotherapy without affecting the overall safety profile. On the basis of these findings, trastuzumab can be considered as a new standard option for patients with HER2-positive advanced gastric or gastro-oesophageal junction cancer when combined with a chemotherapy regimen consisting of capecitabine plus cisplatin or fluorouracil plus cisplatin (Bang et al., 2010).

On the basis of this evidence, in January 2010 the EMEA and on 20 October 2010, the US FDA granted approval for trastuzumab in combination with cisplatin and fluoropyrimidine (either capecitabine or 5FU), for the treatment of metastatic HER-2 positive gastric or gastro-oesophageal junction adenocarcinoma who have not received prior treatment for metastatic disease (Lorenzen & Lordick, 2011).

Further studies are necessary to investigate the role of trastuzumab in curative gastric cancer treatment, as well its role as monotherapy, maintenance therapy and second line treatment in the palliative setting. Furthermore, additional predictive markers are needed besides a HER2-positive status. In addition, there is an urgent need to improve the knowledge of the mechanisms involved in anti-HER2 sensitivity or resistance, in order to develop other rationally targeted agents in the near future (Fornaro et al., 2011).

4.5 Conclusion

Since there is no internationally accepted standard of care for gastric or gastro-esophageal cancer patients and survival remains poor, new therapeutic strategies are needed. There is mounting evidence of the role of HER2 overexpression in patients with gastric cancer. HER2 overexpression has been correlated to poor outcome and more aggressive disease. Furthermore, the positive results of the randomized phase III ToGA trial have opened up new frontiers. Trastuzumab not only represents a new and effective therapeutic option, but has also stimulated the search for predictive marker in order to refine patient selection (Fornaro et al., 2011). Trastuzumab represents a new reference treatment for patients with HER2-positive metastatic gastric or gastro-esophageal cancer. Routine HER2 testing is suggested for all patients with advanced disease. Other agents directed against members of the HER family (like lapatinib) are currently under investigation (Lorenzen & Lordick, 2011).

5. General conclusion

Ample data shows that only a limited portion of patients may benefit from anti-cancer treatments currently used in the clinic. Personalized cancer medicine, based on genetic profiling of individual tumors and biomarkers, is regarded as the treatment strategy of the future. In this review the most promising biomarkers in colorectal, pancreatic and gastric cancer were discussed (table 2). Currently, the only biomarker that has made it into clinical practice for colorectal cancer is *KRAS* mutation for the selection of patients eligible for cetuximab therapy. Furthermore, evidence shows that other molecular alterations, such as *BRAF*, *PIK3CA* (exon-20) mutations or loss of PTEN expression, could preclude response to EGFR moAb.

Location	Predictive	Prognostic	Stage
Colorectal cancer	KRAS		Clinical stage (since 2008)
	PIK3CA exon 20 mut.		Preclinical stage
		BRAF	Preclinical stage
Pancreatic cancer	CA19-9		Clinical stage
	hENT1	hENT1	Preclinical stage
	miRs	miRs	Preclinical stage
		GLUT	Preclinical stage
Gastric cancer	HER2		Clinical stage (since 2010)
		HER2	Preclinical stage

Table 2. The most promising biomarkers in colorectal, pancreatic and gastric cancer summarized.

Improved screening for early diagnosis is essential in order to increase the rate of curatively resectable pancreatic carcinomas, thereby ameliorating patient's prognosis. A relatively non-invasive, cost efficient possibility could be provided by the measurement of disease-specific markers in peripheral blood. However, only a few markers have shown promising results in recent studies with CA19-9 being the most widely investigated and evaluated single marker (Bünger et al., 2011).

In patients with gastric cancer there is mounting evidence of the role of HER2 overexpression since it has been correlated to poor outcome and more aggressive disease. Furthermore, HER 2 overexpression was found to be predictive for treatment of gastric cancer patients with trastuzumab. Routine HER2 testing is now suggested for all patients with advanced disease.

Given the importance of biomarkers in this era of targeted therapies more and especially prospective randomized trials are necessary.

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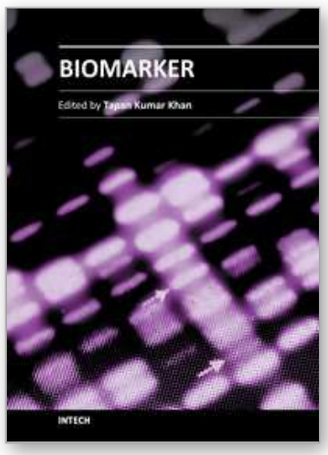

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Clinicians, scientists, and health care professionals use biomarkers or biological markers as a measure of a person's present health condition or response to interventions. An ideal -biomarker should have the following criteria: (I) ability to detect fundamental features of the disease, (II) ability to differentiate from other closely related diseases, (III) ability to detect early stages and stages of progression, (IV) the method should be highly reliable, easy to perform and inexpensive, and (V) sample sources should be easily accessible from body. Most of the chapters in this book follow the basic principle of biomarkers.

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