Abstract

Strong evidence supports the concept of immunosurveillance and immunoeediting in colorectal cancer. In particular, the density of T CD8+ and CD45+ lymphocytes infiltration was recently shown to have a better prognostic value than the classic tumor node metastasis classification factor. Other immune subsets, as macrophages, natural killer cells or unconventional lymphocytes, seem to play an important role. Induction of regulatory T cells (Tregs) or immunosuppressive molecules such as PD-1 or CTLA-4 and downregulation of antigen-presenting molecules are major escape mechanisms to antitumor immune response. The development of these mechanisms is a major obstacle to the establishment of an effective immune response, but also to the use of immunotherapy. Although immunotherapy is not yet routinely used in colorectal cancer, we now know that most treatments used (chemotherapy and biotherapy) have immunomodulatory effects, such as induction of immunogenic cell death by chemotherapy, inhibition of immunosuppression by antiangiogenic agents, and antibody-dependent cytotoxicity induced by cetuximab. Finally, many immunotherapy strategies are being developed and tested in phase I to III clinical trials. The most promising strategies are boosting the immune system with cytokines, inhibition of immunoregulatory checkpoints, vaccination with vectorized antigens, and adoptive cell therapy. Comprehension of antitumor immune response and combination of the different approaches of immunotherapy may allow the use of effective immunotherapy for treatment of colorectal cancer in the near future.

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Key words: Colorectal cancer; Immunotherapy; Immunity; Immunoregulation; Vaccination

Core tip: Immune system is now widely accepted as a key mechanism to prevent occurrence of cancer and intratumoral T CD8+ and CD45+ lymphocytes infiltrate has shown to be a major prognosis factor in colorectal cancer. However, immunity fail in controlling tumor growth, because of strong escape mechanisms to the immune system developed by the tumor. In recent years, several immunotherapy strategies have been tested in colorectal cancer. This review provides an understanding of the mechanisms involved and identifies innovating therapeutic strategies.
INTRODUCTION

With around 1 million new cases every year, colorectal cancer (CRC) is the third most frequent cancer in the world. Despite recent therapeutic advances it causes more than 500000 deaths every year. So there is a real need for therapeutic progress to reduce the risk of recurrence after surgery or prolong survival of patients with metastatic disease. Advances could be provided by understanding the role and mechanisms of the immune response in CRC and by the development of immunotherapy. Indeed there is growing evidence that the immune system may play a role in preventing the occurrence, growth and metastatic diffusion of tumors.

The aim of this review is to provide a comprehensive analysis of known mechanisms of immune response against CRC and immune escape strategies developed by tumor cells, and to present current and future perspectives in immunotherapy for CRC. In particular we will focus on the following questions: (1) What is the clinical and prognostic impact of natural immune response mechanisms? (2) What are the escape mechanisms developed by the tumor which limit the efficiency of the immune system and/or immunotherapy? (3) What is the impact of the immune system in the therapeutic effect of current standard treatments? or (4) Can we in the future develop effective immunotherapy for CRC management?

BASIC CONCEPTS IN ANTITUMOR IMMUNITY

Immune surveillance

The role of immunity in cancer was suspected in 1909 by Ehrlich, who speculated that the immune system can repress the growth of carcinomas. About 50 years later, Macfarlane Burnet and Lewis Thomas elaborated the concept of immunosurveillance, as the capacity of the immune system to promote an effective immunologic reaction to tumor cell-specific neoantigens that eliminates developing cancer before clinical expression.

However, this concept of immunosurveillance has long been questioned. When Hanahan established the six criteria necessary for the development of a tumor in 2000, immunity was not cited.

In humans, the role of immune surveillance was first suspected with observation of increased occurrence of cancer in patients with immunodeficiency. Cohorts of transplanted patients and HIV-infected subjects in particular showed a strong increase in the incidence of cancers. In humans as in murine models the increase in occurrence of neoplasias has long been explained as a consequence of carcinogenesis related to certain infectious pathogens (EBV, HPV, HIV...). However, melanoma, renal, lung, pancreatic and colon cancer are non-pathogen-related and an increased incidence of these tumors was reported in immunocompromised patients. Registries and meta-analyses of solid organ transplant recipients have shown an increased risk of CRC with a standardized incidence ratio of 1.2 to 1.8, this increased risk is more controversial in HIV-infected patients.

The anti-tumor immunosurveillance concept was finally demonstrated in animal models by Shankaran et al., who observed the occurrence of spontaneous neoplasias in immunocompetent or immunodeficient mice. Mice were kept under aseptic conditions for 15 to 21 mo. During this observation period immunocompetent mice did not develop any malignant tumors, while RAG2-/- mice deficient in T and B lymphocytes developed malignant colon and lung tumors (not known to be associated with an infectious agent) in about 50% of cases, and RAG2-/- STAT1-/- mice deficient in T and B lymphocytes and insensitive to IFNγ developed neoplasia in 80% of cases. Since then, many studies have shown the involvement, depending on the model, of the innate and/or adaptive immune response in the protection against the occurrence of malignant neoplasms.

Immunoeediting and immune escape

The immunosurveillance concept was then completed by that of immunoeediting, which describes the interactions between the immune system and the tumor, allowing cancer cells to escape immune surveillance. The selection pressure exerted by the immune system on tumor cells allows the emergence of resistant clones. According to the theory of immunoeediting, immune escape occurs in three phases: the immunosurveillance period with the elimination of tumor cells by the immune system, the latency period, corresponding to a state of equilibrium, and the phase of escape, allowing tumor progression and clinical expression.

ANTITUMOR IMMUNITY IN COLORECTAL CANCER

Innate immunity

Natural killer (NK) cells play a crucial role in preventing recurrence, and are a prognostic factor: NK cells play a major role in the immune response to cancer. They help to prevent tumors, and control tumor growth and dissemination, as shown in murine models and human models. NK cells have 2 types of receptors: activating receptors, including NKG2D, and killer inhibitory receptors (KIR). The NKG2D receptor can bind different activating ligands overexpressed on cancer cells. On the other hand, KIR recognize major histocompatibility (MHC) class I molecules and NK cells can thus also be activated by the decreased expression of MHC class I molecules reported on cancer cells. These two mechanisms can activate NK cells against tumor cells. In
addition, NK cells may exert a cytotoxic effect against cancer cells through other mechanisms such as antibody-dependent cell-mediated cytotoxicity (ADCC), and secretion of cytokines including IFNγ [20].

In CRC, an extensive intratumoral infiltration of NK cells has been reported to be associated with a better prognosis [13]. Moreover, a direct correlation between increased outcome and NK cell infiltrates is suggested [14]. In particular, NK cells could be involved in protection against cancer-initiating cells (CICs) [15]. CICs are characterized by slow growth and resistance to drugs and radiation, and play a crucial role in tumor recurrence. Recent data suggest that CICs are more sensitive to NK cells because they strongly express activating ligands as NKP30 and NKP44 and express low levels of MHC class I molecules.

**Unconventional lymphocyte T cells:** Natural Killer T (NKT) cells share characteristics of both NK cells and T cells. They recognize glycolipid antigens like α-galactosylceramide presented by CD1d, an MHC class 1-like molecule. When activated, NKT cells secrete abundant pro-inflammatory cytokines and effecter molecules involved in cell death (perforin, Fas-L, TRAIL). Increased tumor infiltration of NKT cells is associated in CRC with a better prognosis [10].

Human γδ T cells (γδ T cells) express a receptor to antigens combining a γ chain and a δ chain. This receptor can recognize different antigens usually in a non-MHC-restricted way, such as heat shock proteins or phosphorylated metabolites generated by tumor cells. γδ T cells been demonstrated to have a strong cytotoxic activity against tumor cells in CRC [23].

**Macrophages:** Tumor infiltrating macrophages (TIM) can be divided into two different subtypes with different roles in cancer [20]. M1 TIMs are intimately involved in innate immunity, as they target altered cells, produce pro-inflammatory molecules (IL-6, IL-12, IL-23 and TNFα) and promote adaptive immunity through increased expression of MHC and costimulatory molecules. They may also target tumor cells linked to antibodies because they express a receptor for immunoglobulin constant fragments (ADCC). Activated M2 TIMs are engaged in wound healing and can promote tumor progression through immunosuppressive cytokines (IL-10 and TGFβ). While infiltration by macrophages is generally a poor prognostic factor in different types of cancer, in CRC it seems to be associated with a better prognosis [19], suggesting that antitumorigenic properties dominate in vivo.

**Adaptive immunity**

A specific antitumor response is generated by the adaptive immune system, and in particular by αβ T cells. Briefly, the antigen-presenting cells (APCs), mainly dendritic cells (DCs), capture, process and present tumor antigens to CD4 T cells through MHC class II or to CD8 T cells through MHC class I. Activation of T cells requires 3 signals: (1) recognition of antigenic peptide presented by the APCs; (2) activation of costimulatory molecules (CD80/CD28, CD40/CD40L); and (3) recruitment of cytokines (IL-1, IL-2, IL-6, IL-12, IFNγ). Activated CD8 T cells can recognize and lyse tumor cells. Activated CD4 T cells modulate the antitumor immune response. They differentiate into different cell subgroups: The Th1 response allows secretion of cytokines that promote the antitumor response, as IL-2 or IFNγ, whereas the Th2 response favors tumor growth. The Th17 subset secretes large amounts of IL-17. Its role in the immune response against cancer is controversial. Finally, a subset of CD4+ T cells called regulatory T cells (Tregs) and characterized by the expression of CD25 and Foxp3, inhibit the immune response and represent a widely described mechanism whereby the tumor can escape the immune system.

**Tumor-associated antigens allow recognition of tumor cells by the immune system:** Many cells and molecules are involved in immunosurveillance, they may be linked to the host or the tumor. First, tumor-associated antigens (TAAs) allow an immune response mediated by the humoral and cellular immunity. Several types of TAAs are expressed by the tumor. In CRC, the most frequent TAAs are normal self-antigens, expressed at low levels in normal cells and in embryonic tissues and at high levels in tumor cells. The most famous of them is the carcino-embryonic antigen (CEA), which is normally expressed in fetal tissue, and widely overexpressed in CRC [24]. If it has been shown initially that CEA can lead to a specific cytotoxic response [21], more recent works have shown that CEA may have an immunosuppressive role and that T cells of patients with CRC were not activated by the presentation of this antigen in vitro [22]. Other self-antigens are thought to be immunogenic in CRC, as Ep-Cam HER-2/ neu [25], MUC-1 and p56. Immune responses against some neo-antigens, generated by mutations (tp53, Kras) or against antigen MAGE-3, belonging to the family of “cancer testis antigen” normally expressed by germ cells, have been less frequently identified [21].

TAAs, which likely play an important role in immunosurveillance, are also potential targets for immunotherapy in vaccination strategies.

**Microsatellite instability CRC is associated to immunogenic TAAs:** Microsatellite instability (MSI) is associated with CRC in patients with Lynch syndrome, but also with sporadic cancer, in particular in elderly patients, and is observed in 5% to 25% of CRC patients depending on tumor stage. MSI tumors are associated with a high density of tumor infiltrating lymphocytes (TILs) [20,27], and have a better prognosis than CRC without a microsatellite instability phenotype [28].

MSI induces frameshift somatic mutations within target genes harboring repeated sequences in their coding frame, including TGFβR2, which is mutated in 90% of
cases. These mutations lead not only to the inactivation of these target genes but also to the appearance of potentially immunogenic neoantigens. Indeed, disruption of the reading frame of TGFβR2 results in a new epitope (RLSSCVPVA) and in specific T cells to this epitope in tumors and peripheral blood of patients with MSI tumors.[27] Other MSI-associated mutations, as mutations of OGT[28], MSH3[29] caspase 5, ASTE1 and PTEN, have been shown to induce production of new immunogenic TAA s. Tougeron et al.[30] studied 19 frequently mutated genes in CRC with MSI. In samples of stage II or III MSI tumors, an increased number of mutated genes was correlated with a high density of TILs. Mutations of ASTE1 and PTEN were particularly associated with increased lymphocyte infiltrate. These results suggest an important role of the immune response to specific neoantigens in CRC with MSI, and its potential involvement in the better prognosis of these tumors. Nevertheless, CRC associated with MSI may develop specific mechanisms to escape the immune system as for example particularly high levels of intratumoral Treg described in these patients.[31] Frameshift mutations can also induce inactivation of beta2-microglobulin leading to HLA class I downregulation[32,33] though the association between HLA class I downregulation and MSI is still controversial. Altogether, CRC associated with MSI could lead to a more intense immune response, but also to specific immunoregulatory phenomena, making them good candidates for immunotherapy.

Tumor infiltrate of memory CD8 T cells and CD45RO memory T cells may predict recurrence: The role of cytotoxic CD8 T cells has been widely studied in CRC. Tumor-infiltrating lymphocytes (TILs) are central to the antitumor immune response. The prognostic role of the immune response has been analyzed in a large cohort of resected patients. Pagès et al.[34] showed that the absence of pathological signs of early metastatic invasion (venous, lymphatic and perineural invasion) was associated with increased infiltrates of immune cells and increased levels of messenger RNA (mRNA) for products of Th1 effector T cells. The density of TILs, characterized by CD3 immunostaining, has been reported to be more predictive of overall survival than all the usual histopathologic prognostic factors (i.e., UICC-TNM classification)[35]. Five-year overall survival in patients with high, intermediate or low CD3+ TILs density were of 72.6%, 49.5% and 29.9%, respectively. In multivariate analysis, the density of TILs was an independent prognostic factor, while TNM classification was no longer an independent factor. In multivariate analysis, the density of CD45RO+ cells had a median disease-free survival of respectively 36.5 mo and 11.1 mo, and a median overall survival of respectively 53.2 mo and 20.6 mo (P < 0.001 for all comparisons). In multivariate analysis, the density of CD45RO+ cells was still an independent prognostic factor.

Based on these results, an immune score based on immunostaining has been elaborated, considering 4 densities: density of CD8+ T infiltrates in the center of the tumor (CT), in the invasive margin (IM), and density of memory CD45RO+ cells in the CT and in the IM. This immune score was first studied in early-stage tumors (stages I and II)[36]. Patients with a high density of both CD8+ and CD45RO+ cells in both the CT and IM had a disease-free survival of 95.2%, compared with 25% in patients with a low density of both CD8+ and CD45RO+ cells in both regions. This immune score was validated in a cohort of 599 specimens of stage I to IV CRC[37]. In this study, assessment of immune score was a better predictor of tumor recurrence (HR = 0.64; P < 0.001) than TNM classification. However, the immune infiltrate is highly heterogeneous in a tumor, and quantification is observer-dependent. To simplify and harmonize the quantification of immune infiltrate, automated quantification of CD3+ cells can be used. Linear quantification of lymphocytes has been shown to be predictive of disease-free-survival in multivariate analysis with very good inter-observer reproducibility[38]. However, other teams have not confirmed these results yet and major information are lacking in this large retrospective series such as age, MSI status or the use of adjuvant therapy. Despite these promising results, there is still no immune quantification test in routine practice to use immune infiltrate to guide our therapeutic strategies. This underlines the difficulty to find a standardized and reproducible test that complies with daily practice. Such tests should be of particular interest for clinicians, especially for stage II patients for whom the indication for adjuvant treatment is more controversial.

MECHANISMS OF IMMUNE SYSTEM ESCAPE IN COLORECTAL CANCER

Human leukocyte antigen class I downregulation is associated with a poor prognosis

Expression of Human Leukocyte Antigen class I (HLA-I), the human MHC, class I molecules is downregulated in more than 70% of colorectal tumors[39]. In a few cases there is complete loss of HLA-I on tumor cells. Total loss of HLA-I mainly results from beta2-microglobulin inactivation in MSI tumors and LMP7/TAP2 downregulation in MSI-negative tumors[39]. Downregulation can result from loss of HLA haplotypes due to chromosomal nondisjunction or mitotic recombination, loss of HLA locus expression, or allelic loss due to point mutations or partial deletions of HLA-I genes. The prognostic significance of HLA-I downregulation has
NK cells are activated in the absence of MHC class I expression such as CCL17, CCL22 and CCL28 of Treg in tumors through the production of chemokines. The role of Tregs in cancer was first suspected from the observation of increased Tregs in peripheral blood and tumor tissue. The first mechanism is the preferential recruitment of Tregs into Treg in response to hypoxia seems also to play a crucial role in tumor-induced Treg. VEGF-A secreted by tumor in response to hypoxia also inhibits maturation of DC. Immature DC, which can express TGFβ, can favor the conversion of conventional T cells into Treg. TGFβ can also directly promote expansion of Treg through TGFβ. Recent data suggest that the number of intratumoral FOXP3+/VEGFR-2+ Tregs is more predictive of recurrence and survival than the number of FOXP3+ alone in CRC.

Other escape mechanisms

Other escape mechanisms are suspected in CRC (Figure 1). B7-H1, or PD-L1, is a stimulatory molecule known to regulate T cell function negatively by interaction with PD-1. B7-H1 is strongly expressed in CRC and is associated with poor prognosis. B7-H1 may thus play an important role in tumor cell proliferation, apoptosis, migration and invasion. Other molecules, such as CTLA-4, are involved T lymphocytes inhibition. CTLA-4 is expressed on the surface of T lymphocytes, and its ligands, CD80 and CD86, are expressed on the surface of APCs. Expression of these molecules, called “immune checkpoints”, are important mechanisms of inhibition of antitumor immune response. Recently some monoclonal antibodies targeting these molecules (PD1, CTLA-4) have shown more than promising efficacy results in solid neoplasia such as melanoma and others.

Myeloid-derived suppressor cells (MDSC) are immunosuppressive cells. As Tregs, they contribute to the immune tolerance by inhibiting the function of CD8+ T cells. The prognostic value of MDSC is not well known, but they are thought to be deleterious, as elimination of MDSC in mouse tumor models was shown to enhance antitumor responses, resulting in tumor regression.

IMPACT OF ANTICANCER TREATMENTS ON IMMUNITY IN COLORECTAL CANCER

Chemotherapy induces immunogenic cell death

Some cytotoxic chemotherapy are known to induce immunogenic cell death. In CRC murine models and human tissues, oxaliplatin- but not cisplatin-based chemotherapy can trigger pre-apoptotic calreticulin exposure and the post-apoptotic release of high-mobility group box 1 protein (HMGB1), two signals which are required for immunogenic cell death. DCs have several receptors for HMGB1, including Toll-like receptor 4 (TLR4). In a murine model with CT26 tumor cells, oxaliplatin-treated dying cells failed to elicit an antitumor immune response in TLR4-deficient mice, while TLR4+/+ controls were protected against rechallenge with the same cancer cells. Twelve to 14% of Caucasian patients present the loss-of-function allele of TLR4. In patients from the FFCD 2000-05 randomized trial (Dexeus lancet Oncol) with stage IV CRC and treated with an oxaliplatin-based combination, the TLR4 loss-of-function allele was associated with reduced progression-free and overall survival.
survival, as compared with patients carrying the normal TLR4 allele. This allele, however, was not associated with disease-free survival in another cohort of patients who underwent surgery for CRC stage II and who did not receive chemotherapy, suggesting that TLR4 is predictive of chemotherapy effectiveness, but is not a prognostic factor.

Other checkpoints, such as the P2X7 receptor (P2RX7), which has a high affinity for ATP released by dying tumor cells and carried by DCs, are required for the anticancer immune response induced by chemotherapy and could modulate susceptibility to treatments. Others immune mechanisms could be induced by cytotoxic chemotherapy. It has been shown in murine model that 5-fluorouracil could lead to a decrease of MDSC in the spleen and tumors in vivo, combine to a T cell-dependent antitumor responses, but the therapeutic impact is not well established.

All these data suggest that the immune system may participate to the therapeutic effect of chemotherapy in CRC but should be confirmed in future works prospectively dedicated to this question.

**Anti-VEGF therapy inhibits Treg expansion**

As seen above, tumors can induce immunosuppressive cell populations such as Tregs. It is now well established that antiangiogenic agents decrease Treg numbers in blood and tumors. In peripheral blood of patients with renal carcinoma and different models of tumor-bearing mice, sunitinib reduces Treg numbers, and the decrease in Tregs is associated with overall survival in patients series. In a recent study, we investigated the immunomodulatory effect of antiangiogenic agents in a mouse model of colon cancer. Tregs decrease to their physiological level after treatment with sunitinib or VEGF-A antibody. However, after masitinib treatment, a multi-target tyrosine kinase inhibitor close to sunitinib but not targeting the VEGFR, Tregs were not reduced. VEGFR-2- but not VEGFR-1-specific blockade led to the same results. These results suggest that targeting the VEGF-A/VEGFR-2 pathway is sufficient to decrease Tregs in murine models of CRC. Bevacizumab directly inhibits this pathway and has been widely used in CRC since 2004. In patients with metastatic CRC, we found that bevacizumab inhibited Treg accumulation and proliferation in peripheral blood. Antiangiogenic agents could act on other immunosuppressive cells, such as myeloid-derived suppressor cells and exhausted T cells. Once again it is difficult to argue that the immunomodulating effect of bevacizumab in patients with CRC has an impact on its therapeutic efficacy. But in the future Tregs monitoring could help to predict response to bevacizumab. Furthermore this immunomodulatory effect of anti-angiogenic agents could be used to potentiate immunotherapeutic strategies.

**Activity of cetuximab may depend in part on ADCC**

Monoclonal antibodies used in therapeutics act on specific receptors to inhibit growth pathways. Some may also induce immune phenomena related to the characteristics of natural antibodies. In particular, cetuximab (chimeric IgG1 monoclonal antibody) binds epidermal growth factor (EGFR) and is used in RAS wild type metastatic CRC. It has been suggested that cetuximab, in ad-
Adoptive cell therapy (ACT) is a strategy used in the treatment of CRC. It involves the use of autologous (patient’s own) T cells for treatment. ACT was well tolerated in patients treated with cetuximab.

**Vaccination with autologous tumor cells**

Since 1992, active specific immunotherapy (ASI), consisting of immunization with irradiated autologous tumor cells as adjuvant therapy, has been used, making the procedure labor-intensive and costly. Other adjuvant vaccinations with antigen were studied. Immunization with CEA after curative resection of hepatic metastases did not improve 2-year recurrence-free survival.[77] A pilot study of adjuvant vaccination with a mutant RAS peptide in KRAS mutated stage II and III CRC induced a specific immune response with increased IFN-γ mRNA expression in 4 out of 7 patients and was well tolerated.[78] Several ongoing phase I/II studies are studying antigen vaccines using various peptides, as mucinous glycoprotein 1 (MUC, L-BLP25), MSI, or HER2neu.

**Dendritic cell-based vaccination**

A significant improvement in antitumor vaccination is provided by vectorization of antigens, in particular with DCs.[81] The second strategy used an autologous tumor cell BCG vaccine (OncoVax) in stage II or III CRC.[78,79] In the 3 studies no significant benefit was observed in the overall population, but some subgroups appeared to benefit from vaccination more than others, especially colon cancer (vs rectal) and stage II cancers (vs stage III). Patients with stage II CRC treated with OncoVax had a 5-year recurrence rate of 21.3% vs 37.7% in the control group, leading to a significantly better 5-year recurrence-free survival (P = 0.009), although there was no difference in stage III patients.[80] These results have not yet been confirmed and should lead to a pivotal phase III trial.

**Adoptive cell therapy**

Adoptive cell therapy (ACT) is mostly used in melanoma. Briefly, T cells are collected from the tumor, draining lymph nodes or peripheral blood, and are activated and expanded in vitro. Autologous T cells are then administered intravenously to the patient. To optimize the activity of ACT, some authors have tried lymphodepletion of the host, optimized cytokine cocktails and selection of CD8+ T cell clones with higher affinity for tumor cells/antigens. ACT with T cells from patient lymph nodes has been tested in 16 patients with stage I to IV CRC.[89] ACT was well tolerated.
in all cases with no side effects, and allowed a complete response in 4 out 9 patients with metastatic disease.

Similarly, autologous genetically engineered T cells with high-avidity CEA-specific T cell receptor have been used in CRC\(^\text{[91]}\). In a phase I study, 3 patients were treated and decreased serum CEA levels were observed, but all patients developed severe colitis. Genetically engineered T cells expressing chimeric antigen receptors targeting HER2 also led to severe toxicity in a patient with CRC\(^\text{[92]}\). Similar strategies, such as allogenic lymphocytes and autologous NK therapy, are currently being tested in phase I and II studies.

**CONCLUSION**

The immune system plays a major role in the eradication of tumor cells, but is bypassed by the tumor at the clinical expression phase. Various antitumor immune mechanisms are inhibited by efficient escape mechanisms. The treatments currently used in CRC (cytotoxic chemotherapy, anti-EGFR antibodies, antiangiogenic molecules) are associated with immunomodulating effects shown in vitro and in vivo. However, their clinical impact has not been well evaluated. In some cases the immune escape mechanisms are associated with an aggressive phenotype. In these cases classic treatments clearly fail, and immunotherapeutic approaches is a seducing alternative to try to improve the prognosis of these patients in the future. Several approaches can be considered. First, nonspecific immunotherapy that may use immunostimulatory molecule (GM-CSF, IL-2, IL-7) or inhibit immunosuppressive mechanisms (Treg depletion, anti-PDL1, anti-CTLA4). Second, the purpose of specific immunotherapy is the induction of a specific antitumor immune response. Various vaccination strategies, with peptide, antigen, DNA combined with vectorization techniques, could lead to
the development of effective vaccines, particularly in the advent setting. ACT with T cells or NK cells is a labor-intensive procedure, but advances in genetic engineering raise hope for such treatments. Finally, nearly 40 phase I to III clinical trials testing immunotherapy in CRC are ongoing. This will probably lead in the near future to consider one or a combination of these different strategies in our therapeutic armamentarium to fight CRC.

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**Table 1 Ongoing clinical trials, according to National Cancer Institute registration, using immunotherapy, according to strategy**

<table>
<thead>
<tr>
<th>Principle</th>
<th>Phase</th>
<th>Specificity</th>
<th>Registration number</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Peptide vaccine</td>
<td>I</td>
<td>Targeted peptide(s): Ras mutated MUC-1 HER2/Neu Survivin Frame shift peptides (MSI) Non-MDP MUC-1</td>
<td>NCT01322815 NCT01556789 NCT01730118 NCT00108875 NCT01461418 NCT01376505 NCT01462513</td>
</tr>
<tr>
<td>(B) Whole cell cancer vaccine</td>
<td>I</td>
<td>Characteristic of cancer cells: Allogenic cancer cell</td>
<td>NCT00722228 NCT00656123</td>
</tr>
<tr>
<td>(C) DC-based therapy</td>
<td>I</td>
<td>Characteristic of DCs: Autologous DCs intratumoral injection Loaded with Frame shift antigens (MSI) CEA-pulsed DCs’ IL-2 Autoologous DCs</td>
<td>NCT01882946 NCT01885702 NCT01354713 NCT01348256</td>
</tr>
<tr>
<td>(D) Inhibition of immunoregulation</td>
<td>I</td>
<td>Immunomodulation strategy: Treg depletion Anti-CTLA4 + local radiation therapy</td>
<td>NCT00986518 NCT01769222</td>
</tr>
<tr>
<td>(E) Non specific immunostimulation</td>
<td>I</td>
<td>Recombinant vaccinia virus IFN, Celecoxib + combination of chemokines IL-7 Heat killed whole cell mycobacterium IL-2</td>
<td>NCT01394939 NCT01545141 NCT01339900 NCT01539824</td>
</tr>
<tr>
<td>(F) Cell therapy</td>
<td>I</td>
<td>Characteristic of cells: Allogenic activated lymphocytes Autologous TILs + lymphocyte depletion Engineered autologous anti-ESO-1 lymphocytes Engineered autologous anti-CEA lymphocytes Engineered autologous natural killer T cells</td>
<td>NCT01309126 NCT00149006 NCT00855452 NCT01714212 NCT00670478 NCT01723036 NCT01801852</td>
</tr>
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</table>

Several strategies are used in clinical trials of immunotherapy: (A) Vaccination with direct injection of one or more peptides; (B) Immunization using whole irradiated tumor cells; (C) Vaccination using autologous dendritic cells (DCs) and/or charged DCs with one or more antigens; (D) Inhibition of immunoregulatory mechanisms; (E) Non specific stimulation of the immune system; (F) Adoptive cell therapy using tumor infiltrating lymphocytes (TIL) or lymphocytes from peripheral blood, possibly reworked to target specific antigens. Source: [http://clinicaltrials.gov/](http://clinicaltrials.gov/). IL: Interleukin; IFN: Interferon.


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10.1126/science.1129139


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