ORIGINAL ARTICLE

# Genetic Basis for Clinical Response to CTLA-4 Blockade in Melanoma

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## ABSTRACT

### BACKGROUND

Immune checkpoint inhibitors are effective cancer treatments, but molecular determinants of clinical benefit are unknown. Ipilimumab and tremelimumab are antibodies against cytotoxic T-lymphocyte antigen 4 (CTLA-4). Anti–CTLA-4 treatment prolongs overall survival in patients with melanoma. CTLA-4 blockade activates T cells and enables them to destroy tumor cells.

### METHODS

We obtained tumor tissue from patients with melanoma who were treated with ipilimumab or tremelimumab. Whole-exome sequencing was performed on tumors and matched blood samples. Somatic mutations and candidate neoantigens generated from these mutations were characterized. Neoantigen peptides were tested for the ability to activate lymphocytes from ipilimumab-treated patients.

### RESULTS

Malignant melanoma exomes from 64 patients treated with CTLA-4 blockade were characterized with the use of massively parallel sequencing. A discovery set consisted of 11 patients who derived a long-term clinical benefit and 14 patients who derived a minimal benefit or no benefit. Mutational load was associated with the degree of clinical benefit ($P=0.01$) but alone was not sufficient to predict benefit. Using genomewide somatic neoepitope analysis and patient-specific HLA typing, we identified candidate tumor neoantigens for each patient. We elucidated a neoantigen landscape that is specifically present in tumors with a strong response to CTLA-4 blockade. We validated this signature in a second set of 39 patients with melanoma who were treated with anti–CTLA-4 antibodies. Predicted neoantigens activated T cells from the patients treated with ipilimumab.

### CONCLUSIONS

These findings define a genetic basis for benefit from CTLA-4 blockade in melanoma and provide a rationale for examining exomes of patients for whom anti–CTLA-4 agents are being considered. (Funded by the Frederick Adler Fund and others.)

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Immune checkpoint blockade has led to durable antitumor effects in patients with metastatic melanoma, non–small-cell lung cancer, and other tumor types, but the factors determining whether a patient will have a response remain elusive.[1,2] The fully human monoclonal antibodies ipilimumab and tremelimumab block cytotoxic T-lymphocyte antigen 4 (CTLA-4), resulting in T-cell activation. Some studies have established correlations between outcomes with ipilimumab and peripheral-blood lymphocyte count, markers of T-cell activation,[3] an "inflammatory" microenvironment,[4,5] and maintenance of high-frequency T-cell receptor clonotypes.[6]

The relationship among the genomic landscape of the tumor, the mutational load, and the benefit from treatment remains obscure. The immunogenicity resulting from nonsynonymous melanoma mutations has been shown in a mouse model,[7] and the antigenic diversity of human melanoma tumors has been modeled in silico[8] and in melanoma-specific CD8 T-cell responses after treatment with ipilimumab.[9] Effector and helper T-cell function and regulatory T-cell depletion are necessary for the efficacy of CTLA-4 blockade,[10] but there is not an association between a specific HLA type and a clinical benefit.[11] Melanomas have very high mutational burdens (0.5 to >100 mutations per megabase) as compared with other solid tumors.[12] Elegant studies have shown that somatic mutations can give rise to neoepitopes[13] and that these may serve as neoantigens.[14-16] We conducted a study to determine whether the genetic landscape of a tumor affects the clinical benefit provided by CTLA-4 blocking agents.

## Methods

### Sample Acquisition and DNA Preparation

For the discovery set, we conducted whole-exome sequencing of DNA from tumors and matched normal blood from 25 ipilimumab-treated patients. A validation set included an additional 39 patients, of whom 5 were treated with tremelimumab. Primary tumor samples and matched normal peripheral-blood specimens were obtained after the patients had provided written informed consent. DNA was extracted, and exon capture was performed with the use of the SureSelect Human All Exon 50-Mb kit (Agilent Technologies). Enriched exome libraries were sequenced on the HiSeq 2000 platform (Illumina) to provide a mean exome coverage of more than 100× (Memorial Sloan Kettering Cancer Center Genomics Core and Broad Institute).

### Immunogenicity Analysis of Somatic Mutations

We created a bioinformatic tool to translate all mutations in exomes and then evaluate binding with major histocompatibility complex (MHC) class I molecules. The neoantigen signature was generated from the nonamers containing four amino acid strings of peptides that are common to tumors from patients with a long-term benefit from therapy. Details are provided in the Supplementary Appendix, available with the full text of this article at NEJM.org.

### Intracellular Cytokine Staining

Candidate neoantigen peptides were synthesized (GenScript), cultured with autologous peripheral-blood mononuclear cells (PBMCs), and then analyzed by means of intracellular cytokine staining for interleukin-2, CD107a, macrophage inflammatory protein 1$\beta$, tumor necrosis factor $\alpha$, and interferon-$\gamma$ on restimulation of cells with the candidate peptides.

### Statistical Analysis

The Mann–Whitney test was used to compare mutational loads, and the log-rank test was used to compare Kaplan–Meier curves. The statistical methods used in the study are more fully described in the Supplementary Appendix.

## Results

### Mutational Landscape of Melanomas from the Study Patients

Baseline patient characteristics are shown in Table 1 (for more detailed information, see Tables S1 and S2 in the Supplementary Appendix). The study involved patients with and those without a long-term clinical benefit from therapy (CTLA-4 blockade alone or CTLA-4 blockade with resection of an isolated stable or nonresponding lesion). A long-term clinical benefit was defined by radiographic evidence of freedom from disease or evidence of a stable or decreased volume of disease for more than 6 months. Lack of a long-term ben-



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**Table 1. Clinical Characteristics of the Patients in the Discovery and Validation Sets, According to Clinical Benefit from Therapy.**

| Characteristic | Discovery Set | | Validation Set | |
|---|---|---|---|---|
| | Long-Term Benefit (N=11) | Minimal or No Benefit (N=14) | Long-Term Benefit (N=25) | Minimal or No Benefit (N=14) |
| Age at start of treatment — yr | | | | |
| Median | 63 | 60 | 66 | 57 |
| Range | 39–70 | 48–79 | 33–90 | 18–74 |
| Sex — no. of patients (%) | | | | |
| Female | 3 (27) | 8 (57) | 9 (36) | 5 (36) |
| Male | 8 (73) | 6 (43) | 16 (64) | 9 (64) |
| Disease origin — no. of patients (%) | | | | |
| Acral | 0 | 3 (21) | 1 (4) | 1 (7) |
| Uveal | 0 | 0 | 1 (4) | 0 |
| Cutaneous | 10 (91) | 8 (57) | 15 (60) | 11 (79) |
| Unknown primary | 1 (9) | 3 (21) | 3 (12) | 0 |
| Not available | 0 | 0 | 5 (20) | 2 (14) |
| *BRAF* or *NRAS* mutation — no. of patients (%) | | | | |
| No | 1 (9) | 6 (43) | 17 (68) | 11 (79) |
| Yes | 10 (91) | 8 (57) | 8 (32) | 3 (21) |
| Lactate dehydrogenase level at start of therapy — no. of patients (%) | | | | |
| Normal | 8 (73) | 8 (57) | 8 (32) | 9 (64) |
| Above normal | 2 (18) | 5 (36) | 3 (12) | 3 (21) |
| Not available | 1 (9) | 1 (7) | 14 (56) | 2 (14) |
| Duration of response to therapy — wk | | | | |
| Median | 59 | 14 | 130 | 11 |
| Range | 42–361 | 11–23 | 64–376 | 3–29 |
| Previous therapies — no.* | | | | |
| Median | 1 | 1 | 0 | 0 |
| Range | 0–3 | 0–2 | 0–2 | 0–3 |
| Melanoma stage at time of diagnosis — no. of patients (%) | | | | |
| IIIC | 0 | 0 | 3 (12) | 0 |
| M1a | 0 | 1 (7) | 4 (16) | 1 (7) |
| M1b | 5 (45) | 1 (7) | 2 (8) | 3 (21) |
| M1c | 6 (55) | 12 (86) | 16 (64) | 10 (71) |
| Overall survival — yr† | | | | |
| Median | 4.4 | 0.9 | 3.3 | 0.8 |
| Range | 2.0–6.9 | 0.4–2.7 | 1.6–7.2 | 0.2–2.1 |

* Previous therapies included interleukin-2 and cytotoxic chemotherapy.
† Overall survival was calculated from the date of the first dose of ipilimumab to the date of death or censoring of data.



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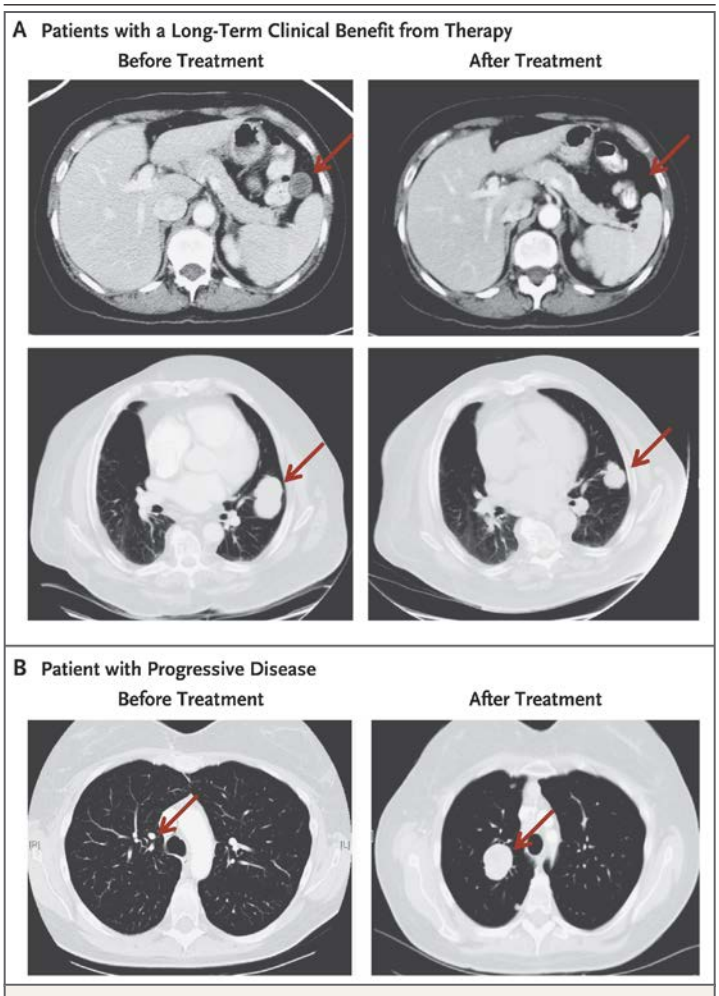


**Figure 1. Paired Pretreatment and Post-Treatment Computed Tomographic Scans.**

In Panel A, the scans on the top were obtained on January 2, 2011, and August 26, 2013, and the scans on the bottom were obtained on September 6, 2011, and January 14, 2013. In Panel B, the scans were obtained on August 13, 2009, and January 9, 2010.

efit was defined by tumor growth on every computed tomographic scan after the initiation of treatment (no benefit) or a clinical benefit lasting 6 months or less (minimal benefit). Representative scans are shown in Figure 1, and Figure S1 in the Supplementary Appendix.

To determine the genetic features associated with a sustained benefit from CTLA-4 blockade, we analyzed DNA in tumor and matched blood samples using whole-exome sequencing. In the discovery set, we generated 6.4 Gb of mapped sequence, with more than 99% of the target sequence covered to at least 10× depth and a mean exome coverage of 103× (Table S3 and Fig. S2 in the Supplementary Appendix). The wide ranges of mutational burdens (Fig. 2A, and Table S3 in the Supplementary Appendix) and recurrent and driver mutations (Fig. S2C and S2D and Table S4 in the Supplementary Appendix) among samples were consistent with previously reported findings.[17-19] The ratio of transitions to transversions (Fig. S2E in the Supplementary Appendix) and the frequency of nucleotide changes (Fig. S2F in the Supplementary Appendix) were similar in the discovery and validation sets.[12] No gene was universally mutated across patients with a sustained benefit.

### ASSOCIATION BETWEEN MUTATIONAL BURDEN AND CLINICAL BENEFIT

We hypothesized that an increased mutational burden in metastatic melanoma samples would correlate with a benefit from CTLA-4 blockade. There was a significant difference in mutational load between patients with a long-term clinical benefit and those with a minimal benefit or no benefit, both in the discovery set ($P=0.01$ by the Mann–Whitney test) and in the validation set ($P=0.009$ by the Mann–Whitney test) (Fig. 2A, and Table S5 in the Supplementary Appendix). In the discovery set, a high mutational load was significantly correlated with improved overall survival ($P=0.04$ by the log-rank test) (Fig. 2B), and there was a trend toward improved survival in the validation set (Fig. S3A in the Supplementary Appendix). The latter set included eight patients with nonresponding tumors who otherwise had systemic disease control, which may confound the relationship between mutational load and survival. Further subdivision into four clinical categories was suggestive of a dose–response relationship in the discovery set (Fig. S3B in the Supplementary Appendix). These data indicate that a high mutational load correlates with a sustained clinical benefit from CTLA-4 blockade but that a high load alone is not sufficient to impart a clinical benefit, because there were tumors with a high mutational burden that did not respond to therapy.

### SOMATIC NEOEPITOPES IN RESPONDING TUMORS AND EFFICACY OF CTLA-4 BLOCKADE

MHC class I presentation and cytotoxic T-cell recognition are required for ipilimumab activity.[10] Because mutational load alone did not explain a clinical benefit from CTLA-4 blockade, we hypoth-



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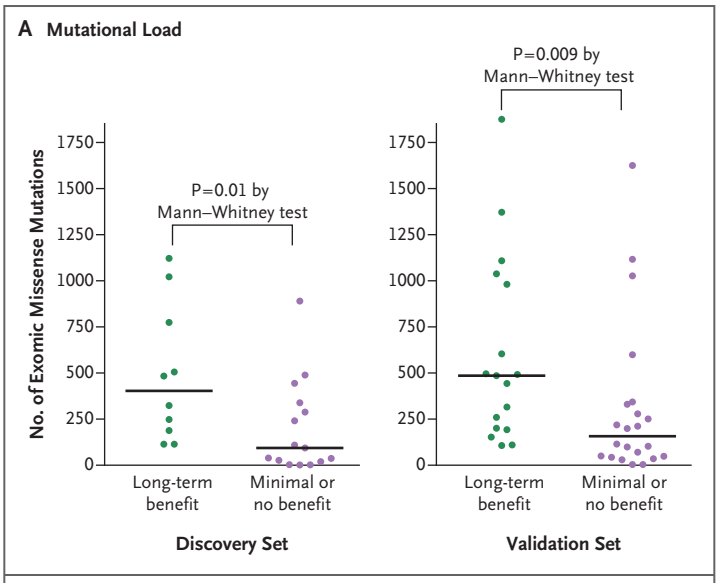
esized that the presence of specific tumor neoantigens might explain the varied therapeutic benefit. To identify these neoepitopes, we developed a bioinformatic pipeline incorporating prediction of MHC class I binding, modeling of T-cell receptor binding, patient-specific HLA type, and epitope-homology analysis (see the Methods section and Fig. S4 in the Supplementary Appendix).

We created a computational algorithm, called NAseek, to translate all nonsynonymous missense mutations into mutant and nonmutant peptides (see the Methods section and Fig. S4 in the Supplementary Appendix). We examined whether a subgroup of somatic neoepitopes would alter the strength of peptide–MHC binding, using patient-specific HLA types (Table S3 in the Supplementary Appendix). We first compared the overall antigenicity trend of all mutant versus nonmutant peptides. In aggregate, the mutant peptides were predicted to bind MHC class I molecules with higher affinity than the corresponding nonmutant peptides (Fig. S5 in the Supplementary Appendix).

Using only peptides predicted to bind to MHC class I molecules (binding affinity, ≤500 nM), we searched for conserved stretches of amino acids shared by multiple tumors. Using the methods described in the Methods section in the Supplementary Appendix, we identified shared, consensus sequences.[20] We identified a number of tetrapeptide sequences that were shared by patients with a long-term clinical benefit but completely absent in patients with a minimal benefit or no benefit (Fig. 3A and 3B, and Table S6 in the Supplementary Appendix). It has been shown that short amino acid substrings comprise conserved regions across antigens recognized by a T-cell receptor.[21] In these experiments, recognition of epitopes was driven by consensus tetrapeptides within the immunogenic peptides, and tetrapeptides within cross-reacting T-cell receptor epitopes were necessary and sufficient to drive T-cell proliferation, findings that are consistent with evidence that this polypeptide length can drive recognition by T-cell receptors.[22] Tetrapeptides are used to model genome phylogeny because they occur relatively infrequently in proteins and typically reflect function.[23]

We used the discovery set to generate a peptide signature from the candidate neoepitopes. This analysis initially pooled the aforementioned discovery and validation sets to remove low-frequency tetrapeptides in the combined exomes. Subsequent analysis is restricted to post-filtering peptides (see the Methods section in the Supple-



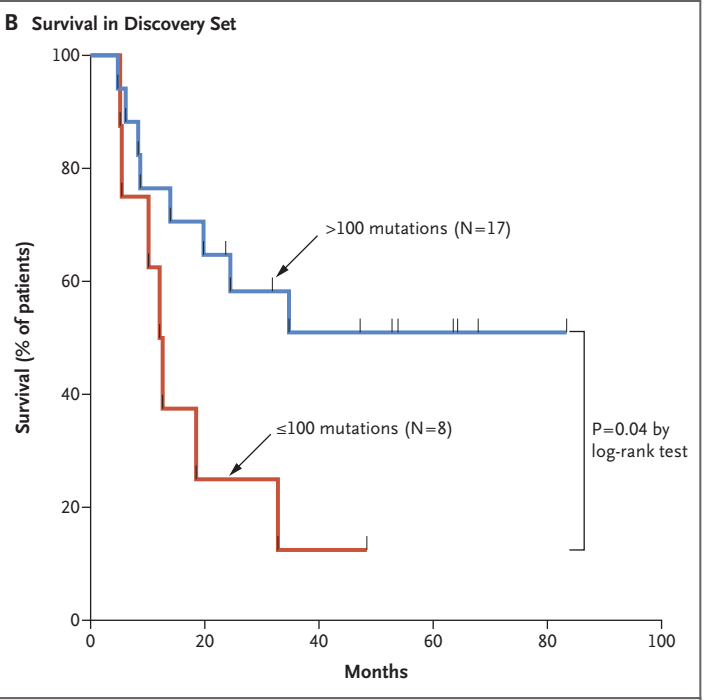




**Figure 2. Mutational Landscape of Tumors According to Clinical Benefit from Ipilimumab Treatment.**

Panel A shows the mutational load (number of nonsynonymous mutations per exome) in the discovery and validation sets, according to status with respect to a clinical benefit from therapy. Panel B depicts the Kaplan–Meier curves for overall survival in the discovery set for patients with more than 100 nonsynonymous coding mutations per exome and patients with 100 or fewer mutations.



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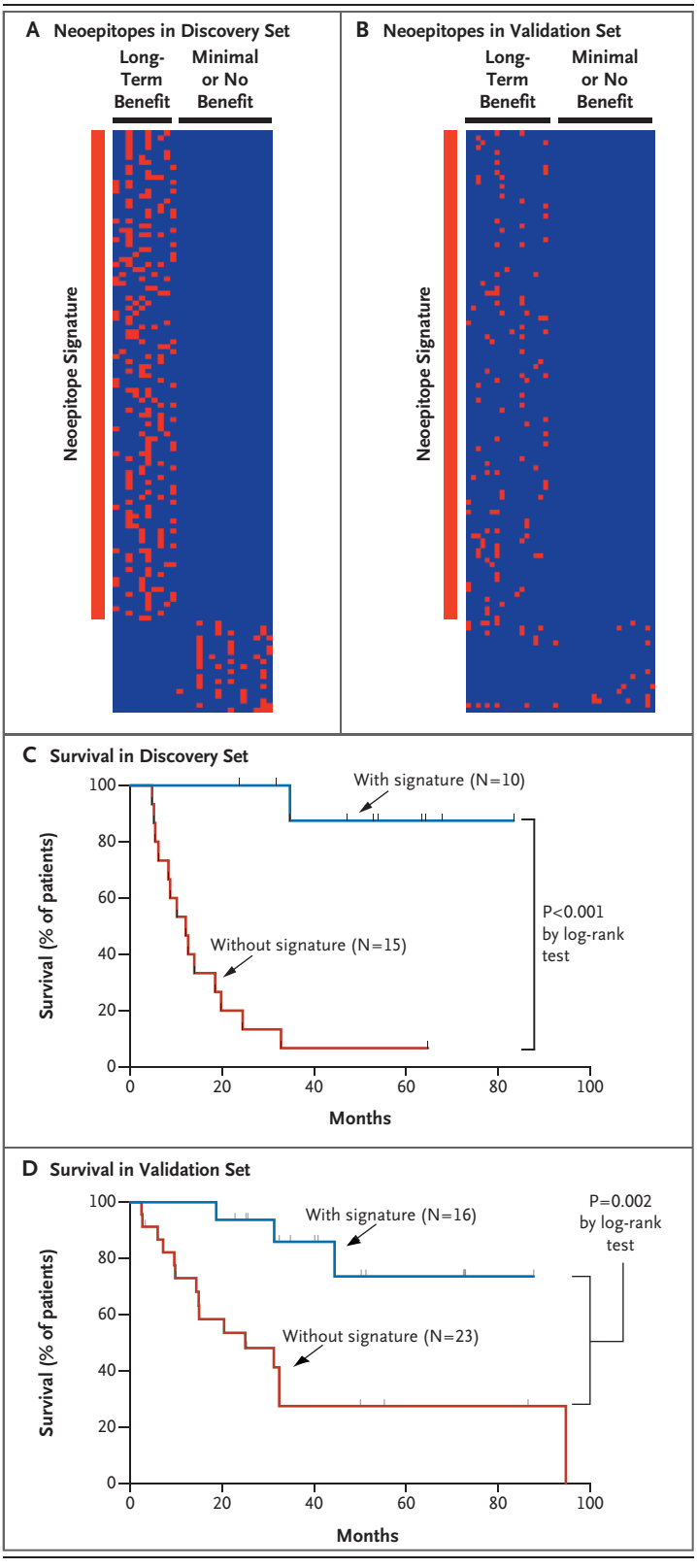


**Figure 3. Association of a Neoepitope Signature with a Clinical Benefit from CTLA-4 Blockade.**

Candidate neoepitopes were identified by means of mutational analysis, as described in the Methods section in the Supplementary Appendix. Panel A shows a heat map of candidate tetrapeptide neoantigens that were present in patients with a long-term clinical benefit but absent in patients with a minimal benefit or no benefit in the discovery set (comprising 25 patients). Each row represents a neoepitope; each column represents a patient. The vertical red line indicates the tetrapeptide signature associated with a response to blockade of cytotoxic T-lymphocyte antigen 4 (CTLA-4). The exact tetrapeptides, chromosomal loci, and nonmutant and mutant nonamers in which they occur are listed in Table S6 in the Supplementary Appendix. Panel B shows the same information for the validation set (comprising 39 patients). Panel C shows the Kaplan–Meier curves for overall survival in the discovery set for patients with the signature and those without the signature. Panel D shows the same data for the validation set.

mentary Appendix). We found that the tetrapeptides common to each group (candidate neoepitopes) included 101 shared exclusively among patients in the discovery set who had a long-term clinical benefit; this was also independently observed in the validation set (Fig. 3A and 3B, and Tables S6 and S7 in the Supplementary Appendix). This set of neoepitopes defines a signature linked to a benefit from CTLA-4 blockade. Because of the size of our discovery set, we cannot exclude the possibility that additional biologically relevant epitopes exist and conversely that there are biologically relevant epitopes that were predicted bioinformatically but were not expressed or presented in patients with a minimal benefit or no benefit (Tables S7A and S7B in the Supplementary Appendix).

Shared tetrapeptide neoepitopes did not simply result from a high mutational load. For example, in the discovery set, the patient with a minimal benefit or no benefit who had the greatest number of mutations (Patient SD7357, who had 1028 mutations) did not share any of the tetrapeptide signatures. This concept was illustrated again in the validation set, in which even tumors from patients with more than 1000 mutations (Patients NR9521 and NR4631) did not respond (Table S3 in the Supplementary Appendix). Simulation testing with five different



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models showed that the association between the neoepitope signature and a long-term clinical benefit was highly significant and was unlikely to have resulted from chance alone ($P<0.001$ for four methods and $P=0.002$ for a fifth method) (Fig. S6 in the Supplementary Appendix). A high mutational load appeared to increase the probability, but not guarantee formation, of a neoepitope signature associated with a benefit. Consensus analysis revealed that the neoepitopes were not random. The frequencies of amino acids that made up the tetrapeptides in the group of patients with a long-term clinical benefit were different from those observed in the group with a minimal benefit or no benefit (Fig. S7A in the Supplementary Appendix).

Presence of the neoepitope signature peptides correlated strongly with survival in both the discovery set and the validation set ($P<0.001$ and $P<0.002$, respectively, by the log-rank test) (Fig. 3C and 3D). The correlation between mutational load and survival was not as strong (Fig. 2B, and Fig. S3A in the Supplementary Appendix).

The shared tetrapeptides were encoded by mutations in diverse genes across the genome (Fig. S7B and Table S6 in the Supplementary Appendix). Using RNA-sequencing data from the Cancer Genome Atlas, we confirmed that the genes harboring our somatic neoepitopes were widely expressed in melanoma (Table S8 in the Supplementary Appendix). In some cases, the amino acid change resulting from the somatic mutation led to a change in the tetrapeptide itself. In others, the mutant amino acid was separate from the tetrapeptide and altered MHC binding, as has been described previously.[24-26]

In addition, candidate neoepitopes common to both clinical groups were analyzed with the use of the Immune Epitope Database (www.iedb.org). This is the most comprehensive database of experimentally validated, published, and curated antigens, and it has been used to develop algorithms to identify antigens with high accuracy.[14] The candidate neoepitopes common to patients with a long-term clinical benefit were homologous to many more viral and bacterial antigens in the database than were the neoepitopes common to patients with a minimal benefit or no benefit (Table S9 in the Supplementary Appendix). For example, the tetrapeptide substring ESSA was shared by patients with a long-term clinical benefit (Fig. 4A) and corresponds to the precise antigenic portion of human cytomegalovirus immediate early epitope (MESSAKRKMDPDNPD).[27] These data suggest that the neoepitopes in patients with strong clinical benefit from CTLA-4 blockade may resemble epitopes from pathogens that T cells are likely to recognize. The cross-reactive peptides defined by short peptide consensus sequences that were discovered by Birnbaum et al. with the use of an unbiased screen also had substantial homology to antigens in microbes.[21] Although tantalizing, these observations will require further study to confirm.

Using a whole-exome sequencing approach, we characterized the predicted antigenic peptide space (see the Methods section in the Supplementary Appendix). As further validation of our study, we reidentified melanoma antigen recognized by T cells (MART-1, also known as MelanA), an experimentally validated melanocytic antigen (Fig. S8).[28] EKLS, which comprises the core amino acids of the MART-1 MHC class II epitope, was shared by patients with a long-term clinical benefit, and the phosphoserine moiety is critical for T-cell receptor recognition.[29] The frequency of leukocyte common antigen–positive cells and ratio of CD8-positive cells to FOXP3-positive cells were substantially different between patients with a long-term clinical benefit from ipilimumab and those with a minimal benefit or no benefit (Fig. S9 in the Supplementary Appendix).

### IN VITRO VALIDATION OF PREDICTED IMMUNOGENIC PEPTIDES

Translation of next-generation sequencing into in vitro validation of peptide predictions has proven challenging, even in expert hands, with very low published validation rates.[15] In vitro assays are hampered by the paucity of clinical samples, the sensitivity of preserved cells to the freeze–thaw process, the low frequency of anti-neoantigen T cells in clinical samples, and the very low sensitivity of T cells in vitro in the absence of the complex in vivo immunogenic microenvironment.

We attempted to optimize prediction by integrating multiple high-throughput approaches (Fig. S4 in the Supplementary Appendix). On the basis of our prediction algorithm, we generated pools of peptides and performed assays of T-cell activation for patients for whom we had suffi-



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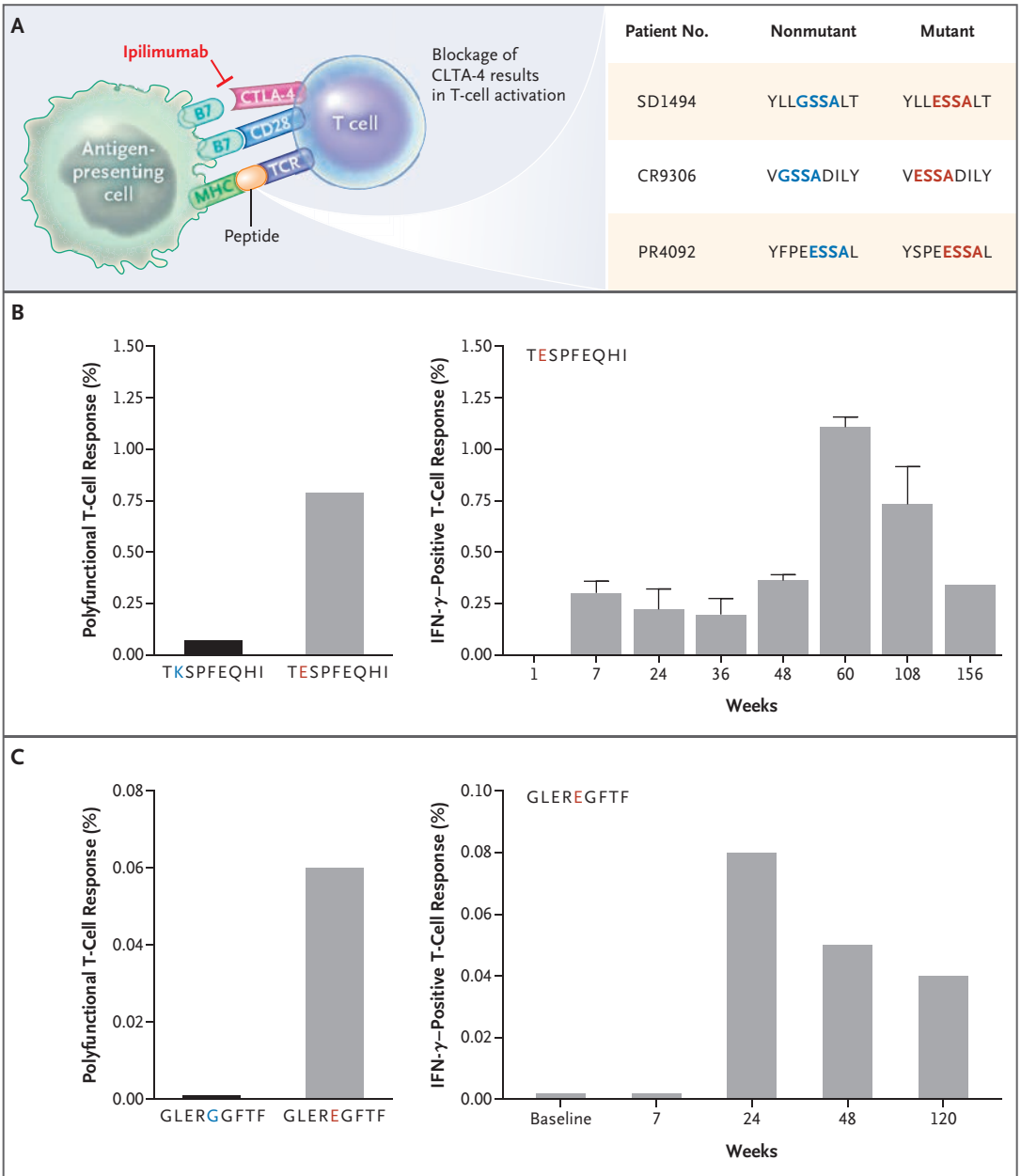


**Figure 4. Role of Neoantigens in Activation of T Cells from Patients Treated with CTLA-4 Blockade.**

Panel A shows an example of a tetrapeptide substring of human cytomegalovirus. In each case, the nonamer containing the mutation is predicted to bind and be presented by a patient-specific HLA. Panel B shows the dual positive (interferon-γ [IFN-γ] and tumor necrosis factor α [TNF-α]) CD8+ T-cell response to T*E*SPFEQHI and nonmutant peptide T*K*SPFEQHI and the increase in IFN-γ+ T cells over time. Data from Patient CR9306 are shown. T bars indicate the standard deviation. Panel C shows the dual positive (IFN-γ and TNF-α) CD8+ T-cell response to GLER*E*GFTF and nonmutant peptide GLER*G*GFTF and illustrates the increase in peptide-specific T cells 24 weeks after the initiation of treatment with ipilimumab relative to baseline. Data from Patient CR0095 are shown. MHC denotes major histocompatibility complex, and TCR T-cell receptor.



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cient lymphocytes (see the Methods section in the Supplementary Appendix). Positives pools were observed for three of five patients (Fig. S10A, S10B, and S10C in the Supplementary Appendix). We identified the exact peptides for patients with adequate PBMCs. We found a polyfunctional T-cell response to the peptide TESPFEQHI in Patient CR9306 (Fig. S10D in the Supplementary Appendix) but not to its nonmutant counterpart, TKSPFEQHI. This response peaked at 60 weeks after the initiation of treatment (Fig. 4B). T-cell responses were absent in healthy donors (Fig. S10E in the Supplementary Appendix). TESPFEQHI had a predicted MHC class I affinity for B4402 of 472 nM, as compared with 18323 nM for TKSPFEQHI. ESPF is a common tetrapeptide found in the response signature and is a substring (positions 176 through 179) of the hepatitis D virus large delta epitope p27 (P*ESPF*A and *ESPF*AR).[30] TESPFEQHI results from a mutation in *FAM3C* (c.A577G;p.K193E), a gene highly expressed in melanoma (Table S8 in the Supplementary Appendix).

We also found that peptide GLER*E*GFTF elicited a polyfunctional T-cell response in Patient CR0095 (Fig. 4C, and Fig. S10F in the Supplementary Appendix), whereas nonmutant GLER*G*GFTF did not. This response peaked at 24 weeks after the initiation of treatment (Fig. 4C). GLER*E*GFTF arises from a mutation in *CSMD1* (c.G10337A;p.G3446E), which is also highly expressed in melanoma (Table S8 in the Supplementary Appendix), and the peptide has 80% homology to a known *Burkholderia pseudomallei* antigen (Immune Epitope Database Reference ID: 1027043). The lack of T-cell activation may not rule out a given neoantigen because in vitro assays are limited in sensitivity, as described above.

## DISCUSSION

Anti–CTLA-4 and anti–programmed cell death 1 antibodies have resulted in long-term disease control in a subgroup of patients with melanoma.[1,2] Here, we have illustrated the importance of tumor genetics in defining the basis of the clinical benefit from CTLA-4 blockade.

Our observations suggest a number of principles relevant to immunotherapy for cancer. Although a high mutational load is associated with a benefit from immune checkpoint abrogation, this factor alone is not sufficient to impart a clinical benefit. Rather, there are somatic neoepitopes that are shared by patients with a prolonged benefit and are absent in those without a prolonged benefit. Owing to somatic mutations, a subset of proteins present in the tumor becomes recognized by the immune system as nonself, given their novelty in the tumor context.[8,14,31,32] These concepts were formulated in the discovery set and confirmed in the validation set and will require further prospective study before use as a definitive biomarker.

It is well known in the field of infectious diseases that an individual amino acid within a peptide can affect immunogenicity by altering peptide–MHC or peptide–T-cell receptor interactions.[33,34] In cancers, the altered amino acid residue resulting from a single missense mutation can create a T-cell epitope from a previously self peptide.[31,32,35] In the patients described here, altered amino acids resulting from tumor mutations caused the tumors to display somatic neoepitopes that elicited an antitumor response augmented by CTLA-4 blockade.

Our study has limitations. Although large for a genomic study (128 exomes), our sample size was limited, patients had received a variety of previous treatments, and tumor samples were obtained at various time points. Furthermore, although the panel of somatic neoepitopes (Fig. 3A and 3B, and Table S6 in the Supplementary Appendix) may constitute the most important ones, the in vivo relative immunologic contribution of each peptide is unclear. However, data showing that functionally important immunogenic epitopes persisted after treatment with expanded tumor-infiltrating lymphocytes suggest that the response to mutations may persist over time.[16] Although the recapitulation of the neoantigen signature in the validation set suggests that this may provide a generally applicable tool for prediction of a benefit from immunotherapy, further studies will be needed to investigate the role of MHC class II molecules and the relative effects and characteristics of neoantigens in different cancers.

Our use of whole-exome sequencing to identify a genetic basis associated with a benefit from



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CTLA-4 blockade provides proof of principle that tumor genomics can inform responses to immunotherapy. For the field of cancer genetics, these data suggest a need for an expanded definition of the previous categories of driver and passenger mutations. Our data show that exonic missense mutations in general confer increased MHC class I binding (Fig. S5A and S5B in the Supplementary Appendix) and confirm the hypothesis[36] that some mutations formerly categorized as passengers may in fact represent "immune determinants."

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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