Research Article

Cancer Immunology Research

Peripheral Blood TCR Repertoire Profiling May Facilitate Patient Stratification for Immunotherapy against Melanoma

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Abstract

Many metastatic melanoma patients experience durable responses to anti-PD1 and/or anti-CTLA4; however, a significant proportion (over 50%) do not benefit from the therapies. In this study, we sought to assess pretreatment liquid biopsies for biomarkers that may correlate with response to checkpoint blockade. We measured the combinatorial diversity evenness of the T-cell receptor (TCR) repertoire (the DE_{50}, with low values corresponding to more clonality and lack of TCR diversity) in pretreatment peripheral blood mononuclear cells from melanoma patients treated with anti-CTLA4 ($n = 42$) or anti-PD1 ($n = 38$) using a multi-N-plex PCR assay on genomic DNA (gDNA). A receiver operating characteristic curve determined the optimal threshold for a dichotomized analysis according to objective responses as defined by RECIST1.1. Correlations between treatment outcome, clinical variables, and DE_{50} were assessed in multivariate regression models and confirmed with Fisher exact tests. In samples obtained prior to treatment initiation, we showed that low DE_{50} values were predictive of a longer progression-free survival and good responses to PD-1 blockade, but, on the other hand, predicted a poor response to CTLA4 inhibition. Multivariate logistic regression models identified DE_{50} as the only independent predictive factor for response to anti-CTLA4 therapy ($P = 0.03$) and anti-PD1 therapy ($P = 0.001$). Fisher exact tests confirmed the association of low DE_{50} with response in the anti-CTLA4 ($P = 0.041$) and the anti-PD1 cohort ($P = 0.0016$). Thus, the evaluation of basal TCR repertoire diversity in peripheral blood, using a PCR-based method, could help predict responses to anti-PD1 and anti-CTLA4 therapies.

Introduction

Despite durable objective responses seen with the FDA-approved immune-checkpoint inhibitor (ICI) drugs anti-PD1 and anti-CTLA4, more than 50% of metastatic melanoma patients do not respond to these treatments (1). ICI therapies aim to modulate the immune system in order to enhance endogenous T-cell–mediated antitumoral responses. This process allows patients to mount an immune response against the tumor with limited side effects. Thanks to the advent of those new therapies, in the last 6 years median survival increased from 9 months to 18 months (2). However, compared with previous treatments (i.e., targeted therapies), the mode of action of ICIs often lengthens the time until objective response can be assessed. As a result, patients who do not respond to those therapies are potentially exposed to unnecessary side effects, are wasting precious time, and may miss their treatment window. Even though few other therapeutic options currently exist, ongoing clinical trials (3–5) may produce a more diverse panel of treatments to choose from in the near future. Thus, a biomarker identifying patients who will respond to anti-PD1 or anti-CTLA4 monotherapies (or combination therapies) would help optimizing patients' stratification in order to achieve the best possible therapeutic outcome given the available drugs. Because tumor-specific T cells present at the tumor site can also be found in the periphery in the absence of metastasis (6, 7), we hypothesized that the peripheral TCR repertoire could be used to detect clonal expansion of reactive T cells in melanoma patients. We used a PCR-based method to detect every possible V–J rearrangement in the CDR3 region of the TCRβ chain in peripheral blood samples from melanoma patients. The aim of this retrospective study was to assess whether the T-cell receptor (TCR) repertoire diversity evenness (DE_{50}), before treatment, initiation could predict which subpopulation of patients was more likely to respond to anti-PD1 or anti-CTLA4 therapy. We showed that patients with a low DE_{50} score did not respond to anti-CTLA4 treatment but did respond to anti-PD1 treatment. Thus, we demonstrated that the quantification of TCR repertoire diversity in the peripheral blood of melanoma patients, prior to treatment initiation, could represent a predictive biomarker to guide the use of immunotherapies to the most appropriate patients. However,

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Note: Supplementary data for this article are available at Cancer Immunology Research Online (http://cancerimmunolres.aacrjournals.org/).

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given the small size of our patient cohorts, these results need to be validated on a larger scale, as well as in cohorts worldwide, before they can be applied in a clinical setting.

Materials and Methods

Patients

All experiments were carried out according to the Declaration of Helsinki principles after approval by the ethics committee (Swissethics, Kantonale Ethikkommission Zurich, vote number ZK.Nr.2014-0425). The study was performed on surplus material from two different cohorts of patients after having obtained written, informed consent approved by the local IRB (EK.647/800, KEK-ZH.2014-0425, N03LAM). The first cohort included 42 patients treated with anti-CTLA4 therapy, 18 were treated at the Netherlands Cancer Institute (NKI) and 24 at the University Hospital Zurich (USZ). Patients from the NKI were randomly assigned to the study, whereas patients from USZ were selected to enrich the responder ratio. Additional clinical variables were collected around baseline (in a range of 30 days from treatment initiation). To harmonize the values of lactate dehydrogenase (LDH) between the two sites, LDH/upper limit of normal (480 U/l and 250U/l for UZH and NKI, respectively) was calculated. The second cohort was composed of 38 patients treated with anti-PD1 at the USZ. As for the CTLA4 cohort, absolute lymphocyte count (ALC), absolute neutrophil count (ANC), LDH, as well as S100 levels and leukocyte count, were documented at baseline (all between −17 and +1 day from treatment start day, except for three LDH and eight S100 concentrations, which were collected between −42 days and +27 days from the start of treatment when their values were stable over time). The objective response was determined by Response Evaluation Criteria in Solid Tumors (RECIST1.1) after 12 weeks of treatment (average = 11.28, median = 11.64, range = 4.7–17.7). Responders were classified as undergoing a complete response (CR) or a partial response (PR), and nonresponders were defined as stable disease (SD) or progressive disease (PD) state. Progression-free survival (PFS) represents the period between the treatment initiation to the date of progression or death, whichever occurred first. Patients' characteristics are shown in Table 1.

Table 1. Patient characteristics for anti-CTLA4 and anti-PD1 cohorts

Patient characteristics	Anti-PD1 *N* (%)	Anti-CTLA4 *N* (%)
Age, years		
<40	2 (5.3)	3 (7.1)
40–55	11 (28.9)	14 (33.3)
56–70	14 (36.8)	18 (42.9)
>70	11 (28.9)	7 (16.7)
Gender		
Male	27 (71.1)	23 (54.8)
Female	11 (28.9)	19 (45.2)
PFS		
<3 months	9 (23.7)	13 (59.1)
3–9 months	11 (28.9)	6 (27.3)
>9 months	18 (47.4)	3 (13.6)
RECIST1.1 at ∼12 weeks		
CR	1 (2.6)	2 (4.8)
PR	11 (28.9)	10 (23.8)
SD	12 (31.6)	3 (7.1)
PD	14 (36.8)	27 (64.3)
BRAF mutation status		
Wild-type	27 (73.0)	
Mutated	10 (27.0)	
NRAS mutation status		
Wild-type	21 (60.0)	
Mutated	14 (40.0)	
Previous treatments		
Chemotherapy	3 (7.9)	
Targeted therapy	2 (5.3)	
Immunotherapy (ipilimumab)	28 (73.7)	
Radiotherapy	4 (10.5)	
Interferon	1 (2.6)	
Lactate dehydrogenase (LDH/ULN)		
<1	20 (54.1)	27 (67.5)
≥1	17 (45.9)	13 (32.5)
S100		
<0.2 μg/L	10 (27.8)	
≥0.2 μg/L	26 (72.2)	
ANC		
<8 g/L	35 (92.1)	40 (95.2)
≥8 g/L	3 (7.9)	2 (4.8)
ALC		
<1.5 g/L	25 (65.8)	28 (66.7)
≥1.5 g/L	13 (34.2)	14 (33.3)
Leukocytes		
<9.6 g/L	34 (89.5)	
≥9.6 g/L	4 (10.5)	
Basophils		
<0.15 g/L	20 (52.6)	
≥0.15 g/L	18 (47.4)	
Eosinophils		
<0.7 g/L	36 (94.7)	
≥0.7 g/L	2 (5.3)	

NOTE: *N*, number of patients in each category. %, representation in percent for each category.

ImmunTraCkeR (multiplex PCR assay)

Peripheral blood mononuclear cell (PBMCs) from melanoma patients, prior to anti-CTLA4 ($n = 42$) or anti-PD1 ($n = 38$) treatment, were obtained by Ficoll-gradient centrifugation (Ficoll-Paque Plus, GE Healthcare). Genomic DNA was extracted from frozen PBMC samples using High Pure PCR Template Preparation Kit (Roche) and concentrated using Amicon ultra 0.5 mL Centrifugal Filters (Millipore). ImmunTraCkeR (8), a TCR repertoire-profiling tool, was used to study the T-cell immune repertoire diversity based on the detection of V-J rearrangements of the CDR3 region of the TCRβ chain, as previously described (9). Briefly, multiplex PCR was performed using upstream primers specific for each V and downstream primer for each J segment, covering all the possible combinations (based on the international ImMunoGeneTics information system, IMGT, http://www.imgt.org). PCR products were separated by microfluidic migration (LabChip GX, PerkinElmer) using a lab-on-a-chip (HT DNA 12 K LabChip Kit, PerkinElmer). The PCR signals obtained were compared using Constel'ID, a software that generates 3D maps representing the immune status of each patient. The diversity of the TCR pool was studied through the evenness of the repertoire based on frequencies of specific V-J rearrangements. For each patient, a diversity evenness score (DE_{50}) was calculated as follows:

$$DE_{50} = \frac{\text{nb rearrangements accounting for 50\% total map intensity}}{\text{total nb rearrangements present}}$$

A high score corresponds to a homogenous occurrence of each V-J rearrangement, whereas a low score represents a skew in the frequency toward specific rearrangements. Figure 1 shows the outline of the analysis pipeline.

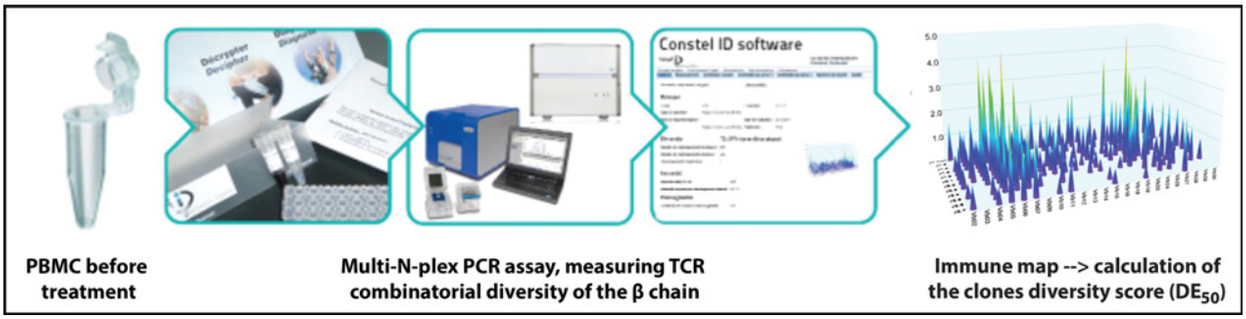

Figure 1.
Outline of the analysis pipeline.

Statistical analysis

The optimal thresholds for each variables were calculated by receiver operating characteristic (ROC) analysis, using the Youden method (ref. 10; *OptimalCutpoints* package in R).

The relationship between OR and each biological/clinical variables was assessed in both cohorts. The prognostic factors, when available, were included in univariate logistic regression analysis with bias adjustment (Firth method; ref. 11), using a previously ROC-determined cutoff. Independent predictive variables of response were identified by a logistic regression analysis using a backward selection procedure. The added value of each selected variable in the models was evaluated using a penalized likelihood ratio χ^2; likelihood scores of the model evaluated with and without the biomarker were compared, considering that lower likelihood scores will indicate better fitting models. The analyses including DE_{50} in the anti-PD1 cohort were performed using bias adjustment (Firth method; ref. 11). The association between response and the high versus low DE_{50} group was assessed by a Fisher exact test.

The relationship between PFS and the available biological/clinical variables was assessed by a univariate Cox proportional hazard regression model using the same previously ROC-determined cutoffs. Independent predictive variables of PFS were identified by a multivariate Cox regression analysis using a backward selection procedure. The added value of each selected variable in the models was evaluated using a likelihood ratio test; likelihood scores of the model evaluated with and without the biomarker were compared, considering that lower likelihood scores will indicate better fitting models. For the Cox regression model assessing the correlation with PFS, NRAS mutation status was excluded from the backward selection process because these data were missing too many pieces of information, which would limit the power of the test.

Results of the regression models are presented in terms of β-coefficients. β-Coefficients describe the change in the natural logarithm of the hazard ratio (HR) or odd ratio (OR) per unit of independent variable: ($e^{\beta coeff}$ = HR or OR).

Results

Multiplex PCR assay

Diversity of the TCR repertoire was evaluated in a blinded fashion, using a multi-N-plex PCR assay, which measures combinatorial diversity between V and J genes of the β chain at the complementarity determining region 3 (CDR3), where most of the diversity is contained. In order to characterize the diversity of the repertoire, we calculated a diversity evenness score (DE_{50}). The DE_{50} represents the ratio of the number of rearrangements among the most frequent necessary to account for 50% of the global map intensity (cumulative sum of each rearrangement's frequency) divided by the total number of rearrangements present. A TCR repertoire that is composed of an even frequency distribution of different V–J rearrangements is considered to have low clonality and thus a high DE_{50} score. A TCR repertoire that is dominated by some specific V–J rearrangements has a low DE_{50} and is considered to be more clonal.

Patients and treatments

In this retrospective study, baseline frozen PBMC samples from two separate cohorts of patients were analyzed. The first of the two cohorts included 42 metastatic melanoma patients who received ipilimumab at 3 mg/kg every 3 weeks, for a maximum of 4 infusions. Only patients who received at least 2 infusions were included. ALC, ANC, and LDH levels were documented at baseline.

The second cohort was composed of 38 patients treated with anti-PD1. Thirty-seven patients received pembrolizumab at 2 mg/kg (or 10 mg/kg for patients participating in the NCT01704287 study) every 3 weeks and 1 patient received nivolumab at 3 mg/kg every 2 weeks. Patients who received fewer than 3 infusions were excluded from the analysis. The median follow-up was 11.38 months (mean = 13.08) for patients who died and 32.75 months (median = 36.49) for patients who were still alive at the last follow-up. As for the CTLA4 cohort, ALC, ANC, LDH, as well as S100 levels, basophil, eosinophil, and leukocyte counts were documented at baseline. We investigated the potential effect of prior treatment on each of the factors and outcome and found no correlation (Supplementary Fig. S1). Patients' characteristics are shown in Table 1.

The OR was determined by RECIST1.1 after at least 12 weeks of treatment (average = 11.28, median = 11.64, range = 4.7–17.7). We evaluated response according to objective response rate, that is, CR and PR are considered to be responders and SD and PD are considered to be nonresponders. Survival plots for PFS and overall survival (OS) are shown in Fig. 2.

Analysis of pretreatment clinical parameters and correlation with response

First, for each clinical parameter, an appropriate threshold for dichotomized analysis was determined by a ROC curve. The

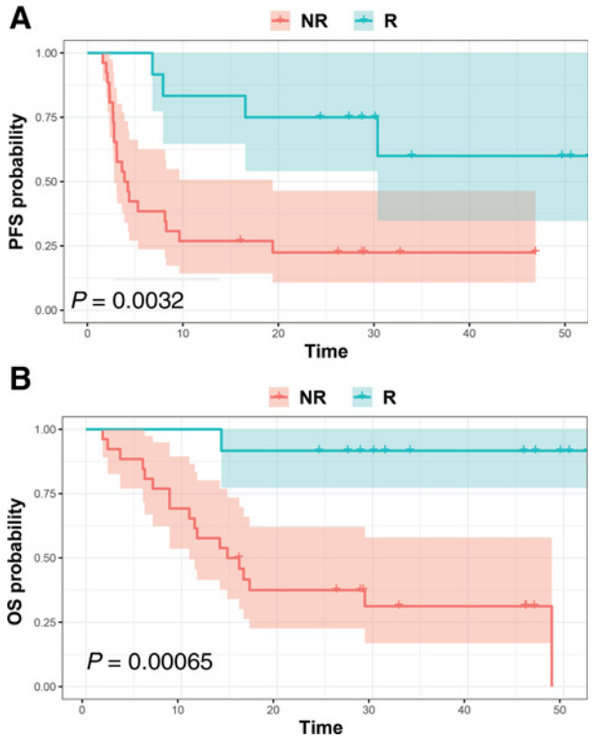

Figure 2.

Kaplan–Meier plots showing survival in R (responders) and NR (nonresponders) to anti-PD1 treatment. Colored bands represent 95% confidence interval (CI). **A,** PFS probability, **B,** OS probability.

optimal cutoff points separating responders from nonresponders were calculated using the Youden method (10). Thresholds are shown next to the variables in each of the tests.

To assess the predictive potential of DE_{50} and the different clinical variables on response to treatment, reliable prognostic factors were included in univariate logistic regression analysis using previously ROC-determined cutoffs (Fig. 3). In the anti–CTLA4-treated cohort, only DE_{50} appears to be significant in the univariate model, and it is the only independent factor that is conserved in the multivariate model (Fig. 3A). Because the only outcome variable available for all the patients in this cohort was OR, no survival analysis could be performed. In the anti–PD1-treated cohort, at the univariate level, both LDH and DE_{50} are significantly correlated with response to treatment. To determine if the candidate predictive factors of our model were independent predictors of response, we performed a multivariate logistic regression analysis using a backward selection procedure. The best fitting model only identified DE_{50} as an independent predictive factor (Fig. 3B).

Correlation between baseline DE_{50} levels and response to treatment

In order to identify the predictive potential of baseline TCR repertoire clonality and its association with clinical benefit, we analyzed the relationship between low and high DE_{50} and response to treatment (Fig. 4). In the anti–CTLA4-treated cohort, all the patients who had $DE_{50} < 20.03\%$ at baseline did not respond to the treatment ($P = 0.041$; Fig. 4A). Conversely, in the anti–PD1-treated cohort, a baseline DE_{50} level below 20.4% was associated with good response to the treatment at 12 weeks ($P = 0.0016$; Fig. 4B).

Identification of clinical parameters linked with PFS in the anti-PD1 cohort

Independent predictive variables of PFS were identified by a Cox regression analysis using the ROC-determined cutoffs. The univariate model identified S100 (β-coeff = −1.814, 95% CI = −3.027 to −0.601, $P = 0.003$) and LDH (β-coeff = −0.945, 95% CI = −1.761 to −0.129, $P = 0.023$) as the only two variables significantly correlated with PFS (Fig. 5A). Backward selection procedure was used to extract the most explanatory variable for the multivariate model. The multivariate Cox regression for PFS retained DE_{50}, ANC/ALC, ALC, and S100; only S100 appears not to be an independent factor associated with PFS ($P > 0.05$; Fig. 5B).

Discussion

The approval of ICIs as first-line treatment for melanoma (in 2011) and subsequent developments represent a huge improvement in cancer management. However, patients' responses are still variable and unpredictable. Few reliable biomarkers are available in clinical practice to optimize patient selection and treatment efficacy for immunotherapy.

The first candidate to be examined for anti-PD1 therapy was PD-L1. However, intratumoral PD-L1 expression failed as a predictive biomarker, because PD-L1–negative patients also responded to the treatment (12, 13). This result may be explained by the lack of a standardized method of detection (different cutoffs and kits were used) or by the fact that the biopsy used for the assay does not account for the full tumor heterogeneity or the dynamic receptor expression (14). The other drawback inherent in PD-L1 or other tumor staining assay is the need for at least part of the tumor to be sampled, which requires an invasive procedure and may not be feasible due to the location of the tumor. Liquid biopsies derived from blood or serum avoid the problem of invasive procedures. In the context of anti-PD1 treatment, some potential predictive markers have been tested in the peripheral blood, namely, LDH level as well as eosinophil, monocyte, and lymphocyte counts (15, 16). In patients treated with anti-CTLA4, studies have investigated lymphocyte, monocyte, eosinophil, or neutrophil counts as predictive markers of patients' responses (17–19). The results of those studies are contradictory and seem to hold more prognostic than predictive information (20, 21). Furthermore, serum levels of C-reactive protein, LDH, soluble CD25, and VEGF have been associated with clinical outcome (22–24). Unfortunately, most of the analyses have been performed on small cohorts, and none of these potential markers have been validated prospectively (25), except for very high LDH levels, which is associated with poor prognosis in anti–CTLA4-treated patients (26). No predictive biomarker for patient stratification prior to treatment other than routine PD-L1 staining (27) has yet proven robust enough to be used in the clinic.

The efficacy of ICIs relies on the activation and effector functions of the adaptive immune system and largely on T cells. However, the exact mechanisms of action are not understood.

Anti-CTLA4 was shown to lower the threshold for T-cell priming and therefore allow expansion of a higher number of effector T cells (28). Anti-CTLA4 also increases clonal expansion of

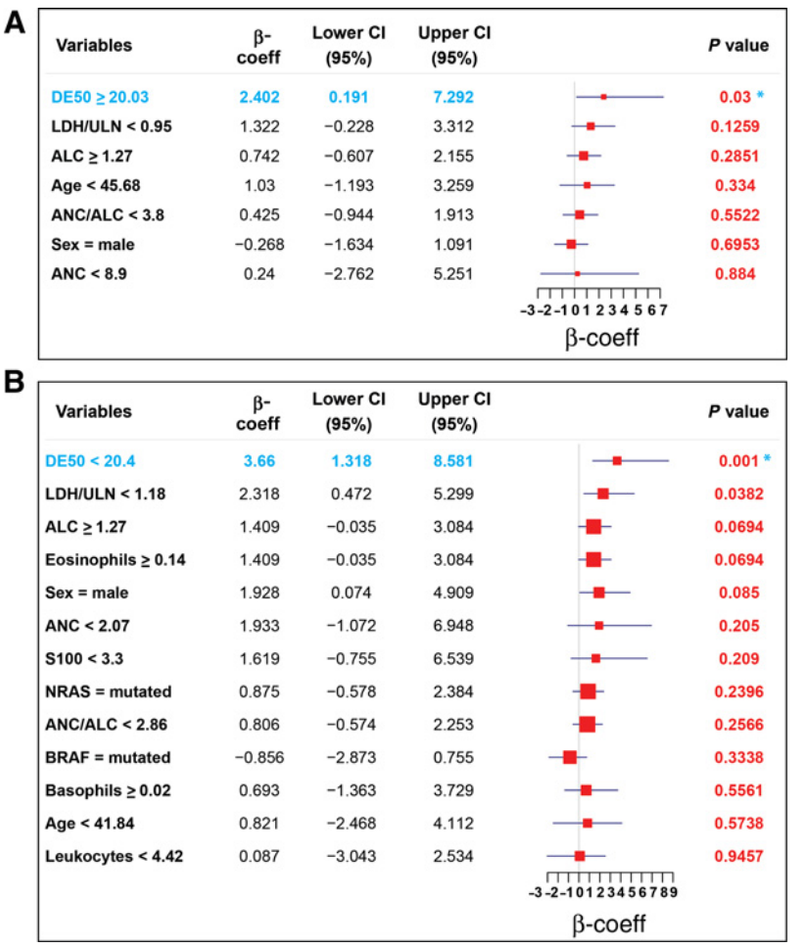

Figure 3.

Logistic regression model assessing the relationship between pretreatment clinical parameters (including DE_{50}) and response to therapy. Forest plots and tables represent the β-coefficients of the clinical variables that may affect response to treatment. Blue lines represent the 95% CI. **A,** Univariate analysis for the anti–CTLA4-treated cohort. *, DE_{50} is the only factor conserved in the multivariate analysis. **B,** Univariate analysis for the anti–PD1-treated cohort. *, DE_{50} is the only factor conserved in the multivariate analysis. In the multivariate analyses, factors were selected using a backward selection process.

memory T cells (29) and depletes regulatory T cells (Treg) in the tumor microenvironment (30), leading to an increase in the effector T-cell/Treg ratio at the tumor site (31).

The major reported effect of PD1 blockade, in the context of cancer treatment, was the restoration of T-cell functions during the effector phase. Anti-PD1 was demonstrated to salvage exhausted $CD8^+$ T cells, restoring their cytotoxic potential, leading to the destruction of tumor cells (32). Anti-PD1 was also reported to affect Treg differentiation and function by preventing PD1 ligation to PD-L1 on antigen-presenting cells, which have been shown to play a key role in Treg development (33). However, PD1 has a broad expression pattern: it is expressed on T cells as well as B cells, natural killer cells, dendritic cells, and myeloid cells. Its ligands, PD-L1 and PD-L2, are also expressed on various hematopoietic and nonhematopoietic cells as well as cancer cells (34, 35). As a result, anti-PD1 therapy can affect various cell types and pathways in ways still being investigated (36, 37). More research is required to understand the exact modes of anti-CTLA4 and anti-PD1 action. Nevertheless, the effect of ICI therapies on the TCR repertoire has been intensively studied. Several groups have shown that anti-CTLA4 and anti-PD1 therapies reshape the TCR repertoire in different ways (38). Anti-CTLA4 broadens the T-cell repertoire, whereas anti-PD1 drives proliferation of a restricted number of clones, giving rise to a skewed peripheral repertoire.

Anti-CTLA4 expands the peripheral TCR repertoire in a non-specific manner by inhibiting Tregs and increasing the $CD8^+$/Treg ratio (39, 40). Improved clinical outcome is associated with reduced clonotype loss and maintenance of high-frequency clonotypes from the start and throughout treatment duration (41). This provides evidence that the baseline TCR repertoire profile might play a role in the response to the treatment.

Investigation of the TCR repertoire in tumors demonstrated a positive association between the clonality of the intratumoral T-cell repertoire at baseline and response to anti-PD1 (42). Other groups confirmed those results and showed that anti-PD1 stimulates oligoclonal expansion of certain T-cell clones at the tumor site, in particular those that were present at a higher frequency (43, 44). Altogether, these results suggest that the initial shape of the T-cell repertoire may guide therapeutic decisions. Other studies have analyzed the TCR repertoire using next-generation sequencing (NGS); however, NGS approaches are currently too expensive and the data processing too time consuming for routine clinical use.

Here, we have aimed to develop a tool that could be useful in the clinic for routine patient stratification. The primary objective of this study was to determine whether baseline TCR repertoire diversity could be used as a predictive biomarker of response to anti-PD1 and anti-CTLA4 and how it compares with other predictive variables described in the literature.

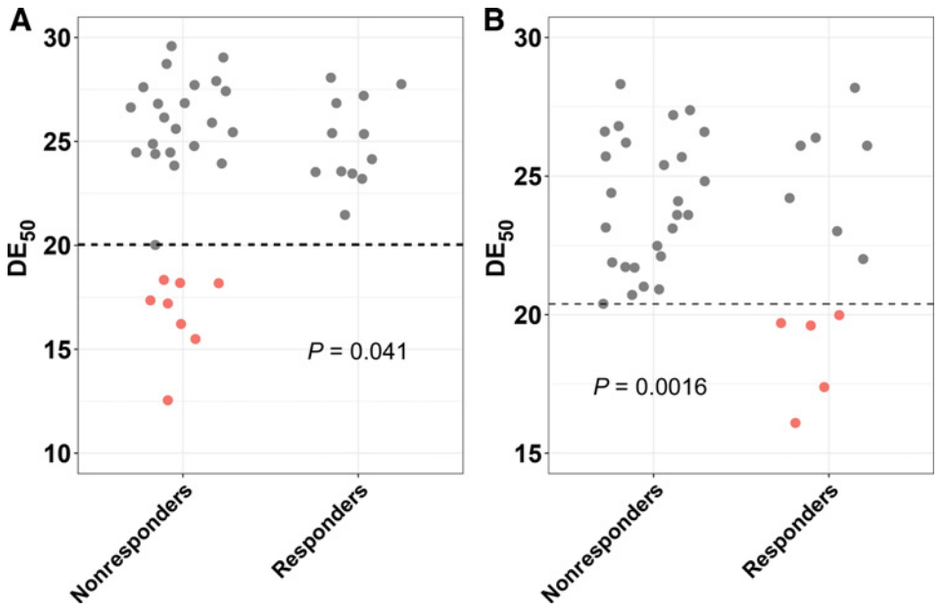

Figure 4.
TCR diversity evenness prior to treatment. **A,** Dot plot showing DE_{50} at baseline in patients who eventually responded or did not respond to anti-CTLA4 therapy. $N = 42$. Dashed line, 20.03%. **B,** Dot plot showing DE_{50} levels at baseline in patients who eventually responded or did not respond to anti-PD1 therapy. $N = 38$. Dashed line, 20.4%. *P* value calculated with the Fisher exact test.

In order to reduce the number of factors included in our analysis, we first confirmed that the previous treatments received by the patients did not affect the clinical variables and was not correlated with response (Supplementary Fig. S1). Then, to compare our predictor's performance to other proposed markers in the literature, we performed regression analyses including other relevant factors whenever available. At the multivariate level, using the ROC-determined threshold, DE_{50} was the only significant predictor of response in our cohorts (Fig. 3). We also observed that in the univariate analyses, LDH level was significantly correlated

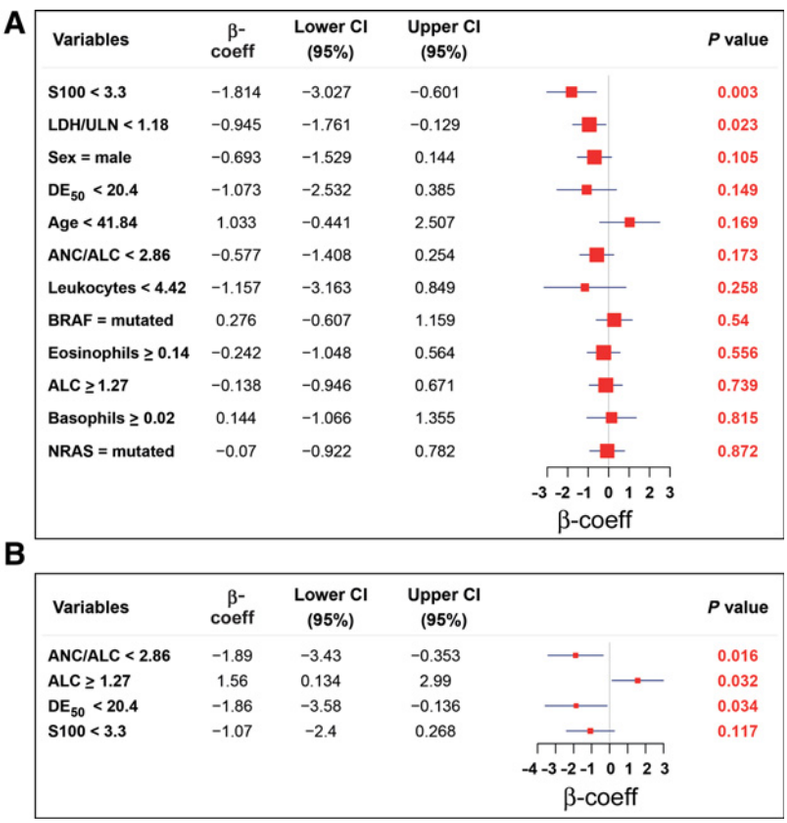

Figure 5.
Cox regression model assessing the correlation of clinical variables with PFS in the anti-PD1 cohort. Forest plots and tables represent the β-coefficients of the clinical variables that may affect PFS. Blue lines represent the 95% CI. **A,** Univariate analysis. **B,** Multivariate analysis using backward selection procedure.

with PFS and response to anti-PD1 therapy. Baseline LDH levels are thought to be indicative of future outcome irrespective of treatment (45–47), but can lead to false-positive predictions of response. For instance, some patients with high baseline LDH levels can also achieve a good response to anti-PD1 (48) and high and low LDH groups show no difference in treatment efficacy (49). This underscores the need to find better early predictors of response to ICI therapies.

The only other factors able to independently predict outcome in one of our cohort were the neutrophil-to-lymphocyte ratio (ANC/ALC) and the ALC, which had PFS predictive power in anti–PD1-treated patients (Fig. 5B). ANC/ALC has already been shown to be a predictive marker for response in anti–PD1-treated melanoma patients (50). Because ANC/ALC is described as a prognostic factor which is also correlated with leukocyte/lymphocyte ratio (51), we investigated this potential association in our cohort (Supplementary Fig. S2). Indeed, we found a similar correlation. Patients with low ANC/ALC expression have more T-cell tumor infiltration compared with the high ANC/ALC group (51). This would support our results because T-cell infiltration is a key element for an appropriate immune response against the tumor. Nevertheless, other studies found a correlation between ANC/ALC and patient outcome regardless of the treatment (52, 53).

Overall, our analysis showed that ALC $\geq$ 1.27 g/L and ANC/ALC < 2.86 are correlated with longer PFS and are also associated with a high lymphocyte-to-leukocyte ratio. We showed that the detection of a basal clonal TCR repertoire characterized by DE_{50} < 20.4% in peripheral blood, via a conventional PCR-based method, is able to predict response to anti-PD1 treatment at 12 weeks. On the other hand, a clonal TCR repertoire prior to anti-CTLA4 therapy characterized by DE_{50} < 20.03% is associated with poor response to the treatment. In line with our results, DE_{50} is also significantly correlated with PFS at 12 months (Supplementary Fig. S3A) and OS at 24 months (Supplementary Fig. S3B) in the anti-PD1 cohort of patients.

We have demonstrated that TCR repertoire clonality, represented by the TCR diversity index DE_{50}, can help predict patient outcome under anti-PD1 and anti-CTLA4 therapy (Fig. 4). A low DE_{50} (i.e., clonal repertoire) was associated with a poor response to anti-CTLA4 and with a better response and a longer PFS in patients treated with anti-PD1, which is consistent with previously published literature (9, 54).

Our results indicate that patients with a repertoire dominated by specific rearrangements may benefit more from anti-PD1. However, our analysis does not allow us to determine the exact composition of the TCR repertoire in terms of cell types, nor to discriminate between protumorigenic (i.e., $CD4^+$ Treg) and antitumorigenic cell types (i.e., cytotoxic $CD8^+$). Yet two hypotheses would support the rationale of our finding. First, in patients whose immune system fails to eliminate the tumor, there is an increase in exhausted T cells (55). Those cells could correspond to the clonal expansion we detect at baseline and explain the subsequent efficient response to anti-PD1 treatment, which restores function to these tumor-specific T cells. Second, the baseline T-cell repertoire of nonresponders has a higher proportion of $CD8^+$-naïve T cells, whereas responders have a larger population of central memory T cells (16). Hence, the higher clonality of the T-cell repertoire seen at baseline could also be due to a higher population of memory T cells, which respond particularly well to PD1 blockade (56).

A clonally expanded TCR repertoire at baseline appears to preclude the efficacy of anti-CTLA4 treatment. A skewed TCR repertoire is the representation of an immune system that is responding to a foreign invasion (57). If, prior to therapy initiation, the patient's immune system has already mounted a response against specific antigens (here, we assume melanoma antigens), treatment with anti-CTLA4 will be of little use. Indeed, the therapy is mainly aimed at eliciting a T-cell response by sequestrating the receptor responsible for inhibition. This means that the treatment has little effect on already activated and expanded T-cell populations (58). If the T-cell clones present before therapy initiation were not able to keep the disease under control, anti-CTLA4 treatment will not improve the outcome. Furthermore, a highly clonal repertoire with no response is most likely associated with immune checkpoints upregulation and T-cell exhaustion. In keeping with this assumption, anti–CTLA4-based treatment is ineffective on melanoma with high PD-L1 expression (40).

Our data need to be further validated in a larger and independent cohort with similar inclusion criteria. Moreover, additional studies are necessary to assess the TCR specificity of the clonally expanded T cells in responding patients in order to determine which epitopes are being recognized. A limitation of our study is that we cannot demonstrate if the expanded clones that were identified in the responding patients were tumor specific. However, the fact that responders to anti-PD1 therapy showed substantial expansion of only a limited number of clones (Supplementary Fig. S4) would corroborate previous studies demonstrating that the antitumor response relies only on a restricted number of specific TCR (59, 60). In summary, with this study we propose the analysis of peripheral blood TCR repertoire as a predictive tool to facilitate patient stratification in the context of ICI therapy for melanoma.

Disclosure of Potential Conflicts of Interest

P. Kvistborg reports receiving commercial research grants from Bristol-Myers Squibb and Merck and is a consultant/advisory board member for Neon Therapeutics and Personalis. J.B.A.G. Haanen reports receiving commercial research funding from Bristol-Myers Squibb, Novartis, and MSD, and is a consultant/advisory board member for Bristol-Myers Squibb, MSD, Pfizer, Roche, Bayer, and Ipsen. R. Dummer is Vice-Chairman Dermatology at University Zurich and reports having intermittent, project-focused consulting and/or advisory relationships with Novartis, Merck Sharp & Dohme, Bristol-Myers Squibb, Roche, Amgen, Takeda, Pierre Fabre, Sun Pharma outside the submitted work. M.P. Levesque reports receiving a commercial research grant from Bristol-Myers Squibb. No potential conflicts of interest were disclosed by the other authors.

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