Established and Potential Predictive Biomarkers in Gastrointestinal Cancer – c-Kit, Her2, Ras and Beyond

Christine Koch    Joerg Trojan
Medizinische Klinik 1, Universitätsklinikum Frankfurt, Frankfurt am Main, Germany

Key Words
Gastrointestinal cancer · Predictive biomarkers · c-Kit · Her2 · Ras · Personalized treatment

Abstract

Background: Gastrointestinal cancers are among the leading causes of cancer-related deaths worldwide. In different tumor types, personalized systemic treatment strategies based upon biomarker-selection were established over the last years. Although there is a flood of targeted agents in clinical development, only a few targeted agents with a predictive biomarker could be established for the treatment of patients with gastrointestinal cancer patients so far. Summary: Currently, predictive biomarkers for gastrointestinal cancers include Her2 overexpression or amplification (gastroesophageal adenocarcinoma), c-Kit overexpression (gastrointestinal stromal tumors) and RAS wild-type (colorectal cancer). Selection of patients based on these biomarkers allows the efficient use of targeted agents. The presence of a BRAF mutation and/or high microsatellite instability is prognostic and rather a predictive marker in CRC. Promising candidate markers in advanced clinical development are MET amplification in gastroesophageal adenocarcinoma, Met overexpression and high AFP serum levels in hepatocellular carcinoma. Key Message: Biomarker-guided systemic treatment is established in a subset of patients with gastrointestinal cancer. Ongoing clinical trials and further advances in high-throughput technologies will hopefully result in more personalized systemic treatment strategies for these patients in the near future.

Gastrointestinal cancers are among the leading causes of cancer morbidity and mortality worldwide. In the western population, colorectal cancer is one the most frequent tumors in women and men, whereas the incidence of hepatocellular carcinoma and gastric cancer is high in Asian populations [1]. Despite approval being obtained of the use of many different classes of chemotherapeutic agents over the last 20 years, the overall prognosis of patients with advanced gastrointestinal cancer still has fatal consequences. Since the approval of imatinib, the first-in-class tyrosine kinase inhibitor (TKI) in 2002, gastrointestinal stromal tumor (GIST) is a model for targeted and multidisciplinary treatment of patients with solid tumors [2]. However, GIST is a rare disease, and in other types of advanced gastrointestinal cancers, targeted agents were used only in selected patients. In this review, we summarized the most relevant data on established treatment strategies for patients with GIST, gastroesophageal (GE) cancer and metastatic colorectal cancer (CRC) based on predictive biomarkers and provide an outlook on MET in GE cancer and hepatocellular carcinoma (HCC).
Inhibition of c-KIT in GIST

GIST is characterized by the expression of the receptor tyrosine kinase c-Kit, the product of the c-KIT proto-oncogene. Activating or gain-of-function mutations in c-KIT have been identified in a majority of GIST patients, mostly involving exon 11 (70%) or exon 9 (10–15%). In 5–7% of GIST, oncogenic mutations in the platelet-derived growth factor receptor (PDGFR) A kinase are found. Imatinib blocks the tyrosine kinase activity of c-Kit as well as the kinase activity of the normal c-ABL gene product, the oncogenic Bcr-Abl chimeric fusion protein of chronic myeloid leukemia, and PDGFR [2]. In 2001, a dramatic response to imatinib was reported in a single patient with advanced, chemotherapy-resistant GIST [3]. Subsequently, a phase 2 trial led to the approval of imatinib as standard treatment in advanced GIST [4]. Today, mutational analysis is strongly recommended to guide treatment. In patients with a c-kit exon 11 mutation, the optimal imatinib dose is 400 mg daily without interruption, whereas in patients harboring an exon 9 mutation 800 mg daily is recommended [5]. In case of further progression or intolerance to imatinib standard, second-line treatment is given using sunitinib, a multitarget TKI inhibiting vascular endothelial growth factor receptors (VEGFRs) 1–3, PDGFR, KIT and Flt-3 [5, 6]. Regorafenib is a novel, oral multikinase inhibitor that blocks the activity of multiple protein kinases, including those involved in the regulation of tumor angiogenesis (VEGFR-1, -2, and -3, and TIE2), oncogenesis (KIT, RET, RAF-1, BRAF, and BRAFV600E), and the tumor microenvironment (PDGFR and FGFR). In Europe, regorafenib was approved in CHECK MONAT 2014 and is recommended for the third-line treatment of patients when imatinib and sunitinib failed [7].

In recent years, it was clearly established that patients with completely resected high-risk GIST, as classified by Miettinen et al. [8], should receive adjuvant treatment with imatinib 400 mg daily, for at least 3 years [9]. However, based on small numbers of patients it seemed that those harboring a GIST with c-kit exon 9 mutation or wild-type did not benefit from a daily dose of 400 mg imatinib [10].

Targeting Her2 in Gastroesophageal Adenocarcinoma

Overexpression or amplification of Her2, a member of the epidermal growth factor receptor family, is present in 15–25% of breast cancers and stimulates cell proliferation by activating the MAP-kinase pathway and by inhibiting apoptosis. Breast cancer patients in whom there is an overexpression of Her2 benefit from treatment with trastuzumab, a fully humanized monoclonal antibody against the Her2-receptor [11, 12]. In gastroesophageal (GE) adenocarcinoma, Her2 is overexpressed or amplified (fig. 1) in 20–30% of tumors with substantial difference according to tumor type (intestinal type 31.8% vs. diffuse-type 6.1%) and tumor location (esophageal adenocarcinoma 32.2% vs. gastric cancer 21.4%) [13]. In the pivotal ToGA trial, the efficacy of trastuzumab in combination with standard chemotherapy was studied in patients with Her2-positive-advanced GE adenocarcinoma as first-line treatment [14]. In this international trial, patients from Asia, Australia,
Europe, Latin America, and South Africa were screened for Her2 overexpression or amplification by immunohistochemistry (score 3+) and fluorescence in situ hybridization (FISH+). Of the screened patients, 810 were found Her2 positive (22.1%) and 594 patients who were eligible were randomly assigned to 6 cycles of fluoropyrimidine (either capecitabine or infusional 5-FU) and cisplatin or fluoropyrimidine and cisplatin plus trastuzumab. In the investigational arm, trastuzumab was given at day 1 (loading dose 8 mg/kg, thereafter 6 mg/kg) every 3 weeks until disease progression. Patients who received chemotherapy and trastuzumab had a longer progression-free survival (PFS) (6.7 vs. 5.5 months) and overall survival (OS) (13.8 vs. 11.1 months) compared to patients treated only with chemotherapy. Moreover, response rates were significantly higher in patients receiving additional trastuzumab (47.3 vs. 34.5%). Furthermore, patients with strong Her2 overexpression and amplification (IHCl+ and FISH+) had a median OS of 17.9 months compared with 10.6 months in patients with amplification of Her2, but lacking immunohistochemical overexpression. The ToGA trial is exceptional in different ways. First, it is a positive trial with an OS benefit of nearly 3 months for the included study population with Her2-positive tumors. Because of the selection of study sites, different ethnic populations were recruited, and the results seem to be transferable to different parts of the world. The most striking benefit was reported for patients with strong Her2 overexpression and amplification, whereas those patients with tumors demonstrating Her2 amplification only did not substantially benefit from additional treatment with trastuzumab. This finding highlighted that selection is essential for personalization of therapeutic improvements. Intra- and interlaboratory validation of Her2 analysis by immunohistochemistry and FISH is crucial to reproduce the results of the ToGA trial in the practice setting. Recently, data from the MAGIC trial were published, showing that Her2 overexpression did not influence the outcome of perioperative chemotherapy [15].

Lapatinib is a TKI, which binds to the intracellular tyrosine kinase domains of the epidermal growth factor receptor (EGFR) and Her2, thereby blocking autophosphorylation and downstream signaling. In first-line treatment of patients with Her2-positive GE adenocarcinoma, the addition of lapatinib to capecitabine and oxaliplatin (CapeOx regimen) did not improve the efficacy as shown in the TRIO-013/LOGiC trial [16]. The OS was 12.2 months in the CapeOx plus lapatinib arm versus 10.5 months in the CapeOx arm (HR 0.91). According to the recently published TyTAN trial, which recruited patients with Her2-amplified gastric cancer from China and Japan, even the addition of lapatinib to paclitaxel as second-line treatment did not improve survival statistical significantly (11.0 vs. 8.9 months, HR 0.84) [17].

For Her2-negative tumors, no targeted treatment strategy with a predictive biomarker has been established so far. In the AVAGAST trial, combining first-line capecitabine, cisplatin, and bevacizumab, an antibody against VEGF-A, did not result in an OS benefit compared to standard chemotherapy, although progression-free survival and tumor response rate were improved [18]. However, patients with high baseline plasma VEGF-A and those with low baseline plasma neuropilin-1 levels showed a trend toward better survival if they received bevacizumab [19]. Recently, data published from two pivotal trials using the VEGFR antibody ramucirumab either alone or in combination with paclitaxel in the second-line treatment of advanced GE cancer showed a survival benefit for patients in the ramucirumab arms [20, 21]. In January 2015, ramucirumab was approved either alone or in combination with paclitaxel by the EMA for second-line treatment of metastatic GE adenocarcinoma. It will be interesting to understand whether plasma VEGF-A and neuropilin-1 levels – or maybe other potential biomarkers for tumor angiogenesis – will help to predict treatment response in this disease.

RAS in Colorectal Cancer

Ras proteins, named after the discovery in rat sarcoma, belong to a protein family acting as GTPase and are ubiquitously involved in cellular signal transduction. Cancer-related mutations in RAS result in constitutive activation, thus permanently activating signal pathways that control proliferation, differentiation, cell adhesion, apoptosis, and cell migration (fig. 2). In humans, three prominent RAS genes have been linked to cancer: HRAS, KRAS and NRAS [22]. Mutations in KRAS (exon 2, 3 and 4) and NRAS (exon 2, 3 and 4) are found in approximately 50% of advanced colorectal cancers (CRC), with the vast majority of mutations involving KRAS exon 2 (90%) [23, 24]. Cetuximab and panitumumab are both monoclonal antibodies that target and inhibit the epidermal growth factor receptor (EGFR), which activates the Ras pathway. Cetuximab was initially approved by the EMA in 2004 without biomarker restriction for patients with metastatic colorectal cancer. The first evidence that treatment with cetuximab favors patients with KRAS wild-type tumors was reported in 2006 [25]. In 2008, the indication was consequently restricted to patients with a KRAS wild-type
Panitumumab was approved by the EMA in 2007 for patients with KRAS wild-type metastatic colorectal cancer [27]. Today, both therapeutic antibodies are indicated for patients with RAS wild-type metastatic colorectal cancer only, since tumors with either a KRAS or NRAS exon 2, 3 or 4 mutation do not benefit from these treatments [28]. In the target population with RAS wild-type metastatic colorectal cancer both cetuximab and panitumumab in combination with backbone chemotherapy increase progression-free survival as well as OS clinically very meaningful (fig. 3). In the PRIME study, combining panitumumab with FOLFOX versus FOLFOX only PFS was 10.1 vs. 7.9 months and OS was 26.0 vs. 20.2 months [23]. In the CRYSTAL trial, combining cetuximab

---

**Fig. 2.** The linkage between the epithelial growth factor receptor (EGFR) and the RAS pathway. Mutation rates of RAS and BRAF in selected tumor types are shown.

<table>
<thead>
<tr>
<th>Study</th>
<th>Biomarker</th>
<th>n</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRYSUL26, 9</td>
<td>KRAS</td>
<td>316</td>
<td>0.80 (0.67–0.95)</td>
</tr>
<tr>
<td></td>
<td>RAS</td>
<td>178</td>
<td>0.69 (0.54–0.88)</td>
</tr>
<tr>
<td>PRIME23</td>
<td>KRAS</td>
<td>325</td>
<td>0.83 (0.70–0.98)</td>
</tr>
<tr>
<td></td>
<td>RAS</td>
<td>259</td>
<td>0.78 (0.62–0.99)</td>
</tr>
<tr>
<td>FIRE-30, 31</td>
<td>KRAS</td>
<td>297</td>
<td>0.77 (0.62–0.96)</td>
</tr>
<tr>
<td></td>
<td>RAS</td>
<td>171</td>
<td>0.70 (0.53–0.92)</td>
</tr>
<tr>
<td>PEAK32</td>
<td>KRAS</td>
<td>142</td>
<td>0.62 (0.44–0.89)</td>
</tr>
<tr>
<td></td>
<td>RAS</td>
<td>88</td>
<td>0.63 (0.39–1.02)</td>
</tr>
<tr>
<td>CALGB33, 34</td>
<td>KRAS</td>
<td>1,137</td>
<td>0.92 (0.78–1.09)</td>
</tr>
<tr>
<td></td>
<td>RAS</td>
<td>526</td>
<td>0.90 (0.70–1.10)</td>
</tr>
</tbody>
</table>

---

**Fig. 3.** Forrest plot of treatment hazard ratios (HR) with 95% confidence intervals (CI) for the OS of selected phase 2 and 3 first-line trials in patients with metastatic colorectal cancer treated with the EGFR antibodies cetuximab [26, 29–31, 33, 34] or panitumumab [23, 32]. For all trials data for KRAS and RAS populations are shown.
imab with FOLFIRI versus FOLFIRI only PFS was 11.4 vs. 8.4 months and OS was 28.4 vs. 20.2 months [29]. Three recent randomized trials addressed the question whether the combination of an EGFR antibody with chemotherapy was more effective than bevacizumab with chemotherapy in the first-line treatment of patients with metastatic colorectal cancer. In the FIRE-3 study, FOLFIRI plus cetuximab was compared to FOLFIRI plus bevacizumab. This trial showed a substantial increase in the OS of 3.7 months for the cetuximab group even in the KRAS exon 2 wild-type population [30]. For patients with a RAS wild-type, the incremental OS benefit was 7.5 months for the cetuximab arm [31]. In the PEAK phase 2 trial, comparing FOLFOX plus panitumumab versus FOLFOX plus bevacizumab, it became evident that the OS was 41.3 vs. 28.9 months, but that PFS was also significantly improved in the panitumumab arm [32]. In the CALGB/SWOG trial, the OS in the RAS group was 32.0 months in the chemotherapy (either FOLFOX or FOLFIRI) plus cetuximab group (n = 270), compared to 31.2 months in the chemotherapy plus bevacizumab group (n = 256), which is statistically not significant [33, 34]. Further data of this trial are awaited, since the RAS group represents less than 50% of the KRAS exon 2 wild-type population and treatment durations were not reported yet.

**Prognostic Value of BRAF Mutations and Microsatellite Instability in CRC**

BRAF is a member of the RAS/RAF/MEK/ERK pathway and the most common activating BRAF mutation in CRC is the V600E mutation. This mutation is present in up to 15% of all tumors and up to 80% of tumors with high microsatellite instability (MSI). Moreover, BRAF mutations occur exclusively in RAS wild-type tumors and are associated with proximal location, high-grade and mucinous histology [35]. The presence of a BRAF mutation is a strong negative prognostic marker with a median OS ranging from 9 to 14 months in patients with metastatic CRC. Although, patients with a BRAF mutation do also benefit from anti-EGFR-based therapy, this benefit seems less significant than in patients with BRAF wild-type tumors [23, 26, 28]. Since BRAF mutations are rare, definitive evidence of which treatment is most beneficial for these patients is currently not available. In stage III patients, BRAF is not a convincing predictive biomarker [36]. However, in patients with metastatic colorectal cancer it seems that triplet chemotherapy plus bevacizumab might be beneficial. In the TRIBE phase 3 trial, treatment with FOLFOXIRI plus bevacizumab seems superior to FOLFIRI plus bevacizumab (mean OS 19.1 vs. 10.8 months, HR 0.55; n = 28) [37]. This is also supported by a non-randomized, prospective trial by the Italian group, reporting a median PFS and OS of 11.8 and 24.1 months, respectively, in a pooled cohort of 25 patients with mutant BRAF treated with FOLFOXIRI plus bevacizumab [38].

The prognostic and predictive value of MSI is still controversial. Some trials demonstrated a survival benefit for stages II and III colorectal cancer patients with MSI tumors [39], while others saw no benefit [40] or an even worse outcome for patients with MSI stage II tumors when treated with adjuvant 5-FU [41]. The later finding provides the base for the ESMO recommendation to include the MSI status in the decision-making process for or against adjuvant chemotherapy in stage II colorectal cancer patients [42].

**MET – A Potential Predictive Biomarker in GE Cancer and HCC**

A potential predictive biomarker in GE cancer is MET. Met is the receptor tyrosine kinase for hepatocyte growth factor (HGF), which is essential for liver regeneration and wound healing. Downstream signaling after Met activation involves MAPK, PI3K/Akt, STAT and NFκB. The Met pathway is frequently activated due to MET gene amplification, transcriptional upregulation, point mutations, or ligand-mediated stimulation in human cancer, which can promote tumor growth and metastasis [43]. Recently, a phase 1/1b trial with AMG 377, an oral inhibitor of Met kinase activity, demonstrated dramatic responses in an extension cohort of patients with MET-amplified GE cancer [44].

In patients with advanced HCC after failure of treatment with sorafenib and with Met overexpression, the novel oral Met inhibitor tivantinib is currently studied in a phase 3 trial [45]. Tivantinib stabilizes the inactive conformation of the Met receptor tyrosine kinase, thus disrupting constitutive and ligand-mediated activation. The overexpression of Met in tumor tissues was a negative prognostic factor in a randomized phase II trial of tivantinib in patients with advanced HCC following progression on one prior treatment regimen [46]. In this study, it was found that tivantinib had promising anti-tumor activity only in the subgroup of Met high tumors (37 patients in total, 22 in the tivantinib and 15 in the placebo arm). Consequently, a pivotal randomized, double-blind study of tivantinib as single-agent therapy in previously treated patients with overexpression of Met, inoperable
HCC was started in the summer of 2013. In this trial, only patients with confirmed Met overexpression, as assessed by immunohistochemistry using the CONFIRM anti-Tot al c-MET (SP44) monoclonal antibody, were included. Of note, recent data indicated, that tivantinib might also be cytostatic, acting through interference with microtubule formation, thus inducing G2/M arrest and promoting apoptosis [47]. Other Met-inhibiting TKIs are currently under investigation in patients with advanced HCC (table 1).

In colorectal cancer, Met overexpression seems to be associated with more advanced stages and metastasis [48], induce VEGF-A production [49] and might be linked to EGFR therapy resistance [50].

**High AFP in HCC – Prognostic and Predictive Value?**

Alphaetoprotein (AFP) is a glycoprotein that is produced in early fetal life by the liver and by a variety of tumors including HCC, hepatoblastoma and germ cell tumors. Among patients with cirrhosis, serum AFP is widely used as a surveillance test for HCC. At the current cut-off value of 10.9 ng/ml, the sensitivity for early HCC is 66% [51]. Several studies indicated that patients with advanced tumors stages have much higher serum AFP levels than those with early stages. Thus, high AFP levels, especially greater than 400 ng/ml, are considered a strong negative prognostic marker [52]. Recently, results of the REACH trial, a randomized phase 3 study of the VEGFR antibody ramucirumab versus placebo as second-line therapy in patients with HCC were presented [53]. Although this study did show a significantly longer OS (9.2 vs. 7.6 months, HR 0.87) in the intent-to-treat population (n = 565), patients with AFP levels greater than 400 ng/ml (n = 250) achieved nearly a doubling in OS in the ramucirumab group (7.8 vs. 4.2 months, HR 0.0059). Therefore, further studies in this group of patients are warranted.

**Evolving Techniques for Molecular Tumor Classification**

Molecular classification and genotyping of tumor tissue should nowadays become routine practice in clinical oncology for many cancers. Today, most routine techniques are based on immunohistochemistry, in-situ hybridization or different sequencing methods, for example, Sanger-sequencing or pyro-sequencing. Advances in high-throughput technologies such as microarray-based techniques for protein expression analysis and next generation sequencing as well as plasma-derived biomarker analysis (e.g. BEAMing) will ease molecular tumor profiling and hopefully result in the establishment of further personalized systemic treatment strategies for patients with gastrointestinal cancers in the near future.

**Conflict of Interest**

J. Trojan received consulting and/or lecture fees from Amgen, Daichi Sankyo, Merck Serono, Lilly Imclone, Roche and Novartis.

---


<table>
<thead>
<tr>
<th>Compound</th>
<th>Phase</th>
<th>Sponsor</th>
<th>Trial name</th>
<th>Setting</th>
<th>Confirmed MET dysregulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tivantinib</td>
<td>3</td>
<td>Daichi Sankyo</td>
<td>Study of tivantinib in subjects with inoperable hepatocellular carcinoma who have been treated with 1 prior therapy (METIV-HCC)</td>
<td>Second-line</td>
<td>Yes</td>
</tr>
<tr>
<td>Cabozantinib</td>
<td>3</td>
<td>Exelisis</td>
<td>Study of cabozantinib (XL184) vs. placebo in subjects with hepatocellular carcinoma who have received prior sorafenib (celestial)</td>
<td>Second- or third-line</td>
<td>No</td>
</tr>
<tr>
<td>INC280</td>
<td>2</td>
<td>Novartis</td>
<td>Study efficacy and safety of INC280 in patients with advanced hepatocellular carcinoma</td>
<td>First-line</td>
<td>Yes</td>
</tr>
<tr>
<td>MSC2156119J</td>
<td>1b/2</td>
<td>Merck Serono</td>
<td>Phase 1b/2, multicenter, single arm trial to assess the efficacy, safety, and pharmacokinetics (PK) of MSC2156119J as monotherapy in subjects with MET+ advanced hepatocellular carcinoma (HCC) with Child-Pugh class A liver function who have failed sorafenib treatment</td>
<td>Second-line</td>
<td>Yes</td>
</tr>
</tbody>
</table>
References


Ciardiello F, Lenz HJ, Innocenzi F, Mahoney MR, O’Neill BH, Shaw JE, Polite BN, Hostcher HS, Atkins JN, Goldberg RM, Mayer RJ, Schilsky RL, Bertagnolli MM, Blanke CD; Cancer and Leukemia Group B (Alliance), SWOG, and ECOG: CALGB/ SWOG 80405: phase III trial of irinotecan/5-FU/leucovorin (FOLFIRI) or oxaliplatin/5-FU/leucovorin (mFOLFOX6) with bevacizumab (BV) or cetuximab (CET) for patients (pts) with KRAS wild-type (wt) untreated metastatic adenocarcinoma of the colon or rectum (MCRC). J Clin Oncol 2014;32(suppl):abstr LBA3.


Venook AP, Niedzwiecki D, Lenz HJ, Innocenzi F, Mahoney MR, O’Neill BH, Shaw JE, Polite BN, Hostcher HS, Atkins JN, Goldberg RM, Mayer RJ, Schilsky RL, Bertagnolli MM, Blanke CD; Cancer and Leukemia Group B (Alliance), SWOG, and ECOG: CALGB/ SWOG 80405: phase III trial of irinotecan/5-FU/leucovorin (FOLFIRI) or oxaliplatin/5-FU/leucovorin (mFOLFOX6) with bevacizumab (BV) or cetuximab (CET) for patients (pts) with KRAS wild-type (wt) untreated metastatic adenocarcinoma of the colon or rectum (MCRC). J Clin Oncol 2014;32(suppl):abstr LBA3.


