Review Article

Neoantigen: A Promising Target for the Immunotherapy of Colorectal Cancer

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At present, there are various treatment strategies for colorectal cancer, including surgery, chemotherapy, radiotherapy, and targeted therapy. In recent years, with the continuous development of immunotherapy, immune checkpoint inhibitors (ICIs) can significantly improve the treatment of advanced colorectal cancer patients with high levels of microsatellite instability. In addition to ICIs, neoantigens, as a class of tumor-specific antigens (TSA), are regarded as new immunotherapy targets for many cancer species and are being explored for antitumor therapy. Immunotherapy strategies based on neoantigens include tumor vaccines and adoptive cell therapy (ACT). These methods aim to eliminate tumor cells by enhancing the immune response of host T-cells to neoantigens. In addition, for MSS colorectal cancer, such “cold tumors” with low mutation rates and stable microsatellites are not sensitive to ICIs, whereas neoantigens could provide a promising immunotherapeutic avenue. In this review, we summarized the current status of colorectal cancer neoantigen prediction and current clinical trials of neoantigens and discussed the difficulties and limitations of neoantigens-based therapies for the treatment of CRC.

1. Introduction

Colorectal cancer (CRC) is the third most diagnosed tumor in the world, accounting for 10% of the total number of cases, and is the second leading cause (9.4%) of cancer-related death [1]. At present, the treatment strategies of CRC include surgery, radiotherapy, chemotherapy, and targeted therapy. Immune checkpoint inhibitors, including nivolumab and pembrolizumab, for programmed death receptor (PD-1) blockade have been approved for the treatment of CRC with deficient mismatch repair (dMMR) or high microsatellite instability (MSI-H) [2, 3]. Based upon the Phase III KEYNOTE-177 study (NCT02563002), pembrolizumab is currently approved for the first-line treatment of MSI-H/dMMR metastatic colorectal cancer [4]. However, immunotherapy with the anti-PD-1 monoclonal antibody did not achieve the desired effect for patients with proficient mismatch repair (pMMR) or microsatellite stability (MSS) [5, 6].

This phenomenon may be related to the fact that colorectal cancer with MSI-H usually has a higher tumor mutation burden (TMB). Due to more frequent insertion and deletion mutations in the DNA sequence and the frameshift mutations of opening read frame, solid tumors with MSI-H may produce and express more neotumor-specific peptides which are completely different from the self [7, 8]. Tumor-specific peptides are defined as neoantigens when they displayed by the major histocompatibility complex (MHC) on the surface of tumor cells, which can trigger a T-cell-mediated cytotoxic anti-tumor immune response and cause T-cell population expansion [9, 10]. These neoantigens are produced by cancer cells bearing mutations that affect the protein sequence and include nonsynonymous point mutations, codon insertion/deletion, frameshift mutations, splicing mutations, and gene fusions. There is also a subgroup of tumor-specific antigens (TSAs) that are derived from viral proteins [11].

The occurrence/progression of tumors is often driven by a series of somatic mutations across the genome. Mutations in genes that regulate cell division and growth, such as protooncogenes and tumor suppressor genes, are significant in the process of tumor formation [12, 13]. Moreover, the type and number of mutations can vary greatly between different tissues and tumor types, which means that neoantigens can
be tumor-type-specific and even individual-specific. Patients with a higher tumor mutation load (TMB) will likely produce a greater number/variety of mutant peptides, which results in a greater abundance and variety of antigens. The mutation frequency of CRC is usually very high compared to most other cancer types, and the average mutation density is ~10 mutations per megabase.

Moreover, ~15% of CRC patients have microsatellite instability caused by defects in mismatch repair proteins, which is a hypermutable phenotype. This phenotype is most often associated with Lynch syndrome and hereditary non-polyposis colorectal cancer (HNPPC) that meet Amsterdam II standards [14–18]. Hypermutable/hypervariable phenotypes with insertion/deletions (INDELs) in these short repetitive DNA sequences can lead to the formation of neoantigens if the mutations occur in coding regions. Some INDELs located within open reading frames are also frameshift mutations, which can lead to the production of vastly different proteins that contain large sections ofmiscellaneous amino acid sequences (depending on the codon sequence). Ultimately, this means that cancers with a hypermutable phenotype are more likely to produce a class of neoantigen that are associated with higher immunogenicity [7].

The neoantigens that are produced through the aforementioned means are expressed on tumor cells, whereas healthy cells will not present such antigens. The tumor-specific nature of neoantigens makes them ideal targets for antitumor immunotherapy and has been investigated for the treatment of CRC in a variety of basic and clinical immunotherapy studies. Nevertheless, the application of neoantigens for clinical immunotherapy is also faced with various challenges. Hence, this article reviews neoantigen technology, its prospects, and the challenges associated with the application of neoantigens for colorectal cancer therapy.

### 2. Identification and Selection of Candidate Neoantigens

Neoantigens need to fulfill two criteria to be useful as the target of immunotherapies, namely, they need to be (1) presented by MHC molecules and (2) induce a CD4+ or CD8+ T-cell immune response. Thus, predicting/identifying neoantigens that can be explored in clinical research is the starting point for exploiting them as targets for tumor immunotherapy. This can be a complex and comprehensive process with three steps: (1) the identification of somatic mutations in DNA or mRNA sequences and the neoepitopes produced from those mutations, (2) an assessment of the neoepitope binding affinity and capacity for presentation by MHC I/II molecules [19], and (3) the determination of whether the neoepitopes can induce T-cell proliferation and their related immune responses. It is notable that different prediction pipelines/algorithms can vary substantially due to features such as the treatment schedule, economic circumstances, and technical limitations [8, 20, 21]. We described the brief flow of neoantigen prediction and the use of neoantigen in the treatment of CRC in Figure 1.

At present, the development of high-throughput sequencing (second-generation sequencing, NGS) technology has increased the feasibility for the accurate identification of mutations using both DNA and RNA. This requires comparing tumor (somatic) whole-genome (WGS) or whole-exon sequencing (WES) data to that from healthy (germline) tissue [19]. Moreover, RNA-sequencing can help infer the expression and therefore viability of mutant peptides with tumors. The sequencing data can also have utility for the identification of HLA genotypes (HLA allele detection), which is an important aspect that can be used to supplement the analysis when determining the neoepitope presentation and binding affinity in later stages of the pipeline [22–24].

Several computational approaches based on machine learning (ML) algorithms that account for HLA alleles have been used to predict neoepitope binding affinity and processing [25, 26]. Such methods are based on a large number of experimental data sets that detail HLA binding. Additionally, immunopeptideomic approaches that utilize state of the art mass spectrometry (MS) analyses can directly assess neoepitopes that are displayed by class I and class II MHC molecules, which can be useful for the verification of the results that were predicted via in silico analyses [27–30]. For example, NetMHCPan and NetMHCIIpan can integrate information form in silico and MS analyses to predict the presentation of neoepitopes and their HLA binding affinity [31–34]. There are also computational pipelines, such as pVACtools and MuPeXi, which predict and prioritize neoepitopes by integrating WGS, mutant cloning, mRNA expression, peptides processing, and HLA binding affinity data [24, 35–38].

A major difficulty for the application of neoantigens as immunotherapy targets is the need to predict whether the neoantigens will be recognized by T-cell receptors (TCRs) and stimulate T-cell activation and infiltration. There are a number of T-cell-based assays that can be used to measure tumor-related T-cell responses, including enzyme-linked immunosorbent spot (ELISpot) detection [39] and multicolor tetramer-based flow cytometry [40]. Peng et al. reported that nanoparticle- (NP-) barcoded nucleic acid cell sorting (NACS) can be used to enumerate and isolate neoantigen-specific CD8+ T-cells. Additionally, a mutation-related neoantigen-specific function extension (MANAFEST) analysis has the ability to sensitively monitor neoantigen-related antitumor immune responses via the molecular characterization of related TCR sequences [41].

A global community with researchers from many institutions has established a Tumor Neoantigen Selection Alliance (TESLA). Different teams in the alliance independently mine shared data, predict potential neoantigens, and prioritize candidate neoantigens. The results from each team are crossmatched and combined, with the aim to understand the immunogenicity of tumor epitopes and improve neoantigen prediction algorithms [42]. For example, Bai et al. proposed the "NP" rule based on the conservative mutation direction of anchor residues from immunogenic neoantigens and integrated the rule within...
existing prediction algorithms to improve neoantigen immunogenicity prediction [43].

3. Neoantigens and Immune Response in Colorectal Cancer

The median TMB of colorectal cancer ranked seventh among 30 of the most common types of malignant tumors. About 16% of CRCs have a TMB of >12 mutations per 106 base pairs, which are classified as highly mutated tumors [44]. Patients with higher TMB may present more candidate neoantigens that can be used for clinical treatment. However, the type of mutation can also have a great impact upon the clinical applicability of the neoantigen. We list the mutated antigens that were found or studied in CRC in Table 1.

3.1. Frameshift Peptide. For MSI-H CRC, frameshift mutations caused by INDELs can lead to the production of new frameshift peptides (FSP), which are the main source of neoantigens in these tumors [17]. Frameshift protein sequences represent a novel tumor-specific antigen subclass that can induce FSP-specific immune response [45]. Frameshift mutations can be commonly found in genes with important biological functions in most MSI-H colorectal tumors. These genes have functions including epigenetic regulation (HDAC2, ARID1A), DNA repair (MSH3 and MSH6), signal transduction (TGFβRII, IGF2, ACVR2A), cell apoptosis (BAX), and miRNA processing (TARBP2, XPO5). Moreover, there is a correlation between the density of CD8+ tumor infiltrating T lymphocytes and the number of mutations [46–49]. Tumor growth factor β receptor II (TGFβRII) mutations are commonly found in MSI-H CRC (90% of HNPCC) but can also be present in ~15% of MSS CRC [17]. The short peptide produced by a frameshift mutation within TGFβRII can cause the proliferation of CD4+ T-cells in the tumor infiltrating lymphocyte (TIL) population [7, 50]. This is associated with the specific killing of cells bearing this mutation in an HLA-A2-restricted manner, which makes it a prime target for tumor vaccine therapy [7]. Indeed, Inderberg et al. have reported that the immunogenic neopeptide produced by a -1A mutation within the TGFβRII microsatellite A [10] tract can induce the HLA-A2-restricted TGFβRII mutation-specific T-cell immune response and increase survival in a CRC mouse model [51].

A number of other promising neoantigens arising from frameshift mutations have also been described that can cause related cytotoxic T lymphocyte antitumor responses, such as the HLA-A0201-restricted neoantigen caused by frameshift mutations within O-linked N-acetylglucosamine transferase (OGT) and the -1A mutation within the A [8] tract of MSH3 [47, 52]. It has been reported that tumor-specific peptides produced by frameshift mutations in the coding region of CDX2 in patients with colorectal cancer can cause relative antibody immune responses in serum [53]. Speetjens et al. reported immunogenicity tests for 15 peptides with microsatellite frameshift mutations, of which 8 antigens (TGFβRII-1, MARCKS-1, MARCKS-2, CDX2-2, BAXα+1, PCNXL2-2, TCF7L2-2, and TAF1B-1) can be combined with MHC molecules and presented to T-cells (the four foremost antigens being the most significant) [54].

3.2. Single-Nucleotide Variants and Shared Mutation Peptides. In addition to specific neoantigens produced by individual mutations, driver gene mutations in genes such as KRAS, TP53, and BRAF can be commonly found in multiple tumor types [55, 56]. Point mutations and single amino acid substitutions in KRAS can cause its activation and affect cell proliferation, division, and apoptosis via the intracellular signaling cascade in about 40% of CRC patients [57–60]. The most frequent KRAS mutation is located in its second exon (most commonly resulting in G12D, G12V, and G13D) [60, 61]. It has been reported that peptides produced by high-frequency point mutations in KRAS can stimulate the proliferation of cytotoxic T lymphocytes (CTLs) in vitro and in CRC patients.
[61–63]. Iizumi et al. found that mutant peptides produced by driver gene mutations are immunogenic, including KRAS-G12D, KRAS-G12R, KRAS-G13D, and PIK3CA-H1047R. And the results show the neoantigens that stimulate the response of CD4+ T-cells (rather than CD8+), which indicate that a greater number of the neoantigens are HLA-II-restricted [64]. This phenomenon has also been previously reported [65]. For TP53, the most commonly mutant gene in multiple tumors, Lo et al. found that TP53 p.R175H can trigger a TCR-mediated immunogenic response, and that the immunogenicity was HLA-A*0201-restricted. This feature has also been recognized in ovarian cancer, uterine cancer, and myeloma cell lines. Transduction with a retrovirus encoding HLA-A*0201 can also cause the recognition of colorectal cancer cells with TP53 p.R175H [66]. In a humanized mouse colorectal cancer model, a vaccine produced by using a mixture of long peptides derived from KRAS and TP53 mutant proteins combined with MHC molecules was found to induce strong cytotoxicity and T helper cell immune responses to multiple mutations at the same time [67]. Recently, it has been reported that single-nucleotide variants (SNVs) in genes, such as KRAS, PIK3CA, PCBP1, and CHEK2, are associated with the production of the 10 most frequent neoantigens. An analysis of the published CRC WES data has enabled a more complete map of CRC mutations to be produced, which indicated that high-frequency mutations such as KRAS G12D, KRAS G12V, PIK3CA E545K, PIK3CA H1047R, and BMPR2 N583Tfs∗44 can combine with HLA and be presented

| Gene     | Type of mutation | Epitope                          | HLA    | Reference |
|----------|------------------|----------------------------------|--------|-----------|
| OGT      | Frameshift mutation | SLYKFSFPL (FSP06) p523, SLRLSCCV p573, RLSSCVPA p577, SSCPVALM p578, LSSCVVAL p579, VPVALMSAM | HLA-A0201 | [48]      |
| TGFβRII  | Frameshift mutation | p537, AMTSSQKNITPAILTCC p538, SLRLSSCVVALMSAMTSSSQ p539, ALMSAMTSSQKNITPAILTCC p540, SPKCIMKEKKSLRLSSCV p541, PKCIMKEKKSKLRLSSCV p542, SPKCIMKEKKKAW p543, PKCIMKEKKKAW p547, LSLRLSSCVVALMSAMT | —       | [51], [52], [53] |
| Bax      | Frameshift mutation | —                                 | —      | [51]      |
| MSH3     | Frameshift mutation | —                                 | HLA-A0201 | [54]      |
| CDX2     | Frameshift mutation | —                                 | —      | [55]      |
| FTO      | Frameshift mutation | —                                 | HLA-A0201 | [56], [92] |
| Caspase 5| Frameshift mutation | TLSPGWASAV                        | HLA-A0201 | [56], [93] |
| KRAS     | SNVs              | G12D, VVGADGVGK                   | HLA-A1101 | [65], [70] |
| PIK3CA   | SNVs              | G12V, VVAGVGVGK                   | —      | [70]      |
| PARVA    | SNVs              | NPLSLIPPELREDTMLLENEVRT           | —      | [76]      |
| G3BP1    | SNVs              | NCHTKIRHVDAHTLNGVQVMG IRHVDAHTTL | —      | [76]      |
| ACTR10   | SNVs              | SVEPGVLEDIAHTCFVSDLKRGLK          | —      | [76]      |
| RAE1     | SNVs              | WWLETLAQPEFLSTLPHLCTNLGP          | —      | [76]      |
| PDP1     | SNVs              | PKSEAKSVQKDVWLGLLMLMPFRAFG SEAKSVQQVQDW SEAKSVQQVQDW | —      | [76]      |
| QRICHT1  | SNVs              | VHVSGSPTALAAAFKLEDDEKMVGT         | —      | [76]      |

Table 1: List of mutated antigens that were found or studied in CRC.
3.3. Microsatellite Stability. The neoantigens mentioned above all appear in MSI-H CRC, which is associated with a higher tumor mutation burden. However, most CRCs have relatively lower mutation burden (MMR-p/MSS CRC) and do not benefit from treatment with immune checkpoint inhibitors [6].

Nevertheless, studies have focused upon the possibility and potential for neoantigen immunotherapy in CRC patients with a low TMB. Ovarian cancer, glioblastoma, metastatic cholangiocarcinoma, and other tumors with low TMB have been reported to have positive effects following treatment with personalized vaccines against neoantigens and adoptive cell transfer (ACT) therapy with reactive T-cells [69–73]. CRC patients with a low TMB are affected less by immune escape events (such as antigen presentation defects) and may benefit from neoantigen vaccines or ACT. Bulk et al. investigated the autologous neoantigen-specific T-cell immune response of patients with MSS CRC. The results showed that there were specific T-cell immune responses against multiple neoepitopes in three patients, and the existence of neoantigen-specific T-cells in the CD39 + CD103 + T-cell subset was confirmed by TIL sorting [74]. The study of Tran et al. confirmed that TILs can be used to detect neoantigen-directed T-cell reactivity in gastrointestinal tumors (including those with moderate mutation burden) [75]. In addition, studies have found that there are immune responses to new epitopes by TILs from metastatic tumors (including MSS CRC) [76].

4. Clinical Trial of Neoantigens as Targets in Colorectal Cancer

4.1. Neoantigen Vaccine. The phenomenon of eliciting effective neoantigen-specific antitumor T-cell immune responses and inhibiting tumor growth has been observed in vivo. Such preclinical experiments have highlighted the potential of neoantigen-based immunotherapy as a new therapeutic strategy. The efficacy of neoantigen vaccines has also been confirmed in the mouse models of CRC [30, 65]. Clinical trials of neoantigen vaccines have been conducted for melanoma and glioblastoma, which proved that the vaccine is a safe method for eliciting tumor-specific T-cell responses [71, 77–79]. Moreover, there are currently several clinical trials exploring the efficacy and safety of vaccines against different kinds of neoantigens for CRC patients (Table 2).

A pilot study targeting a KRAS mutant peptide demonstrated that only two of the seven CRC patients showed a positive immune response after vaccination [80]. Kloor et al. recently conducted a phase I clinical trial to evaluate the safety and immunogenicity of frameshift peptide neoantigen-based vaccines for dMMR CRC patients. The trial used a vaccine based on FSP neoantigens derived caused by AILM2, HT001, and TAF1B mutations. Their results showed that humoral and cellular immune responses were induced by at least one of the frameshift peptide vaccines for all 22 of the dMMR CRC patients, and there were no serious vaccine-related adverse reactions [81].

Attempts to enhance the immune response generated against neoantigen vaccines through the use of GM-CSF as an adjuvant were successful. However, the vaccine was not found to be beneficial for patient’s disease progression, which may be related to an increase of immune regulatory cells [82]. In addition, many neoantigen vaccines have been tested in preclinical mouse models. Ni et al. developed a neoantigen (Adpgk) nanovaccine (banNV) with a Toll-like receptor 7/8 agonist R848 and TLR9 agonist CpG as a dual adjuvant. It was found that the dual adjuvant neoantigen vaccine increased the immunogenicity of the neoantigen and elicited a good antitumor response together with anti-PD-1 therapy [83]. It has also been reported that the use of multiple neoantigen DNA vaccines and anti-PD-1 therapy can synergistically control the growth of the MC38 colon cancer cell line [84]. Kim et al. reported that the combination of a neoantigen-based EpiGVAX vaccine and 5-aza-2′-deoxycytidine can increase the antitumor efficacy of an irradiated whole-cell CRC vaccine by inducing neoantigen-specific antitumor T-cell response [85]. Leoni et al. recently selected 209 shared FSPs in the MSI CRC genome map database to produce a viral vector vaccine, Nous-209, which was confirmed to activate human CD8+ T-cells in vitro experiments [86].

4.2. Adoptive Cell Transfer. ACT is a type of immunotherapy that transfers immune cells to patients. T-cells that specifically recognize neoantigens and are capable of inducing antitumor response can be ideal carriers for ACT. Inderberg et al. used T-cells transduced with HLA-A2-restricted TGFβRII mutation-specific TCR in a mouse colorectal cancer model and found that it reduced tumor growth and improved survival [51]. A clinical trials reported that the autologous transfer of a HLA-C*08:02-restricted KRAS G12D reactive polyclonal CD8+ T-cell population successfully treated patients with metastatic CRC [87]. Tran et al. expanded KRAS G12D-specific CD8+ T-cells isolated from the lung metastasis in CRC patients, and the subsequent infusion caused the metastases to complete resolve for six out of the seven patients [88]. These studies suggest the potential of neoantigen-specific T-cells in the treatment of CRC, and other clinical trials of ACT based on neoantigens are currently underway (Table 2).

5. Challenges and Conclusion

Studies have proven that MSI-H CRC patients have a higher survival rate and therapeutic effectiveness following immune checkpoint inhibitor therapy [5, 6, 89, 90]. The neoantigens produced by gene mutations can cause antitumor immune responses, and the specific T-cells that recognize neoantigens are not affected by thymus central tolerance [91]. This feature makes neoantigen-based vaccines, ACT, and other immune-based treatments a promising strategy for the treatment of colorectal cancer. These treatments may even be used to prevent cancer formation in tumor-free Lynch syndrome mutation carriers. MSS patients with a low TMB
| Type of therapy | Study phase | Tumor | Status of CRC | Strategy | Combination therapy | Number of patients | Status | Trail number |
|-----------------|-------------|-------|---------------|----------|---------------------|--------------------|--------|--------------|
| Vaccine         | Phase I     | Pancreatic cancer, metastatic | MMR-p | Neoantigen vaccine with poly-ICLC adjuvant | Retifanlimab | 12 | Not yet recruiting | NCT04799431 |
| Vaccine         | Phase I     | Colorectal cancer | Vaccination with frameshift-derived neoantigen-loaded DC | — | 25 | Active, not recruiting | NCT01885702 |
| Vaccine         | Phase I     | Colorectal cancer | — | Neoantigen-primed DC cell vaccine | — | 80 | Recruiting | NCT04147078 |
| Vaccine         | Phase I/II  | Colorectal cancer | Gastroesophageal adenocarcinoma, Urothelial carcinoma, Non-small-cell lung cancer | GRT-C901/GRT-R902, a neoantigen cancer vaccine | Nivolumab and ipilimumab | 214 | Recruiting | NCT03639714 |
| Vaccine         | Phase I/II  | Colorectal cancer | MSS | Nivolumab and ipilimumab | 144 | Recruiting | NCT03953235 |
| Vaccine         | Phase I     | Colorectal cancer | MMR-p | Pooled mutant-KRAS peptide vaccine with poly-ICLC | Nivolumab and ipilimumab | 30 | Recruiting | NCT04117087 |
| Vaccine         | Not applicable | — | Personalized mRNA tumor vaccine encoding neoantigen | — | 24 | Recruiting | NCT03468244 |
| Vaccine         | Phase I     | Colorectal cancer, Pancreatic cancer, Advanced esophageal squamous carcinoma, Gastric adenocarcinoma, Pancreatic adenocarcinoma, Colorectal adenocarcinoma, Colorectal adenocarcinoma, Pancreatic ductal adenocarcinoma, Colorectal cancer, Breast cancer, Head and neck squamous cell carcinoma, Melanoma, Non-small-cell lung cancer, Pancreatic cancer, Liver cancer | — | — | — | — | — | — |

Table 2: Clinical trials of neoantigens in colorectal cancer.
are currently considered unsuitable for immunotherapy strategies. However, some studies have found that there are still more than one neoantigen (related to the CRC molecular subtype) that can produce an immune response in such patients [76]. Neoantigen-related immunotherapy strategies have also achieved a good level immunoreactivity in other tumors with low TMB, such as glioblastoma.

Most neoantigens are patient-specific, and the process for clinical testing of individual neoantigens in patients is limited by prediction technology, economic costs, and other aspects. Moreover, tumor sequencing generally only reveals the mutations of some cells within the tumor; hence, intratumor heterogeneity usually is not considered. Heterogeneity between the primary lesion and the metastasis may also hinder the neoantigen based therapies. Tumor heterogeneity as a mechanism of treatment failure and disease recurrence/progression should therefore be considered during the identification, selection, and clinical application of neoantigens.

Among patients with CRC, especially those with MSI-H phenotype, neopeptides derived from frameshift mutations account are predominant. However, the current neoantigen prediction technologies are mostly used for the prediction of neoantigens derived from SNVs; thus, the prediction of neoantigens generated by frameshift mutations remains problematic. It is notable that if the binding affinity of driver gene neoantigens (e.g., TP53 and KRAS) resulting hotspot mutations with MHC was improved, they could be used as therapeutic targets for a larger number of patients with a variety of cancer types, thus enabling more patients to receive immunotherapy.

The ability of a neoantigen to induce an adaptive immune response after binding to MHC molecules is affected by many factors, including the HLA presentation function of the antigen, mutations that regulate the HLA expression, peptide transport, and the characteristics of HLA itself.

Even though neoantigens can cause local or systemic increase in specific T-cells, the immune function is still affected by inhibitory factors in the immune microenvironment (such as Tregs and M2 macrophages). The use of chemotherapy, radiotherapy, and oncolytic viruses can enhance the inflammatory response and may be a promising method to support immunotherapy.

In short, neoantigens are a new immunotherapeutic strategy for treatment of various types of CRC. However, there are still many challenges in ranging from the clinical application to the neoantigen prediction/screening, which still need to be further explored.

**Conflicts of Interest**

The authors declare that there is no conflict of interest regarding the publication of this paper.

**References**

[1] H. Sung, J. Ferlay, R. L. Siegel, M. Laversanne, I. Soerjomataram, and A. Jamal, "Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality
worldwide for 36 cancers in 185 countries,” CA: a Cancer Journal for Clinicians, 2021.

[2] D. T. Le, J. N. Uram, H. Wang, B. R. Bartlett, H. Kemberling, and A. D. Eyring, “PD-1 blockade in tumors with mismatch-repair deficiency,” The New England Journal of Medicine, vol. 372, no. 26, pp. 2509–2520, 2015.

[3] M. J. Overman, R. McDermott, J. L. Leach et al., “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, vol. 18, no. 9, pp. 1182–1191, 2017.

[4] T. André, K.-K. Shiu, T. W. Kim, B. V. Jensen, L. H. Jensen, and C. Punt, “Pembrolizumab in microsatellite-instability-high advanced colorectal cancer,” The New England Journal of Medicine, vol. 383, no. 23, pp. 2207–2218, 2020.

[5] M. Yarchoan, B. A. Johnson, E. R. Lutz, D. A. Laheru, and E. M. Jaffee, “Targeting neoantigens to augment antitumour immunity,” Nature reviews Cancer., vol. 17, no. 4, pp. 209–222, 2017.

[6] D. T. Le, J. N. Durham, K. N. Smith, H. Wang, B. R. Bartlett, and L. K. Aulakh, “Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade,” Science, vol. 357, no. 6349, pp. 409–413, 2017.

[7] M. Linnebacher, J. Gebert, W. Rudy, S. Woerner, Y. P. Yuan, and P. Bork, “Frameshift peptide-derived T-cell epitopes: a source of novel tumor-specific antigens,” International Journal of Cancer, vol. 93, no. 1, 2001.

[8] M. M. Gubin, M. N. Artyomov, E. R. Mardis, and R. D. Schreiber, “Tumor neoantigens: building a framework for personalized cancer immunotherapy,” The Journal of Clinical Investigation, vol. 125, no. 9, pp. 3413–3421, 2015.

[9] T. N. Schumacher and R. D. Schreiber, “Neoantigens in cancer immunotherapy,” Science, vol. 348, no. 6320, pp. 69–74, 2015.

[10] C. H. Lee, R. Yeleisky, K. Jooss, and T. A. Chan, “Update on tumor neoantigens and their utility: why it is good to be different,” Trends in Immunology, vol. 39, no. 7, pp. 536–548, 2018.

[11] C. C. Smith, S. R. Selitsky, S. Chai, P. M. Armistead, B. G. Vincent, and J. S. Serody, “Alternative tumour-specific antigens,” Nature Reviews. Cancer, vol. 19, no. 8, pp. 465–478, 2019.

[12] M. S. Lawrence, P. Stojanov, P. Polak, G. V. Kryukov, K. Cibulskis, and A. Sivachenko, “Mutational heterogeneity in cancer and the search for new cancer-associated genes,” Nature, vol. 499, no. 7457, pp. 434–442, 2013.

[13] G. Ciriello, M. L. Miller, B. A. Aksoy, Y. Senbabaoglu, N. Schultz, and C. Sander, “Emerging landscape of oncogenic signatures across human cancers,” Nature Genetics, vol. 45, no. 10, pp. 1127–1133, 2013.

[14] G. Kurzawski, J. Suchy, T. Debnik, J. Kladny, and J. Lubinski, “Importance of microsatellite instability (MSI) in colorectal cancer: MSI as a diagnostic tool,” Annals of Oncology, vol. 15, pp. iv283–iv284, 2004.

[15] C. R. Boland and A. Goel, “Microsatellite instability in colorectal cancer,” Gastroenterology, vol. 138, no. 6, pp. 2073–2087.e3, 2010.

[16] Y. Ionov, M. A. Peinado, S. Malkhosyan, D. Shibata, and M. Perucho, “Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis,” Nature, vol. 363, no. 6429, pp. 558–561, 1993.

[17] B. Liu, R. Parsons, N. Papadopoulos, N. C. Nicolaides, H. T. Lynch, and P. Watson, “Analysis of mismatch repair genes in hereditary non-polyposis colorectal cancer patients,” Nature Medicine, vol. 2, no. 2, pp. 169–174, 1996.

[18] E. Vilari and S. B. Gruber, “Microsatellite instability in colorectal cancer-the stable evidence,” Nature Reviews. Clinical Oncology, vol. 7, no. 3, pp. 153–162, 2010.

[19] H. Hackl, P. Charoentong, F. Finotello, and Z. Trajanoski, “Computational genomics tools for dissecting tumour-immune cell interactions,” Nature Reviews. Genetics, vol. 17, no. 8, pp. 441–458, 2016.

[20] S. Hutchison and A. L. Pritchard, “Identifying neoantigens for use in immunotherapy,” Mammalian Genome, vol. 29, no. 11-12, pp. 714–730, 2018.

[21] A. L. Pritchard, J. G. Burel, M. A. Neller et al., “Exome Sequencing to Predict Neoantigens in Melanoma,” Cancer Immunology Research, vol. 3, no. 9, pp. 992–998, 2015.

[22] B. Bulik-Sullivan, J. Busby, C. D. Palmer et al., “Deep learning using tumor HLA peptide mass spectrometry datasets improves neoantigen identification,” Nature Biotechnology, vol. 37, 2019.

[23] S. A. Shukla, M. S. Rooney, M. Rajasagi, G. Tiao, P. M. Dixon, and M. S. Lawrence, “Comprehensive analysis of cancer-associated somatic mutations in class I HLA genes,” Nature Biotechnology, vol. 33, no. 11, pp. 1152–1158, 2015.

[24] J. Hundal, S. Kiwala, J. McMichael, C. A. Miller, H. Xia, and A. T. Wollam, “pVACtools: a computational toolkit to identify and visualize cancer neoantigens,” Cancer Immunology Research, vol. 8, no. 3, pp. 409–420, 2020.

[25] T. Trolle, I. G. Metusshi, J. A. Greenbaum, Y. Kim, J. Sidney, and O. Lund, “Automated benchmarking of peptide-MHC class I binding predictions,” Bioinformatics, vol. 31, no. 13, pp. 2174–2181, 2015.

[26] M. Bonnack, S. Hoppe, J. Winter et al., “Performance Evaluation of MHC Class-I Binding Prediction Tools Based on an Experimentally Validated MHC–Peptide Binding Data Set,” Cancer Immunology Research, vol. 7, no. 5, pp. 719–736, 2019.

[27] M. Bassani-Sternberg and G. Coukos, “Mass spectrometry-based antigen discovery for cancer immunotherapy,” Current Opinion in Immunology., vol. 41, pp. 9–17, 2016.

[28] M. Bassani-Sternberg, “Mass spectrometry based Immuno-peptidomics for the discovery of cancer Neoantigens,” in Pep-tidomics: Methods and Strategies, M. Schrader and L. Fricker, Eds., pp. 209–221, Springer New York, New York, NY, 2018.

[29] L. K. Freudemann, A. Marcu, and S. Stavenvoci, “Mapping the tumour human leukocyte antigen (HLA) ligandome by mass spectrometry,” Immunology, vol. 154, no. 3, pp. 331–345, 2018.

[30] M. Yadav, S. Jhusjhuwala, Q. T. Phung, P. Lupardus, J. Tanguay, and S. Bumbaca, “Predicting immunogenic tumour mutations by combining mass spectrometry and exome sequencing,” Nature, vol. 515, no. 7528, pp. 572–576, 2014.

[31] V. Jurtz, S. Paul, M. Andreotta, P. Marcattili, B. Peters, and M. Nielsen, “NetMHCpan-4.0: Improved Peptide–MHC Class I Interaction Predictions Integrating Eluted Ligand and Peptide Binding Affinity Data,” The Journal of Immunology, vol. 199, no. 9, pp. 3360–3368, 2017.

[32] M. Andreotta, E. Karosiene, M. Rasmussen, A. Stryhn, S. Buus, and M. Nielsen, “Accurate pan-specific prediction of peptide-MHC class II binding affinity with improved binding core identification,” Immunogenetics, vol. 67, no. 11-12, pp. 641–650, 2015.
[33] M. Nielsen, C. Lundegaard, T. Blicher et al., “NetMHCpan, a method for quantitative predictions of peptide binding to any HLA-A and -B locus protein of known sequence,” PLoS One, vol. 2, no. 8, article e796, 2007.

[34] K. K. Jensen, M. Andreatta, P. Marcattili, S. Buus, J. A. Greenbaum, and Z. Yan, “Improved methods for predicting peptide binding affinity to MHC class II molecules,” Immunology, vol. 154, no. 3, pp. 394–406, 2018.

[35] J. Hundal, B. M. Carreno, A. A. Petti, G. P. Linette, O. L. Griffith, and E. R. Mardis, “pVAC-Seq: a genome-guided in silico approach to identifying tumor neoantigens,” Genome Medicine, vol. 8, no. 1, p. 11, 2016.

[36] A. Miller, Y. Asmann, L. Cattaneo, E. Braggio, J. Keats, and V. P. Balachandran, M. Ł Slota, J.-B. Lim, Y. Dang, and M. L. Disis, “High somatic mutation and neoantigen burden are correlated with decreased progression-free survival in multiple myeloma,” Cancer Journal, vol. 7, no. 9, p. e612, 2017.

[37] V. P. Balachandran, M. Łuksza, J. N. Zhao, V. Makarov, J. A. Moral, and R. Remark, “Identification of unique neoantigen qualities in long-term survivors of pancreatic cancer,” Nature, vol. 551, no. 7681, pp. 512–516, 2017.

[38] A.-M. Bjerregaard, M. Nielsen, S. R. Hadrup, Z. Szallasi, and A. C. Eklund, “MuPxi: prediction of neo-epitopes from tumor sequencing data,” Cancer Immunology, Immunotherapy, vol. 66, no. 9, pp. 1123–1130, 2017.

[39] M. Slota, J.-B. Lim, Y. Dang, and M. L. Disis, “ELISpot for measuring human immune responses to vaccines,” Expert Review of Vaccines, vol. 10, no. 3, pp. 299–306, 2011.

[40] S. R. Hadrup, A. H. Bakker, C. J. Shu, R. S. Andersen, J. van Veluw, and P. Hombriek, “Parallel detection of antigen-specific T-cell responses by multidimensional encoding of MHC multimers,” Nature Methods, vol. 6, no. 7, pp. 520–526, 2009.

[41] L. Danilova, V. Anagnostou, J. X. Caushi et al., “The mutation-associated Neoantigen functional expansion of specific T cells (MANAFEST) assay: a sensitive platform for monitoring anti-tumor Immunity,” Immunity, vol. 6, no. 8, pp. 888–899, 2018.

[42] D. K. Wells, M. M. van Buuren, K. K. Dang, V. M. Hubbard-Lucey, K. C. F. Sheehan, K. M. Campbell et al., “Key parameters of tumor epitope immunogenicity revealed through a consortia approach improve Neoantigen prediction,” Cell, vol. 183, no. 3, pp. 818–34.e13, 2020.

[43] P. Bai, Y. Li, Q. Zhou et al., “Immune-based mutation classification enables neoantigen prioritization and immune feature discovery in cancer immunotherapy,” Oncoimmunology, vol. 10, no. 1, 2021.

[44] L. B. Alexandrov, S. Nik-Zainal, D. C. Wedge, S. A. J. R. Aparicio, S. Behjati, and A. V. Biankin, “Signatures of mutational processes in human cancer,” Nature, vol. 500, no. 7463, pp. 415–421, 2013.

[45] Y. Schvitalle, M. Kloor, S. Eiermann, M. Linnebacher, P. Kienle, and H. P. Kneabel, “Immune response against frameshift-induced neopeptides in HNPPC patients and healthy HNPPC mutation carriers,” Gastroenterology, vol. 134, no. 4, pp. 988–997, 2008.

[46] P. Mahy, D. Tougeron, M. Hamieh, B. Mlecnik, H. Kora, and G. Bindea, “Correlation between density of CD8+ T-cell infiltrate in microsatellite unstable colorectal cancers and frameshift mutations: a rationale for personalized immunotherapy,” Cancer Research, vol. 75, no. 17, pp. 3446–3455, 2015.

[47] E. Ripberger, M. Linnebacher, Y. Schwitalla, J. Gebert, and D. M. von Knebel, “Identification of an HLA-A0201-restricted CTL epitope generated by a tumor-specific frameshift mutation in a coding microsatellite of the OGT gene,” Journal of Clinical Immunology, vol. 23, no. 5, pp. 415–423, 2003.

[48] S. M. Woerner, A. Benner, C. Sutter, Y. P. Yuan, and G. Keller, “Pathogenesis of DNA repair-deficient cancers: a statistical meta-analysis of putative real common target genes,” Oncogene, vol. 22, no. 15, pp. 2226–2235, 2003.

[49] A. Duval and R. Hamelin, “Mutations at coding repeat sequences in mismatch repair-deficient human cancers: toward a new concept of target genes for instability,” Cancer Research, vol. 62, no. 9, pp. 2447–2454, 2002.

[50] I. Saeterdal, J. Bjoerheim, K. Lislrud, M. K. Gjertsen, I. K. Bukholm, and O. C. Olsen, “Frameshift-mutation-derived peptides as tumor-specific antigens in inherited and spontaneous colorectal cancer,” Proceedings of the National Academy of Sciences of the United States of America, vol. 98, no. 23, pp. 13255–13260, 2001.

[51] E. M. Inderberg, S. Wachli, M. R. Myhre et al., “T cell therapy targeting a public neoantigen in microsatellite instable colon cancer reduces inivotumor growth,” Oncoimmunology, vol. 6, no. 4, article e1302631, 2017.

[52] Y. Garbe, C. Maletzki, and M. Linnebacher, “An MSI tumor specific frameshift mutation in a coding microsatellite of MSH3 encodes for HLA-A0201-restricted CD8+ cytotoxic T cell epitopes,” PLoS One, vol. 6, no. 11, p. e26517, 2011.

[53] T. Ishikawa, T. Fujita, Y. Suzuki, S. Okabe, Y. Yuasa, and T. Iwai, “Tumor-specific Immunological Recognition of Frameshift-mutated Peptides in Colon Cancer with Microsatellite Instability,” Cancer Research, vol. 63, no. 17, pp. 5564–5572, 2003.

[54] F. M. Speetjens, M. M. Lauwen, K. L. Franken et al., “Prediction of the immunogenic potential of frameshift-mutated antigens in microsatellite instable cancer,” International Journal of Cancer, vol. 123, no. 4, pp. 838–845, 2008.

[55] B. Vogelstein, N. Papadopoulos, V. E. Velculescu, S. Zhou, L. A. Diaz, and K. W. Kinzler, “Cancer genome landscapes,” Science, vol. 339, no. 6127, pp. 1546–1558, 2013.

[56] E. R. Fearon and B. Vogelstein, “A genetic model for colorectal tumorigenesis,” Cell, vol. 61, no. 5, pp. 759–767, 1990.

[57] M. Dobre, M. Comanescu, D. Arsen, C. Iosif, and G. Bussolati, “K-ras gene mutation status in colorectal cancer: comparative analysis of pyrosequencing and PCR-RFLP,” Romanian Journal of Morphology and Embryology, vol. 54, no. 3, pp. 567–574, 2013.

[58] C. Tan and X. Du, “KRAS mutation testing in metastatic colorectal cancer,” World Journal of Gastroenterology, vol. 18, no. 37, pp. 5171–5180, 2012.

[59] C. P. Vaughn, S. D. Zobell, L. V. Furtado, C. L. Baker, and W. S. Samowitz, “Frequency of KRAS, BRAF, and NRAS mutations in colorectal cancer,” Genes, Chromosomes & Cancer, vol. 50, no. 5, pp. 307–312, 2011.

[60] H. J. Andreyev, A. R. Norman, D. Cunningham, J. R. Oates, and P. A. Clarke, “Kirsten ras mutations in patients with colorectal cancer: the multicenter “RASCAL” study,” Journal of the National Cancer Institute, vol. 90, no. 9, pp. 675–684, 1998.

[61] J. L. Bos, E. R. Fearon, S. R. Hamilton, M. Verlaan-de Vries, J. H. van Boom, and A. J. van der Eb, “Prevalence of ras gene
mutations in human colorectal cancers,” *Nature*, vol. 327, no. 6120, pp. 293–297, 1987.

[62] D. J. Peace, W. Chen, H. Nelson, and M. A. Cheever, “T cell recognition of transforming proteins encoded by mutated ras proto-oncogenes,” *Journal of Immunology*, vol. 146, no. 6, pp. 2059–2065, 1991.

[63] Y. Shono, H. Tanimura, M. Iwashashi, T. Tsunoda, M. Tani, H. Tanaka et al., “Specific T-cell immunity against Ki-ras peptides in patients with pancreatic and colorectal cancers,” *British Journal of Cancer*, vol. 88, no. 4, pp. 530–536, 2003.

[64] S. Iiizumi, J. Ohtake, N. Murakami et al., “Identification of Novel HLA Class II-Restricted Neoantigens Derived from Driver Mutations,” *Cancers*, vol. 11, no. 2, p. 266, 2019.

[65] S. Kreiter, M. Vormehr, N. van de Roemer, M. Diken, W. Lo, M. Parkhurst, P. F. Robbins, E. Tran, Y.-C. Lu, and M. Löwer, “Mutant MHC class II epitopes drive therapeutic immune responses to cancer,” *Nature*, vol. 520, no. 7549, pp. 692–696, 2015.

[66] W. Lo, M. Parkhurst, P. F. Robbins, E. Tran, Y.-C. Lu, and L. Jia, “Immunologic recognition of a shared p53 mutated Neoantigen in a patient with metastatic colorectal cancer,” *Cancer immunology research*, vol. 7, no. 4, pp. 534–543, 2019.

[67] J. Quandt, C. Schlude, M. Bartoschek et al., “Long-peptide vaccination with driver gene mutations in p53 and Kras induces cancer mutation-specific effector as well as regulatory T cell responses,” *Oncoimmunology*, vol. 7, no. 12, p. e1500671, 2018.

[68] C. Chen, S. Liu, R. Qu, and B. Li, “Recurrent neoantigens in colorectal cancer as potential immunotherapy targets,” *BioMed Research International*, vol. 2020, 2020.

[69] S. Bobisse, R. Genolet, A. Roberti, J. L. Taniy, J. Racle, and B. J. Stevenson, “Sensitive and frequent identification of high avidity neo-epitope specific CD8+ T cells in immunotherapy-naive ovarian cancer,” *Nature Communications*, vol. 9, no. 1, p. 1092, 2018.

[70] J. L. Taniy, S. Bobisse, E. Ophir, S. Tuyaerts, A. Roberti, and R. Genolet, “Personalized cancer vaccine effectively mobilizes antitumor T cell immunity in ovarian cancer,” *Science translational medicine*, vol. 10, no. 436, 2018.

[71] D. B. Keskin, A. J. Anandappa, J. Sun, I. Tirosch, N. D. Mathewson, and S. Li, “Neoantigen vaccine generates intratumoral T cell responses in phase Ib glioblastoma trial,” *Nature*, vol. 565, no. 7738, pp. 234–239, 2019.

[72] N. Hilf, S. Kuttruff-Coqui, K. Frenzel, V. Bukur, S. Stevanović, and C. Gouttefangeas, “Actively personalized vaccination trial for newly diagnosed glioblastoma,” *Nature*, vol. 565, no. 7738, pp. 240–245, 2019.

[73] E. Tran, S. Turcotte, A. Gros, P. F. Robbins, Y.-C. Lu, and M. E. Dudley, “Cancer immunotherapy based on mutation-specific CD4+ T cells in a patient with epithelial cancer,” *Science*, vol. 344, no. 6184, pp. 641–645, 2014.

[74] J. van den Bulk, E. M. E. Verdegaal, D. Ruano, M. E. Ijsjesstein, M. Visser, and R. van der Breggen, “Neoantigen-specific immunity in low mutation burden colorectal cancers of the consensus molecular subtype 4,” *Genome Medicine*, vol. 11, no. 1, p. 87, 2019.

[75] E. Tran, M. Ahmadzadeh, Y.-C. Lu, A. Gros, S. Turcotte, and P. F. Robbins, “Immunogenicity of somatic mutations in human gastrointestinal cancers,” *Science*, vol. 350, no. 6266, pp. 1387–1390, 2015.

[76] M. R. Parkhurst, P. F. Robbins, E. Tran, T. D. Prickett, J. J. Gartner, and L. Jia, “Unique neoantigens arise from somatic mutations in patients with gastrointestinal cancers,” *Cancer Discovery*, vol. 9, no. 8, pp. 1022–1035, 2019.

[77] B. M. Carreno, V. Magrini, M. Becker-Hapak, S. Kaabinejadjan, J. Hundal, and A. A. Petti, “Cancer immunotherapy. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells,” *Science*, vol. 348, no. 6236, pp. 803–808, 2015.

[78] P. A. Ott, Z. Hu, D. B. Keskin, S. A. Shukla, J. Sun, D. J. Bozym et al., “An immunogenic personal neoantigen vaccine for patients with melanoma,” *Nature*, vol. 547, no. 7662, pp. 217–221, 2017.

[79] U. Sahin, E. Derhovanessian, M. Miller, B.-P. Kloke, P. Simon, and M. Löwer, “Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer,” *Nature*, vol. 547, no. 7662, pp. 222–226, 2017.

[80] A. Toubaji, M. Achtar, M. Provenzano, V. E. Herrin, R. Behrens, and M. Hamilton, “Pilot study of mutant ras peptide-based vaccine as an adjuvant treatment in pancreatic and colorectal cancers,” *Cancer Immunology, Immunotherapy*, vol. 57, no. 9, pp. 1413–1420, 2008.

[81] M. Kloos, M. Reuschbach, C. Pauligk, J. Karbach, M.-R. Rafiyan, and S.-E. Al-Batran, “A frameshift peptide neoantigen-based vaccine for mismatch repair-deficient cancers: a phase I/IIa clinical trial,” *Clinical Cancer Research*, vol. 26, no. 17, pp. 4503–4510, 2020.

[82] O. E. Rahma, J. M. Hamilton, M. Wojtowicz et al., “The immunological and clinical effects of mutated ras peptide vaccine in combination with IL-2, GM-CSF, or both in patients with solid tumors,” *Journal of translational medicine*, vol. 12, no. 1, 2014.

[83] Q. Ni, F. Zhang, Y. Liu et al., “A bi-adjuvant nanovaccine that potentiates immunogenicity of neoantigen for combination immunotherapy of colorectal cancer,” *Science Advances*, vol. 6, no. 12, p. eaaw6071, 2020.

[84] E. Tondini, T. Arakelian, K. Oosterhuis et al., “A poly-neoantigen DNA vaccine synergizes with PD-1 blockade to induce T cell-mediated tumor control,” *Oncoimmunology*, vol. 8, no. 11, 2019.

[85] V. M. Kim, X. Pan, K. C. Soares et al., “Neoantigen-based EpigVAX vaccine initiates antitumor immunity in colorectal cancer,” *JCI Insight*, vol. 5, no. 9, article e136368, 2020.

[86] G. Lenzi, A. M. Aline, G. Cotugno, F. Langone, I. Garzia, and M. De Lucia, “A genetic vaccine encoding shared cancer Neoantigens to treat tumors with microsatellite instability,” *Cancer Research*, vol. 80, no. 18, pp. 3972–3982, 2020.

[87] E. Tran, P. F. Robbins, Y.-C. Lu, T. D. Prickett, J. J. Gartner, and L. Jia, “T-cell transfer therapy targeting mutant KRAS in cancer,” *The New England Journal of Medicine*, vol. 375, no. 23, pp. 2255–2262, 2016.

[88] M. M. Richters, H. Xia, K. M. Campbell, W. E. Gillanders, O. L. Griffith, and M. Griffith, “Best practices for bioinformatic characterization of neoantigens for clinical utility,” *Genome Medicine*, vol. 11, no. 1, p. 56, 2019.

[89] E. M. Van Allen, D. Miao, B. Schilling, S. A. Shukla, C. Blank, and L. Zimmer, “Genomic correlates of response to CTLA-4 blockade in metastatic melanoma,” *Science*, vol. 350, no. 6257, pp. 207–211, 2015.

[90] N. A. Rizvi, M. D. Hellmann, A. Snyder, P. Kvistborg, V. Makarov, and J. J. Havel, “Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in nonsmall cell lung cancer,” *Science*, vol. 348, no. 6230, pp. 124–128, 2015.
[91] M. E. Ijsselsteijn, F. Petitprez, L. Lacroix, D. Ruano, R. van der Breggen, C. Julie et al., “Revisiting immune escape in colorectal cancer in the era of immunotherapy,” British Journal of Cancer, vol. 120, no. 8, pp. 815–818, 2019.

[92] M. Linnebacher, A. Wienck, I. Boeck, and E. Klar, “Identification of an MSI-H tumor-specific cytotoxic T cell epitope generated by the (-1) frame of U79260 (FTO),” Journal of Biomedicine & Biotechnology, vol. 2010, article 841451, pp. 1–6, 2010.

[93] Y. Schwitalle, M. Linnebacher, E. Ripberger, J. Gebert, and D. M. von Knebel, “Immunogenic peptides generated by frameshift mutations in DNA mismatch repair-deficient cancer cells,” Cancer Immunity, vol. 4, 2004.