The handle http://hdl.handle.net/1887/39812 holds various files of this Leiden University dissertation

**Author**: Buuren, Marit M. van  
**Title**: Analysis of the neo-antigen specific T cell response : and consequences for personalized immunotherapy  
**Issue Date**: 2016-06-01
Tumor exome analysis reveals neoantigen specific T-cell reactivity in ipilimumab-responsive melanoma

Nienke van Rooij*, Marit M. van Buuren1*, Daisy Philips1, Arno Velds2, Mireille Toebes1, Bianca Heemskerk1, Laura J.A.van Dijk1, Sam Behjati5, Henk Hilkmann4, Dris el Atmioui8, Marja Nieuwland2, Michael R. Stratton3, Ron M. Kerkhoven2, Can Kesmir5, John B. Haanen1, Pia Kvistborg1# and Ton N.M. Schumacher1#

1 Division of Immunology, The Netherlands Cancer Institute, Amsterdam, The Netherlands
2 Genomics Core Facility, The Netherlands Cancer Institute, Amsterdam, The Netherlands
3 Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Cambridge, United Kingdom
4 Peptide Synthesis Facility, The Netherlands Cancer Institute, Amsterdam, The Netherlands
5 Theoretical Biology and Bioinformatics, Utrecht University, The Netherlands
* These authors contributed equally
# Both are corresponding authors

Journal of Clinical Oncology 2013 Nov 10;31(32):e439-42
Tumor exome analysis reveals neoantigen specific T-cell reactivity in ipilimumab-responsive melanoma

Introduction

The evidence for T cell mediated regression of human cancers such as non-small cell lung carcinoma, renal cell carcinoma and in particular melanoma following immunotherapy is strong. Anti-CTLA4 (Ipilimumab) treatment has been approved for treatment of metastatic melanoma (1), and antibody-mediated blockade of PD-1, a second inhibitory receptor on T cells, has shown highly encouraging results in early clinical trials (2;3). While the clinical activity of these treatments is apparent, it is unknown which T cell reactivities are involved in immunotherapy-induced cancer regression (4). T cell reactivity against non-mutated tumor-associated self antigens has been analyzed in patients treated with ipilimumab or with autologous tumor-infiltrating T cells, but the magnitude of the T cell responses observed has been relatively modest (5;6). In part on the basis of such data, recognition of patient-specific mutant epitopes (hereafter referred to as neo-antigens) has been suggested to be a potentially important component (7). A potential involvement of mutated epitopes in T cell control would fit well with the observation that the mutation load in sun-exposed melanomas is particularly high (8-10).

Intriguingly, on the basis of animal model data it has recently been suggested that (therapy-induced) analysis of T cell reactivity against patient-specific neo-antigens may be feasible through exploitation of cancer genome data (11;12). However, human data have thus far been lacking. Here we report a case of a stage IV melanoma patient who exhibited a clinical response to ipilimumab treatment. Cancer exome-guided analysis of T cell reactivity in this patient revealed reactivity against 2 neo-antigens, including a dominant T cell response against a mutant epitope of the ATR (Ataxia Telangiectasia and Rad3 related) gene product that increased strongly after ipilimumab treatment. These data provide the first demonstration of cancer exome-guided analysis to dissect the effects of melanoma immunotherapy.

Case Report

A 56-year-old male was diagnosed in 2003 with a nodular melanoma with a Breslow thickness of 1.5 mm on the left upper arm. In April 2009, he developed lymph node metastases in both axillae, and underwent dissection of involved nodes at the right side. Positron emission tomography (PET) showed 18FDG uptake in both axillae, in soft tissue at the right scapula, in the left liver lobe, and mesenterially cranial of the transverse colon. He was treated with dacarbazine, but clearly progressed after six courses. At that time (October 2009), due to discomfort, a palliative dissection of the left axillary nodes was performed. In June 2010, prior to enrollment in the ipilimumab Expanded Access Program (EAP), a brain MRI showed three lesions, of which one was resected and two others were treated with stereotactic radiotherapy. In August 2010 he started ipilimumab treatment
(3 mg/kg) and received four infusions. All four courses of ipilimumab were tolerated well, except for grade one dermatitis. After completion, the patient displayed a marked regression of the tumor load (-25%), as shown by computed tomography (CT, Fig. 1A) and close to normalization (ULN=0.10 ug/L) of the S100b tumor marker after ipilimumab treatment (Fig. 1B).

**Figure 1. Patient characteristics**

To determine whether exome-guided analysis of antigen specific T cell responses against mutated antigens was feasible, we obtained both tumor cells and tumor-infiltrating lymphocytes from the lesion resected in 2009. Whole-exome sequencing of tumor cells and autologous healthy tissue was performed to identify tumor specific mutations. This revealed a total of 1,657 somatic mutations, consisting of 1,075 non-
synonymous (1,036 single nucleotide- and 39 non-sense variants) and 573 synonymous mutations with a false discovery rate of 0.07. Additionally, the tumor harbored seven frame shifts and two codon-deletions. Consistent with prior data, C>T/G>A mutations, reflective of UV induced DNA damage, predominated (Fig. 1C) (8-10). To predict potential neo-antigens from this set of mutations, the data were first filtered for gene expression using RNAseq data. Subsequently, predictions for proteasomal processing and HLA class I binding were performed on stretches of amino acid sequences that contained non-synonymous mutations, using the NetChop Cterm3.0 and NetMHC3.2 algorithms (13-15). This analysis yielded a set of 448 potential CD8 T cell epitopes (9-11 amino acids in length) with a predicted medium-to-high affinity binding for each HLA-A and -B allele (HLA-A*03:01, HLA-A*32:01, HLA-B*35:03 and HLA-B*40:02). Predicted peptides were synthesized and HLA multimers containing these ligands were produced by micro-scale parallel UV-induced peptide exchange reactions (16;17). Subsequently, tumor-infiltrating lymphocytes from this patient were analyzed for reactivity against these predicted T cell epitopes by a multiplexed MHC multimer staining strategy (16;18;19). An overview of the complete screening procedure can be found in Fig. 2. Additionally, an overview of detailed materials and methods can be found in Supplementary data 1.

This analysis revealed a T cell response against two patient-specific neo-antigens (both confirmed in independent analyses); a minor T cell response against a mutated epitope in the ZNF462 gene (0.003% of CD8+ cells) that was not pursued further, and a dominant response (3.3% of CD8+ cells, Fig. 3A, pMHC, peptide MHC, PE-Cy7, R-Phycoerythrin-cyanine 7; QD 625, Qdot 625; WT, wildtype) against a mutated epitope in the ATR serine/threonine protein kinase that functions to signal DNA damage (20). This mutation in ATR, resulting in an S>L change at position eight of a nonameric HLA-A*03:01 restricted epitope (ATR_{86}, = KLYEPLLK) was heterozygous within the tumor (66% of reads obtained, n=298) but absent in healthy control tissue (0%, n=189).
In order to assess whether the observed T cells were specific for the mutant ATR S>L epitope, bulk TIL were co-cultured with HLA-A*03:01-matched melanoma cells (526-cells). In the absence of added peptide, a low level of T cell reactivity was observed, presumably reflecting recognition of shared (i.e. non-mutated) HLA-A*03:01-restricted antigens (Fig. 3B). Importantly, whereas addition of wild-type ATR peptide was without effect, addition of the ATR S>L peptide led to a substantial increase in T cell recognition. Furthermore, sorted ATR S>L MHC multimer+ T cells likewise showed strong reactivity against target cells loaded with the mutant epitope but not with the wild-type peptide (Fig. 3C).

Having established the presence of neo-antigen specific T cell reactivity on the basis of cancer exome data, we subsequently assessed whether this could be utilized to follow treatment-induced T cell reactivity. To this purpose, PBMC samples collected before and during ipilimumab therapy (-341 days, -216 days, -28 days and +33 days after start of ipilimumab treatment) were analyzed by ATR S>L MHC multimer staining. HLA-A*03:01 ATR S>L specific T cells could be detected in peripheral blood at the earliest time point available and remained stable for a 10 month period preceding the start of ipilimumab treatment. Remarkably, within 5 weeks post start of ipilimumab, the magnitude of this neo-antigen-specific T cell response increased 5-fold (Fig. 3D), demonstrating that autologous cancer exome data can not only be used to predict neo-antigens in patients, but that these can also be utilized to follow the effects of immunotherapy.
Tumor exome analysis reveals neoantigen specific T-cell reactivity in ipilimumab-responsive melanoma

3.3%

Figure 3. Functional characterization of ATR_{S>L} specific T cells

Discussion

To our knowledge, this is the first report to show how autologous cancer exome data can be employed to reveal T cell responses against patient-specific neo-antigens in humans. The ATR_{S>L} specific-T cell response was identified in purposively comprehensive analysis, in which all genes with RNA expression above 0 were used for epitope prediction, and in which all neo-antigens with at least an intermediate predicted HLA affinity were retained. Interestingly, analysis of RNA expression of the ATR gene and the predicted HLA binding affinity of the ATR_{S>L} mutant epitope revealed that ATR was in the top 28% of expressed genes and that the ATR_{S>L} epitope was in the top 4% of predicted HLA-A*03:01 restricted
epitopes. In case this reflects a more general bias towards recognition of neo-antigens from highly expressed genes and with a high predicted HLA binding affinity, it may in future studies be feasible to analyze patient-specific T cell reactivity with even relatively small peptide sets. Analysis of larger groups of patients will be useful to address this issue.

Recent work of the groups of Schreiber and Sahin (11, 12) in animal model systems have provided the first indications how cancer exome data may be utilized for immunotherapy, by demonstrating that vaccination against neo-antigens within a mouse melanoma model can be used to increase tumor control, but also by demonstrating that immune-based selection against such neo-antigens can lead to epitope loss in vivo. Here we provide the first demonstration of the feasibility of exome-driven analysis of tumor-specific T cell reactivity in human cancer. In the coming years it will be interesting to assess how such information can be utilized as a potential diagnostic strategy, but also for the development of personalized cancer immunotherapy (21).

Acknowledgement

After acceptance of this manuscript, Robbins et al (22) demonstrated how mutated antigens that are recognized by adoptively transferred tumor-reactive T cells can also be identified with the use of exomic sequencing data.

Supported by grants from the Danish Strategic Research Council and the Cutch Cancer Society (Netherlands Cancer Institute 2012-5463). We thank members from the Schumacher and Haanen laboratories for useful discussions.

N.V.R. and M.M.V.B. contributed equally to this work.

Authors’ Disclosures of Potential Conflicts of Interest

John B. Haanen: received research funding from Bristol-Myers Squibb.
Tumor exome analysis reveals neoantigen specific T-cell reactivity in ipilimumab-responsive melanoma

References