EXPERT OPINION

Monoclonal antibodies in gastrointestinal cancers

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Introduction: Among gastrointestinal cancers, colorectal and gastric neoplasms are the most frequent. The development of new targeted drugs improved the efficacy of systemic therapy in advanced stages of those malignancies.

Areas covered: This review highlights the main biological processes implicated in gastrointestinal cancer development and progression, such as angiogenesis and epidermal growth factor receptor (EGFR) signaling pathway. On these bases, anti-EGFR and anti-vascular endothelial growth factor (VEGF) monoclonal antibodies in colorectal and gastric cancer are discussed. Data about further monoclonal antibodies in development are also reported.

Expert opinion: The use of monoclonal antibodies in colorectal and gastric cancers showed the best outcomes when combined with chemotherapy, even though single agent anti-EGFR antibodies seem active in particular setting of metastatic colorectal cancer (CRC) patients. It is not well defined whether the addition of anti-VEGF and anti-EGFR to chemotherapy could improve outcome in those patients susceptible to CRC-related metastases resection. Little and conflicting data are available about the role of these drugs in adjuvant setting. Tests are available to select patients with higher probability to get benefit from these treatments. Further biomarkers need to be evaluated to improve this selection and achieve "tailorization" of systemic therapy.

Keywords: antibody, cancer, colorectal, gastric, monoclonal

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1. Introduction

Gastrointestinal cancers cover a great part of all malignancies in world population. Some of them, such as colorectal cancer (CRC), are more frequent in Western countries and represent some of the leading causes of cancer-related death. In the last decades, the therapeutical approaches have been changed by the integration of improved surgical procedures, radiation and systemic therapy. This improvement was mainly influenced by the combination of different cytotoxic drugs and the introduction of targeted drugs, in particular monoclonal antibodies (mAbs). Those agents target the biological pathways, which are already known to drive the tumor development and progression. To date, angiogenic process and epidermal growth factor receptor (EGFR) pathway are recognized as the most relevant hallmarks of gastrointestinal cancers. The addiction of cancer cell function to oncogenes implicated in those pathways seems to be struck by specific mAbs, such as anti-EGFR, anti-human epidermal growth receptor 2 (HER2) and anti-vascular endothelial growth factor (VEGF).

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Article highlights.

- The mAbs in gastrointestinal cancer are directed against VEGF-related and EGFR/HER2-related pathways.
- Gastrointestinal cancers, like other tumors, develop 'oncogene addiction', which could be inhibited by specific mAbs.
- Bevacizumab is an anti-VEGF mAb used in mCRC treatment in combination with chemotherapy.
- Cetuximab and panitumumab are anti-EGFR mAbs delivered alone or in combination with chemotherapy.
- Trastuzumab is an anti-HER2 mAb, which was recently approved for advanced GC overexpressing HER2.
- New mAbs are under development for gastrointestinal cancers.

This box summarizes key points contained in the article.

2. Biological pathways in gastrointestinal carcinogenesis

2.1 Angiogenic pathway

Among the hallmarks of cancer, angiogenesis has a predominant role in gastrointestinal cancers. This is a process of the formation of new blood vessel within the tumor, essentially to provide nutrients and oxygen. VEGF is an endothelial cell-specific mitogen and an angiogenesis-inducer released by a variety of tumor cells and expressed in human tumors *in situ* [1]. VEGF was first isolated in 1983 as a factor leading to increased vascular permeability in tumors and was thus also called vascular permeability factor [2]. VEGF (also designated as VEGF-A) is the founding member of a family of homodimeric glycoproteins that are structurally related to the platelet-derived growth factors; this family also includes placenta growth factor, VEGF-B, VEGF-C, VEGF-D and VEGF-E. This VEGF family of proteins selectively binds with different affinities to at least five distinct receptors: VEGF-A binds to receptors, VEGFR-1 and VEGFR-2. Recently, the neuropilins have been identified as co-receptors for specific VEGF isoforms [3]. Targeted inactivation of VEGFR-1 and VEGFR-2 as well as neuropilin-1 in mice resulted in defects in the formation of blood vessel and embryonic lethality, demonstrating further the importance of VEGF-A for appropriate vascular development [4-6]. A variety of physiological and pathological processes are associated with upregulation of components of the VEGF/VEGFR-system, including embryogenesis, the female reproductive cycle, pregnancy, wound healing, tumor growth, diabetic retinopathy and ischemic diseases [7]. In the search for mechanisms and factors that are able to influence the VEGF expression during these processes, many cytokines and growth factors have been shown to modulate *VEGF* gene expression [8]. For example, TNF-α and bFGF are able to induce *VEGF* gene expression *in vitro*; also, glucose deficiency has been shown to increase *VEGF* expression [9]. The most important and intensively studied inducer of *VEGF* gene expression, however, is hypoxia [10-14]. Under hypoxic conditions, *VEGF* expression is mediated through the activation of specific hypoxia-inducible transcription factors, HIF-1 and HIF-2. In addition, *VEGF* upregulation during hypoxia is also achieved by an increase in the stability of its mRNA51 and by the efficient hypoxic translation of the VEGF mRNA which is mediated by an internal ribosomal entry site [15-18]. In endothelial cells, VEGFR-2 is considered to be the major signaling receptor, while VEGFR-1 acts as a sink to trap an excess of VEGF. Endothelial proliferation is mediated via the Ras-Raf-MAP (mitogen-activated protein)-kinase pathway, while protein kinase C activation is involved in endothelial migration and vascular permeability. The role of VEGF as a survival factor for endothelial cells is mediated by the phosphoinositol-3 (PI3) kinase-AKT signaling pathway [19].

2.2 EGFR/HER2 pathway

The ErbB family of receptors (also known as type I receptor tyrosine kinases [TKs]) includes four homologous receptors: the EGFR (ErbB1/EGFR/HER1); ErbB2 (HER2/*neu*); ErbB3 (HER3) and ErbB4 (HER4) [20]. The EGFR is a membrane-bound TK that contributes to signaling cascades with multiple procarcinogenic effects, including cell proliferation, motility, adhesion, invasion, cell survival and angiogenesis. EGFR overexpression has been detected in several human cancers, including CRC, and correlates with progression and metastasis. HER2 overexpression or gene amplification has been observed in multiple cancer types, including gastric tumors. As with EGFR, HER2 overexpression has been observed in aberrant crypt foci in human colon [21,22]. These receptors are composed of an extracellular binding domain, a transmembrane lipophilic segment, and an intracellular protein TK domain with a regulatory COOH-terminal segment. There is a rich crosstalk among the ErbB family that regulates the cellular effects mediated by these receptors. At least six different ligands, known as EGF-like ligands, bind to the EGFR. These ligands include EGF, transforming growth factor α, amphiregulin, heparin-binding EGF, betacellulin and epiregulin. A second class of ligands, collectively termed heregulins, binds directly to HER3 and/or HER4. After ligand binding, the ErbB receptors become activated by dimerization between two identical receptors (homodimerization) or between different receptors of the same family (heterodimerization). After receptor dimerization, activation of the intrinsic protein kinase activity and tyrosine autophosphorylation occur, recruiting and phosphorylating several intracellular substrates involving the Ras-Raf-MAPK, the PI3k-Akt and other signaling pathways that regulate multiple biological processes, including apoptosis and cellular proliferation. RAS protein function is normally regulated by cycling between inactive GDP bound and active GTP-bound forms. Signaling is terminated when RAS-GTP is hydrolyzed to the RAS–GDP inactive complex by GTPase-activating proteins. Activated RAS recruits Raf protein to the cell membrane and phosphorylates it, triggering serine-threonine kinase activity of various

proteins. Finally active mitogen-activated protein kinases (MAPKs) can translocate to the nucleus, where they regulate the activity of several transcription factors for the expression of multiple genes of survival and proliferation [22].

3. Approved mAbs for gastrointestinal cancers

3.1 Bevacizumab

The introduction of molecules, such as oxaliplatin and irinotecan, in the treatment of metastatic CRC (mCRC) achieved improved response rate and overall survival (OS). Recently the addition of targeted drugs to standard chemotherapy regimens has improved these results. Bevacizumab is a humanized mAb that inhibits VEGF as key modulator of angiogenesis. It was initially tested in a Phase II study, comparing the combination of 5-fluorouracil/Leucovorin (5FU/LV) and bevacizumab with 5FU/LV alone [23]. Subsequently, a Phase III trial evaluated patients with previously untreated mCRC who were randomly assigned to receive irinotecan, bolus fluorouracil and leucovorin (Saltz' IFL regimen) plus bevacizumab or IFL plus placebo. The median duration of survival was 20.3 months in the group treated with IFL plus bevacizumab as against 15.6 months in the group treated with IFL plus placebo. These results were very significant if considered that the control arm was a standard first-line chemotherapy regimen [24]. Other recent trials have also failed to demonstrate the same statistically significant results in survival, particularly with other backbone regimens, such as isolated capecitabine or oxaliplatin-containing regimens, such as FOLFOX or XELOXs plus placebo or bevacizumab in first-line mCRC (mOS 19.9 vs 21.3 months in bevacizumab group) [25]. Subsequently, a three-arm multicenter randomized study (E3200 trial) assigned 829 mCRC patients previously treated with a fluoropyrimidine and irinotecan to one of three treatment groups: oxaliplatin, fluorouracil, and leucovorin (FOLFOX4) with bevacizumab; FOLFOX4 without bevacizumab or bevacizumab alone. It showed that the combination of chemotherapy plus bevacizumab was better than chemotherapy alone (median progression-free survival [mPFS] was 7.2 vs 4.8 months) but even that bevacizumab alone does not have the same effect as combination regimen (mPFS 2.7 vs 7.2 months). These observations suggested that VEGF pathway is a probable mainstream. This phenomenon could be explained by the effect of bevacizumab to improve tumor vascular architecture and let chemotherapy have a higher cytotoxicity on cancer cells, as shown in some preclinical studies [26]. Moreover, as demonstrated in the first BEAT trial, bevacizumab plus conventional first-line chemotherapy regimens had an important role before surgery to improve R0 liver metastases resections rate safely in patients originally deemed unresectable giving a rationale to make prospective randomized trials evaluating the use of bevacizumab before resection of liver metastases [27]. Another point of discussion is the probable role of bevacizumab to prevent recurrence after liver metastasectomy. We know that most patients will develop local or distant recurrences after surgery for colorectal liver metastases, that adjuvant chemotherapy with 5FU-based chemotherapy has shown to improve the prognosis of these patients and that bevacizumab prolongs PFS and improves the response rate of chemotherapy in metastatic setting. For these reasons, recent controversial studies have explored a hypothetical role of anti-VEGF by adding the angiogenesis inhibitor to an adjuvant standard regimen chemotherapy to improve disease-free survival (DFS) for patients after resection of colorectal liver metastases. Different results were found: the HEPATICA Phase III trial demonstrated a favorable not-statistically significant trend in 2-year DFS (52 vs 70%) by adding bevacizumab to CAPOX chemotherapy after resection of colorectal liver metastases ($p = 0.074$). This study does not clarify the role of bevacizumab in combination with chemotherapy as adjuvant treatment for those mCRC patients resected for liver metastases [28]. A trial presented during ASCO 2011 annual meeting in which patients after complete resection of liver-confined metastases from CRC were treated with cytotoxic regimens (fluoropyrimidines alone or irinotecan or oxaliplatin-based) with or without bevacizumab. After metastasectomy, the median follow-up time was 21 months, the median OS and DFS were 54 and 13 months, respectively, meaning that adding bevacizumab to cytotoxic regimens has no impact on clinical outcomes with respect to DFS and OS after complete resection of liver-confined metastases for CRC [29].

3.2 Cetuximab

About use of cetuximab in gastrointestinal tumors, in 2004, the FDA has tried to use the cetuximab on the basis of the EPIC trial data, a multicenter open-label Phase III study, which showed an advantage in terms of time-to-progression and response rate when cetuximab was associated with irinotecan compared to irinotecan alone. OS was similar between study groups, possibly influenced by the large number of patients in the irinotecan arm who received cetuximab and irinotecan at progression [30]. Moreover, in the BOND trial, a randomized, open-label Phase II study, authors were able to demonstrate the effectiveness of cetuximab either in monotherapy or in combination with irinotecan in patients with irinotecan-refractory CRC. Although both schedules show activity in this subset of patients, combination therapy had a significantly higher response rate (22.9 vs 10.8%) and longer time to progression (4.1 vs 1.5 months), suggesting that the combination of irinotecan and cetuximab should be preferred for patients with irinotecan-refractory cancer [31]. Other two important trials, CRYSTAL and OPUS, investigated the use of cetuximab associated with chemotherapy regimens as first-line treatment for mCRC. About CRYSTAL, a randomized, open-label, multicenter trial, authors verified the efficacy of cetuximab plus irinotecan, fluorouracil and leucovorin (FOLFIRI) versus FOLFIRI alone as first-line treatment for mCRC and sought associations between the mutation status of the *KRAS* gene in tumors and clinical response to

cetuximab. The primary endpoint was PFS. Although benefit in terms of OS between the two treatment groups (hazard ratio [HR] = 0.93, p = 0.31) is not shown in this study, the data also showed that the treatment FOLFIRI + cetuximab when compared with FOLFIRI alone reduces the risk of disease progression (HR = 0.85, p = 0.048) and that benefit of cetuximab was limited to patients with KRAS wild-type tumors (HR = 0.68) [32].

Subsequent retrospective subgroup analysis of the study data revealed an advantage in terms of OS (23.5 v 20 months, HR = 0796, p = 0.0093) in the subgroup of patients KRAS wild type. Of these, in particular, the BRAF wild type had a significantly reduced risk of disease progression (HR = 0637, p = 0.0013) and significantly increased odds of response (odds ratio = 2.175, p < 0.001) compared with those who received FOLFIRI alone demonstrating how BRAF V600E mutation probably indicated poor prognosis in patients with KRAS wild-type disease in both treatment groups [33].

In the OPUS trial, a multicenter, not controlled, open-label, Phase II study, authors investigated the efficacy of cetuximab in combination with oxaliplatin-based standard chemotherapy (FOLFOX-4), compared to chemotherapy alone, in previously untreated mCRC patients. The primary endpoint was response rate. In cetuximab arm a better response rate was achieved (46 vs 36%). Median PFS and median duration of response were 12.3 and 10.8 months, respectively. The results from the OPUS trial prompted the European approval for the extension of the use of cetuximab to first-line treatment in mCRC patients expressing EGFR and KRAS wild-type [34].

But these interesting results are conflicting with other results from COIN trial, a randomized controlled trial, in which patients who had not received previous chemotherapy for advanced CRC were randomly assigned to oxaliplatin and fluoropyrimidine chemotherapy (arm A), the same combination plus cetuximab (arm B) or intermittent chemotherapy (arm C). The comparison of arm A and arm B, for which the primary outcome was OS in patients with KRAS wild-type tumors, confirmed that OS did not differ between treatment groups and control group (17.0 vs 17.9 months). Similarly, there was no effect on PFS. Overall response rate increased from 57% with chemotherapy alone to 64% with addition of cetuximab. However, toxicity rates were higher: grade 3 – 4 skin and gastrointestinal side effects were increased with cetuximab (14 vs 114 and 67 vs 97 patients in the control group vs the cetuximab group with KRAS wild-type tumors, respectively). This trial did not confirm a benefit of addition of cetuximab to oxaliplatin-based chemotherapy in first-line treatment of patients with advanced CRC and for this reason the use of cetuximab in combination with oxaliplatin and capecitabine in first-line chemotherapy in patients with widespread metastases was not recommended by authors [35].

Results are also interesting from CELIM trial in which patients underwent resection with curative intent of previously unresectable metastases. The aim of this study was to explore if the addition of cetuximab to neoadjuvant chemotherapy in patients with CRC with unresectable liver metastases can lead to a decrease in tumor size and a subsequent higher probability for curative (R0) resection. The study involved those patients who had undergone resection of the primary tumor, but who later developed liver metastases deemed unresectable. Enrolled patients were divided into two groups, one treated with cetuximab plus oxaliplatin, fluorouracil and folinic acid (FOLFOX6; group A) and the other with cetuximab plus irinotecan, fluorouracil and folinic acid (FOLFIRI; group B). In 68% of patients in group A and in 57% of those in group B a complete or partial response was achieved. The response was higher (70%) in patients whose tumors had KRAS wild-type (70%) than those with mutated KRAS (41%), remarking the predictive role of this mutation for resistance to cetuximab. Curative surgery was possible in 34% of patients. Results have shown that the addition of cetuximab achieved a higher tumor response rate and also a higher rate of radically resectable liver metastases [36].

3.3 Panitumumab

On 2006, US Food and Drug Administration granted approval to panitumumab for the treatment of patients with EGFR-expressing, mCRC with disease progression on or following fluoropyrimidine-, oxaliplatin-, and irinotecan-containing chemotherapy regimens. The efficacy as single agent was studied in randomized controlled trial (463 patients). In this study, patients were randomized to receive best supportive care (BSC) with or without panitumumab, administered until disease progression or intolerable toxicity. At progression, patients in the BSC-alone arm were eligible to receive panitumumab. The primary endpoint was PFS. Although median PFS was similar in both treatment arms (~ 8 weeks), the mean PFS was ~ 50% longer among patients receiving panitumumab than among those receiving BSC alone (96 vs 60 days, respectively) [37]. After this approval, many studies tested panitumumab in combination with chemotherapy. When administered as first- or second-line treatment in combination with chemotherapy with FOLFOX as first-line treatment, the PRIME trial, panitumumab plus chemotherapy prolonged PFS to a significantly greater extent than chemotherapy alone (9.6 vs 8 months) in patients with wild-type KRAS tumors; no significant difference between groups in OS was seen. This lack of impact on OS suggests that panitumumab is not as powerful as cetuximab, a perception that probably continues to be taken into account among clinicians and which probably has limited the acceptance of panitumumab in clinical practice [38]. In patients with mutant KRAS tumors, PFS was significantly shorter with panitumumab plus oxaliplatin-based chemotherapy than with oxaliplatin-based chemotherapy alone in the first-line treatment trial, with no significant difference between patients receiving panitumumab plus irinotecan-based chemotherapy (FOLFIRI) and those receiving FOLFIRI alone in the second-line treatment trial [39]. The

Table 1. Main studies regarding the combination of approved mAbs in gastrointestinal cancers and chemotherapy.

Study	Treatment	Patients (n)	Line of treatment	RR (%)	Median PFS (months)	Median OS
AVF2192 g	5FU/LV vs 5FU/LV + BEVACIZUMAB	209	Second	15.2	5.5	12.9
				26	9.2	16.6
AVF2107 g	IFL vs IFL + BEVACIZUMAB	813	First	34.8	6.2	15.6
				44.8	10.6	20.3
NO16966	FOLFOX-4 or CAPOX vs FOLFOX-4 or CAPOX + BEVACIZUMAB	1,400	First	49.2	8	19.9
				46.5	9.4	21.2
E3200	FOLFOX vs FOLFOX + BEVACIZUMAB	585	Second	8.6	4.5	10.8
				22.2	7.5	13
CRYSTAL	FOLFIRI vs FOLFIRI + CETUXIMAB (KRAS WT)	666	First	40	8.4	20
				57	9.9	23.5
OPUS	FOLFOX vs FOLFOX + CETUXIMAB (KRAS WT)	179	First	34	7.3	19.5
				57	8.3	22.8
COIN	XELOX/FOLFOX vs XELOX/FOLFOX + CETUXIMAB (KRAS WT)	729	FIRST	50	8.6	17.9
				59	8.6	17
EPIC	IRINOTECAN vs IRINOTECAN + CETUXIMAB	1,298	SECOND	4.2	2.6	9.9
				16.4	4	10.7
PRIME	FOLFOX vs FOLFOX + PANITUMUMAB (KRAS WT)	656	FIRST	48	8.6	19.7
				57	10	23.9
20100007	BSC vs BSC + PANITUMUMAB (KRAS WT)	329	THIRD	0	8 weeks	ND
				17	16 weeks	ND
TOGA	CISPLATIN – 5FU or CAPECITABINE vs CISPLATIN – 5FU or CAPECITABINE + TRASTUZUMAB	584	FIRST	34.5	5.5	11.1
				47.3	6.7	13.8

RR: Response rate.

possibility that panitumumab was effective in patients with KRAS Wild-Type Colorectal Cancer after Progression on Cetuximab (PANERB trial – Matges *et al.*) was also explored. Authors concluded, however, that as long as markers predictive of response to treatment with panitumumab were not generated, this option should not be adopted as effective because of its limited efficacy only in a small subset of patients [40].

3.4 Trastuzumab

Chemotherapy improved survival compared to BSC in patients with advanced gastric cancer (GC) and combination chemotherapy was superior to monotherapy [41]. About 20% of gastric and esophagogastric junction tumors overexpress HER2, providing a rationale in the past 20 years to investigate a biological target therapy as trastuzumab, a mAb directed against HER2, in this neoplasm [42]. The efficacy of trastuzumab was demonstrated through the ToGA trial, an open-label, international, Phase III, randomized controlled trial, that investigated trastuzumab in combination with chemotherapy for first-line treatment of HER2-positive advanced gastric or gastroesophageal junction cancer. Median OS was favorable for trastuzumab plus chemotherapy arm (13.8 vs 11.1 months), that corresponded to a 26% reduction in the death rate. The results showed that this combination could represent a new standard therapy for patients affected by inoperable/metastatic gastric or gastroesophageal junction cancer and this use of modern biological targeted therapies as trastuzumab will represents a new way of conceiving the chemotherapy of GC (**Table 1**) [43].

4. Molecular tests for patient selection

4.1 KRAS and BRAF mutational status in CRC

KRAS proto-oncogene is a key component of the system of signal transduction downstream of EGFR and it plays a critical role in the regulation of cell growth and proliferation. The presence of activating mutations in KRAS stimulates the RAS/MAPK signaling pathway independent of EGFR giving the advantage to the cell to avoid apoptosis and to grow and proliferate. It has been demonstrated that 40 – 45% of all colorectal adenocarcinomas are carriers of those mutations of *KRAS* and the most of them are located on codon 12 (70%) and codon 13 (30%) and less in codon [44]. Several studies showed that the activity in mCRC of anti-EGFR mAbs (cetuximab and panitumumab) is strongly linked to the wild type mutational status of Kirsten-Ras gene. For this reason KRAS-testing has become mandatory to choose the most appropriate therapy for these patients [45]. Moreover, the treatment with anti-EGFR mAbs in combination with FOLFOX-based chemotherapy in mCRC patients, carriers of mutations in the *KRAS* gene, has been shown to be even detrimental [46]. Some studies report that colorectal adenocarcinomas with *KRAS* WT gene showed a better prognosis then KRAS mutated ones. Today the relation between prognosis and KRAS mutational status remains controversial, and further studies are needed to establish the real clinical role of this biomolecular marker in mCRC. Mutational status of *KRAS* can be assessed with several laboratory techniques but the most commonly used are direct sequencing and real-time PCR. Direct sequencing is the most diffuse method and is

Table 2. Correlation between KRAS mutation status and efficacy endpoints of cetuximab-based treatment from CRYSTAL trial.

EMR 62 202-013 (CRYSTAL) [32]	KRAS wild type		KRAS MUT	
	FOLFIRI + cetuximab (n = 316)	FOLFIRI (n = 350)	FOLFIRI + cetuximab (n = 214)	FOLFIRI (n = 183)
Time of OS				
Median OS (months)	23.5	20	16.2	16.7
HR	0.796		1.035	
(95% CI)	[0.670 – 0.946]		[0.834 – 1.284]	
p value	0.0094		0.7551	
Time of PFS				
Median PFS (months)	9.9	8.4	7.4	7.7
HR	0.696		1.171	
(95% CI)	[0.558 – 0.867]		[0.887 – 1.544]	
p value	0.0012		0.2661	
Tumor response				
ORR (%)	57.3	39.7	31.3	36.1
Odds ratio	2.0693		0.8220	
(95% CI)	[1.5154 – 2.8258]		[0.5441 – 1.2419]	
p value	< 0.001		0.3475	

CI: Confidence interval; FOLFIRI: Irinotecan plus infusional 5-FU/FA; ORR: Objective response rate (patients with complete response or partial response); OS: Overall survival; PFS: Progression-free survival.

able to detect all mutations in exons 2 and 3 of *KRAS* gene despite low sensitivity. On the other hand, real-time PCR uses primers, having sufficient level of sensitivity, that bind the most common mutations in codon 12 and 13. The detection limit of these two techniques is around 20% of the mutation rate. Pyrosequencing is a technology which operates by synthesis. It represents another important method to detect KRAS mutations. It allows a real-time monitoring of DNA synthesis by the detection of the bioluminescence produced at the end of a cascade of enzymatic reactions triggered by the incorporation of a nucleotide. This technique has some advantages in comparison with standard sequencing techniques including a higher sensitivity and the possibility of sequencing shorter fragments of DNA, thus solving problems related to DNA over-fragmentation. The p.G13D mutation represents another target of *KRAS* gene, whose prognostic significance must still be well determined, in comparison with other KRAS mutations. It seems that the use of cetuximab in addition to first-line chemotherapy gives benefits to patients with *KRAS* G13D-mutant tumors. These data have been confirmed by a recent analysis by Tejpar *et al.* in which the associations between tumor *KRAS* mutation status (wild-type, G13D, G12V or other mutations) and PFS, survival, and response were investigated in pooled data from 1,378 evaluable patients from the CRYSTAL and OPUS studies (**Tables 2** and **3**). Of 533 patients with *KRAS*-mutant tumors, 83 (16%) had G13D, and these patients, treated with cetuximab in addition to chemotherapy showed an improved PFS (median = 7.4 vs 6 months; HR = 0.47, p = 0.039) and tumor response (40.5 vs 22%; odds ratio = 3.38, p = 0.042) but not survival (median = 15.4 vs 14.7 months; HR = 0.89, p = 0.68), in comparison with chemotherapy alone [9]. BRAF mutation represents another genetic target to evaluate for establishing tumor's response to anti EGFR mAbs. *BRAF* gene codificates for a kinase that regulates part of MAP kinase/ERKs signaling pathway and it is involved in many cells functions such as mitosis, differentiation and secretion. Mutations in B-raf have been described as an alternative oncogenic event in patients affected with mCRC without KRAS mutations. The most common of them is a DNA missense which leads to a valine to glutamic acid substitution (V600E). These mutations have been found ~ 10% of CRCs and it is mutually exclusive with KRAS mutations. A retrospective analysis conducted by Di Nicolantonio *et al.*, showed that a *BRAF* wild-type status is required for response to panitumumab or cetuximab and could be used to select patients who are eligible for the treatment. They analyzed responses, KRAS and BRAF status, time to tumor progression and OS of cohort of 113 mCRCs treated with cetuximab or panitumumab. In 30% of the patients, KRAS mutations were present and showed resistance to cetuximab or panitumumab. None of the V600E BRAF mutated/KRAS WT patients (11 of 79 KRAS WT patients) responded to the treatment and had significantly shorter PFS (p = 0.011) and OS (p < 0.0001) than wild-type patients [47].

4.2 HER2 expression in GC

The detection of *HER2* overexpression, or *HER2* gene amplification, represents the most important goal of the biological characterization of gastric and gastroesophageal junction cancers. The ToGA trial, a randomized, open, international, multicentric Phase III trial, showed a significant benefit with the introduction of trastuzumab administered in combination with chemotherapy, for the treatment of HER2-positive metastatic gastric and gastroesophageal junction cancer, with a

Table 3. Correlation between KRAS mutation status and efficacy endpoints of cetuximab-based treatment from OPUS trial.

EMR 62 202-047 (OPUS) [34]	KRAS wild type		KRAS MUT	
	FOLFOX4 + cetuximab (n = 82)	FOLFOX4 (n = 97)	FOLFOX4 + cetuximab (n = 77)	FOLFOX4 (n = 59)
Time of OS				
Median OS (months)	22.8	18.5	13.4	17.5
HR	0.855		1.290	
(95% CI)	[0.599 – 1.219]		[0.873 – 1.906]	
p value	0.3854		0.2004	
Time of PFS				
Median PFS (months)	8.3	7.2	5.5	8.6
HR	0.567		1.720	
(95% CI)	[0.375 – 0.856]		[1.104 – 2.679]	
p value	0.0064		0.0153	
Tumor response				
ORR (%)	57.3	34	33.8	52.5
Odds Ratio	2.5512		0.4591	
(95% CI)	[1.3799 – 4.7169]		[0.2280 – 0.9244]	
p value	0.0027		0.0290	

CI: Confidence interval; FOLFOX4: Oxaliplatin plus infusional 5-FU/FA; ORR: Objective response rate (patients with complete response or partial response); OS: Overall survival; PFS: Progression-free survival.

26% reduction of the risk of death (HR = 0.74) and a median OS of 3 months [43]. The correct determination of HER2 expression by immunohistochemistry (IHC) is in fact indicated in patients affected by gastric and gastroesophageal junction cancers in the metastatic and locally advanced settings, and it is very important to establish a valid therapeutic strategy for the treatment of these malignancies. A modified scoring system has been developed to identify HER2+ patients to treat with trastuzumab. IHC examination defines membrane status of HER2 with three levels of expression: 0+ (negative) with no reactivity or membranous reactivity in < 10% of tumor cells, 1+ (negative) with a faint/barely perceptible membranous reactivity in ≥ 10% tumor cells (or cells are reactive only in part of the membrane), 2+ (equivocal) with a weak-to-moderate complete basolateral or lateral membranous reactivity in ≥ 10% tumor cells, 3+ (positive) with a strong complete basolateral or lateral membranous reactivity in ≥ 10% tumor cells, according to the HER2 scoring system for GC (**Table 4**). A HER2 0 – 1+ score excludes the patient from the treatment with trastuzumab, whereas a 3+ score establish the membrane overexpression of HER2 receptor and leads to the possibility of using trastuzumab in combination with chemotherapy. An equivocal data is represented by a 2+ score which requires a second-level test with *in situ* hybridization techniques (FISH or CISH) to assess the state of amplification of *HER2* gene. There are some differences between breast and gastric/junction cancer in HER2 testing: more tumor heterogeneity in GC than in breast cancer cells (4.8 vs 1.4%); incomplete membrane immunoreactivity due to higher frequency of glandular formations in gastric tissue; a higher frequency of FISH+/IHC 0 – 1 in GC cells than in breast cancer [48].

5. Monoclonal antibodies in development for clinical use

Despite advances in clinical diagnostics, surgical techniques, chemotherapy and immunotherapy regimes, the prognosis of gastrointestinal cancers in the metastatic phase remains critical and new studies are needed to identify new molecules in order to offer new opportunities to patients. In this section we will look at the preclinical and clinical studies on new mAbs.

5.1 Edrecolomab

Edrecolomab is a murine mAb directed against the transmembrane glycoprotein epithelial cell adhesion molecule (EpCAM). This antigen is normally expressed on many human epithelia and overexpressed in many malignancies, including CRC. Preclinical data showed that it works by antibody-dependent cell-mediated cytotoxicity [49]. Early clinical data indicate an antitumor activity and could be beneficial for advanced cancer patients. A small Phase III trial in patients with resected stage III CRC using edrecolomab was designed, before its efficacy was formally demonstrated in cancer patients at advanced stage [50].

Edrecolomab achieved a significant improvement of relapse-free and OS, similar to that observed for FU plus leucovorin. These results yielded the approval of edrecolomab for adjuvant therapy in colon cancer in Germany [51]. Four large prospective randomized trials were prompted in patients with stage II and stage III colon cancer to confirm the results of this pivotal trial.

Two of these trials compared edrecolomab alone with no treatment in stage II disease and the others edrecolomab alone versus chemotherapy with 5-FU plus leucovorin or the combination of chemotherapy and edrecolomab in stage III colon

Table 4. HER2 assessment by IHC.

HER2 score	Surgical specimen pattern	Biopsy specimen pattern	HER2 overexpression assessment
0	With no reactivity or membranous reactivity in < 10% of tumor cells	No reactivity or no membranous reactivity in any tumor cell	Negative
1+	Faint/barely perceptible membranous reactivity in ≥ 10% tumor cells (or cells are reactive only in part of the membrane)	Tumor cell cluster with a faint or barely perceptible membranous reactivity irrespective of percentage of tumor cells stained	Negative
2+	Weak-to-moderate complete basolateral or lateral membranous reactivity in ≥ 10% tumor cells	Tumor cell cluster with a weak-to-moderate complete, basolateral or lateral membranous reactivity irrespective of percentage of tumor cells stained	Equivocal
3+	Strong complete basolateral or lateral membranous reactivity in ≥ 10% tumor cells	Tumor cell cluster with a strong complete, basolateral or lateral membranous reactivity irrespective of percentage of tumor cells stained	Positive

cancer. These studies showed a lack of efficacy of edrecolomab in the adjuvant setting of colon cancer patients [52,53].

5.2 Catumaxomab

Catumaxomab is a hybrid, trifunctional and bispecific mAb. It combines two half antibodies of mouse anti-EpCAM IgG2a and rat anti-CD3 IgG2b. Catumaxomab is defined as bispecific because it can bind two different antigens and trifunctional because it is active through three different events. Preclinical studies have, in fact, shown that one antigen binding site recognizes the EpCAM on tumor cells, the other antigen binding site binds to CD3, a component of the T-cell receptor complex, and the Fc-fragment binds to FcgR Types I and III-positive cells, including macrophages, dendritic cells and natural killer cells [54-56].

Catumaxomab has been studied for the intraperitoneal treatment of malignant ascites in patients with EpCAM-positive epithelial tumors, when standard therapy is not available or no longer feasible. Treatment consists of four constant-rate intraperitoneal infusions via intraperitoneal catheter at doses of 10, 20, 50 and 150 μg of catumaxomab on days 0, 3, 7 and 10 as proposed by the result of a Phase I/II trial. This treatment was compared with paracentesis alone in a pivotal Phase II/III study [57-58].

Catumaxomab can prolong puncture-free survival in patients with malignant ascites requiring symptomatic therapeutic paracentesis. Side effects are explained by the cytokine release induced by the drug and are usually reversible. These commonly include fever, chills, nausea and vomiting.

5.3 Anti-VEGFR mAbs

Ramucirumab (IMC-1121B) (Ram) is a fully human mAb which binds the extracellular domain of VEGFR-2 with a high affinity. It binds the ligand-binding domain of VEGFR-2, thus blocking the interaction with VEGF [59]. In a Phase I trial, Ram has been administered weekly and the most important side effects were hypertension, thrombosis, proteinuria and bleeding. The maximum weekly tolerated dose was 13 mg/kg [60]. Another Phase I study evaluated the q2-3W administration, with a similar profile of toxicity [61]. Many studies (Phases II and III) are now testing Ram for various solid tumors and most of them focus on gastrointestinal cancers, both as single agent and in combination with chemotherapy. A Phase III trial (NCT00917384 – REGARD) investigated Ram plus BSC in comparison with placebo plus BSC in patients affected by metastatic GC following disease progression after first-line platinum- or fluoropyrimidine- containing combination therapy. Final results of this study have been presented as a late-breaking poster abstract at ASCO GI 2013 Congress. The addition of Ram obtained an improvement of 0.8 months in OS (median OS: 5.2 vs 3.8 months; HR = 0.776, p = 0.0473) and 0.8 months in PFS (median PFS = 2.1 vs 1.3 months; HR = 0.483, p < 0.0001) [62]. Another Phase III study (NCT01170663 – RAIMBOW) is evaluating the use of Ram in association with paclitaxel versus paclitaxel alone as second-line treatment of metastatic gastric adenocarcinoma. A Japanese Phase Ib study (NCT01286818) is also studying the association of FOLFIRI regimen plus Ram in patients with mCRC. Partial or final results of these and other studies are expected to be available in the months to come and it is hoped that they will meet the high expectations regarding the efficacy of this new drug.

Icrucumab (IMC-18F1) is a fully human IgG1 mAb developed against human VEGFR-1/FLT-1 with antiangiogenetic and antineoplastic activities. It binds VEGFR-1 with high affinity and blocks its activity preventing the second messengers cascade and so inhibiting tumor neoangiogenesis. A Phase I study by Krishnamurthi *et al.* has been published as abstract for the 2008 ASCO meeting. In this trial designed to evaluate the safety, pharmacokinetics, pharmacodynamics and immunogenicity of IMC-18F1, 14 patients were treated with the drug and received it at the weekly dose of 2, 3, 6, 12 mg/kg, 15 mg/kg every 2 weeks, and 20 mg/kg every 3 weeks. At the date of submission no grade > 2 adverse events or dose-limiting toxicities has been observed [63]. A Phase II randomized trial (NCT01111604), which is still recruiting

patients, is evaluating safety and efficacy of modified FOLFOX 6 regimen in combination with ramucirumab (IMC-1121B) or icrucumab (IMC-18F1) or without investigational therapy as second-line therapy in patients with mCRC after disease progression on first-line irinotecan-based therapy. The primary objective of the study is PFS and first partial results are expected by the end of 2013.

6. Expert opinion

This review article aimed to highlight the effects of using mAbs targeting specific molecules in the treatment of gastrointestinal cancers.

At present, the mAbs adopted in gastrointestinal tumors therapy are directed against VEGF (bevacizumab), EGFR (cetuximab and panitumumab) or HER2 (trastuzumab).

In particular, in mCRC the addition of bevacizumab improved clinical outcomes either in first-line chemotherapy (in association with IFL regimen only) or in second-line treatment (in association with FOLFOX4). Moreover, controversial results are still available regarding the impact of bevacizumab addition to chemotherapy on liver metastases.

On the other hand, evidence has been provided that the addition of cetuximab to irinotecan-based chemotherapy in gastrointestinal cancer improved the efficacy of treatment in selected KRAS wild-type patients. In particular, the effectiveness of cetuximab was shown either in monotherapy or associated with irinotecan in patients with irinotecan-refractory CRC. On the contrary, contrasting reports are available about the benefits of associating cetuximab to FOLFOX regimen. Finally, the addition of cetuximab to neoadjuvant treatment improved the efficacy on liver metastases.

In wild type KRAS mCRC, panitumumab has shown an efficacy against BSC in patients with disease progression after different chemotherapy treatments. Furthermore, a better efficacy than first- or second-line FOLFOX treatment alone has been shown by the association with panitumumab. In KRAS-mutated patients, the association with panitumumab did not show significant difference in comparison to irinotecan-based treatment alone, whereas worse clinical outcomes were evidenced in association with oxaliplatin-based treatment. At present, panitumumab is considered to exert a less powerful action than cetuximab, and its efficacy in patients with disease progression on cetuximab remains still unproven.

The combination of trastuzumab with chemotherapy for first-line treatment of HER2-positive advanced gastric or gastroesophageal junction cancer could represent a new standard therapy for patients affected by inoperable/metastatic gastric or gastroesophageal junction cancer.

As a general observation, the highest efficacy of mAbs in gastric and CRC has been shown in combination with chemotherapy, although in selected mCRC patients, anti-EGFR antibodies have proven to be efficacious in monotherapy. Moreover, no clear results are still available about an improved outcome induced by the addition of anti-VEGF or anti-EGFR antibodies to chemotherapy in patients with resectable CRC metastases. Furthermore, a univocal evidence of efficacy in adjuvant setting was not shown.

At present, the prognosis of gastrointestinal cancers in the metastatic phase remains critical and new studies are needed to identify new molecules in order to offer new opportunities to patients. Among these, edrecolomab and catumaxomab, both directed against the EpCAM, are the most promising agents. Early clinical data on edrecolomab indicate an antitumor activity and could be beneficial for advanced cancer patients. However, a lack of efficacy of edrecolomab was shown in the adjuvant setting of colon cancer patients. Catumaxomab has been studied for the intraperitoneal treatment of malignant ascites in patients with EpCAM-positive epithelial tumors, increasing the intervals between symptomatic therapeutic paracentesis.

The main mechanism of resistance for oncogene-directed mAbs seems to be the development of 'escape routes', through the activation of alternative signaling pathways bypassing the effects of mAbs on receptors. A further mechanism of resistance may be the acquisition of mutations by which the cell becomes independent from the drug-mediated signaling block.

Contributions from basic research could help in identifying new target molecules. Interesting results have been obtained by aflibercept, a VEGF trap. Till now molecules directed against VEGFR had limited development because of related toxicity. To date some anti-VEGFR mAbs are under development to overcome resistance of standard treatment and to provide alternative strategies.

Even though the combination of mAbs with chemotherapy allowed an advancement of treatment-induced survival benefit, further strategies of combination with different doses need to be developed to optimize management of particular patient settings.

The research effort aims to find more 'tailored' systemic therapy protocols, through a selection of patients on the basis of biomarkers useful as prognostic indicators of response to treatment. Although some tests are available in order to perform such a selection, further biomarkers need to be evaluated to improve the prognostic predictability.

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Declaration of interest

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