Progress of CTLA-4 and PD-1 Immune Checkpoint Inhibitors in Treatment for Colorectal Cancer

Zhangyi Yang [1,*,†], Yifan Zhang [2, †]

[1] Life science and Biopharmaceutical College of Shenyang Pharmaceutical University, China

[2] YK Pao School, Shanghai, China

* Corresponding Author Email: maom@kean.edu

[†] These authors contributed equally.

Abstract. Colorectal cancer (CRC) is a common type of cancer, with approximately 149,500 new cases in 2021. Colorectal cancer can be caused by genetic mutations, bacterial and viral infections, and second-hand smoke. In metastatic CRC (mCRC), only patients with deficient mismatch repair (dMMR) or microsatellite instability-high (MSI-H) benefit from current therapeutic approaches. 95% of the patients with proficient mismatch repair (pMMR) or microsatellite stable (MSS) mCRC still have poor prognostic outcomes. Conventional surgical treatment cannot meet patients' expectations for treatment effect and prognosis. Therefore, innovative approaches are needed to develop effective immunotherapy for these patients. This article introduces the mechanisms of action and clinical application of immune checkpoint inhibitors (ICIs) in colorectal cancer, especially CTLA-4 and PD-1.

Keywords: Cancer Immunology, Immune Checkpoint Inhibitor, CTLA-4, PD-1, Colorectal Cancer.

1. Introduction

Immune checkpoints are a type of targeted treatment that regulates the immune system, allowing self-tolerance and preventing the immune system from attacking self-cells. They also invigorate anti-tumor immune responses by interrupting the signaling pathways and blocking different checkpoint proteins. However, cancer cells can use immune checkpoint inhibitors to escape immunosurveillance by stimulating the checkpoints [1].

In 1987, the first immune checkpoint molecule cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) was discovered [2] and caused a revolution in cancer biology. In 1996, Dr. James Allison developed and tested CTLA-4 inhibitors on mice [2]. The first antibody blocking the immune checkpoint CTLA-4, Ipilimumab (Yervoy), was approved by the FDA for treatment against advanced melanoma in 2011 [3]. Since then, Ipilimumab has also been approved for several other malignancies [3]. The results were significant, more than 20% of the patients receiving CTLA-4 checkpoint inhibitors survived for 10 years, before, the 10-year survival rate was only half as high. In 2014, the first programmed cell death protein 1 (PD-1) inhibitor, Nivolumab (Opdivo) was approved by the FDA for patients with MSI metastatic Colorectal cancer (mCRC). In the next four years following the approval of PD-1, inhibitors such as PD-L1 and PD-L2 were approved [4]. Ipilimumab in combination with Nivolumab for the treatment of colorectal cancer with MSI-H and dMMR aberrations, for patients aged 12 years and older, was approved by the FDA in July 2018 [5].

Colorectal cancer (CRC) is the third most common cause of cancer mortality with more than 850,000 deaths annually [6]. Out of all the patients diagnosed, about 20% of them have metastatic colorectal cancer. Among those diagnosed with metastatic colorectal cancer, 70% ~ 75% survive for more than 1 year, and less than 20% of the patients survive for more than 5 years [7]. Colorectal cancer can be categorized into 2 types: microsatellite instability, and microsatellite stable. Microsatellites are DNA segments that contain multiple short tandem repeats of nucleotide base pairs. During DNA synthesis, these sequences are highly likely to mutate due to errors caused by DNA polymerase. The mismatch repair system functions to recognize and correct these errors, but a deficient one would lead to further tumor mutations, thus more sensitive to immune checkpoint inhibitors [8]. Colorectal cancer can also be classified into four subtypes according to Consensus

Molecular Subtype (CMS) classification. CMS1 are hyper-mutated tumors that mostly carry microsatellite instability-high (MSI-H) features, and take up to approximately 14% of the cases. CMS2 are tumors with active Wnt and Myc pathways, they make up nearly 37 % of all the cases. CMS3 takes up to 13% of cases. They are tumors with Kristen rat sarcoma virus (KRAS) mutations and exhibit downregulation in cancer cell metabolic pathways. CMS4 are tumors that display activation of the transforming growth factor-beta (TGF-β) pathway, enhanced angiogenesis, stroll activation and inflammatory infiltrate, this subtype makes up approximately 23% of the cases [9].

Combining chemotherapy with molecular-targeted agents seems to be the optimal treatment for colorectal cancer, but the prognosis of patients remains poor. The development of immune checkpoint inhibitors revolutionized anti-tumor treatments for several cancer types, and are associated with the increasing survival rate in some patients with advanced or metastatic cancers, giving positive prognostic outcomes [11]. However, immunotherapy is less effective in colorectal cancer. This review aims to support further research in combination immunotherapy of CTLA-4 and PD-1 for colorectal cancer by introducing the mechanisms of CTLA-4 and PD-1 inhibitors, their applications in the treatment of colorectal cancer, and discussing some of the challenges that lay ahead.

2. Mechanisms of CTLA-4 and PD-1 inhibitors

CTLA-4 and PD-1 are two of the most paradigmatic immune checkpoints that downregulate immune responses. Overexpression of CTLA-4 and PD-1 are associated with better therapeutic responses. During phases of T-cell activation, they downregulate T-cell immune function. CTLA-4, also known as CD152, is a protein receptor of the B7/CD28 family [12]. It is expressed on the surface of regulatory T cells (Treg) and upregulated in conventional T cells after activation [13]. As shown in Figure 1, when CTLA-4 binds to CD80 or CD86 on the surface of antigen-presenting cells, CTLA-4 sends an “off” signal to the T cells, preventing the immune system from destroying the cancer cells.

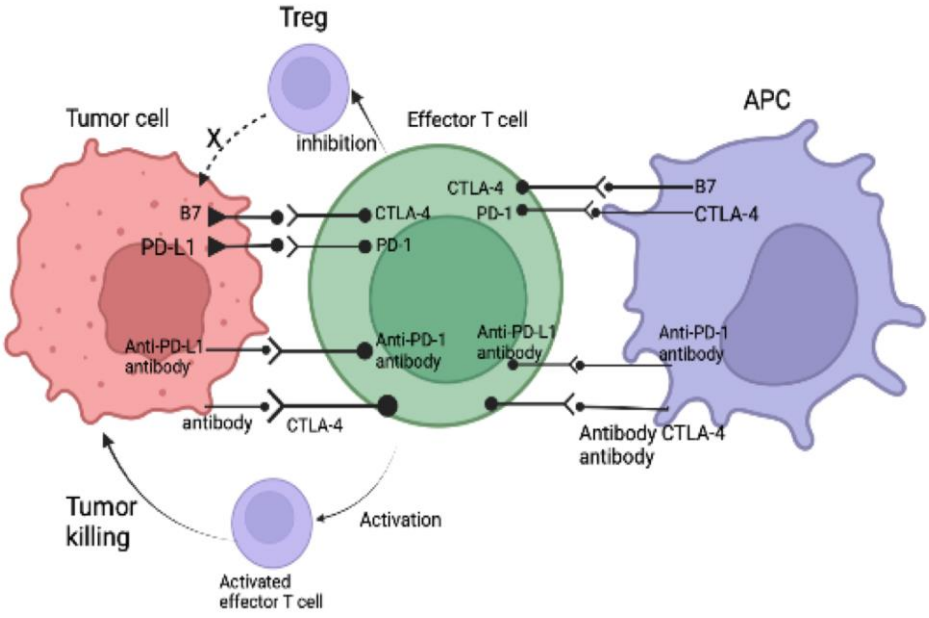


Figure 1. CTLA-4 target special molecular to inhibit some activity, and PD-1 strengthen this procedure [16]

PD-1 also comes from the B7/CD28 family, regulating T-cell activation by binding to its ligands PD-L1 and PD-L2 [14]. Its mechanisms of regulation include genetic alteration, epigenetic regulation, inflammatory signaling, and oncogenic signaling. Similar to CTLA-4, binding of PD-1 expressed on antigen-stimulated T cells to PD-L1 and PD-L2 expressed on cancer cells inhibits T-cell proliferation and activity of AKT and MEK signaling pathways, prevents the production of interferon-γ, tumor necrosis factor-α, and IL-2, and decreases T-cell survival [14]. PD-L1 is also being over-expressed

on tumor-infiltrating lymphocytes and many other cells, causing T cells to become exhausted after experiencing high levels of stimulation. T-cell exhaustion contributes to creating an immunosuppressive tumor microenvironment (TME) that promotes tumor progression, thus making immune-based treatment approaches more beneficial [15].

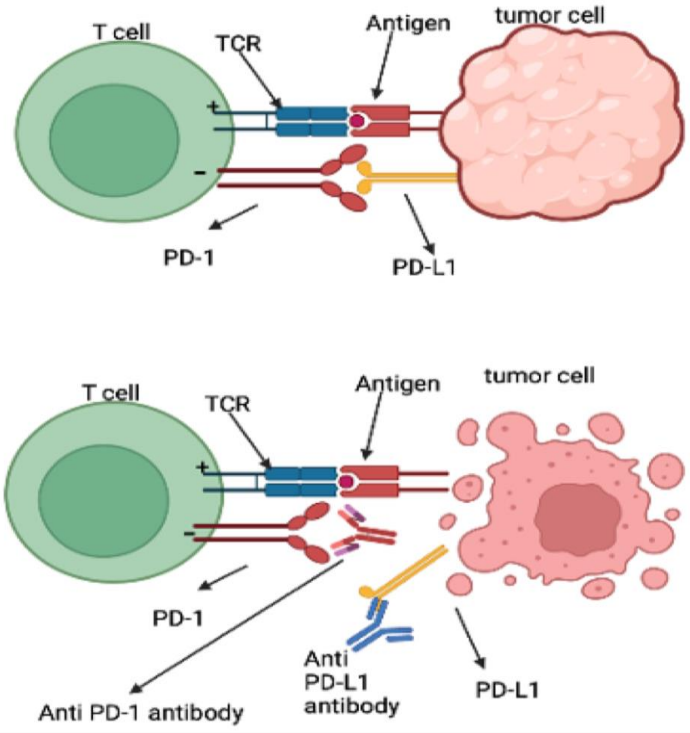


Figure 2. An explanation of PD-1 in binding tumour cell's antigen [16].

3. Application of CTLA-4 in colorectal cancer

CTLA-4 belongs to the immunoglobulin superfamily, its expression is associated with many immune responses. Some molecular signs can activate T cells to present CTLA-4 molecular transitorily [17]. CTLA-4 halts the production of cytokines in T cells. CTLA-4 and CD28 have homology characteristics so they competitively bind to the B7 ligand. According to the three-dimensional structure, CTLA-4 and CD28 have homology characteristics so they competitively bind to the B7 ligand [18]. Genetic variations in chromosome 2q33 increase the risk of cancer inside the human body. Chromosome 2q33, which is also known as immunoregulatory gene regions, includes the genes that code for CTLA-4.

The poor prognosis coming from preliminary assessments of several tumor types is associated with the expression of CTLA-4, making it a decisive part of the response to tumors [19]. Apart from this, inhibiting CTLA-4 has access to reactive T cells and restores their ability to attack cancer cells, so self-protect may take the lead again.

Patients who have suffered from MSI CRC have been treated with Nivolumab combined with Ipilimumab. The beneficial add-on characterized by CTLA-4 blockade is the key to approval in these patients, who have a high response to PD-1 blockade therapy. CTLA-4 occupies an important position in the inhibition of dendritic cells' normal physiologic activities in lymphoid tissue, while the T-cell inhibition which is influenced by PD-1 and NK cell activation in peripheral tissues, is the same as CTLA-4 that promotes regulatory T cell (Treg) differentiation [20]. The rationale of many clinical trials which have evaluated the good prognosis attributing to the monotherapy of PD-1 or CTLA-4 blockades or a combination of them is built on the effect they caused.

A multicenter study, Checkmate-142, recruited 119 patients into clinical observation, those patients receiving either Nivolumab alone or a combination of two. The ORR and the OS demonstrated the benefit of novel therapy dominated by Ipilimumab addition in previous patients.

Tremelimumab is a monoclonal antibody (MAB) that has been authorized for clinical use against CTLA-4. In a randomized controlled trial, the combination of Tremelimumab and Durvalumab, a PD-1 inhibitor acting as the alternative group to previously taken, proved that these have a more effective consequence than the control group.

CTLA-4 has fewer side effects than other ICIs; however, there remain many challenges. CTLA-4 alone is only effective well in special subtypes of CRC, so the generality should be improved in the next step. The side effects of anti-CTLA-4 occur in the early 12 weeks. When used alone, Nivolumab may cause damage to the kidney and liver. It could also lead to autoimmunity in the liver and acute kidney injury. CTLA-4 is also a good marker for prognostic impacts. High CTLA-4 expression in CRC suggests a shorter OS and disease-free survival (DFS) in some patients. So, it provides a new thought to shed a light on their clear relationship.

4. Application of PD-1 in colorectal cancer

CTLA-4 and CD28 are transmembrane proteins, along with PD-1 and CD279 Type I. There are two classes of PD-L1 molecules A and B, forming asymmetric units. PD-L1 domain 1 (D1) has β chains connected to 310 helical short regions, forming a V-set topology, which is of high research value. The crystal structure of the human PD-1 / PD-L1 complex has been analyzed and showed clear differences due to binding tendency. The TME of CRC infiltrates different immune cells, including B and T lymphocytes. The B and T cell infiltration share the same malignancies. The patient's clinical course has a strong relation to the accumulation of memory B cells and plasma cells in the CRC TME and partial tissues, which marks an anti-tumor response rate.

Following the discovery of Ipilimumab, Nivolumab has become another artificial inhibitor that blocks PD-1. Not only was it used in non-small cell lung cancer (NSCLC), but also phase I clinical trials for CRC patients. The blocking of PD-1/PD-L1 signaling pathways has fewer side effects in preliminary assessments [21]. The results from phase II of the study Checkmate-142 backed up this theory, with the overall response rate (OOR) reaching 30% in mCRC patients whose treatment scheme consists of prior fluoropyrimidine, oxaliplatin, and irinotecan. The results from this study helped to speed up the approval of Nivolumab.

Atezolizumab is a PD-L1-targeting IgG1 MAB characterized by PD-1 and B7 (CD80) receptors. In an open-label clinical trial number NCT01633970, the researchers examined the safety, pharmacology, and preliminary efficacy of atezolizumab combined with bevacizumab and chemotherapy. The results from the clinical trial have proven that atezolizumab in combination with bevacizumab has great potential for CRC treatment. Chemotherapy can upregulate or downregulate the expression of PD-1 in different periods of the cell cycle [22]. However, whether the direction of expression is upregulation or downregulation depends on the period of the cell cycle and the ancillary drug used in the chemotherapy. In a recent study, researchers examined the relationship between chemotherapy and the expression of PD-1. The results showed that PD-L1 has a 27.5%-57.5% expression rate in human CRC [23], and a 17.6% expression rate in normal cancer [24]. The expression rate has a positive association with the development of tumors.

In the research conducted by Magile et al., researchers designed a method to evaluate the ability of chemotherapies to potentiate anti-PD-1 combination therapy. They found that among all the treatments, Folfox was the only one that led to a complete and long-lasting cancer cure with a low recurring rate. Data obtained from the research supports that the TME dominated by Folfox provides colorectal tumors with enough sensibility to PD-1 inhibitors. It is because Folfox promotes high levels of PD-1, TIM-3, and CD8TILs [22].

Overall, data from studies have proven that PD-L1 expression and MSI tumors have shown more anti-tumor signals during monotherapy using PD-1 inhibitors due to cell infiltration. Combination

with PD-1 has shown high efficacy. In colorectal cancer, PD-L1 is likely to be expressed on tumor-infiltration cells instead of tumor cells, so it may provide novel insights into immune therapies. However, a low response rate still exists in some CRC patients with pMMR or non-MSI subtypes, so further research is needed to combat this problem.

Preclinical prevention is much more important when compared with a positive prognosis. Cancer vaccines that have common applications may be useful in the future when treating patients with mCRC because autologous cancer vaccination can strengthen the sensitivity to PD-1 in human bodies.

Combination therapy and oncolytic viruses will gradually play a more important role in CRC. Chemoreceptor TlpC in the bacteria Helicobactor pylori tends to a lactic environment, which is the environment cancer cells prefer. Researchers around the world have been designing a specific synthetic protein in oncolytic viruses to target cancer cells living in that environment. This specific synthetic protein would help Helicobactor pylori to enter the lactate and bind to specific molecules, making it an ideal targeted drug for patients. Tumor cells will change the oxygen percentage in TME. Helicobacter pylori, which can adapt to low oxygen environments, can survive for a long time in this environment, ensuring that the ICIs can get to the precise location where they are needed. What's more important is that this bacterium can be infused into the body through intravenous injection, making it more convenient when compared with past treatments that needed surgery. Combination therapy does not only apply to anti-CTLA-4 but also to proteins such as CD40, which have shown great synergistic effect in CRC patients. NK cells may lose their ability to secrete interferon-gamma (IFN-γ). When Helicobacter pylori are delivered into the tumors, it helps to re-activate NK cells to secrete IFN-γ, which terminate

5. Challenges and Improvements

The development of ICIs has revolutionized anti-tumor treatments. Before, treatments for colorectal cancer usually include chemotherapy, radiotherapy, and surgeries. Despite all the benefits and achievements in immunotherapy brought by the discovery and development of immune checkpoint inhibitors, it also faces many issues such as immune-related adverse events (irAEs) and low efficacy. As suggested by clinical evidence from recent years, immune checkpoint inhibitors play a therapeutic role in highly microsatellite instable metastatic colorectal cancer. However, about 95% of all the patients diagnosed with colorectal cancer have microsatellite stable tumors, and they do not benefit from current immunotherapy treatments [12].

Certain irAEs can be caused by the use of ICIs, most occurring due to the immune system being over-stimulated. Of all the patients treated with PD-1 inhibitors, approximately 15% have developed irAEs [25]. While immune checkpoint inhibitors such as Ipilimumab and Nivolumab have significant effects on boosting the immune cells to fight against cancer cells, it also causes the T cells in the immune system to become overactive and can lead to inflammation in different parts of the body. This might lead to the patient experiencing side effects. For instances: feeling tired or sick, fever, skin rash, loss of appetite, diarrhea (which occurs in 60% of the cases), increased risk of infection, and dry cough (caused by inflammation in the lungs) [26]. Immune checkpoint inhibitors immune-related adverse events tend to be organ-specific, so some serious side effects include disruption of the normal functions of the liver, kidneys, and thyroid [27].

In a study conducted by Michael J. Overman et al., The results demonstrated that out of all the 74 patients identified to have dMMR/MSI-H positive tumors and treated with the drug Nivolumab, the ORR was 31.1%, and the rate of disease control past 12 weeks was 69%. The combined treatment of Ipilimumab with Nivolumab increased the ORR to 55% and disease control rate to 80%, demonstrating that it has a better efficacy when compared to monotherapies and is a desirable treatment for patients with metastatic colorectal cancer [20].

6. Conclusion

In recent years, ICIs have revolutionized anti-tumor treatment for colorectal cancer. Treatments of anti-CTLA-4 and anti-PD-1 monoclonal antibodies have been proven to have high efficacy and play a therapeutic role for the patients with dMMR / MSI-H mCRC. However, this approach is effective for over 10% of those patients with mCRC, who have pMMR / MSS, and biomarker selection is needed to optimize the treatments. Improvements targeting this approach are needed, and several ongoing clinical trials have combined PD-1 inhibitors with other ICIs and chemotherapy to assess their efficacy. Although the trials demonstrated promising preliminary clinical results, there still lacks clear evidence that ICIs have a high efficacy in these patients. More in-depth knowledge about the mechanisms of CTLA-4 and PD-1 and the complex functions of the immune system within the TME are needed to develop effective therapeutic strategies and predictive biomarkers so patients can be effectively treated with ICIs.

References

[1] Esfahani, K., Roudaia, L., Del Rincon, S. V., Papneja, N., & Miller, W. H. (2020). A Review of Cancer Immunotherapy: From the Past, to the Present, to the Future. Current Oncology, 27(12), 87–97. https://doi.org/10.3747/co.27.5223

[2] Dobosz, P., & Dzieciątkowski, T. (2019). The Intriguing History of Cancer Immunotherapy. Frontiers in Immunology, 10. https://www.frontiersin.org/article/10.3389/fimmu.2019.02965

[3] Ipilimumab (Yervoy) | Cancer information | Cancer Research UK. (n.d.). Retrieved February 17, 2022, from https://www.cancerresearchuk.org/about-cancer/cancer-in-general/treatment/cancer-drugs/drugs/ipilimumab-yervoy

[4] Nivolumab (Opdivo) | Cancer information | Cancer Research UK. (n.d.). Retrieved February 17, 2022, from https://www.cancerresearchuk.org/about-cancer/cancer-in-general/treatment/cancer-drugs/drugs/nivolumab

[5] Km, H., Ce, J., & Cj, W. (2018). Immune checkpoint blockade therapy for cancer: An overview of FDA-approved immune checkpoint inhibitors. International Immunopharmacology, 62. https://doi.org/10.1016/j.intimp.2018.06.001

[6] Ferlay, J., Colombet, M., Soerjomataram, I., Parkin, D. M., Piñeros, M., Znaor, A., & Bray, F. (2021). Cancer statistics for the year 2020: An overview. International Journal of Cancer, 149(4), 778–789. https://doi.org/10.1002/ijc.33588

[7] Siegel, R. L., Miller, K. D., Fedewa, S. A., Ahnen, D. J., Meester, R. G. S., Barzi, A., & Jemal, A. (2017). Colorectal cancer statistics, 2017. CA: A Cancer Journal for Clinicians, 67(3), 177–193. https://doi.org/10.3322/caac.21395

[8] Ciardiello, D., Vitiello, P. P., Cardone, C., Martini, G., Troiani, T., Martinelli, E., & Ciardiello, F. (2019). Immunotherapy of colorectal cancer: Challenges for therapeutic efficacy. Cancer Treatment Reviews, 76, 22–32. https://doi.org/10.1016/j.ctrv.2019.04.003

[9] Fontana, E., Eason, K., Cervantes, A., Salazar, R., & Sadanandam, A. (2019). Context matters—Consensus molecular subtypes of colorectal cancer as biomarkers for clinical trials. Annals of Oncology, 30(4), 520–527. https://doi.org/10.1093/annonc/mdz052

[10] Thanki, K., Nicholls, M. E., Gajjar, A., Senagore, A. J., Qiu, S., Szabo, C., Hellmich, M. R., & Chao, C. (2017). Consensus Molecular Subtypes of Colorectal Cancer and their Clinical Implications. International biological and biomedical journal, 3(3), 105–111.

[11] Gupta, A. K., Melton, L. J., Petersen, G. M., Timmons, L. J., Vege, S. S., Harmsen, W. S., Diehl, N. N., Zinsmeister, A. R., & Ahlquist, D. A. (2005). Changing trends in the incidence, stage, survival, and screen-detection of colorectal cancer: A population-based study. Clinical Gastroenterology and Hepatology, 3(2), 150–158. https://doi.org/10.1016/S1542-3565(04)00664-0

[12] Ooki, A., Shinozaki, E., & Yamaguchi, K. (2021). Immunotherapy in Colorectal Cancer: Current and Future Strategies. Journal of the Anus, Rectum and Colon, 5(1), 11–24. https://doi.org/10.23922/jarc.2020-064

[13] Zhang, H., Dai, Z., Wu, W., Wang, Z., Zhang, N., Zhang, L., Zeng, W.-J., Liu, Z., & Cheng, Q. (2021). Regulatory mechanisms of immune checkpoints PD-L1 and CTLA-4 in cancer. Journal of Experimental & Clinical Cancer Research, 40(1), 184. https://doi.org/10.1186/s13046-021-01987-7

[14] Wu, X., Gu, Z., Chen, Y., Chen, B., Chen, W., Weng, L., & Liu, X. (2019). Application of PD-1 Blockade in Cancer Immunotherapy. Computational and Structural Biotechnology Journal, 17, 661–674. https://doi.org/10.1016/j.csbj.2019.03.006

[15] Combination of CTLA-4 and PD-1 blockers for treatment of cancer | Journal of Experimental & Clinical Cancer Research | Full Text. (n.d.). Retrieved April 1, 2022, from https://jeccr.biomedcentral.com/articles/10.1186/s13046-019-1259-z

[16] Seidel, J. A., Otsuka, A., & Kabashima, K. (2018). Anti-PD-1 and Anti-CTLA-4 Therapies in Cancer: Mechanisms of Action, Efficacy, and Limitations. Frontiers in oncology, 8, 86. https://doi.org/10.3389/fonc.2018.00086

[17] Pardoll D. M. (2012). The blockade of immune checkpoints in cancer immunotherapy. Nature reviews. Cancer, 12(4), 252–264. https://doi.org/10.1038/nrc3239

[18] Linsley, P. S., Greene, J. L., Tan, P., Bradshaw, J., Ledbetter, J. A., Anasetti, C., & Damle, N. K. (1992). Coexpression and functional cooperation of CTLA-4 and CD28 on activated T lymphocytes. The Journal of experimental medicine, 176(6), 1595–1604.

[19] Chen, L., Ashe, S., Brady, W. A., Hellström, I., Hellström, K. E., Ledbetter, J. A., McGowan, P., & Linsley, P. S. (1992). Costimulation of antitumor immunity by the B7 counterreceptor for the T lymphocyte molecules CD28 and CTLA-4. Cell, 71(7), 1093–1102. https://doi.org/10.1016/s0092-8674(05)80059-5

[20] Overman, M. J., McDermott, R., Leach, J. L., Lonardi, S., Lenz, H. J., Morse, M. A., Desai, J., Hill, A., Axelson, M., Moss, R. A., Goldberg, M. V., Cao, Z. A., Ledeine, J. M., Maglinte, G. A., Kopetz, S., & André, T. (2017). Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. The Lancet. Oncology, 18(9), 1182–1191. https://doi.org/10.1016/S1470-2045(17)30422-9

[21] Zitvogel L, Apetoh L, Ghiringhelli F, André F, Tesniere A, Kroemer G. The anticancer immune response: indispensable for therapeutic success? J Clin Invest. 2008 Jun;118(6):1991-2001. doi: 10.1172/JCI35180. PMID: 18523649; PMCID: PMC2396905.

[22] Kang, M. J., Kim, K. M., Bae, J. S., Park, H. S., Lee, H., Chung, M. J., Moon, W. S., Lee, D. G., & Jang, K. Y. (2013). Tumor-infiltrating PD1-Positive Lymphocytes and FoxP3-Positive Regulatory T Cells Predict Distant Metastatic Relapse and Survival of Clear Cell Renal Cell Carcinoma. Translational oncology, 6(3), 282–289.

[23] Brahmer, J. R., Drake, C. G., Wollner, I., Powderly, J. D., Picus, J., Sharfman, W. H., Stankevich, E., Pons, A., Salay, T. M., McMiller, T. L., Gilson, M. M., Wang, C., Selby, M., Taube, J. M., Anders, R., Chen, L., Korman, A. J., Pardoll, D. M., Lowy, I., & Topalian, S. L. (2010). Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. Journal of clinical oncology: official journal of the American Society of Clinical Oncology, 28(19), 3167–3175.

[24] Dosset, M., Vargas, T. R., Lagrange, A., Boidot, R., Végran, F., Roussey, A., Chalmin, F., Dondaine, L., Paul, C., Lauret Marie-Joseph, E., Martin, F., Ryffel, B., Borg, C., Adotévi, O., Ghiringhelli, F., & Apetoh, L. (2018). PD-1/PD-L1 pathway: an adaptive immune resistance mechanism to immunogenic chemotherapy in colorectal cancer. Oncoimmunology, 7(6), e1433981.

[25] Hussaini, S., Chehade, R., Boldt, R. G., Raphael, J., Blanchette, P., Maleki Vareki, S., & Fernandes, R. (2021). Association between immune-related side effects and efficacy and benefit of immune checkpoint inhibitors – A systematic review and meta-analysis. Cancer Treatment Reviews, 92, 102134.

[26] De Miguel, M., & Calvo, E. (2020). Clinical Challenges of Immune Checkpoint Inhibitors. Cancer Cell, 38(3), 326–333.

[27] S, B., R, Y., & Eg, E. (2021). Immune Checkpoint Inhibitors for the Treatment of Cancer: Clinical Impact and Mechanisms of Response and Resistance. Annual Review of Pathology, 16. https://doi.org/10.1146/annurev-pathol-042020-042741