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Immunotherapy in gastrointestinal cancer: recent results, current studies and future perspectives

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Abstract

The new therapeutic approach of using immune checkpoint inhibitors as anticancer agents is a landmark innovation. Early studies suggest that immune checkpoint inhibition is also effective in patients with gastrointestinal cancer. To improve immunotherapy in these and other settings, different strategies are currently under evaluation. This review summarizes the discussion during the European Organisation for Research and Treatment of Cancer (EORTC) Gastrointestinal Tract Cancer translational research meeting being held in November 2014 and gives an update on the most recent results of immune therapy in gastrointestinal cancers. The knowledge of the potential relationship between tumor cells and tumor microenvironment including the immune system will be essential in gastrointestinal malignancies. Thus, the density of T-cell infiltrates within colorectal (CRC) cancer metastases has been associated with response to chemotherapy, and a high expression of programmed cell death (PD) 1 ligand (PD-L1) in gastric cancer (GC) has been related with a poor prognosis. Effective targets might include neo-antigens encoded from genes carrying tumor-specific somatic mutations. Tailored immunotherapy based on such mutations could enable the effective targeting of an individual patient's tumor with vaccines produced on demand. Other strategies considering checkpoints inhibitors have shown efficacy by targeting cytotoxic T-lymphocyte–associated protein 4 (CTLA-4) and PD-1 or PD-L1. DNA mismatch repair (MMR)-deficient tumors appear to be the potential best candidates for these therapies. Finally, the combination of oncolytic viruses with immunotherapy might as well boost antitumor activity. Further evaluation of these promising immunological therapeutic approaches will require large prospective clinical studies.

Introduction

The use of immune checkpoint inhibitors in the treatment of patients with malignant melanoma and non-small cell lung cancer (NSCLC) represents a landmark therapeutic innovation which at the same time led to a renaissance of immune-mediated anticancer strategies. Initial results suggest that this approach may also be effective in the treatment of gastrointestinal carcinomas (1-5).

At the beginning of the 1990s the first human tumor-associated antigens (TAAs) were discovered in melanoma (6) enabling the evaluation of autologous, tumor cell-specific cytotoxic $CD8^+$ T-lymphocytes as a therapeutic approach (7). However, the activation of the immune system via TAAs is not sufficient to induce strong and durable antitumor immune responses in many patients. This is due to immune escape mechanisms, such as loss of antigen expression by the tumor cells, upregulation of regulatory T cells (Tregs), or the establishment of a tumor-induced protective microenvironment (8, 9). In contrast, enhanced maturation and activation of antigen-presenting cells (APCs; e.g., dendritic cells, DCs) can result in an enhanced immune response (10, 11). The characterization of tumor antigens from an individual patient basis has the potential of an adoptive personalized immunotherapy, based on the production and amplification of natural or genetically modified tumor-specific T cells (12-14). The efficacy of such an immunotherapy approach correlates with the patient's existing individual T cell repertoire (15).

To improve immunotherapy in oncology, many different strategies are currently being evaluated. Active cellular immunotherapy includes tearing down immunological barriers. Furthermore, the combination of chemotherapy or radiotherapy with immunotherapy may enhance immune response (16). This review summarizes discussions which took place at the last EORTC Gastrointestinal Tract Cancer Group translational research meeting in Mainz, November 2014 and gives an update on the most recent results of immune therapy in gastrointestinal cancers (Suppl. Table 1).

Immune cell infiltration and new molecular targets

The qualitative and quantitative analysis of tumor immune infiltration has stimulated the use of molecularly targeted agents as well as of clinical and molecular biomarkers in gastrointestinal cancers. Tumors consist of tumor cells and intratumoral stroma (ITS). (17). Wu et al. showed that a stromal gene expression signature as well as the ITS proportion quantified by morphometry in tissue sections of patient samples, were correlated and could both serve as potential prognostic markers. GC patients with high ITS were found to have poorer cancer-specific survival compared with patients with low ITS proportion (Figure 1). Measuring the relative amount of ITS may offer the possibility of identifying subgroups of GC patients that might respond to tumor stroma-directed therapies (18). Recently, tumor-infiltrating immune cells (TILs) were assessed in Epstein-Barr virus (EBV)-associated gastric cancer demonstrating the association of a better disease-free survival (DFS) in those patients with a larger percentage of TILs in ITS (19).

Recently, Halama et al. discussed the infiltrating immune cell phenotypes in the primary tumor versus metastases within the perspective of maintenance strategies in CRC. They demonstrated that the type, density and location of immune cells within primary CRC predict survival (20). The same group developed a score to investigate the prognostic and predictive significance of TIL densities at the invasive margins of CRC liver metastases (21) and described an association of high density TIL values with better outcome was confirmed in a validation cohort of 68 patients; the assessment had a sensitivity of 79% and a specificity of 100% (Figure 2). In CRC, $CD3^+$ TILs appear to be associated with prolonged survival and could potentially be more relevant for prognosis prediction than the conventional International Union Against Cancer-Tumor-Node-Metastasis (UICC-TNM) classification (20). Tanis et al. evaluated immune response in 82 patients included in EORTC study 40983, in which patients with CRC liver metastases were treated by either resection or resection with perioperative FOLFOX (22). In response to chemotherapy, increased intratumoral $CD3^+$ lymphocyte and mast cell counts were seen ($p<0.01$), underscoring previous data that an

immunogenic oxaliplatin-based chemotherapy triggers a tumoral immune infiltration. A high number of $CD3^+$ TIL and mast cells was found to be correlated with tumor regression grade. This study is the first study to suggest that the presence of mast cells in the metastasis could have a prognostic value (HR 0.54, 95% CI 0.32-0.93, p=0.03).

Response to therapy seems to be closely linked with lymphocyte infiltration in metastatic sites, indicating a more immunogenic disease state (21). Promising data in relation to disease control were observed in a phase II trial using MGN1703, a synthetic DNA-based immunomodulator, and TLR-9 agonist as maintenance treatment in patients with advanced CRC (23, 24). Subsequently, IMPALA, a phase III trial of MGN1703 in the first-line/maintenance setting in patients with metastatic CRC, is currently ongoing and aims to enroll 540 patients who have had tumor reduction after receiving first-line chemotherapy with or without biological agents (NCT02077868).

The increasing use of immune therapeutic agents such as pembrolizumab or MGN1703enhanced interest in biomarkers in CRC (Table 1). Recent data from Reissfelder et al. support the measurement of intratumoral TNF-α levels as a prognostic biomarker (25). In particular, they postulated that TNF-α might predict the activity of tumor specific in situ cytotoxic T-lymphocyte (CTL) activity. Furthermore, a retrospective multivariate analysis showed that increased TNF-α concentration was an independent prognostic factor for survival.

Gastric cancers showed PD-L1 expression in particular in tumor-infiltrating immune cells. Here, immune cell infiltrates ranged from sparse to quite dense in the tumor and/or surrounding of the tumor; (Moehler unpublished data, Figure 3). First analyses particularly in melanoma and lung cancer suggest that PD-L1-negative tumors treated with agents targeting this pathway may have a lower response rate compared with PD-L1–positive tumors (26-28). In considering expression level as a potential biomarker, the stability of PD-L1 protein in processed biopsy tissue is an important question. Even more, the prognosis and development of metastases of several different types of gastrointestinal malignancies

has been related to the expression levels of the chemokine CXC motif receptor 4 (CXCR4) (29, 30). Furthermore, CXCR4 expression levels may predict tumor recurrence (31). Recently, Thomaidis et al. showed that patients with CXCR4-positive advanced esophagogastric cancer derived a greater overall survival (OS) benefit from first-line cisplatin/leucovorin/5-fluorouracil (5-FU) (FLP) than from oxaliplatin/leucovorin/5-FU (FLO), while patients with CXCR4- and VEGFR-3-negative tumors benefited most from FLO suggesting that these markers should be further assessed as predictive biomarkers for gastrointestinal cancers (32).

Personalization of immunotherapy

The investigation of tissues and serum of different patient populations will maximize the possibility of finding clinically relevant immune-related biomarkers and may facilitate the development of diagnostic tests for patient stratification and treatment monitoring (33). The ongoing multinational translational research oriented vaccination “LICC”-trial, might serve as a model of a trial for future projects. The “LICC” trial (L-BLP25 in patients after curative resection of hepatic CRC metastases, NCT01462513) is a randomized phase II trial using BLP25 Liposome Vaccine (Tecemotide®) which targets the MUC1 glycoprotein as adjuvant immunotherapy in CRC patients with liver metastases resected with curative intent (33). This trial investigates whether L-BLP25 extends the recurrence-free survival time compared with placebo in 120 patients with CRC following R0/R1 resection of hepatic metastases. Important translational aims are to identify predictive biomarkers for the vaccination efficacy. Therefore, the incidence of MUC1-specific T cells, tumor-associated immune suppression, auto-antibody signatures in plasma, levels of tumor infiltrating immune cells, micro-RNA patterns and DNA mutations in tumors will be analysed (Figure 4) (34). Furthermore, MUC1 expression levels in tumors as well as differences in cytokine/chemokine levels in the blood will be tested as biomarkers.

Another class of targets for cancer immunotherapy are tumor-specific somatic mutations, which by their nature, are not present in normal tissues. Epitopes of the protein products of genes carrying such mutations may be recognized by the mature T cell repertoire as neo-antigens. Tailored immunotherapy approaches could exploit the substantial cancer neo-epitope repertoire with a patient specific de novo vaccine produced on demand (35). Exploring such a personalized vaccination approach with Individualized Cancer Immunotherapies (IVAC) MUTANOME (a poly-neo-epitope mRNA cancer vaccine) will confirm whether 'just in time' production of tailored cancer vaccines is feasible. By determination of patient-specific tumor mutation patterns and flexible mutation-targeting drug platforms, individualized RNA-based cancer vaccines may be rapidly and affordably synthesized, leading to potential benefits for each single patient through a personalized therapy approach (36). Mutanome individualized vaccine is currently been investigated in phase I clinical trial for the treatment of melanoma (37).

Checkpoint inhibitors

Recent results with immune checkpoint inhibitors such as ipilimumab and tremelimumab (CTLA-4 antibodies), and PD-1 or PD-L1 antibodies (e.g. nivolumab and pembrolizumab) in particular, indicate that these drugs enhance the local immune response (Figure 5, Table 2) (1). Whereas the inhibitory CTLA-4 antibodies mediate an increased activation of T cells, through antigen-presenting cells (APC) in lymphatic tissue, modulation of signaling pathways associated with PD-1 can lead to a more effective action of effector T cells by interfering with tumor-mediated immune blockade in the tumor microenvironment (Figure 6) (38).

A randomized phase II trial aimed to compare the efficacy of ipilimumab with standard of care immediately after first-line chemotherapy in the treatment of unresectable or metastatic gastric and gastro-esophageal junction cancer (NCT01585987). Furthermore, a phase I/IIb study of the PD-L1 antibody durvalumab (MEDI4736) in combination with tremelimumab in patients with gastric adenocarcinoma is ongoing (NCT02340975). In addition to the PD-1-

directed checkpoint inhibitors, nivolumab and pembrolizumab (both already approved for melanoma and NSCLC in the US), new other PD-1 or PD-L1 antibodies (e.g., atezolizumab, avelumab, etc.) are currently being evaluated alone or in combination for different tumor types, including

gastrointestinal cancers. Currently, a phase I/II study of nivolumab monotherapy or nivolumab combined with ipilimumab in four tumor types, including gastric cancer, is ongoing (NCT01928394).

Muro and Bang et al. showed that pembrolizumab is active in pretreated patients with PD-L1–expressing (>1% PD-L1 positive tumor cells and/or tumor stroma) tumors, with a response rate of 22%, 6-month progression-free survival (PFS) rate of 24%, 6-months overall survival rate of 69% and manageable side effects (39). Thus, pembrolizumab will be tested shortly in pivotal phase III studies.

Biomarkers - such as the immunohistochemical PD-L1 expression level in tumors or in the tumor environment - must be further evaluated to better define patient populations for whom immunotherapy is appropriate. A further consideration is if and under which circumstances patients might benefit from the combination of two checkpoint inhibitors in order to better overcome the immunosuppressive tumor environment. Results obtained in melanoma suggest that a combination of CTLA-4 and PD-1 inhibitors might be appropriate for PD-L1 negative tumors (40).

Defining genetic markers for immune therapy

In 2014, the Cancer Genome Atlas Research Network described a new molecular classification of gastric cancer, suggesting that the disease could be divided into four genomic subtypes: Epstein-Barr virus (EBV)-positive tumors, microsatellite (MSI)-unstable tumors, tumors with chromosomal instability (CIN), and genomically stable (GS) tumors (41). Since PD-L1 may have higher expression levels within the EBV and MSI subgroups - in the first case derived by virus stimulation and in the second case encouraged by an elevated

mutational rate – it is important to further prospectively analyse these subtypes whether they really enable selection for targeted or immunotherapies.

Comparably for quite a large CRC collection, Dienstmann et al. identified 4 biologically distinct CRC molecular subtypes (CMS1-4) enriched for key clinical, pathway and molecular traits (42). Thus, not only clinicopathologic and molecular markers (e.g., microsatellite instability, BRAF or KRAS mutations) will be used to direct CRC patients' prognostic stratification in future adjuvant and palliative therapies (43).

At the 2015 American Society of Clinical Oncology (ASCO) Annual Meeting, Le et al. presented the results of the first study on the correlation of immunotherapy with markers for MMR deficiency (44). MMR deficiency is found in approximately 15% of sporadic CRCs and in almost all hereditary non-polyposis colorectal cancers (HNPCC), a genetic predisposition syndrome which is also associated with an increased risk for gastric cancer (45). MMR-deficient tumors produce a relatively large number of aberrant protein, which may be recognized as "non-self" antigens, restimulating and triggering an antitumor response (46). Le et al. conducted a phase II study evaluating the clinical activity of pembrolizumab in 41 intensively treated patients with progressive metastatic cancers with or without MMR deficiency. The MMR-deficient CRC-group showed an objective response rate of 40% and a disease control rate of 90%. The side effects (any grade, >10%) were in accordance with previously reported safety data for pembrolizumab (44). A phase 2 study of nivolumab and nivolumab plus ipilimumab in recurrent and metastatic microsatellite high (MSI-H) colon cancer is ongoing (NCT02060188). In addition, a phase III study of pembrolizumab vs. chemotherapy in MSI-H or mismatch repair deficient (dMMR) stage IV colorectal carcinoma is planned (NCT02563002) to confirm the early observations.

Potential new combinations for checkpoint inhibitors and oncolytic viruses

To enhance antitumor activity, the combination of an engineered oncolytic virus with a checkpoint inhibitor may allow a boost over either agent alone. Oncolytic viruses are able to induce tumor cell lysis and inflammation (47). By increasing T cell numbers and activity, they

may thereby strengthen the antitumor response following treatment with anti–CTLA-4 or anti–PD-1 therapies (48). Furthermore, such combinations may enhance therapeutic effects by blocking the capacity of the local tumor microenvironment to suppress the resultant immune response (49). Puzanov et al. showed that the combination of talimogene laherparepvec (T-VEC) and the checkpoint inhibitor ipilimumab obtained promising anti-tumor activity (14). Other preclinical data are already available for Coxsackie virus (Cavatak; Viralytics) in combination with anti-PD-1 agents (50). In addition, preclinical data relating to the combined administration of Newcastle disease virus with CTLA-4 blockade have provided a strong rationale for the investigation of such approaches in the clinic (51). In both of these cases, the antitumor effect of the combination therapy was greater than that seen for the individual agents. Furthermore, ColoAd1, a chimeric oncolytic adenovirus, is currently being tested in a dose escalation study in metastatic epithelial solid tumors leading into a phase II randomized stage in metastatic CRC (NCT02028442). This phase I clinical trial that enrolled 26 CRC patients among total of 34 patients enrolled showed early safety data (52).

By observation of the shift in tumor environment (e.g., T cell number and activation state) before and after treatment with oncolytic viruses and checkpoint inhibitors, combination strategies might be further optimized (53). Many randomized trials with oncolytic virus therapy have reported considerable response rates providing promising indications for clinically effective cancer treatments (47, 54). It is interesting to note that granulocyte-macrophage colony-stimulating factor (GM-CSF) can successfully act as an immune-activating transgene in such engineered viruses (55). This provides support for the argument that the activation and targeting of an immune response is the primary mode of action of oncolytic viruses (56). To further assess the use of these viruses as anticancer agents, the development of more complex treatment strategies will be necessary (57). Moehler et al. showed that the oncolytic Parvovirus H-1 (H-1PV) combined with chemotherapeutic or targeted agents induced effective immunostimulation via a pronounced DC maturation, better cytokine release and CTL activation compared with agents alone (58).

Furthermore, activation of TLRs by H-1PV infection elicited an immune response in human DC (59).

The oncolytic and immunotherapeutic vaccinia virus Pexa-Vec (Pexastimogene devacirepvec; JX-594), which expresses GM-CSF, has also been evaluated. A randomized phase II dose-ranging study in patients with advanced HCC (n = 30) demonstrated that overall survival was significantly longer in the high-dose arm compared with the low-dose arm (14.1 versus 6.7 months, HR 0.39; p-value 0.020). In contrast, a randomized phase IIb clinical trial of this engineered virus in a poor-prognosis patient population with HCC who had failed sorafenib therapy (n = 120) did not achieve the primary endpoint of prolonging overall survival in Pexa-Vec treated patients when compared with patients treated with best supportive care (Traverse; NCT01387555) suggesting that less advanced patients may be more likely to benefit from an oncolytic immunotherapy (60, 61). A phase I/IIa study to evaluate the safety, tolerability, and efficacy of Pexa-Vec administered intravenously either alone or in combination with irinotecan in patients with CRC who are refractory to or intolerant to standard therapy is ongoing (NCT01394939).

Outlook

Promising but preliminary data support the rationale for immunotherapy in gastrointestinal cancer. Therefore, it appears advisable to perform translational studies and identify biomarkers which can select patients for clinical trials with checkpoint inhibitors to optimize benefit. More innovative combinations with checkpoint modulation will be needed to improve the initiating immune response and increase the response rates to either targeted vaccines or nonspecific cytotoxic or radio-oncological therapies (chemotherapy, radiation, transarterial chemoembolization [TACE], selective internal radiotherapy [SIRT], oncolytic viruses, etc.) (62, 63). To optimize immune-mediated tumor control, alternative combination partners like antibody modulating, co-inhibitory or co-stimulatory agonists (e.g., from the TNF receptor family), are of some interest, however, they are still in early clinical development (e.g., OX40, CD137, CD27). New toxicity profiles of immunotherapy agents are an important clinical

consideration and oncology practitioners will need to familiarize themselves with the treatment spectrum and mainly mechanism-based and/or T cell-mediated adverse events. Most of these are induced by a hyper-activated T cell response with non-tissue specific cross-reactivity against normal tissue. Herein, cytokines seem to generate diffuse (such as capillary leakage) and nonspecific events, whereas checkpoint protein inhibition, vaccines, and adaptive cell therapy seem to activate more specific T cells that interact directly with normal tissues, potentially causing specific organ damage and auto-inflammatory in nature (64).

The evaluation of the various immunological therapeutic approaches will require larger, prospective clinical studies, particularly in the context of investigator initiated studies. These studies should be designed with care and may require other criteria of evaluation than Response Evaluation Criteria In Solid Tumors (RECIST) (5, 65), and the assessment of different endpoints and parameters (66, 67) compared with the classical chemotherapeutic combination protocols. Furthermore Immune-Related Response Criteria (irRC) which is criteria based on the total tumor burden and conformation with imaging at least 4 weeks apart can be used (5).

Conflict of interest statement

MM's institution received research support from Amgen, Boehringer, Bayer and Merck Serono. MM advised or lectured to Amgen, AstraZeneca, Bayer, BTG, MSD, Merck Serono, Biontech, Lilly, Nordic, Roche and Taiho. CS received research support from Merck Serono. FL's institution received research support from GSK, Fresenius Biotech and Merck Serono. FL gave scientific advise to Amgen, Biontech, Lilly, Nordic, Ribological, Roche and Taiho. FL lectured for Amgen, Celgene, Lilly, Merck Serono, Roche and Taiho sponsored symposia. NL holds a patent on the quantification of immune cells for treatment response prediction (WO2012038068). DW gave scientific advice to Amgen, Roche, Celgene, Merck Serono and Lilly and lectured for Taiho-sponsored symposia.

All other authors did not record on any conflicts of interests.

Figure legends

Fig. 1. Tumor composition. Kaplan–Meier analysis demonstrates that gastric cancers with high intratumoral stroma (ITS) proportion have poorer cancer-specific survival compared to patients with low ITS proportion (18).

Fig. 2. Tumor-infiltrating lymphocytes (TIL) densities. (A) density score of 0 to 2 (low infiltrate density), (B) metastases with a density score of 3 (high density), (C) metastases with a density score of 4 (high density), (D) Kaplan–Meier curves of estimated progression-free survival (PFS) and (E) overall survival (OS) in all groups with scores 0-2. (low density) and scores 3-4 (high density), (F) PFS of patients according to the density of the scoring system (21).

Fig. 3. PD-L1 as a biomarker for selection. Gastric cancers show PD-L1-expression, particularly in tumor-infiltrating immune cells. Immune cells infiltrate from spare to quite dense in the tumor and/or surrounding of the tumor. Red arrow indicates tumor cells; green arrow indicates tumor-infiltrating immune cells.

Fig. 4. Biomarker discovery strategie. Collected Blood and tumor tissue samples enable identification of candidate biomarkers by use of the broad search program described in the table, with the final goal to develop diagnostic tests for patient stratification and treatment monitoring.

Fig. 5. Therapeutic approaches to targeting the cancer immunity cycle. The cancer immunity cycle is regulated by a number of checkpoint molecules. Cancer immunotherapy can be used to target these checkpoint molecules and initiate or re-initiate the cycle, re-invigorating the immune system to recognise and kill cancer cells. To promote cancer antigen presentation vaccines, administration of IFN-α or GM-CSF, and antibodies targeting CD40 or TLR cen be

used. Application of anti-CTLA-4 and anti-PDL1/PD1 antibodies can primarily promote T cell priming and activation. Stimulating (agonist) anti-CD137, anti-OX40 and anti-CD27 antibodies, or administration of IL-2 and IL-12 can promote activation and priming of T cells. VEGF inhibitors can potentially promote immigration of T cells into the tumor. Anti-PD-L1 or anti-PD-1 antibodies can primarily promote killing of tumor cells.

Fig. 6. Checkpoint inhibitors. The immune checkpoint inhibitors particularly prove the strengthening of the local immune response. They mediate an increased activation of T cells through antigen-presenting cells (APCs). CTLA-4 on tumors can bind to CD80 or CD86 on DCs and inhibit their capability to activate T cells. The use of the monoclonal anti-CTLA-4 and anti-PD-1 or anti-PD-L1 antibodies can overcome CTLA-4 or PD-L1 induced immune suppression.

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