

Immunotherapy of Colorectal Cancer

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Summary

It is known that the immune response, reflected by high T cell infiltrates in primary tumors and metastases, influences the clinical course of colorectal cancer (CRC). Therefore, immunotherapy concepts have been adapted from other tumor entities, which typically rely on the activation of T cells in the tumor microenvironment (e.g. blockade of the immune checkpoint molecules PD-1 and CTLA-4). However, most of the strategies using the approved checkpoint inhibitors and/or combination strategies have more or less failed to produce impressive results in early phase trials in CRC. Therefore, a number of novel targets for checkpoint inhibition are currently in early phase clinical testing (TIM-3, Lag-3, OX40, GITR, 4-1BB, CD40, CD70). A simple activation of infiltrating T cells will not, however, lead to a meaningful anti-tumor response without modulating the environmental factors in CRC. Thus, it is absolutely necessary to improve our understanding of the complex regulation of the tumor microenvironment in CRC to design individual combination treatments leading to effective immune control.

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Currently, colorectal cancer (CRC) is 1 of the 3 most commonly diagnosed cancer types in Europe and worldwide. In early stage CRC, overall survival rates of more than 70% for localized disease are observed. However, almost 50% of all CRC patients are diagnosed with metastatic disease or experience recurrent metastatic disease after treatment of the initial local disease. The only available curative treatment remains surgery for localized disease; in the setting of metastatic disease a small proportion of patients can be

cured by multimodal treatment. Genetically, about 10–20% of all CRC cases develop on a familiar background of mismatch repair deficiency, whereas the majority of CRCs develop sporadically.

The prognosis of patients with CRC depends on the tumor stage (Union Internationale Contre le Cancer, UICC). The current guidelines use tumor stage, grade and other risk factors, such as emergency surgery for primary disease, and perforation, to define treatment indications for adjuvant chemotherapy in stage II and III disease.

We know that the prognosis of CRC patients is strongly influenced by tumor-host interactions reflected by T cell infiltrates in primary tumors and metastatic lesions [1, 2]. Although T cell densities in the primary tumor represent a strong prognostic marker and T cell densities at the invasive margin of metastatic lesions constitute a strong predictive marker for chemotherapy response, the analysis of T cell infiltrates is not as yet part of routine diagnostic work up.

Current immunotherapies typically rely on the activation of T cells in this microenvironment. Immunotherapy using checkpoint inhibitors such as antibodies directed against cytotoxic T lymphocyte antigen-4 (CTLA-4) and programmed cell death protein (PD-1)/PD-1 receptor (PD-L1) has shown impressive results in melanoma, non-small cell lung cancer (NSCLC), renal cancer, bladder cancer, Hodgkin's lymphoma and other tumor types, and is already approved as standard treatment in a variety of indications [3–6].

PD-1, also known as CD279, is an inhibitory receptor expressed on CD4⁺ T cells, CD8⁺ T cells, NKT cells and B cells as well as monocytes and macrophages. The natural ligands for PD-1 are PD-L1 (B7-H1) and PD-L2 (B7-DC). PD-L1 is expressed on a variety of immune cells, and is significantly overexpressed in tumors. The PD-L1/PD-1 pathway is associated with immune evasion and T cell exhaustion, resulting in impaired T cell proliferation, cytokine production and cytotoxicity [7].

In CRC, PD-1 is frequently upregulated on tumor-infiltrating T cells (TIL) compared to T cells from lymph nodes, and these T cells have a decreased ability to produce cytokines and perforin [7]. The expression of PD-L1 was shown to be high in the environ-

ment, suggesting that the PD-L1/PD-1 axis plays an important role in creating an immunosuppressive environment in CRC. It was thought that in CRC anti-PD-1/PD-L1 antibodies might have similar therapeutic effects to those seen in melanoma or NSCLC, but unfortunately early phase trials using PD-1/PD-L1 blockade alone failed to induce objective responses in patients with metastatic mismatch-repair stable (MSS) CRC [8]. In that trial, pembrolizumab induced remarkable responses in patients with mismatch-repair deficient CRC (MSI). Currently, several ongoing clinical phase II and phase III trials are testing PD-1 antibodies in MSI CRC patients with pretreated and untreated metastatic disease.

CTLA-4 is another inhibiting receptor expressed on T cells and other immune cells. CTLA-4 has similar binding affinities for B7-1 and B7-2, and transmits inhibitory signals. Ipilimumab is the only approved monoclonal antibody that targets CTLA-4 and is being used as monotherapy or combination therapy in melanoma. CTLA-4 antibodies have been shown to deplete regulatory T cells (Tregs). The anti-CTLA-4 antibody tremelimumab was tested in a phase II trial in refractory metastatic CRC patients without showing any clinical efficacy [9]. Currently, several ongoing trials are testing combinations of ipilimumab with PD-1/PD-L1 antibodies as well as combinations of different checkpoint inhibitors with chemotherapy or radiotherapy in CRC.

A number of novel targets for checkpoint inhibition are currently in early phase clinical testing. One novel monoclonal antibody targeting TIM-3 (T cell immunoglobulin and mucin containing protein-3) was identified on T cells that express interferon (IFN)- γ . The ligand for TIM-3, galactin-9, inhibits Th1 T cell responses and induces apoptosis [10]. TIM-3-mediated signaling together with PD-1 induces T cell exhaustion. In animal models, TIM-3 blockade reinduced potent T cell immunity and synergistic effects have been shown for the combination with anti-PD-1. It was shown that high TIM-3 and high PD-1 expression on TILs is a marker for T cell exhaustion and dysfunction [11]. TILs in CRC seem to express higher levels of TIM-3 and PD-1 compared to T cells in the adjacent normal tissue, suggesting that TIM-3 represents a dominant inhibitory receptor on T cells in CRC. The ongoing trials will show if TIM-3 blockade might play a role in CRC patients [12].

The lymphocyte activation gene-3 (LAG-3) is a cell surface protein belonging to the immunoglobulin superfamily. LAG-3 negatively regulates T cell proliferation by interacting with MHC class II molecules. LAG-3 is expressed on T cells, NK cells, B cells and plasmacytoid dendritic cells (DC) [13]. LAG-3 expression on T cells was recognized as one of the markers indicating T cell exhaustion. A subgroup of Tregs also strongly expresses LAG-3. Blocking LAG-3 in combination with PD-1 blockade resulted in higher response rates in preclinical models [14]. Several ongoing trials are testing anti-LAG-3 in monotherapy or combination therapy with anti-PD-1 in advanced malignancies.

OX40 (CD134) represents another checkpoint molecule that belongs to the TNF receptor superfamily. OX40 is expressed transiently on activated T cells following activation of the T cell receptor (TCR), and on other cells belonging to the innate immune sys-

tem, such as NK cells, NK-T cells and neutrophils. Stimulation of these cells via OX40 results in a pro-inflammatory and pro-survival effect. The OX40 ligand (OX40L) is expressed on activated antigen-presenting cells (APCs), activated endothelial cells, epithelial cells and B cells. Agonistic OX40 antibodies and soluble forms of OX40L have been shown to induce T cell differentiation, survival, expansion and cytotoxicity. OX40 antibodies suppress Treg function. OX40 was shown to be highly expressed on TILs in CRC patients. OX40 agonistic antibodies induce objective responses in some patients [15]. However, OX40 agonists failed to induce an adequate anti-tumor response in poorly immunogenic tumors. Therefore, OX40 antibodies will probably be developed in combination with inhibitory antibodies such as anti-PD-1 or anti-CTLA-4.

The glucocorticoid-induced TNF receptor (TNFR)-related protein (GITR, CD357) is expressed at low levels on resting CD4⁺ and CD8⁺ T cells and is upregulated after TCR engagement. GITR is constitutively expressed on Tregs, resulting in an inhibitory function of Tregs. The GITR ligand (GITRL) is expressed on activated APCs and endothelial cells.

In preclinical models, agonistic GITR antibodies have induced lineage instability in Tregs and have shown costimulatory effects on effector T cells. In such models, impressive tumor responses have been observed, although it is unclear whether the costimulatory effects on T cells or the effect on the environment are responsible for these responses. Preclinical model systems with CRC have also shown impressive effects of GITR antibody in combination with CTLA-4 blockade. Ongoing trials with agonistic GITR antibodies as monotherapy or combination treatment with PD-1 antibody are ongoing, and are also recruiting CRC patients.

4-1BB (CD137) is a member of the TNFR superfamily and is known as a costimulatory receptor induced after T cell recognition. Binding of the 4-1BB ligand 4-1BBL induces T cell growth and differentiation. 4-1BB is expressed at low levels on most cells of the hematopoietic system. Agonistic 4-1BB antibody will have effects on many different immune cell subsets. In preclinical models with CRC, responses of agonistic monoclonal 4-1BB antibodies have been observed. 2 monoclonal antibodies have been tested in clinical trials showing acceptable toxicity and significant increase in activated circulating T cells. Recently, a phase II trial with urelumab in melanoma was stopped after fatal liver toxicity. The other 4-1BB antibody PF-05082566 did not show significant toxicity in early trials and is being developed in combination with antibody-dependent cellular cytotoxicity-inducing monoclonal antibodies such as cetuximab and in combination with PD-1 blockade such as nivolumab.

CD40, again a member of the TNFR superfamily, was detected on B cells and found to be expressed on DCs, monocytes, platelets and macrophage populations. There is also expression in fibroblasts, endothelial and epithelial cells. The CD40 ligand (CD40L) is expressed by activated T cells, B cells, and platelets. CD40 activation leads to enhanced antigen presentation, expression of costimulatory molecules and maturation of DCs, which triggers T cell activation. It was shown that CD40 is expressed in CRC cells, and

similar expression patterns were identified for CD40L expression [16]. There are currently 5 different agonistic monoclonal antibodies with different affinities and Fc-fragments in early phase clinical testing. Since response rates in monotherapy were low, combination treatments with checkpoint inhibitors or chemotherapy are being developed clinically.

CD70, another member of the TNFR superfamily, is expressed on T and B cells. Expression on tumor cells has also been described. CD27-CD70 interaction can lead to immune escape by inducing apoptosis in T cells, inducing T cell exhaustion and supporting Treg function. Expression levels of CD70 in CRC are reported to be not that frequent, therefore clinical development of anti CD70 antibodies will focus on other solid tumor types and hematological indications.

An agonistic CD27 antibody is being tested clinically in monotherapy and combination treatment. Objective responses in CRC have been reported.

Specific Situation in CRC

With the exception of MSI high CRC, all strategies using the approved checkpoint inhibitors and/or combination strategies with newer generation agonistic or antagonistic checkpoint molecules have more or less failed to produce impressive results in early phase trials in CRC. There are several potential reasons for this. Firstly, MSS CRC is a poorly immunogenic tumor with no or low preexisting immunity, and T cell immunity does not play a significant role. Therefore, checkpoint molecules aiming at enhancing immunity are not effective.

What argue against this hypothesis are the findings from Galon et al., Halama et al. and others that clearly demonstrate that T cells play a highly significant prognostic role in primary CRC and represent the strongest predictive parameter in metastatic disease for chemotherapy response. Also, the mutational landscape shows relevant frequencies of mutations in CRC compared to other tumor entities.

Secondly, the local tumor environment in metastatic MSS CRC is highly immunosuppressive and inhibits influx of activated T cells. Although treatment-naïve patients with metastatic disease CRC do have T cell infiltrates at the tumor site, all T cells seem to be trapped at the invasive margin (fig. 1). A simple activation of such infiltrating T cells will not lead to a meaningful anti-tumor response without modulating the environmental factors that keep the T cells outside of the tumor lesion.

Our group has analyzed the tumor environment in more detail, looking at immune cell densities and their spatial distribution as well as at cytokine/chemokine concentrations in different areas of the local environment. The tumor stroma is rich in macrophages as the dominant type of immune cells, especially M2-polarized macrophages [2, 17]. Several pathways that tumors use to create a T cell suppressive environment are activated. The CCL5/CCR5 axis seemed to be a potential target for modulating the tumor microenvironment (TME) into a more accessible milieu.

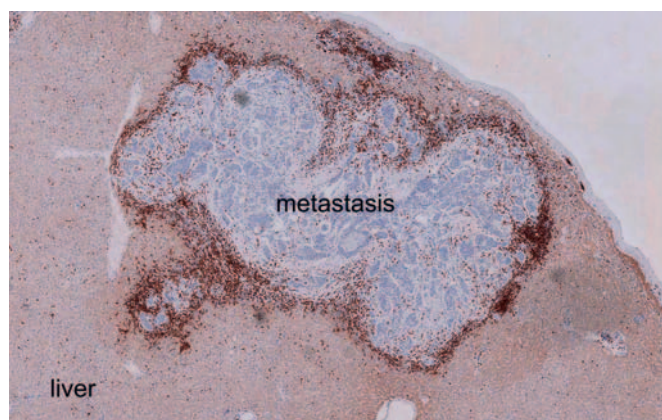


Fig. 1. CD3 staining showing intense T cell infiltrates at the invasive margin in metastatic colorectal cancer.

Preclinical human tissue models as well as a phase I clinical trial utilizing CCR5 inhibition induced a dramatic change of the environment towards a more T cell friendly environment, with redistribution of T cells, repolarization of M2 macrophages towards M1 macrophages and regression of metastatic lesions in chemotherapy refractory CRC patients [17]. This trial is the first to utilize repolarization of macrophages to modify the microenvironment and clearly shows the promising potential for this approach in CRC and other cancer entities. Based on these findings, a phase II trial testing the combination of CCR5 inhibition and pembrolizumab will start soon.

In CRC, innovative immunotherapy approaches have to address the specific immunosuppressive composition of the TME in combination with a T cell approach. Potential combination partners are drugs that specifically target the dominant suppressive cytokines/chemokines of the environment (such as CCR5 blockade) and strategies that target dominant suppressive immune cell subtypes in the environment (e.g. chemotherapy, M2 targeting/depletion, radiotherapy or other immunomodulators). There is evidence from a phase II trial that combination treatment with atezolizumab plus FOLFOX plus Avastin in refractory disease leads to objective responses and high disease control rates (Bendell, ASCO GI 2015).

To explore this strategy further, we will start a clinical trial prospectively testing the combination of atezolizumab plus FOLFOX plus Avastin in first-line metastatic CRC. The primary goal is to analyze the specific changes in the TME under treatment associated with response or resistance to treatment. Only if we understand the complex regulation of the TME in CRC, we can intelligently design combination treatments targeting the patient's individual suppressive elements leading to effective immune control. This strategy will change the conceptual design of clinical trials. Instead of giving the same treatment to the entire study population, we treat different patients in the same trial differently (different combinations, different sequences).

We rely on immunomonitoring platforms that allow us to get real-time information regarding the individual composition of the patient's TME, including immune cell densities, their activation status and their spatial distribution as well as cytokine/chemokine concentrations in different tumor areas.

Companies are developing bispecific antibodies or bite molecules that enrich cytokines such as IL-2 or IL-12 in tumors. Such molecules are also promising candidates for specific modulation of the environment.

Disclosure Statement

The authors declare that they have no conflict of interest.

References

- 1 Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pages C, Tosolini M, Camus M, Berger A, Wind P, Zinzindohoue F, Bruneval P, Cugnenc PH, Trajanoski Z, Fridman WH, Pages F: Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science* 2006;313:1960–1964.
- 2 Halama N, Michel S, Kloor M, Zoernig I, Benner A, Spille A, Pommerenke T, von Knebel DM, Folprecht G, Lubber B, Feyen N, Martens UM, Beckhove P, Gnajatic S, Schirmacher P, Herpel E, Weitz J, Grabe N, Jaeger D: Localization and density of immune cells in the invasive margin of human colorectal cancer liver metastases are prognostic for response to chemotherapy. *Cancer Res* 2011;71:5670–5677.
- 3 Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, Chow LQ, Vokes EE, Felip E, Holgado E, Barlesi F, Kohlhaufl M, Arrieta O, Burgio MA, Fayette J, Lena H, Poddubskaya E, Gerber DE, Gettinger SN, Rudin CM, Rizvi N, Crino L, Blumenschein GR, Jr., Antonia SJ, Dorange C, Harbison CT, Graf Finckenstein F, Brahmer JR: Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. *N Engl J Med* 2015;373:1627–1639.
- 4 Brahmer J, Reckamp KL, Baas P, Crino L, Eberhardt WE, Poddubskaya E, Antonia S, Pluzanski A, Vokes EE, Holgado E, Waterhouse D, Ready N, Gainor J, Aren Frontera O, Havel L, Steins M, Garassino MC, Aerts JG, Domine M, Paz-Ares L, Reck M, Baudelet C, Harbison CT, Lestini B, Spigel DR: Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. *N Engl J Med* 2015;373:123–135.
- 5 Motzer RJ, Escudier B, McDermott DF, George S, Hammers HJ, Srinivas S, Tykodi SS, Sosman JA, Procopio G, Plimack ER, Castellano D, Choueiri TK, Gurney H, Donskov F, Bono P, Wagstaff J, Gaurer TC, Ueda T, Tomita Y, Schutz FA, Kollmannsberger C, Larkin J, Ravaud A, Simon JS, Xu LA, Waxman IM, Sharma P, CheckMate 1: Nivolumab versus everolimus in advanced renal-cell carcinoma. *N Engl J Med* 2015;373:1803–1813.
- 6 Robert C, Schachter J, Long GV, Arance A, Grob JJ, Mortier L, Daud A, Carlino MS, McNeil C, Lotem M, Larkin J, Lorigan P, Neyns B, Blank CU, Hamid O, Mateus C, Shapira-Frommer R, Kosh M, Zhou H, Ibrahim N, Ebbinghaus S, Ribas A, investigators K: Pembrolizumab versus ipilimumab in advanced melanoma. *N Engl J Med* 2015;372:2521–2532.
- 7 Wu X, Zhang H, Xing Q, Cui J, Li J, Li Y, Tan Y, Wang S: PD-1(+) CD8(+) T cells are exhausted in tumours and functional in draining lymph nodes of colorectal cancer patients. *Br J Cancer* 2014;111:1391–1399.
- 8 Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, Skora AD, Lubber BS, Azad NS, Laheru D, Biedrzycki B, Donehower RC, Zaheer A, Fisher GA, Crocenzi TS, Lee JJ, Duffy SM, Goldberg RM, de la Chapelle A, Koshiji M, Bhajee F, Huebner T, Hruban RH, Wood LD, Cuka N, Pardoll DM, Papadopoulos N, Kinzler KW, Zhou S, Cornish TC, Taube JM, Anders RA, Eshleman JR, Vogelstein B, Diaz LA, Jr.: PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med* 2015;372:2509–2520.
- 9 Chung KY, Gore I, Fong L, Venook A, Beck SB, Dorazio P, Criscitiello PJ, Healey DI, Huang B, Gomez-Navarro J, Saltz LB: Phase II study of the anti-cytotoxic T-lymphocyte-associated antigen 4 monoclonal antibody, tremelimumab, in patients with refractory metastatic colorectal cancer. *J Clin Oncol* 2010;28:3485–3490.
- 10 Zhu C, Anderson AC, Schubart A, Xiong H, Imitola J, Khoury SJ, Zheng XX, Strom TB, Kuchroo VK: The TIM-3 ligand galectin-9 negatively regulates T helper type 1 immunity. *Nat Immunol* 2005;6:1245–1252.
- 11 Sakuishi K, Apetoh L, Sullivan JM, Blazar BR, Kuchroo VK, Anderson AC: Targeting TIM-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity. *J Exp Med* 2010;207:2187–2194.
- 12 Shin DS, Ribas A: The evolution of checkpoint blockade as a cancer therapy: What's here, what's next? *Curr Opin Immunol* 2015;33:23–35.
- 13 Goldberg MV, Drake CG: LAG-3 in cancer immunotherapy. *Curr Top Microbiol Immunol* 2011;344:269–278.
- 14 Blackburn SD, Shin H, Haining WN, Zou T, Workman CJ, Polley A, Betts MR, Freeman GJ, Vignali DA, Wherry EJ: Coregulation of CD8+ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. *Nat Immunol* 2009;10:29–37.
- 15 Curti BD, Kovacsocics-Bankowski M, Morris N, Walker E, Chisholm L, Floyd K, Walker J, Gonzalez I, Meeuwse T, Fox BA, Moudgil T, Miller W, Haley D, Coffey T, Fisher B, Delanty-Miller L, Rymarchyk N, Kelly T, Crocenzi T, Bernstein E, Sanborn R, Urba WJ, Weinberg AD: OX40 is a potent immune-stimulating target in late-stage cancer patients. *Cancer Res* 2013;73:7189–7198.
- 16 Georgopoulos NT, Merrick A, Scott N, Selby PJ, Melcher A, Trejdosiewicz LK: CD40-mediated death and cytokine secretion in colorectal cancer: A potential target for inflammatory tumour cell killing. *Int J Cancer* 2007;121:1373–1381.
- 17 Halama N, Zoernig I, Berthel A, Kahlert C, Klupp F, Suarez-Carmona M, Suetterlin T, Brand K, Krauss J, Lasitschka F, Lerchl T, Luckner-Minden C, Ulrich A, Koch M, Weitz J, Schneider M, Buechler MW, Zitvogel L, Herrmann T, Benner A, Kunz C, Luecke S, Springfield C, Grabe N, Falk CS, Jaeger D: Tumoral immune cell exploitation in colorectal cancer metastases can be targeted effectively by anti-CCR5 therapy in cancer patients. *Cancer Cell* 2016;29:587–601.