

2016 Colorectal Cancer: Global view

Immunotherapy in human colorectal cancer: Challenges and prospective

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Abstract

Human colorectal cancer (CRC) is the third most commonly diagnosed malignancies and the prognosis for patients with recurrent or metastatic disease is extremely poor. Although new chemotherapeutic regimen improves survival rates, therapy with better efficacy and less adverse effects is drastically needed. Immunotherapy has been investigated in human CRC for decades with limited success. However, recent developments of immunotherapy, particularly immune checkpoint inhibitor therapy, have achieved promising clinical benefits in many types of cancer and revived the hope for utilizing such therapy in human CRC. In this review, we will discuss important immunological landscape within the CRC microenvironment and introduce immunoscore system to better describe immunophenotyping in CRC. We will also discuss different immunotherapeutic approaches currently utilized in different phases of clinical trials. Some of those completed or ongoing trials are summarized. Finally, we provide a brief prospective on the future human CRC immunotherapy.

Key words: Immunotherapy; Human colorectal cancer; Adoptive cell therapy; Immune checkpoint inhibitor therapy; Immunosuppression

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Core tip: Immunotherapy has recently achieved great clinical objective response in multiple cancer types. However, immunotherapy in human colorectal cancer (CRC) is still in its infancy. Identifying CRC patients who are responding to different forms of immunotherapy is drastically needed. In this review, we will discuss important immunological landscape within the CRC microenvironment and introduce immunoscore system

to better describe immunophenotyping in CRC. Knowledge gained from these studies may provide rational design for immunotherapy in human CRC patients.

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INTRODUCTION

Human colorectal cancer (CRC) is the third most commonly diagnosed malignancy and is the leading cause of death worldwide^[1]. The projected global burden of CRC is expected to increase by 60% to more than 2.2 million new cases and 1.1 million deaths by 2030^[2]. The combined use of irinotecan, oxaliplatin and oral form of 5-fluorouracil (5-FU) has shown significant therapeutic efficacy in human CRC. In addition, the neoadjuvant treatment of radiotherapy with/without chemotherapy and radical surgery with complete mesorectal excision has become the standard treatment for locally advanced rectal cancer. However, the toxicity of radiotherapy and chemotherapy such as actinic colitis is substantial and is associated with considerable morbidity and mortality. Adverse effects including chronic diarrhea and potential intestinal obstructions seriously impair the quality of life of the patients with CRC^[3].

Tumor immunotherapy has gained momentum in recent years and shown significant clinical benefits for many types of cancer. For example, Ipilimumab, a specific antibody (Ab) for cytotoxic T lymphocyte associated antigen 4 (CTLA-4) has been used as the first or second line of immunotherapy for advanced melanoma patients^[4]. In addition, anti-program death-1 (PD-1) Ab (nivolumab and pembrolizumab) was approved by the FDA in 2015 to treat patients with advanced non-small cell lung carcinoma (NSCLC) including squamous and non-squamous NSCLC^[5]. Combined anti-CTLA-1 and anti-PD-1 mAbs to treat metastatic melanoma have shown remarkable clinical benefits^[6,7]. The overall response rate reached over 60%. Therefore, tumor immunotherapy was proclaimed recently as "the Advance of the Year" by the American Society of Clinical Oncology (<http://www.asco.org/press-center/asco-names-advance-year-cancer-immunotherapy>). Despite great successes achieved in other cancer types, immunotherapy in human CRC is still in its infancy. This may be due to many factors such as lack of good ways to study CRC in humans and colorectal animal models are inadequate. In this review, we will examine an immunological landscape within the CRC microenvironment and summarize current major immunotherapies in human CRC. In addition, we will

discuss potential challenges for CRC immunotherapies and offer our own perspective on this issue.

IMMUNOLOGICAL LANDSCAPE IN HUMAN CRC

The prognostic value of tumor infiltrating immune cells within the tumor microenvironment has been extensively studied and appreciated^[8,9]. A molecular classification of human CRC has characterized the group of patients with microsatellite-unstable (MSI) and microsatellite-stable (MSS) tumors. In CRC, MSI is due to a DNA mismatch repair deficiency leading to accumulation of insertions and deletions in DNA repeat sequence. Consequently, this defect results in many mutations that may generate potential immunogenic neoantigens which can be recognized by the immune system^[10]. Studies analyzing patients with MSS tumors have shown associations of T cell subpopulations with prognosis. In addition, MSI CRC which comprises approximately 15% of sporadic CRC shows high levels of tumor-infiltrating T cells^[11]. This is correlated with high mutational load in MSI tumors, typically 10-50 times more than those of MSS tumors. The high gene mutational load may lead to more tumor-specific neoantigens which may provoke potent anti-tumor T cell responses despite their inability to eradicate cancer naturally. Indeed, recent studies have shown that cancers with high gene mutational load have better therapeutic outcomes in response to immune checkpoint inhibitor therapy^[12-14]. MSI tumors display more infiltrations of CD8⁺ cytotoxic T lymphocytes (CTLs) as well as activated Th1 cells with more IFN- γ production and the Th1 transcription factor T-bet. In contrast, MSI tumors have less Th17 or Th2 cells. Therefore, in general, patients with MSI tumors have better survival than patients with MSS CRC. On the other hand, MSI CRC has high expression levels of immune checkpoint molecules including PD-1, PD-L1, CTLA-4, LAG-3, and IDO leading to an immunosuppressive microenvironment. This is also a good candidate for immune checkpoint inhibitor therapy^[15]. In addition to DNA mismatch repair status, recent studies show that immunoscore is a better predictor for patient survival than microsatellite instability in human CRC^[16,17]. Immunoscore is a scoring system based on the quantitated numbers of cytotoxic and memory T cells infiltrating in the core of the tumor and in the invasive margins of the tumor^[8,18]. Previous studies have shown that immunoscore is the strongest survival prognostic factor in CRC^[19]. More importantly, immunoscore may be used as a criteria parameter to choose patients with CRC for immunotherapy since a pre-existing anti-tumor T cells in the tumor is critical for tumor immunotherapy such as anti-PD-1 immune checkpoint inhibitor therapy. Indeed, it appears that patients with MSI CRC could be more responsiveness to such therapy than MSS patients^[15]. However, a recent study using integrative

Table 1 Gene mutations in human colorectal cancer

Pathways	Cell cycle	WNT	MAPK	TGF	P53	PI3K-Akt	Apoptosis
Genes	MLH1 ^[71] MSH2 ^[71] MSH6 ^[81] RAD54B ^[85] CCND1 ^[88] BUB1B ^[90] AURKA ^[91] EP300 ^[92]	APC ^[71] CTNNB1 ^[77] MCC ^[82] AXIN2 ^[86] ARID1A SOX9	KRAS ^[72] PLA2G2A ^[78] PTPN12 ^[83]	SMAD4 ^[73]	P53 ^[74]	PTEN ^[75] NRAS ^[79] PIK3CA ^[84] FGFR3 ^[87] AKT1 ^[89]	DCC ^[76] BAX ^[80]

MLH1: MutL homolog 1; MSH2: MutS homolog 2; MSH6: MutS homolog 6; RAD54B: RAD54 homolog B (*S. Cerevisiae*); CCND1: Cyclin D1; BUB1B: BUB1 mitotic checkpoint serine/threonine kinase B; AURKA: Aurora kinase A; EP300: E1A binding protein P300; PC: Adenomatous polyposis coli; CTNNB1: Catenin (cadherin-associated protein), Beta 1, 88 kDa; MCC: Mutated in colorectal cancers; AXIN2: Axin 2; ARID1A: AT rich interactive domain 1A (SWI-Like); SOX9: SRY (sex determining region Y)-Box 9; KRAS: Kirsten rat sarcoma viral oncogene homolog; PLA2G2A: Phospholipase A2, group IIA (platelets, synovial fluid); PTPN12: Protein tyrosine phosphatase, non-receptor type 12; SMAD4: SMAD family member 4; P53: Tumor protein P53; PTEN: Phosphatase and tensin homolog; NRAS: Neuroblastoma RAS viral (V-Ras) oncogene homolog; PIK3CA: Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha; FGFR3: Fibroblast growth factor receptor 3; AKT1: V-Akt murine thymoma viral oncogene homolog 1; DCC: DCC netrin 1 receptor; BAX: BCL2-associated X protein.

analyses in CRC found that patients with MSI CRC and a subgroup of MSS have high intratumoral immune gene expression which correlates with prolonged survival, suggesting that immunoscore may be a better indicator to predict patient survival^[16].

GENETIC MUTATIONS IN HUMAN CRC

Genetic mutational loads have been linked to immune checkpoint inhibitors therapeutic efficacy^[14,20]. In human NSCLC patients, anti-PD-1 therapeutic efficacy is associated with a higher number of mutational alterations in the tumors^[12]. As described above, the MSI CRC microenvironment displays highly infiltrated activated CD8⁺ cytotoxic T cells. In human CRC, it also shows that mismatch repair-deficient tumors are more responsive to PD-1 blockade treatment than mismatch repair-proficient tumors. This is correlated with mutational loads in the tumors as mismatch repair-deficient tumors have a mean of 1782 somatic mutations while 73 mutations in mismatch repair-proficient tumors^[21]. Table 1 summarizes different gene mutations through different pathways in human CRC.

IMMUNOTHERAPY IN HUMAN CRC

Currently, there are many immunotherapies under clinical investigation in human CRC. Immunotherapy in CRC contains different approaches, including monoclonal antibody (mAb) therapy, cancer vaccines, chemoimmunotherapy, immune checkpoint inhibitors therapy, adoptive cell therapy, immune modulators, oncolytic virus therapy, adjuvant immunotherapy and cytokines treatment. Most immunotherapies are still in early-stage clinical development (phase I and II) for CRC treatment and some of these trials showed promising results. Until now, more than 15 immunotherapy clinical trials for human CRC have been completed and more than 20 clinical trials are

recruiting or about to recruit patients (https://www.clinicaltrials.gov/ct2/results?term=colorectal+cancer+and+immunotherapy&no_unk=Y&pg=3). Some of those completed trials are summarized in Table 2 and ongoing trials are listed in Table 3. We will also briefly discuss five major immunotherapeutic approaches in CRC.

mAb therapy

Cetuximab and Panitumumab which target the epidermal growth factor receptor (EGFR) are humanized Ab and have been approved to treat metastatic CRC either alone or in combination with chemotherapeutic drugs^[22]. However, approximately 4 out of 10 CRC patients have *KRAS* or *NRAS* mutations which make these Ab therapy ineffective. Therefore, testing for these gene mutations before treatment is necessary^[23]. The mechanism of action for Cetuximab is mainly *via* blockade of EGFR signaling which is vital for tumor cell growth. In addition, Cetuximab also engages immune mechanisms such as antibody-dependent cellular cytotoxicity and/or complement-dependent cytotoxicity for tumor killing^[24,25]. Vascular endothelial growth factor Ab (Bevacizumab) was also approved initially for the first-line treatment of patients with metastatic CRC in combination with 5-FU-based chemotherapy^[26]. However, the overall response rate is limited and adverse effects are substantial including increased risk for cardiac ischemic events. There are more humanized Abs currently in different phases of clinical trials such as alectumumab against EpCAM, labetuzumab against carcinoembryonic antigen (CEA), and pemtumomab against Mucins.

IMMUNE CHECKPOINT INHIBITORS THERAPY

CTLA-4 is an immune checkpoint molecule that down-regulates T cell activation by binding to CD80/CD86

Table 2 Completed clinical trials of immunotherapy in human colorectal cancer

Ref.	Clinical phase	No. patients	Clinical setting	Immunotherapy	Comments
Morse <i>et al</i> ^[93]	II	74	Liver or lung metastases from CRC removed by surgery	PANVAC-V + PANVAC-F + DC/ PANVAC-V + PANVAC-F + GM-CSF	Good safety record
Vermorken <i>et al</i> ^[44]	II and III	254	Colon cancer	Active specific immunotherapy (ASI) with an autologous tumor cell- bacillus Calmette-Guérin (BCG) vaccine with surgical resection/ resection alone	ASI gave significant clinical benefit in surgically resected patients in stage II colon cancer
Hanna <i>et al</i> ^[94]	Dukes' stage B2-C3	80	Colon or rectal cancer	ASI with an autologous tumor cell- BCG vaccine	ASI may be beneficial to patients with colon cancer
	Stage II and Stage III		Colon cancer	ASI consisting of autologous tumor cells mixed with BCG (OncoVAX™)	Increase 5-yr survival rate and 5-yr disease-free survival rate, reduce recurrence rate
Marshall <i>et al</i> ^[95]	Stage IV		Colon cancer	ALVAC-CEA	Safe and can elicit CEA-specific CTL responses
Harris <i>et al</i> ^[96]	Stage II and stage III	412	Colon cancer	Adjuvant active specific immunotherapy with an autologous tumor cell-BCG vaccine	More beneficial than resection alone

Table 3 Ongoing immunotherapy clinical trials in human colorectal cancer

Type	Cancer	Phase	Name	Identifier	Start time
Monoclonal antibodies	Untreated metastatic colorectal cancer	II	RO5520985 (a bispecific anti-ANG-2/anti-VEGF-A)	NCT02141295	5/15/2014
	Colorectal cancer	I / II	IMMU-132 (an Ab-drug conjugate targeting Trop-2)	NCT01631552	6/26/2012
	Metastatic colorectal cancer	I / II	IMMU-130 (an Ab-drug conjugate targeting CEACAM5)	NCT01605318	5/22/2012
	Metastatic colorectal carcinoma	I	MGD007 (a dual-affinity re-targeting DART protein designed to target the glycoprotein A33)	NCT02248805	9/18/2014
	Metastatic colorectal cancer	I	OMP-131R10 (an anti-RSPO3 Ab)	NCT02482441	6/19/2015
Immune checkpoint inhibitors	MSI positive/negative colorectal cancer	II	MK-3475 (an Ab that blocks negative signals on T cells)	NCT01876511	6/10/2013
	MSI positive colorectal cancer	I	Ipilimumab	NCT02060188	12/18/2013
	MSI negative colorectal cancer	II	Nivolumab	NCT02060188	12/18/2013
	Colorectal cancer	I	MED14736	NCT01975831	10/29/2013
	Colon cancer	I / II	Anti-CD27 (Varlilumab) and Nivolumab	NCT02335918	12/18/2014
Cancer vaccines	Metastatic colorectal cancer	II	DC vaccine	NCT02615574	11/24/2015
	Colorectal cancer	I	AVX701 (targets CEA)	NCT01890213	6/26/2013
	Metastatic colorectal carcinoma	I	SGI-110 in combination with an allogeneic colon cancer cell vaccine (GVAX) and cyclophosphamide (CY)	NCT01966289	10/10/2013
Adoptive cell therapy	Colorectal cancer	I	HER-2 vaccine	NCT01376505	6/9/2011
	Metastatic colorectal cancer	II	TIL (tumor-infiltrating lymphocytes)	NCT01174121	7/31/2010
	Colorectal cancer	I / II	Anti-MAGE-A3-DP4 TCR	NCT02111850	4/9/2014
	Colorectal carcinoma	I / II	CAR T cells	NCT02617134	11/26/2015
Oncolytic virus therapies	Colorectal cancer	I	NK cells + CliniMACs CD3 and CD56 systems	NCT00720785	7/22/2008
	KRAS mutant metastatic colorectal cancer	I	REOLYSIN in combination with FOLFIRI and Bevacizumab	NCT01274624	1/7/2011
Adjuvant immunotherapies	Recurrent colorectal cancer	I / II	Chemokine modulatory regimen	NCT01545141	2/29/2012
Cytokines	Colorectal carcinoma	I	AM0010 (a recombinant human IL-10)	NCT02009449	12/2/2013
	Colorectal carcinoma	I	Rh IL-15	NCT01572493	4/5/2012

molecules on antigen-presenting cells (APC). Programmed death receptor ligand 1/2 (PD-L1/L2) also negatively regulates effector T cell function by binding to PD-1 receptor on T cells. Generally induced by their respective ligands that are expressed on either tumor cells (e.g., PD-L1/ L2→PD-1) or APCs (e.g., CD80/86→

CTLA-4; PD-L1/L2→PD-1), activated CTLA-4 and PD-1 immune checkpoint pathways are potent inhibitors of tumor-reactive T cell activation, clonal expansion and subsequent tumor rejection^[27,28]. Anti-CTLA-4 and anti-PD-1 mAbs effectively block these pathways resulting in the re-activation and clonal expansion of

tumor-reactive lymphocytes. Until recently, clinical trials with immune checkpoint inhibitors showed remarkable success in patients with different cancers, including metastatic melanoma and NSCLC patients^[5,29]. Anti-CTLA mAb Yervoy (ipilimumab) was originally approved to treat non-resectable, late stage melanoma in 2011. Anti-PD-1 Ab (nivolumab and pembrolizumab) was approved by the FDA in 2015 to treat patients with advanced NSCLC including squamous and non-squamous NSCLC. Intriguingly, a new Phase 1/2 study (CheckMate 069) evaluating concurrent ipilimumab/nivolumab vs ipilimumab alone in chemotherapy naïve advanced melanoma patients reported a remarkable 61% objective response rate (34). Thus, combined ipilimumab and nivolumab was approved by the FDA for the frontline treatment of advanced melanoma in 2015.

In human CRC, a phase II clinical trial was conducted to use pembrolizumab for the treatment of mismatch repair-deficient CRC and mismatch repair-proficient CRC^[21]. Pembrolizumab was administered intravenously at a dose of 10 mg/kg every 14 d. A total of 32 patients with CRC were enrolled in this trial. Among them, 10 patients had mismatch repair-deficient tumors and 18 had mismatch repair-proficient tumors. In the patients with mismatch repair-deficient CRC, the immune-related objective response rate was 40% and the immune-related progression-free survival rate at 30 wk was 78%. In a sharp contrast, patients with mismatch repair-proficient tumors had 0% immune-related objective response rate and the immune-related progression free survival rate was 11%. More importantly, in the patients with mismatch repair-deficient CRC, the median progression-free survival and median overall survival were not reached while among the patients with mismatch repair-proficient tumors, the median progression-free survival was only 2.2 mo and the median overall survival was 5.0 mo. Although this trial has a relative small cohort of CRC patients, the findings are significant and further support the idea that mutation-associated neoantigen recognition is a critical component of the endogenous antitumor immune response. Thus patients with mismatch repair-deficient tumors may benefit greatly with anti-PD-1 immune checkpoint inhibitor therapy. In this trial, the investigators also analyzed the number of somatic mutations in tumors using whole-exome sequencing. They suggested that patients with mismatch repair-deficient tumors that have more than 20 times higher of mutation-associated neoantigens than in tumors without this deficiency should be the basis for the anti-PD-1 therapy. However, more studies need to be done to further support this conclusion.

Tremelimumab, another CTLA-4 inhibitor, is presently under clinical investigation in patients with advanced melanoma, hepatocellular carcinoma, non-small cell lung cancer and metastatic CRC^[30-33]. However, the results of one study did not show clinical benefit from the single-agent administration to the patients with

treatment-refractory CRC^[33]. Previous studies also indicate that administrate anti-CTLA-4 Ab combined with other agents significantly improve the treatment effect in colon cancer^[34-37]. However, CTLA-4 Ab treatment has reported previously that 43% of patients suffered from grades 3 to 4 autoimmune responses, such as enterocolitis, hypophysitis, dermatitis and hepatitis^[38]. A phase II clinical trial of Nivolumab and Nivolumab plus Ipilimumab in recurrent and metastatic microsatellite high colon cancer is underway (ClinicalTrials.gov Identifier: NCT02060188).

CANCER VACCINES

Cancer vaccines are designed to stimulate antigen-specific T-cell or B-cell response against cancer by providing antigens to professional APC such as dendritic cells (DCs). In addition, vaccines also include components intended to activate DCs pulsed with antigens and program them to migrate to a local lymph node.

DC vaccine

DC-based cancer vaccine has been previously approved by the FDA for the treatment of metastatic castrate-resistant prostate cancer^[39]. Since most of CRCs express CEA which is a tumor-associated antigen (TAA), DCs can be pulsed with CEA mRNA^[40] or CEA peptides^[41]. In these early clinical trials, CEA-specific T cell immune responses were elicited in most of DC vaccinated CRC patients. The vaccines were well-tolerated and safe administration. Despite induced T cell responses, these clinical trials did not show significant objective tumor regression^[42]. It may be rationale to combine DC-based vaccine with immune checkpoint inhibitor therapy to boost both endogenous and TAA-specific antitumor T cell response. In addition, some limitations of DC vaccines need to overcome, such as DC vaccine quality control, migration after vaccine injection, the specificity of tumor targets, and the expenses of clinical utilization^[43].

OncoVAX

OncoVAX is designed to utilize patients' own cancer cells with an immunostimulating adjuvant to elicit antitumor immune responses against the recurrence of colon cancer after surgery. This patient-specific vaccine is composed of metabolically-active, sterile, irradiated, and non-tumorigenic autologous cancer cells, with or without fresh frozen *Bacillus Calmette-Guerin* (BCG) bacteria as an adjuvant. In this phase III study, 254 patients were enrolled and randomized into two groups: active specific immunotherapy (ASI) with autologous tumor cell-BCG vaccine with tumor resection and resection alone. ASI was given three weekly vaccinations starting 4 wk after surgery. Patients were boosted at 6 mo with irradiated autologous tumor cells. It appears that OncoVAX has the major impact on stage II disease with a significantly

longer recurrence-free period and 61% risk reduction for recurrences. Recurrence-free survival was also significantly longer with ASI. However, the overall survival was not significantly improved^[44]. Longer follow-up studies reveal that a beneficial effect of OncoVAX is statistically significant for all endpoints including recurrence-free interval, overall survival, and recurrence-free survival but only in Stage II colon cancer but not in Stage III patients^[45]. A recent meta-analysis also suggests that combined ASI therapy with surgery showed a significantly survival benefit^[46]. However, it is unclear whether this benefit is associated with CRC patients with different stages.

ADOPTIVE T CELL THERAPY

Adoptive T cell therapy has the potential to enhance antitumor immunity and augment vaccine efficacy. Indeed adoptive cell immunotherapy based on the transfusion of genetically re-directed autologous T cells has demonstrated clinical promise for the treatment of both hematologic malignancies and solid tumors. Particularly, recent developments have been focused on gain-of-function strategy to endow effector T cells with desired antigen receptors, such as chimeric antigen receptor (CAR) T cells^[47]. Interestingly, recent studies showed that modulating T cell metabolic pathways significantly augments effect T cell antitumor responses^[48] and synergizes with immune checkpoint inhibitor therapy in cancer^[49].

In a phase I clinical trial in CRC patients, genetically engineered T cells were adoptively transferred into three patients with metastatic CRC refractory to standard treatment. Those autologous T cells were genetically engineered to express murine TCR against human CEA^[50]. Serum CEA levels dramatically decreased after treatment in all three patients (74%-99%) and one patient had an objective regression of tumor metastatic to the lung and liver. However, severe transient inflammatory colitis developed in all three patients. More clinical studies need to be done to demonstrate true benefit of CAR T cells in human CRC.

In addition to adoptive $\alpha\beta$ T cell therapy in cancer, innate $\gamma\delta$ T cells have also been shown to elicit potent antitumor immunity^[51,52]. Innate $\gamma\delta$ T cells have two major subsets with one predominately producing IFN- γ and another one secreting large amounts of IL-17. Although IL-17-producing $\gamma\delta$ T cells play critical roles in cancer progression and metastasis^[53-56], IFN- γ -producing $\gamma\delta$ T cells have potent antitumor effect. Indeed, adoptive transfer of V γ 4 $\gamma\delta$ T cells into tumor-bearing mice has shown a therapeutic effect^[57]. In addition, we showed recently that *ex vivo* expanded human V δ 1 $\gamma\delta$ T cells exhibited therapeutic effect in human colon cancer xenografted mouse model^[58]. These expanded $\gamma\delta$ T cells predominately express granzyme B, perforin, and IFN- γ . Adoptive transfer of $\gamma\delta$ T cell therapy has been done in many types of cancer with varying efficacy^[59].

CHEMOIMMUNOTHERAPY

Chemoimmunotherapy is chemotherapy combined with immunotherapy. Although chemotherapy and immunotherapy appear to be antagonistic as chemotherapeutic drugs not only kill tumor cells leading to dying tumor cells but also impact on immune cells, chemotherapy also depletes immune suppressive cells such as regulatory T cells, potentially enhancing antitumor immune response. In addition, lymphodepletion stimulates homeostatic T cell proliferation thus providing a unique opportunity for tumor immunotherapy. Chemoimmunotherapy has been widely used for hematological malignancy treatment^[60]. The first chemoimmunotherapy clinical trial conducted in human colon cancer patients was reported in 2008^[61]. Total 46 CRC patients with advanced disease were enrolled and received chemodrugs gemcitabine, oxaliplatin, levofolinic acid, and 5-FU followed by granulocyte macrophage colony-stimulating factor (GM-CSF) and IL-2 (GOLFIG-1 trial). In this trial, six patients showed a prolonged time to progression and survival. In addition, these patients had decreased Treg cells but increased central memory T cells as well as colon cancer-specific cytotoxic T cells. Interestingly, it appears that tumor-infiltrating Treg cells were associated with a better prognosis in patients received chemoimmunotherapy^[62]. Recently, a multicenter open label phase III trial was conducted as a frontline treatment of metastatic CRC^[63]. Patients receiving chemoimmunotherapy (GOLFIG-2 trial) showed prolonged progression-free survival and increased overall response rate. However, this study was discontinued due to poor recruitment in the control arm. Thus it is too early to make any recommendation whether chemoimmunotherapy has any therapeutic efficacy for advanced CRC treatment. Nevertheless, these clinical studies provide "proof-of-principle" suggesting that GOLFIG chemoimmunotherapy may be used as a novel regimen for the first-line treatment of advanced CRC.

CONCLUSION

Immunotherapies have been investigated in human CRC for decades^[64] although there is no approved immunotherapy for human CRC up to date. As described above, different immune-directed approaches have been developed such as DC-based vaccines and genetically engineered CAR T cells. However, these developments are still in the early stages. One promising approach for CRC immunotherapy may lie in immune checkpoint inhibitor therapy given the encouraging phase II clinical trial data^[21]. This is particularly important for patients harboring MSI tumors since more mutational loads lead to more neoantigens that can be recognized by effector T cells. Therefore, identifying proper criteria to choose eligible patients thus getting maximum therapeutic benefits is essential. It may be critical to assess immunological

profiles of human CRC. In this context, immunoscore system seems critical, which should be included in the eligibility criteria for patients receiving such therapy. In addition, more research needs to be done to identify more Ag targets that can be used for cancer vaccine development and/or CAR T cells. Increasing studies have identified new immune components which maybe the potential targets for CRC immunotherapy, such as GUCY2C, HHLA2, OR7C1, and MAGE-D4^[53,65-70]. Furthermore, combined immunotherapies such as combined anti-CTLA-4 with anti-PD-1 or combined cancer vaccines with immune checkpoint inhibitor therapy may yield better therapeutic efficacy. Certainly, cautions are needed to avoid severe adverse effects that could be elicited by such combination therapy. Nevertheless, this is an exciting time for immunotherapy in human CRC.

REFERENCES

- 1 Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. *CA Cancer J Clin* 2015; **65**: 5-29 [PMID: 25559415 DOI: 10.3322/caac.21254]
- 2 Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global patterns and trends in colorectal cancer incidence and mortality. *Gut* 2016; Epub ahead of print [PMID: 26818619 DOI: 10.1136/gutjnl-2015-310912]
- 3 Pérez-Ruiz E, Berraondo P. Immunological Landscape and Clinical Management of Rectal Cancer. *Front Immunol* 2016; **7**: 61 [PMID: 26941741 DOI: 10.3389/fimmu.2016.00061]
- 4 Robert C, Thomas L, Bondarenko I, O'Day S, Weber J, Garbe C, Lebbe C, Baurain JF, Testori A, Grob JJ, Davidson N, Richards J, Maio M, Hauschild A, Miller WH, Gascon P, Lotem M, Harmankaya K, Ibrahim R, Francis S, Chen TT, Humphrey R, Hoos A, Wolchok JD. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N Engl J Med* 2011; **364**: 2517-2526 [PMID: 21639810 DOI: 10.1056/NEJMoa1104621]
- 5 Garon EB, Rizvi NA, Hui R, Leigh N, Balmanoukian AS, Eder JP, Patnaik A, Aggarwal C, Gubens M, Horn L, Carcereny E, Ahn MJ, Felip E, Lee JS, Hellmann MD, Hamid O, Goldman JW, Soria JC, Dolled-Filhart M, Rutledge RZ, Zhang J, Luceford JK, Rangwala R, Lubiniecki GM, Roach C, Emancipator K, Gandhi L. Pembrolizumab for the treatment of non-small-cell lung cancer. *N Engl J Med* 2015; **372**: 2018-2028 [PMID: 25891174 DOI: 10.1056/NEJMoa1501824]
- 6 Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, Schadendorf D, Dummer R, Smylie M, Rutkowski P, Ferrucci PF, Hill A, Wagstaff J, Carlino MS, Haanen JB, Maio M, Marquez-Rodas I, McArthur GA, Ascierto PA, Long GV, Callahan MK, Postow MA, Grossmann K, Sznol M, Dreno B, Bastholt L, Yang A, Rollin LM, Horak C, Hodi FS, Wolchok JD. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. *N Engl J Med* 2015; **373**: 23-34 [PMID: 26027431 DOI: 10.1056/NEJMoa1504030]
- 7 Postow MA, Chesney J, Pavlick AC, Robert C, Grossmann K, McDermott D, Linette GP, Meyer N, Giguere JK, Agarwala SS, Shaheen M, Ernstoff MS, Minor D, Salama AK, Taylor M, Ott PA, Rollin LM, Horak C, Gagnier P, Wolchok JD, Hodi FS. Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. *N Engl J Med* 2015; **372**: 2006-2017 [PMID: 25891304 DOI: 10.1056/NEJMoa1414428]
- 8 Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pagès C, Tosolini M, Camus M, Berger A, Wind P, Zinzindohoué F, Bruneval P, Cugnenc PH, Trajanoski Z, Fridman WH, Pagès F. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science* 2006; **313**: 1960-1964 [PMID: 17008531]
- 9 Bindea G, Mlecnik B, Tosolini M, Kirilovsky A, Waldner M, Obenauf AC, Angell H, Fredriksen T, Lafontaine L, Berger A, Bruneval P, Fridman WH, Becker C, Pagès F, Speicher MR, Trajanoski Z, Galon J. Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. *Immunity* 2013; **39**: 782-795 [PMID: 24138885 DOI: 10.1016/j.immuni.2013.10.003]
- 10 Williams DS, Bird MJ, Jorissen RN, Yu YL, Walker F, Zhang HH, Nice EC, Burgess AW. Nonsense mediated decay resistant mutations are a source of expressed mutant proteins in colon cancer cell lines with microsatellite instability. *PLoS One* 2010; **5**: e16012 [PMID: 21209843 DOI: 10.1371/journal.pone.0016012]
- 11 Smyrk TC, Watson P, Kaul K, Lynch HT. Tumor-infiltrating lymphocytes are a marker for microsatellite instability in colorectal carcinoma. *Cancer* 2001; **91**: 2417-2422 [PMID: 11413533]
- 12 McGranahan N, Furness AJ, Rosenthal R, Ramskov S, Lyngaa R, Saini SK, Jamal-Hanjani M, Wilson GA, Birkbak NJ, Hiley CT, Watkins TB, Shafi S, Murugaesu N, Mitter R, Akarca AU, Linares J, Marafioti T, Henry JY, Van Allen EM, Miao D, Schilling B, Schadendorf D, Garraway LA, Makarov V, Rizvi NA, Snyder A, Hellmann MD, Merghoub T, Wolchok JD, Shukla SA, Wu CJ, Peggs KS, Chan TA, Hadrup SR, Quezada SA, Swanton C. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. *Science* 2016; **351**: 1463-1469 [PMID: 26940869 DOI: 10.1126/science.aaf1490]
- 13 Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, Bignell GR, Bolli N, Borg A, Børresen-Dale AL, Boyault S, Burkhardt B, Butler AP, Caldas C, Davies HR, Desmedt C, Eils R, Eyfjörð JE, Foekens JA, Greaves M, Hosoda F, Hutter B, Ilicic T, Imbeaud S, Imielinski M, Jäger N, Jones DT, Jones D, Knappskog S, Kool M, Lakhani SR, López-Otin C, Martin S, Munshi NC, Nakamura H, Northcott PA, Pajic M, Papaemmanuil E, Paradiso A, Pearson JV, Puente XS, Raine K, Ramakrishna M, Richardson AL, Richter J, Rosenthal P, Schlesner M, Schumacher TN, Span PN, Teague JW, Totoki Y, Tutt AN, Valdés-Mas R, van Buuren MM, van 't Veer L, Vincent-Salomon A, Waddell N, Yates LR, Zucman-Rossi J, Futreal PA, McDermott U, Lichter P, Meyerson M, Grimmond SM, Siebert R, Campo E, Shibata T, Pfister SM, Campbell PJ, Stratton MR. Signatures of mutational processes in human cancer. *Nature* 2013; **500**: 415-421 [PMID: 23945592 DOI: 10.1038/nature12477]
- 14 Van Allen EM, Miao D, Schilling B, Shukla SA, Blank C, Zimmer L, Sucker A, Hillen U, Geukes Foppen MH, Goldinger SM, Utikal J, Hassel JC, Weide B, Kaehler KC, Loquai C, Mohr P, Gutzmer R, Dummer R, Gabriel S, Wu CJ, Schadendorf D, Garraway LA. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. *Science* 2015; **350**: 207-211 [PMID: 26359337 DOI: 10.1126/science.aad0095]
- 15 Xiao Y, Freeman GJ. The microsatellite unstable subset of colorectal cancer is a particularly good candidate for checkpoint blockade immunotherapy. *Cancer Discov* 2015; **5**: 16-18 [PMID: 25583798 DOI: 10.1158/2159-8290.CD-14-1397]
- 16 Mlecnik B, Bindea G, Angell HK, Maby P, Angelova M, Tougeron D, Church SE, Lafontaine L, Fischer M, Fredriksen T, Sasso M, Bilocq AM, Kirilovsky A, Obenauf AC, Hamieh M, Berger A, Bruneval P, Tuech JJ, Sabourin JC, Le Pessot F, Mauillon J, Rafii A, Laurent-Puig P, Speicher MR, Trajanoski Z, Michel P, Sesboué R, Frebourg T, Pagès F, Valge-Archer V, Latouche JB, Galon J. Integrative Analyses of Colorectal Cancer Show Immunoscore Is a Stronger Predictor of Patient Survival Than Microsatellite Instability. *Immunity* 2016; **44**: 698-711 [PMID: 26982367 DOI: 10.1016/j.immuni.2016.02.025]
- 17 Mlecnik B, Bindea G, Kirilovsky A, Angell HK, Obenauf AC, Tosolini M, Church SE, Maby P, Vasaturo A, Angelova M, Fredriksen T, Mauger S, Waldner M, Berger A, Speicher MR, Pagès F, Valge-Archer V, Galon J. The tumor microenvironment and Immunoscore are critical determinants of dissemination to distant metastasis. *Sci Transl Med* 2016; **8**: 327ra26 [PMID: 26912905 DOI: 10.1126/scitranslmed.aad6352]

- 18 **Galon J**, Pagès F, Marincola FM, Thurin M, Trinchieri G, Fox BA, Gajewski TF, Ascierto PA. The immune score as a new possible approach for the classification of cancer. *J Transl Med* 2012; **10**: 1 [PMID: 22214470 DOI: 10.1186/1479-5876-10-1]
- 19 **Galon J**, Angell HK, Bedognetti D, Marincola FM. The continuum of cancer immunosurveillance: prognostic, predictive, and mechanistic signatures. *Immunity* 2013; **39**: 11-26 [PMID: 23890060 DOI: 10.1016/j.immuni.2013.07.008]
- 20 **Skoulidis F**, Byers LA, Diao L, Papadimitrakopoulou VA, Tong P, Izzo J, Behrens C, Kadara H, Parra ER, Canales JR, Zhang J, Giri U, Gudikote J, Cortez MA, Yang C, Fan Y, Peyton M, Girard L, Coombes KR, Toniatti C, Heffernan TP, Choi M, Frampton GM, Miller V, Weinstein JN, Herbst RS, Wong KK, Zhang J, Sharma P, Mills GB, Hong WK, Minna JD, Allison JP, Futreal A, Wang J, Wistuba II, Heymach JV. Co-occurring genomic alterations define major subsets of KRAS-mutant lung adenocarcinoma with distinct biology, immune profiles, and therapeutic vulnerabilities. *Cancer Discov* 2015; **5**: 860-877 [PMID: 26069186 DOI: 10.1158/2159-8290.CD-14-1236]
- 21 **Le DT**, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, Skora AD, Luber 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, Bhaijee 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. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. *N Engl J Med* 2015; **372**: 2509-2520 [PMID: 26028255 DOI: 10.1056/NEJMoa1500596]
- 22 **Cunningham D**, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, Bets D, Mueser M, Harstrick A, Verslype C, Chau I, Van Cutsem E. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N Engl J Med* 2004; **351**: 337-345 [PMID: 15269313 DOI: 10.1056/NEJMoa033025]
- 23 **Sorich MJ**, Wiese MD, Rowland A, Kichenadasse G, McKinnon RA, Karapetis CS. Extended RAS mutations and anti-EGFR monoclonal antibody survival benefit in metastatic colorectal cancer: a meta-analysis of randomized, controlled trials. *Ann Oncol* 2015; **26**: 13-21 [PMID: 25115304 DOI: 10.1093/annonc/mdl378]
- 24 **Trotta AM**, Ottaiano A, Romano C, Nasti G, Nappi A, De Divitiis C, Napolitano M, Zanolta S, Casaretti R, D'Alterio C, Avallone A, Califano D, Iaffaioli RV, Scala S. Prospective Evaluation of Cetuximab-Mediated Antibody-Dependent Cell Cytotoxicity in Metastatic Colorectal Cancer Patients Predicts Treatment Efficacy. *Cancer Immunol Res* 2016; **4**: 366-374 [PMID: 26817995 DOI: 10.1158/2326-6066.CIR-15-0184]
- 25 **Hsu YF**, Ajona D, Corrales L, Lopez-Picazo JM, Gurpide A, Montuenga LM, Pio R. Complement activation mediates cetuximab inhibition of non-small cell lung cancer tumor growth in vivo. *Mol Cancer* 2010; **9**: 139 [PMID: 20529262 DOI: 10.1186/1476-4598-9-139]
- 26 **Kabbinavar F**, Hurwitz HI, Fehrenbacher L, Meropol NJ, Novotny WF, Lieberman G, Griffing S, Bergsland E. Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. *J Clin Oncol* 2003; **21**: 60-65 [PMID: 12506171]
- 27 **Sharma P**, Allison JP. Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. *Cell* 2015; **161**: 205-214 [PMID: 25860605 DOI: 10.1016/j.cell.2015.03.030]
- 28 **Sharma P**, Allison JP. The future of immune checkpoint therapy. *Science* 2015; **348**: 56-61 [PMID: 25838373 DOI: 10.1126/science.aaa8172]
- 29 **Gettinger SN**, Horn L, Gandhi L, Spigel DR, Antonia SJ, Rizvi NA, Powderly JD, Heist RS, Carvajal RD, Jackman DM, Sequist LV, Smith DC, Leming P, Carbone DP, Pinder-Schenck MC, Topalian SL, Hodi FS, Sosman JA, Sznol M, McDermott DF, Pardoll DM, Sankar V, Ahlers CM, Salvati M, Wigginton JM, Hellmann MD, Kollia GD, Gupta AK, Brahmer JR. Overall Survival and Long-Term Safety of Nivolumab (Anti-Programmed Death 1 Antibody, BMS-936558, ONO-4538) in Patients With Previously Treated Advanced Non-Small-Cell Lung Cancer. *J Clin Oncol* 2015; **33**: 2004-2012 [PMID: 25897158 DOI: 10.1200/JCO.2014.58.3708]
- 30 **Ribas A**, Kefford R, Marshall MA, Punt CJ, Haanen JB, Marmol M, Garbe C, Gogas H, Schachter J, Linette G, Lorigan P, Kendra KL, Maio M, Trefzer U, Smylie M, McArthur GA, Dreno B, Nathan PD, Mackiewicz J, Kirkwood JM, Gomez-Navarro J, Huang B, Pavlov D, Hauschild A. Phase III randomized clinical trial comparing tremelimumab with standard-of-care chemotherapy in patients with advanced melanoma. *J Clin Oncol* 2013; **31**: 616-622 [PMID: 23295794 DOI: 10.1200/jco.2012.44.6112]
- 31 **Sangro B**, Gomez-Martin C, de la Mata M, Iñarrairaegui M, Garralda E, Barrera P, Riezu-Boj JI, Larrea E, Alfaro C, Sarobe P, Lasarte JJ, Pérez-Gracia JL, Melero I, Prieto J. A clinical trial of CTLA-4 blockade with tremelimumab in patients with hepatocellular carcinoma and chronic hepatitis C. *J Hepatol* 2013; **59**: 81-88 [PMID: 23466307 DOI: 10.1016/j.jhep.2013.02.022]
- 32 **Zatloukal P**, Heo D, Park K, Kang J, Butts C, Bradford D, Graziano S, Huang B, Healey D. Randomized phase II clinical trial comparing tremelimumab (CP-675,206) with best supportive care (BSC) following first-line platinum-based therapy in patients (pts) with advanced non-small cell lung cancer (NSCLC). Proceedings of the ASCO Annual Meeting Proceedings, 2009: 8071
- 33 **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 [PMID: 20498386 DOI: 10.1200/jco.2010.28.3994]
- 34 **Lute KD**, May KF, Lu P, Zhang H, Kocak E, Mosinger B, Wolford C, Phillips G, Caligiuri MA, Zheng P, Liu Y. Human CTLA4 knock-in mice unravel the quantitative link between tumor immunity and autoimmunity induced by anti-CTLA-4 antibodies. *Blood* 2005; **106**: 3127-3133 [PMID: 16037385 DOI: 10.1182/blood-2005-06-2298]
- 35 **Pedersen AE**, Buus S, Claesson MH. Treatment of transplanted CT26 tumour with dendritic cell vaccine in combination with blockade of vascular endothelial growth factor receptor 2 and CTLA-4. *Cancer Lett* 2006; **235**: 229-238 [PMID: 15927356 DOI: 10.1016/j.canlet.2005.04.012]
- 36 **Jure-Kunkel MN**, Masters G, Girit E, Dito G, Lee FY. Antitumor activity of anti-CTLA-4 monoclonal antibody (mAb) in combination with ixabepilone in preclinical tumor models. *J Clin Oncol* (Meeting Abstracts) 2008; **26**: 3048
- 37 **Kocak E**, Lute K, Chang X, May KF, Exten KR, Zhang H, Abdessalam SF, Lehman AM, Jarjoura D, Zheng P, Liu Y. Combination therapy with anti-CTL antigen-4 and anti-4-1BB antibodies enhances cancer immunity and reduces autoimmunity. *Cancer Res* 2006; **66**: 7276-7284 [PMID: 16849577 DOI: 10.1158/0008-5472.can-05-2128]
- 38 **Phan GQ**, Yang JC, Sherry RM, Hwu P, Topalian SL, Schwartzentruber DJ, Restifo NP, Haworth LR, Seipp CA, Freezer LJ, Morton KE, Mavroukakis SA, Duray PH, Steinberg SM, Allison JP, Davis TA, Rosenberg SA. Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma. *Proc Natl Acad Sci USA* 2003; **100**: 8372-8377 [PMID: 12826605 DOI: 10.1073/pnas.1533209100]
- 39 **Fishman M**. A changing world for DCvax: a PSMA loaded autologous dendritic cell vaccine for prostate cancer. *Expert Opin Biol Ther* 2009; **9**: 1565-1575 [PMID: 19916735 DOI: 10.1517/14712590903446921]
- 40 **Nair SK**, Hull S, Coleman D, Gilboa E, Lysterly HK, Morse MA. Induction of carcinoembryonic antigen (CEA)-specific cytotoxic T-lymphocyte responses in vitro using autologous dendritic cells loaded with CEA peptide or CEA RNA in patients with metastatic malignancies expressing CEA. *Int J Cancer* 1999; **82**: 121-124 [PMID: 10360830]
- 41 **Fong L**, Hou Y, Rivas A, Benike C, Yuen A, Fisher GA, Davis

- MM, Engleman EG. Altered peptide ligand vaccination with Flt3 ligand expanded dendritic cells for tumor immunotherapy. *Proc Natl Acad Sci USA* 2001; **98**: 8809-8814 [PMID: 11427731 DOI: 10.1073/pnas.141226398]
- 42 **O'Rourke MG**, Johnson M, Lanagan C, See J, Yang J, Bell JR, Slater GJ, Kerr BM, Crowe B, Purdie DM, Elliott SL, Ellem KA, Schmidt CW. Durable complete clinical responses in a phase I/II trial using an autologous melanoma cell/dendritic cell vaccine. *Cancer Immunol Immunother* 2003; **52**: 387-395 [PMID: 12682787 DOI: 10.1007/s00262-003-0375-x]
 - 43 **De Vries IJ**, Krooshoop DJ, Scharenborg NM, Lesterhuis WJ, Diepstra JH, Van Muijen GN, Strijk SP, Ruers TJ, Boerman OC, Oyen WJ, Adema GJ, Punt CJ, Figdor CG. Effective migration of antigen-pulsed dendritic cells to lymph nodes in melanoma patients is determined by their maturation state. *Cancer Res* 2003; **63**: 12-17 [PMID: 12517769]
 - 44 **Vermorken JB**, Claessen AM, van Tinteren H, Gall HE, Ezinga R, Meijer S, Scheper RJ, Meijer CJ, Bloemena E, Ransom JH, Hanna MG, Pinedo HM. Active specific immunotherapy for stage II and stage III human colon cancer: a randomised trial. *Lancet* 1999; **353**: 345-350 [PMID: 9950438 DOI: 10.1016/S0140-6736(98)07186-4]
 - 45 **Uyl-de Groot CA**, Vermorken JB, Hanna MG, Verboom P, Groot MT, Bonsel GJ, Meijer CJ, Pinedo HM. Immunotherapy with autologous tumor cell-BCG vaccine in patients with colon cancer: a prospective study of medical and economic benefits. *Vaccine* 2005; **23**: 2379-2387 [PMID: 15755632 DOI: 10.1016/j.vaccine.2005.01.015]
 - 46 **Cao JX**, Zhang XY, Liu JL, Li JL, Liu YS, Wang M, Xu BL, Wang ZX. Validity of combination active specific immunotherapy for colorectal cancer: a meta-analysis of 2993 patients. *Cytotherapy* 2015; **17**: 1746-1762 [PMID: 26455275 DOI: 10.1016/j.cyt.2015.08.009]
 - 47 **June CH**, Riddell SR, Schumacher TN. Adoptive cellular therapy: a race to the finish line. *Sci Transl Med* 2015; **7**: 280ps7 [PMID: 25810311 DOI: 10.1126/scitranslmed.aaa3643]
 - 48 **Kawalekar OU**, O'Connor RS, Fraietta JA, Guo L, McGettigan SE, Posey AD, Patel PR, Guedan S, Scholler J, Keith B, Snyder N, Blair I, Milone MC, June CH. Distinct Signaling of Coreceptors Regulates Specific Metabolism Pathways and Impacts Memory Development in CAR T Cells. *Immunity* 2016; **44**: 380-390 [PMID: 26885860 DOI: 10.1016/j.immuni.2016.01.021]
 - 49 **Yang W**, Bai Y, Xiong Y, Zhang J, Chen S, Zheng X, Meng X, Li L, Wang J, Xu C, Yan C, Wang L, Chang CC, Chang TY, Zhang T, Zhou P, Song BL, Liu W, Sun SC, Liu X, Li BL, Xu C. Potentiating the antitumor response of CD8(+) T cells by modulating cholesterol metabolism. *Nature* 2016; **531**: 651-655 [PMID: 26982734 DOI: 10.1038/nature17412]
 - 50 **Parkhurst MR**, Yang JC, Langan RC, Dudley ME, Nathan DA, Feldman SA, Davis JL, Morgan RA, Merino MJ, Sherry RM, Hughes MS, Kammula US, Phan GQ, Lim RM, Wank SA, Restifo NP, Robbins PF, Laurencot CM, Rosenberg SA. T cells targeting carcinoembryonic antigen can mediate regression of metastatic colorectal cancer but induce severe transient colitis. *Mol Ther* 2011; **19**: 620-626 [PMID: 21157437 DOI: 10.1038/mt.2010.272]
 - 51 **Van Acker HH**, Anguille S, Van Tendeloo VF, Lion E. Empowering gamma delta T cells with antitumor immunity by dendritic cell-based immunotherapy. *Oncoimmunology* 2015; **4**: e1021538 [PMID: 26405575 DOI: 10.1080/2162402X.2015.1021538]
 - 52 **Chien YH**, Meyer C, Bonneville M. $\gamma\delta$ T cells: first line of defense and beyond. *Annu Rev Immunol* 2014; **32**: 121-155 [PMID: 24387714 DOI: 10.1146/annurev-immunol-032713-120216]
 - 53 **Wu P**, Wu D, Ni C, Ye J, Chen W, Hu G, Wang Z, Wang C, Zhang Z, Xia W, Chen Z, Wang K, Zhang T, Xu J, Han Y, Zhang T, Wu X, Wang J, Gong W, Zheng S, Qiu F, Yan J, Huang J. $\gamma\delta$ T17 cells promote the accumulation and expansion of myeloid-derived suppressor cells in human colorectal cancer. *Immunity* 2014; **40**: 785-800 [PMID: 24816404 DOI: 10.1016/j.immuni.2014.03.013]
 - 54 **Yan J**, Huang J. Innate $\gamma\delta$ T17 cells convert cancer-elicited inflammation into immunosuppression through myeloid-derived suppressor cells. *Oncoimmunology* 2014; **3**: e953423 [PMID: 25610744 DOI: 10.4161/21624011.2014.953423]
 - 55 **Ma S**, Cheng Q, Cai Y, Gong H, Wu Y, Yu X, Shi L, Wu D, Dong C, Liu H. IL-17A produced by $\gamma\delta$ T cells promotes tumor growth in hepatocellular carcinoma. *Cancer Res* 2014; **74**: 1969-1982 [PMID: 24525743 DOI: 10.1158/0008-5472.can-13-2534]
 - 56 **Coffelt SB**, Kersten K, Doornebal CW, Weiden J, Vrijland K, Hau CS, Verstegen NJ, Ciampricotti M, Hawinkels LJ, Jonkers J, de Visser KE. IL-17-producing $\gamma\delta$ T cells and neutrophils conspire to promote breast cancer metastasis. *Nature* 2015; **522**: 345-348 [PMID: 25822788 DOI: 10.1038/nature14282]
 - 57 **He W**, Hao J, Dong S, Gao Y, Tao J, Chi H, Flavell R, O'Brien RL, Born WK, Craft J, Han J, Wang P, Zhao L, Wu J, Yin Z. Naturally activated V gamma 4 gamma delta T cells play a protective role in tumor immunity through expression of eomesodermin. *J Immunol* 2010; **185**: 126-133 [PMID: 20525896 DOI: 10.4049/jimmunol.0903767]
 - 58 **Wu D**, Wu P, Wu X, Ye J, Wang Z, Zhao S, Ni C, Hu G, Xu J, Han Y, Zhang T, Qiu F, Yan J, Huang J. Ex vivo expanded human circulating V δ 1 $\gamma\delta$ T cells exhibit favorable therapeutic potential for colon cancer. *Oncoimmunology* 2015; **4**: e992749 [PMID: 25949914 DOI: 10.4161/2162402X.2014.992749]
 - 59 **Fisher JP**, Heuvelink J, Yan M, Gustafsson K, Anderson J. $\gamma\delta$ T cells for cancer immunotherapy: A systematic review of clinical trials. *Oncoimmunology* 2014; **3**: e27572 [PMID: 24734216 DOI: 10.4161/onci.27572]
 - 60 **Ferreri AJ**, Cwynarski K, Pulczynski E, Ponzoni M, Deckert M, Politi LS, Torri V, Fox CP, Rosée PL, Schorb E, Ambrosetti A, Roth A, Hemmaway C, Ferrari A, Linton KM, Rudà R, Binder M, Pukrop T, Balzarotti M, Fabbri A, Johnson P, Gørlov JS, Hess G, Panse J, Pisani F, Tucci A, Stilgenbauer S, Hertenstein B, Keller U, Krause SW, Levis A, Schmoll HJ, Cavalli F, Finke J, Reni M, Zucca E, Illerhaus G. Chemotherapy with methotrexate, cytarabine, thiopeta, and rituximab (MATRix regimen) in patients with primary CNS lymphoma: results of the first randomisation of the International Extranodal Lymphoma Study Group-32 (IELSG32) phase 2 trial. *Lancet Haematol* 2016; **3**: e217-e227 [PMID: 27132696 DOI: 10.1016/S2352-3026(16)00036-3]
 - 61 **Correale P**, Tagliaferri P, Fioravanti A, Del Vecchio MT, Remondo C, Montagnani F, Rotundo MS, Ginanneschi C, Martellucci I, Francini E, Cusi MG, Tassone P, Francini G. Immunity feedback and clinical outcome in colon cancer patients undergoing chemoimmunotherapy with gemcitabine + FOLFOX followed by subcutaneous granulocyte macrophage colony-stimulating factor and aldesleukin (GOLFIG-1 Trial). *Clin Cancer Res* 2008; **14**: 4192-4199 [PMID: 18593999 DOI: 10.1158/1078-0432.CCR-07-5278]
 - 62 **Correale P**, Rotundo MS, Del Vecchio MT, Remondo C, Migali C, Ginanneschi C, Tsang KY, Licchetta A, Mannucci S, Loiacono L, Tassone P, Francini G, Tagliaferri P. Regulatory (FoxP3+) T-cell tumor infiltration is a favorable prognostic factor in advanced colon cancer patients undergoing chemo or chemoimmunotherapy. *J Immunother* 2010; **33**: 435-441 [PMID: 20386463 DOI: 10.1097/CJI.0b013e3181d32f01]
 - 63 **Correale P**, Botta C, Rotundo MS, Guglielmo A, Conca R, Licchetta A, Pastina P, Bestoso E, Ciliberto D, Cusi MG, Fioravanti A, Guidelli GM, Bianco MT, Misso G, Martino E, Caraglia M, Tassone P, Mini E, Mantovani G, Ridolfi R, Pirtoli L, Tagliaferri P. Gemcitabine, oxaliplatin, levofolinate, 5-fluorouracil, granulocyte-macrophage colony-stimulating factor, and interleukin-2 (GOLFIG) versus FOLFOX chemotherapy in metastatic colorectal cancer patients: the GOLFIG-2 multicentric open-label randomized phase III trial. *J Immunother* 2014; **37**: 26-35 [PMID: 24316553 DOI: 10.1097/CJI.0000000000000004]
 - 64 **Evans JT**. Letter: Immunotherapy for colorectal cancer. *Lancet* 1976; **1**: 1248 [PMID: 58300]
 - 65 **Xiao Y**, Freeman GJ. A New B7: CD28 Family Checkpoint Target for Cancer Immunotherapy: HHLA2. *Clin Cancer Res* 2015; **21**: 2201-2203 [PMID: 25869386 DOI: 10.1158/1078-0432.ccr-14-2658]
 - 66 **Morita R**, Hirohashi Y, Torigoe T, Ito-Inoda S, Takahashi A, Mariya T, Asanuma H, Tamura Y, Tsukahara T, Kanaseki T, Kubo

- T, Kutomi G, Mizuguchi T, Terui T, Ishitani K, Hashino S, Kondo T, Minagawa N, Takahashi N, Taketomi A, Todo S, Asaka M, Sato N. Olfactory Receptor Family 7 Subfamily C Member 1 Is a Novel Marker of Colon Cancer-Initiating Cells and Is a Potent Target of Immunotherapy. *Clin Cancer Res* 2016; **22**: 3298-3309 [PMID: 26861454 DOI: 10.1158/1078-0432.ccr-15-1709]
- 67 **Zhang QM**, He SJ, Shen N, Luo B, Fan R, Fu J, Luo GR, Zhou SF, Xiao SW, Xie XX. Overexpression of MAGE-D4 in colorectal cancer is a potentially prognostic biomarker and immunotherapy target. *Int J Clin Exp Pathol* 2014; **7**: 3918-3927 [PMID: 25120768]
- 68 **Sawada Y**, Komori H, Tsunoda Y, Shimomura M, Takahashi M, Baba H, Ito M, Saito N, Kuwano H, Endo I, Nishimura Y, Nakatsura T. Identification of HLA-A2 or HLA-A24-restricted CTL epitopes for potential HSP105-targeted immunotherapy in colorectal cancer. *Oncol Rep* 2014; **31**: 1051-1058 [PMID: 24366042 DOI: 10.3892/or.2013.2941]
- 69 **Itoh T**, Ueda Y, Kawashima I, Nukaya I, Fujiwara H, Fuji N, Yamashita T, Yoshimura T, Okugawa K, Iwasaki T, Ideno M, Takesako K, Mitsuhashi M, Orita K, Yamagishi H. Immunotherapy of solid cancer using dendritic cells pulsed with the HLA-A24-restricted peptide of carcinoembryonic antigen. *Cancer Immunol Immunother* 2002; **51**: 99-106 [PMID: 11904734 DOI: 10.1007/s00262-001-0257-z]
- 70 **Snook AE**, Magee MS, Waldman SA. GUCY2C-targeted cancer immunotherapy: past, present and future. *Immunol Res* 2011; **51**: 161-169 [PMID: 22038530 DOI: 10.1007/s12026-011-8253-7]
- 71 **Fearon ER**. Human cancer syndromes: clues to the origin and nature of cancer. *Science* 1997; **278**: 1043-1050 [PMID: 9353177]
- 72 **Samowitz WS**, Holden JA, Curtin K, Edwards SL, Walker AR, Lin HA, Robertson MA, Nichols MF, Gruenthal KM, Lynch BJ, Leppert MF, Slattery ML. Inverse relationship between microsatellite instability and K-ras and p53 gene alterations in colon cancer. *Am J Pathol* 2001; **158**: 1517-1524 [PMID: 11290569 DOI: 10.1016/s0002-9440(10)64102-8]
- 73 **Miyaki M**, Iijima T, Konishi M, Sakai K, Ishii A, Yasuno M, Hishima T, Koike M, Shitara N, Iwama T, Utsunomiya J, Kuroki T, Mori T. Higher frequency of Smad4 gene mutation in human colorectal cancer with distant metastasis. *Oncogene* 1999; **18**: 3098-3103 [PMID: 10340381 DOI: 10.1038/sj.onc.1202642]
- 74 **Baker SJ**, Fearon ER, Nigro JM, Hamilton SR, Preisinger AC, Jessup JM, vanTuinen P, Ledbetter DH, Barker DF, Nakamura Y, White R, Vogelstein B. Chromosome 17 deletions and p53 gene mutations in colorectal carcinomas. *Science* 1989; **244**: 217-221 [PMID: 2649981]
- 75 **Goel A**, Arnold CN, Niedzwiecki D, Carethers JM, Dowell JM, Wasserman L, Compton C, Mayer RJ, Bertagnolli MM, Bolland CR. Frequent inactivation of PTEN by promoter hypermethylation in microsatellite instability-high sporadic colorectal cancers. *Cancer Res* 2004; **64**: 3014-3021 [PMID: 15126336]
- 76 **Cho KR**, Oliner JD, Simons JW, Hedrick L, Fearon ER, Preisinger AC, Hedge P, Silverman GA, Vogelstein B. The DCC gene: structural analysis and mutations in colorectal carcinomas. *Genomics* 1994; **19**: 525-531 [PMID: 8188295 DOI: 10.1006/geno.1994.1102]
- 77 **Morin PJ**, Sparks AB, Korinek V, Barker N, Clevers H, Vogelstein B, Kinzler KW. Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. *Science* 1997; **275**: 1787-1790 [PMID: 9065402]
- 78 **Nimmrich I**, Friedl W, Kruse R, Pietsch S, Hentsch S, Deuter R, Winde G, Müller O. Loss of the PLA2G2A gene in a sporadic colorectal tumor of a patient with a PLA2G2A germline mutation and absence of PLA2G2A germline alterations in patients with FAP. *Hum Genet* 1997; **100**: 345-349 [PMID: 9272153]
- 79 **Haigis KM**, Kendall KR, Wang Y, Cheung A, Haigis MC, Glickman JN, Niwa-Kawakita M, Sweet-Cordero A, Sebolt-Leopold J, Shannon KM, Settleman J, Giovannini M, Jacks T. Differential effects of oncogenic K-Ras and N-Ras on proliferation, differentiation and tumor progression in the colon. *Nat Genet* 2008; **40**: 600-608 [PMID: 18372904 DOI: 10.1038/ng.115]
- 80 **Rampino N**, Yamamoto H, Ionov Y, Li Y, Sawai H, Reed JC, Perucho M. Somatic frameshift mutations in the BAX gene in colon cancers of the microsatellite mutator phenotype. *Science* 1997; **275**: 967-969 [PMID: 9020077]
- 81 **Hendriks YM**, Wagner A, Morreau H, Menko F, Stormorken A, Quehenberger F, Sandkuijl L, Möller P, Genuardi M, Van Houwelingen H, Tops C, Van Puijenbroek M, Verkuijlen P, Kenter G, Van Mil A, Meijers-Heijboer H, Tan GB, Breuning MH, Fodde R, Wijnen JT, Bröcker-Vriends AH, Vasen H. Cancer risk in hereditary nonpolyposis colorectal cancer due to MSH6 mutations: impact on counseling and surveillance. *Gastroenterology* 2004; **127**: 17-25 [PMID: 15236168]
- 82 **Starr TK**, Allaei R, Silverstein KA, Staggs RA, Sarver AL, Bergemann TL, Gupta M, O'Sullivan MG, Matise I, Dupuy AJ, Collier LS, Powers S, Oberg AL, Asmann YW, Thibodeau SN, Tessarollo L, Copeland NG, Jenkins NA, Cormier RT, Largaespada DA. A transposon-based genetic screen in mice identifies genes altered in colorectal cancer. *Science* 2009; **323**: 1747-1750 [PMID: 19251594 DOI: 10.1126/science.1163040]
- 83 **Espejo R**, Rengifo-Cam W, Schaller MD, Evers BM, Sastry SK. PTP-PEST controls motility, adherens junction assembly, and Rho GTPase activity in colon cancer cells. *Am J Physiol Cell Physiol* 2010; **299**: C454-C463 [PMID: 20519451 DOI: 10.1152/ajpcell.00148.2010]
- 84 **Samuels Y**, Diaz LA, Schmidt-Kittler O, Cummins JM, Delong L, Cheong I, Rago C, Huso DL, Lengauer C, Kinzler KW, Vogelstein B, Velculescu VE. Mutant PIK3CA promotes cell growth and invasion of human cancer cells. *Cancer Cell* 2005; **7**: 561-573 [PMID: 15950905 DOI: 10.1016/j.ccr.2005.05.014]
- 85 **Hiramoto T**, Nakanishi T, Sumiyoshi T, Fukuda T, Matsuura S, Tauchi H, Komatsu K, Shibasaki Y, Inui H, Watatani M, Yasutomi M, Sumii K, Kajiyama G, Kamada N, Miyagawa K, Kamiya K. Mutations of a novel human RAD54 homologue, RAD54B, in primary cancer. *Oncogene* 1999; **18**: 3422-3426 [PMID: 10362364 DOI: 10.1038/sj.onc.1202691]
- 86 **Liu W**, Dong X, Mai M, Seelan RS, Taniguchi K, Krishnadath KK, Halling KC, Cunningham JM, Boardman LA, Qian C, Christensen E, Schmidt SS, Roche PC, Smith DI, Thibodeau SN. Mutations in AXIN2 cause colorectal cancer with defective mismatch repair by activating beta-catenin/TCF signalling. *Nat Genet* 2000; **26**: 146-147 [PMID: 11017067 DOI: 10.1038/79859]
- 87 **Jang JH**, Shin KH, Park JG. Mutations in fibroblast growth factor receptor 2 and fibroblast growth factor receptor 3 genes associated with human gastric and colorectal cancers. *Cancer Res* 2001; **61**: 3541-3543 [PMID: 11325814]
- 88 **Jirawatnotai S**, Hu Y, Michowski W, Elias JE, Becks L, Bienvenu F, Zagodzdzon A, Goswami T, Wang YE, Clark AB, Kunkel TA, van Harn T, Xia B, Correll M, Quackenbush J, Livingston DM, Gygi SP, Sicinski P. A function for cyclin D1 in DNA repair uncovered by protein interactome analyses in human cancers. *Nature* 2011; **474**: 230-234 [PMID: 21654808 DOI: 10.1038/nature10155]
- 89 **Carpten JD**, Faber AL, Horn C, Donoho GP, Briggs SL, Robbins CM, Hostetter G, Boguslawski S, Moses TY, Savage S, Uhlik M, Lin A, Du J, Qian YW, Zeckner DJ, Tucker-Kellogg G, Touchman J, Patel K, Mousses S, Bittner M, Schevitz R, Lai MH, Blanchard KL, Thomas JE. A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. *Nature* 2007; **448**: 439-444 [PMID: 17611497 DOI: 10.1038/nature05933]
- 90 **Cahill DP**, Lengauer C, Yu J, Riggins GJ, Willson JK, Markowitz SD, Kinzler KW, Vogelstein B. Mutations of mitotic checkpoint genes in human cancers. *Nature* 1998; **392**: 300-303 [PMID: 9521327 DOI: 10.1038/32688]
- 91 **Pino MS**, Chung DC. The chromosomal instability pathway in colon cancer. *Gastroenterology* 2010; **138**: 2059-2072 [PMID: 20420946 DOI: 10.1053/j.gastro.2009.12.065]
- 92 **Gayther SA**, Batley SJ, Linger L, Bannister A, Thorpe K, Chin SF, Daigo Y, Russell P, Wilson A, Sowter HM, Delhanty JD, Ponder BA, Kouzarides T, Caldas C. Mutations truncating the EP300 acetylase in human cancers. *Nat Genet* 2000; **24**: 300-303 [PMID: 10700188 DOI: 10.1038/73536]
- 93 **Morse MA**, Niedzwiecki D, Marshall JL, Garrett C, Chang

- DZ, Aklilu M, Crocenzi TS, Cole DJ, Dessureault S, Hobeika AC, Osada T, Onaitis M, Clary BM, Hsu D, Devi GR, Bulusu A, Annechiarico RP, Chadaram V, Clay TM, Lyster HK. A randomized phase II study of immunization with dendritic cells modified with poxvectors encoding CEA and MUC1 compared with the same poxvectors plus GM-CSF for resected metastatic colorectal cancer. *Ann Surg* 2013; **258**: 879-886 [PMID: 23657083 DOI: 10.1097/SLA.0b013e318292919e]
- 94 **Hanna MG**, Hoover HC, Vermorken JB, Harris JE, Pinedo HM. Adjuvant active specific immunotherapy of stage II and stage III colon cancer with an autologous tumor cell vaccine: first randomized phase III trials show promise. *Vaccine* 2001; **19**: 2576-2582 [PMID: 11257395]
- 95 **Marshall JL**, Hawkins MJ, Tsang KY, Richmond E, Pedicano JE, Zhu MZ, Schlom J. Phase I study in cancer patients of a replication-defective avipox recombinant vaccine that expresses human carcinoembryonic antigen. *J Clin Oncol* 1999; **17**: 332-337 [PMID: 10458251]
- 96 **Harris JE**, Ryan L, Hoover HC, Stuart RK, Oken MM, Benson AB, Mansour E, Haller DG, Manola J, Hanna MG. Adjuvant active specific immunotherapy for stage II and III colon cancer with an autologous tumor cell vaccine: Eastern Cooperative Oncology Group Study E5283. *J Clin Oncol* 2000; **18**: 148-157 [PMID: 10623705]

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