

Cancer Immunotherapy Research

Selected publications featuring next-generation sequencing technology

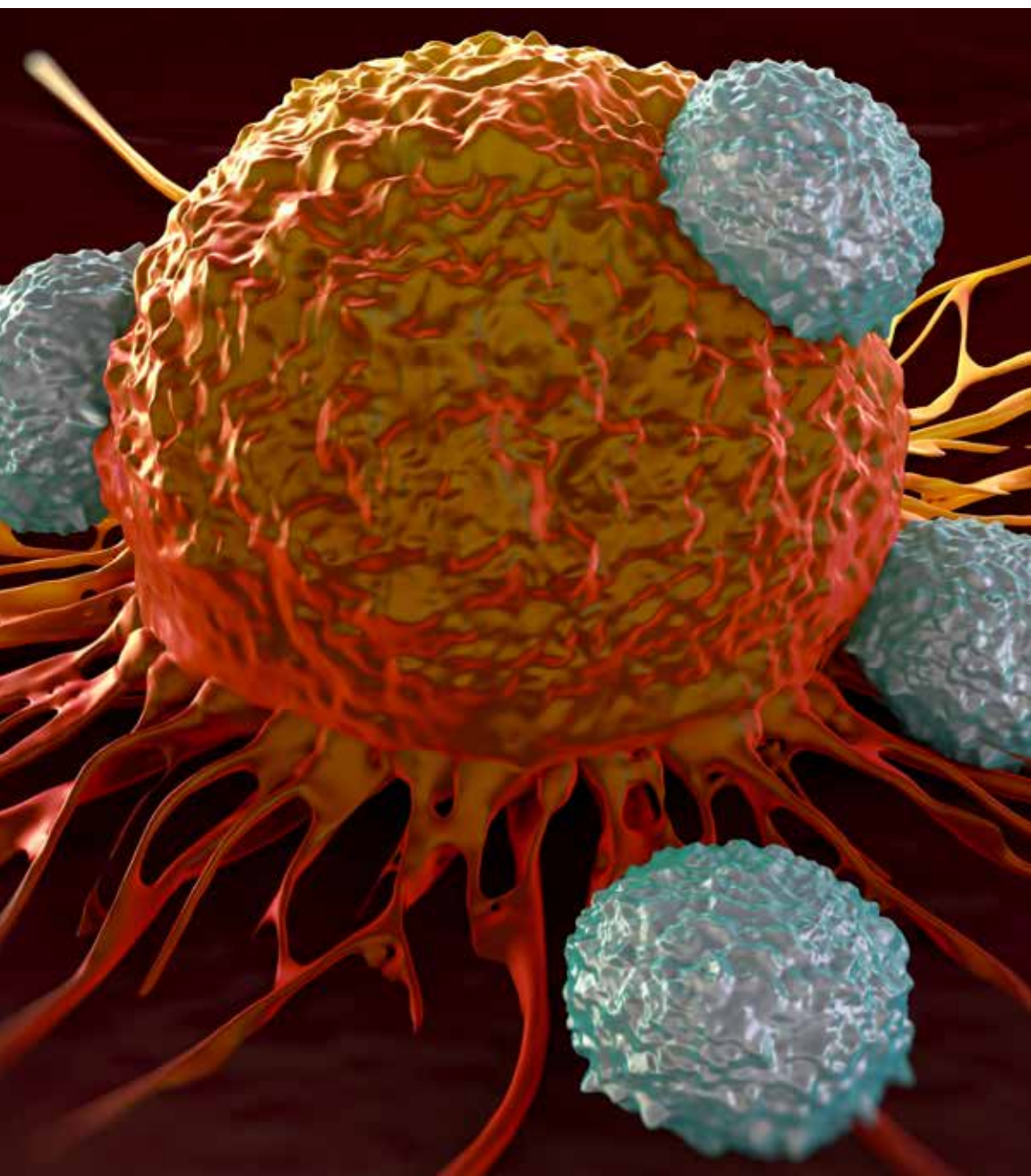


TABLE OF CONTENTS

04	Introduction
06	Vaccine immunotherapy
08	Adoptive cell transfer
	Chimeric antigen receptor (CAR) T cells
	Tumor infiltrating lymphocytes (TILs)
14	Checkpoint inhibitors
21	Role of the microbiome
23	Bibliography

INTRODUCTION

High-throughput sequencing has shown remarkable utility in cancer and immunology research, as well as in the development of individualized immunotherapy. For example, high-throughput sequencing has dramatically improved our knowledge of the cancer genome and the intracellular mechanisms involved in tumor progression. In addition, careful analysis of the cancer genome can also reveal new epitopes that could be targeted by the immune system.¹ Sequencing can also be used to determine the immune repertoire as a real-time, highly sensitive monitor of clonal expansion and contraction of the cell populations in response to tumor growth or treatment.^{2,3,4}

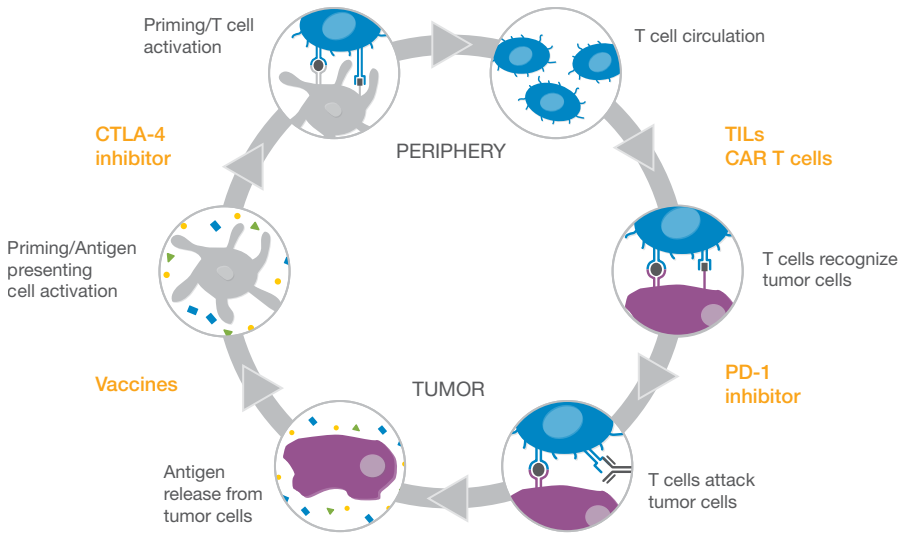


Figure 1: T Cell Mediated Immunity—T Cell Mediated Immunity—Many steps are necessary for establishing a successful immune response, which may be augmented with immunotherapies. Tumor-specific antigens are released from dead tumor cells. Neoantigens that are recognized by antigen presenting cells are presented to T-cells, and activation of T-cells occurs when they bypass immune checkpoints. Activated T-cells circulate in the bloodstream until tumor infiltration. When tumor recognition occurs, additional checkpoints must be surpassed before systemic T-cell response is established. Orange text indicates immune modulatory treatments that are described in this publication review. This figure was adapted from Chen and Mellman.⁵

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1. Kreiter S, Vormehr M, van de Roemer N, et al. Mutant MHC class II epitopes drive therapeutic immune responses to cancer. *Nature*. 2015;520(7549):692-696.
 2. Robinson WH. Sequencing the functional antibody repertoire—diagnostic and therapeutic discovery. *Nat Rev Rheumatol*. 2015;11(3):171-182.
 3. Ribas A, Wolchok JD. Combining cancer immunotherapy and targeted therapy. *Curr Opin Immunol*. 2013;25:291-296.
 4. Kvistborg P, van Buuren MM, Schumacher TN. Human cancer regression antigens. *Curr Opin Immunol*. 2013;25:284-290.
 5. Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. *Immunity*. 2013;39(1):1-10.
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VACCINE IMMUNOTHERAPY

Carreno BM, Magrini V, Becker-Hapak M, et al. Cancer immunotherapy. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells. *Science*. 2015;348 (6236):803-808.

The genome of melanoma cells, as for any cancerous cell, displays several somatic mutations. Amino acid substitutions (AASs) are the result of missense mutations, and they can provide patient-specific antigens that trigger immune response and tumor-specific T cell immunity. The aim of this study was to evaluate these putative neoantigens as targets of anti-tumor activity and to verify whether a vaccination could augment such immune response. To do so, the authors performed exome sequencing on excised tumors from 3 patients with stage III resected cutaneous melanoma to identify AASs. They then enrolled the 3 patients in a phase 1 clinical trial, administering a dendritic cell vaccine, directed at tumor-specific amino acids. They found that the vaccine increased the naturally occurring neoantigen-specific immunity and revealed previously undetected human leukocyte antigen (HLA) class I-restricted neoantigens. Vaccination also promoted a diverse neoantigen-specific T cell receptor (TCR) repertoire in terms of both TCR- β and clonal composition.

Technology used: the HiSeq® 2000 System (sequencing) by Illumina

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Diken M, Kreiter S, Kloke B, et al. Current developments in actively personalized cancer vaccination with a focus on RNA as the drug format. *Prog Tumor Res.* 2015;42:44-54.

Sequencing technologies are enabling the rapid characterization of genomic and transcriptional data from cancer patients, revealing the high degree of cancer-specific variability. This is a breakthrough in personalized medicine and cancer diagnostics, which sets great expectations in personalized therapies. The several mutations in protein sequences from cancerous cells can provide a valuable source of antigens that can be recognized by the immune system and used in the development of personalized vaccines. Of the various forms of possible cancer vaccines, mRNA-based vaccine encoding for the mutated epitopes has given promising results and been proven safe in preclinical and clinical settings. After the preclinical proof of concept in mice, actively personalized mRNA cancer vaccination was first introduced to the clinic in 2013, in a phase I study. The approach consists in, first, the use of next-generation sequencing (NGS) to characterize a cancer's mutanome, immunome, and transcriptome by comparing healthy and cancer tissues from each patient. Then, patients receive 2 polypeptide-encoding RNA molecules, which are patient specific. This approach is aimed at targeting multiple epitopes in a specific patient's tumor to address cancer heterogeneity, and is therefore, applicable to any cancer that carries more than 1 mutation.

Technology used: NA (review)

ADOPTIVE CELL TRANSFER

Chimeric Antigen Receptor (CAR) T Cells

Morrison C. CAR-T field booms as next-generation platforms attract big players. *Nat Biotechnol.* 2015(6);33:571-572.

Chimeric antigen receptor (CAR) T cell (CAR-T) treatments are next-generation therapies that belong to the adoptive cell therapy class of treatments. These treatments comprise the *ex vivo* modification of T cells to direct a response to a tumor antigen. Recently, more biopharma companies have been licensing deals to access CAR-based cellular immunotherapies, following the example initially set by Novartis in 2012, when it acquired the exclusive rights to a CAR-T program developed at the University of Pennsylvania by Dr Carl June. Although this technology still presents challenges, these come with high-improvement opportunities. Examples are the ability to regulate T cell gene expression and response after they have been infused back into the patient using small molecules as activators, and the simplification of the procedure itself.

Technology used: NA (review)

Kalos M, Levine BL, Porter DL, et al. T cells with chimeric antigen receptors have potent anti-tumor effects and can establish memory in patients with advanced leukemia. *Sci Transl Med.* 2011;3(95):95ra73.

Tumor immunotherapy using T lymphocytes can recognize and destroy malignant cells. T cells can be engineered to express chimeric antigen receptors (CARs), which are composed by an antibody-binding domain and domains that activate the T cell. The expression of CAR domains could overcome tolerance by allowing T cells to respond to cell surface antigens. To test CD19 as a potential tumor target in B cell malignancies, the authors treated 3 patients with chemotherapy-resistant lymphocytic leukemia (CLL) with autologous T cells expressing an anti-CD19 CAR domain that included both the CD3- ζ and the 4-1BB costimulatory domain. They reported that such engineered cells expanded more than thousandfold *in vivo*, trafficked to bone marrow; showed potent non-cross-resistant clinical activity; and kept expressing CARs at high levels for 6 months. Furthermore, immune profiling revealed a CD19-specific immune response accompanied by complete remission in 2 of 3 patients. A portion of these cells persisted as memory cells and retained anti-CD19 effector functionality.

Technology used: NA (sequencing)

Grupp SA, Kalos M, Barrett D, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *N Engl J Med.* 2013;368:1509-1518.

Engineered T cells expressing CARs with specificity for CD19 represent a promising therapy for chemotherapy-resistant lymphocytic leukemia (CLL). To verify whether chimeric antigen receptor T cell (CAR-T) treatments have clinical activity in acute lymphoblastic leukemia (ALL) as well, the authors treated 2 children with relapsed and refractory pre-B cell ALL (B-ALL) with infusions of T cells transduced with an anti-CD19 antibody and a T cell-signaling molecule. In both of the patients, infused cells expanded thousandfold and were found in bone marrow and cerebrospinal fluid for up to 6 months after infusion. Although both patients presented cytokine release syndrome as a side effect, cytokine blockade treatment was effective without inhibiting neither the proliferation of CAR-T nor the anti-cancerous effect of the therapy. Both patients had complete remission from the disease. For 1 of the 2 patients, remission lasted at least 9 months, which was confirmed at the molecular level by DNA sequencing. The second patient had a relapse after 2 months, where blast cells no longer expressed CD19. DNA sequencing allowed the identification of the malignant clone in peripheral blood and bone marrow on day 23, hence assessing the relapse earlier than the clinical presentation of symptoms as well as before blast cells were identifiable in the circulation through flow cytometry.

Technology used: NA (sequencing)

Sotillo E, Barrett DM, Black KL, et al. Convergence of acquired mutations and alternative splicing of CD19 enables resistance to CART-19 immunotherapy. *Cancer Discov.* 2015;5(12):1282-1295.

CART-19 targets the CD19 antigen, expressed in most cases of pre-B cell acute lymphoblastic leukemia (B-ALL). Treatment with CART-19 antigen yields 70% response in patients of B-ALL, but it also can lead to escape variants that result in relapse with epitope loss. The aim of this study was to identify the underlying mechanism of this resistance. To do this, the authors analyzed CD19-positive leukemia cells pre- and post-CART-19 therapy, as well as relapsed cells. They detected hemizygous deletions in spanning the CD19 locus, *de novo* mutations in exon 2 of the gene, and alternatively spliced CD19 mRNA isoforms lacking exon 2. They identified SRSF3 as splicing factor involved in the retention of exon 2. With the use of genome-editing techniques, they provided evidence suggesting a mechanism of resistance based on a combination of deleterious mutations and selecting for alternatively spliced RNA isoforms.

Technology used: NA (sequencing)

Tumor Infiltrating Lymphocytes (TILs)

Rosenberg SA, Restifo NP. Adoptive cell transfer as personalized immunotherapy for human cancer. *Science*. 2015;348(6230):62-68.

Adoptive cell transfer (ACT) consists of the administration of cancer-bearing host immune cells. While other therapies rely on sufficient expansion of anti-tumor cell types *in vivo*, in ACT, lymphocytes are grown *in vitro*, activated, and selected on the basis of their anti-tumor activity before injection into patient. Once injected, they can expand more than thousandfold. Lymphocytes infiltrating the stroma of tumors are capable of recognizing cancer cells. It was demonstrated in the 1980s that adoptive transfer of such tumor infiltrating lymphocytes (TILs) can mediate regression in certain tumors. Such populations of TILs are usually CD8+ and CD4+ T cells. In early studies, the effect of the transferred cells was often of short duration, and cells were rarely found in circulation a few days after treatment. An important observation in 2002 demonstrated that lymphodepletion using a nonmyeloablative chemotherapy regimen administered prior to TIL transfer led to an improvement in cancer regression, as well as oligoclonal population of the host. TILs can be grown from several tumors; however, melanoma is the 1 cancer that has given the most results. In fact, ACT using TILs is the most effective way to treat metastatic melanoma. Thanks to exome studies on tumor vs control screening to identify patients' mutanomes, it is now known that TILs recognize and target products of cancer mutations. This is true for melanoma, but recent studies have demonstrated the existence of TILs from other epithelial cancers that can recognize cancer mutations. Also, lately, the ability to engineer lymphocytes to express conventional T cell receptors or chimeric antigens has further advanced the applications of ACT in cancer treatment.

Technology used: NA

Robbins PF, Lu YC, El-Gamil M, et al. Mining exomic sequencing data to identify mutated antigens recognized by adoptively transferred tumor-reactive T cells. *Nat Med.* 2013;19(6):747-752.

The positive outcome for up to 70% of melanoma patients receiving tumor infiltrating lymphocytes (TILs) encourages the use of TILs to mediate durable regressions. To identify the ability of TILs to recognize potent antigens of mutated gene products, this study examined cancer genomes using whole-exome sequencing (WES) data from the HiSeq 2000 System to identify mutated proteins expressed in patient tumors. T cell epitopes were selected using an algorithm evaluating the recognition by TILs. The authors successfully identified mutated antigens expressed on tumor cells that were recognized by 3 bulk TIL lines from 3 melanoma patients.

Technology used: the TruSeq® DNA Library Prep Kit (sequencing) and the HiSeq 2000 System (sequencing) by Illumina

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CHECKPOINT INHIBITORS

Sharma P, Allison JP. The future of immune checkpoint therapy. *Science*. 2015;348(6230):56-61.

Immune checkpoint therapy targets specific regulatory pathways in T cells, enhancing immune response. This approach has provided significant and durable benefit to a subset of patients and has led to the development and release of 2 new cancer treatments: ipilimumab, an anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) antibody, approved in 2011 by the US Food and Drug Administration (FDA), and pembrolizumab and nivolumab, antibodies against programmed cell death protein (PD-1) that were approved by the FDA in 2014. Although in some cases, these treatments have led to long-term remission with no clinical signs of cancer for several years, cancer and patient characteristics that will lead to successful use of immune checkpoint therapy still need to be defined. Recent studies are proving that mutational load is likely associated to treatment outcome for certain cancers. The identification of prognostic biomarkers, as well as the identification of new pathways and development of new therapies, will lie in our ability to understand the immune system and its responses in the tumor microenvironment. This will also be useful in understanding what therapies, or combination of therapies, will be appropriate for which patients.

Technology used: NA (review)

Sharma P, Allison JP. Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential.

***Cell.* 2015;161(2):205-214.**

Somatic mutation events in oncogenesis inactivate genes normally involved in regulation of cell division and programmed cell death, as well as DNA repair. The identification of these mutations has triggered the study and development of therapies that can lead to clinical response but not provide durability in time. Memory, as well as specificity, are the characteristics that make the use of the immune system particularly advantageous in cancer treatment. Thanks to advances in DNA sequencing and genomic technologies, several tumor antigens defined by tumor-specific cells have been identified in mice and humans. However, the activation of T cells requires antigen presentation by antigen-presenting cells (APCs), which provide the co-stimulatory molecule B7 that is normally lacking in tumors. B7 molecules will engage their ligand CD28 expressed on the T cell to activate it. This priming elicits both a program to activate the response and a program that will eventually inactivate it. This inhibitory program is mediated by cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), which is homologous to CD28 but much more affine to the B7 activator molecules. Expression of CTLA-4 is fundamental in healthy tissues, but its accumulation in the immunological synapse in the context of tumor response will eventually attenuate it. CTLA-4 blockade is a promising therapy for certain cancers, and it was approved as a treatment for melanoma in 2011. This success has opened a new field called “immune checkpoint therapy” that has identified other pathways such as programmed cell death protein (PD-1) and its ligand PD-L1, also expressed in activated T cells only. Anti-PD-L1 antibodies have shown promising results in multiple cancers. As any cancer treatment, checkpoint inhibitors have side effects, mainly related to immune-related adverse events. Many other pathways are showing promising results, and it is likely that in the future, combination treatments—as in chemotherapy—will hold the key to better results in cancer treatments.

Technology used: NA (review)

Le DT, Uram JN, Wang H, et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med.* 2015;372(26):2509-2520.

Antibodies to the programmed cell death protein (PD-1) pathway lead to a remarkable response in certain cancers, such as melanomas, non-small-cell lung cancer, renal cell carcinoma, bladder cancer, and Hodgkin's lymphoma. The authors of this study hypothesized that this treatment is particularly effective in patients whose tumors have large numbers of somatic mutations. To prove their hypothesis, they conducted a phase 2 study in 41 patients with progressive metastatic carcinoma, either repair-deficient or repair-proficient. To estimate the number of mutation-associated antigens in each tumor, they performed exome sequencing and human leukocyte antigen (HLA) haplotyping from a primary tumor sample and matched peripheral blood, and used the data for an epitope prediction algorithm. They observed that in repair-deficient cancer patients, the immune-related objective response rate and immune-related progression-free survival rate were 40% (4 out of 10 patients) and 78% (7 out of 9 patients). On the other side, these percentages were as low as 0% and 11% (2 out of 18 patients) in mismatch repair-proficient patients. Furthermore, mutation loads were significantly associated with prolonged progression-free survival ($p = 0.02$).

Technology used: the TruSeq Sample Prep Kit, the HiSeq 2000 System, and the HiSeq 2500 System by Illumina

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Rizvi NA, Hellmann MD, Snyder A, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science*. 2015;348(6230):124-128.

Immune checkpoint inhibitors use a patient's own T cells to kill tumors. The aim of this study was to characterize the genomic determinants of response to therapy with antibody targeting programmed cell death protein (PD-1). To do so, they used exome sequencing data from two cohorts of non-small-cell lung cancers and the matched normal DNA (n = 16 and n = 18). In both cohorts, higher nonsynonymous mutation burden in tumors was associated with improved response, durable clinical benefit, and progression-free survival. These results are consistent with a genomic-shaped response to anti-PD-1 therapy.

Technology used: the HiSeq 2000 System by Illumina

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Snyder A, Makarov V, Merghoub T, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. N Engl J Med. 2014;371(23):2189-2199.

Ipilimumab and tremelimumab are monoclonal antibodies that block cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), resulting in T cell activation. Treatment of melanoma patients with such antibodies enables T cells to kill cancer cells and prolongs overall survival. The authors of this study sequenced exomes of 64 patients treated with CTLA-4; the discovery set consisted of 25 patients treated with ipilimumab, and the validation set consisted of 39 patients treated with ipilimumab, except for 5 treated with tremelimumab. In the discovery set, mutational load was associated with clinical benefit, but it was not sufficient to predict benefit alone. The authors identified tumor neoantigens for each patient using patient-specific human leukocyte antigen (HLA) typing and genome-wide somatic neopeptide analysis. By doing so, they elucidated a neoantigen landscape that was specific to tumors that responded to treatment. They then validated such signature in the validation set, where predicted neoantigens activated T cells from the patients treated with ipilimumab. These results prove and define a genetic basis for benefit from CTLA-4 blockade in melanoma and provide a rationale for screening exomes from patients who are being considered for CTLA-4 treatment.

Technology used: the HiSeq 2000 System by Illumina

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Yadav M, Jhunjhunwala S, Phung Q, et al. Predicting immunogenic tumour mutations by combining mass spectrometry and exome sequencing. *Nature*. 2014;515(7528):572-576.

Although CD8 T cells can recognize tumor cells and mediate tumor regression following immunotherapy, the antigens driving effective anti-tumor CD8 T cell responses remain largely unknown. This study used a combination of Illumina whole-exome and transcriptome sequencing with mass spectrometry to identify neoepitopes in 2 widely used murine tumor models. More than 1300 amino acid changes were identified using this approach; 13% of those were predicted to bind major histocompatibility complex class I molecules (MHC I), and a small fraction of them were confirmed by mass spectrometry. The authors demonstrated how vaccination of mice confirmed the approach, with each predicted immunogenic peptide yielding therapeutically active T cell responses.

Technology used: the TruSeq RNA Library Prep Kit (sequencing) and the HiSeq System (sequencing) by Illumina

For Research Use Only. Not for use in diagnostic procedures.

Van Allen EM, Miao D, Schilling B, et al. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. *Science*. 2015;350(6257):207-211.

Inhibition of immune checkpoints through monoclonal antibodies against cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) is a promising therapy for metastatic melanoma. The aim of this study was to characterize the role of tumor-specific neoantigens and alterations of the tumor microenvironment in response to anti-CTLA-4 antibodies. To do so, the authors sequenced exomes from pre-treatment melanoma biopsies and matched normal DNA from 110 patients. They found that overall mutation load, neoantigen load, and expression of cytolytic markers in the immune microenvironment were significantly associated with clinical benefit. However, no recurrent neoantigen peptide was able to predict response in the population.

Technology used: the TruSeq Sample Prep Kit and the HiSeq System by Illumina

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ROLE OF THE MICROBIOME

Sivan A, Corrales L, Hubert N, et al. Commensal *Bifidobacterium* promotes anti-tumor immunity and facilitates anti-PD-L1 efficacy. *Science*. 2015;350(6264):1084-1089.

The mechanism underlying variable immune response to cancer is not well understood. In this study, the authors aimed at assessing the role of the intestinal microbiota in modulating this response. To do so, they compared subcutaneous melanoma growth in 2 lines of C57BL mice, which are genetically similar but differ in their commensal microbes. They observed significant differences in spontaneous anti-tumor immunity and melanoma growth rate. These differences were eliminated after cohousing or fecal transfer. They sequenced the 16S ribosomal RNA of mice subjected to administration of fecal permutations and identified *Bifidobacterium* as significantly associated with anti-tumor immune-mediated response. They then administered *Bifidobacterium* alone to treated mice, improving tumor control at the same extent as programmed cell death protein 1 ligand (PD-L1)-specific antibody therapy. A combination of both *Bifidobacterium* and anti-PD-L1 treatment nearly abolished tumor outgrowth. These results highlight an important role for the gut microbiome in anti-tumor immunity.

Technology used: the MiSeq® System and the HiScan® System by Illumina

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Vetizou M, Pitt JM, Dailere R, et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science*. 2015;350(6264):1079-1084.

The use of antibodies against cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) is a promising treatment in cancer immunotherapy. The aim of this study was to address the role of the gut microbiota in the immunomodulatory effects of CTLA-4 blockade. To do so, the authors compared the therapeutic effects on sarcoma model mice housed in a pathogen-free vs germ-free environment, and demonstrated that the effect of CTLA-4 blockade was effective in mice that were hosted in a pathogen-free environment but not in a germ-free environment. By sequencing of the 16S ribosomal RNA subunit, they then identified T cell responses specific to *Bacteroides thetaiotaomicron* and *Bacteroides fragilis* as associated with the response to against CTLA-4 treatment. Furthermore, antibiotic administration also inhibited the effect of therapy. This inhibition was overcome by gavage administration of *B. thetaiotaomicron*, immunization with *B. fragilis* polysaccharides, or adoptive cell transfer of *B. fragilis*-specific T cells. These results were confirmed by fecal transplantation from human to mice, as melanoma patients with anti-CTLA-4 antibodies favored the outgrowth of *B. fragilis* having anticancer properties. These results demonstrated a key role for *Bacteroides* in the immunostimulatory effects of CTLA-4 blockade.

Technology used: NA

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