Circulating CD137$^+$ T Cells Correlate with Improved Response to Anti-PD1 Immunotherapy in Patients with Cancer

Ilaria Grazia Zizzari[1], Alessandra Di Filippo[1], Andrea Botticelli[2], Lidia Strigari[3], Angelina Pernazza[4], Emma Rullo[4], Maria Gemma Pignataro[4], Alessio Ugolini[1,5], Fabio Scirocchi[1], Francesca Romana Di Pietro[6], Ernesto Rossi[7], Alain Gelibter[2], Giovanni Schinzari[7,8], Giulia D'Amati[4], Aurelia Rughetti[1], Paolo Marchetti[6,9,10], Marianna Nuti[1], and Chiara Napoletano[1]

ABSTRACT

Purpose: CD137 molecule is expressed by activated lymphocytes, and in patients with cancer identifies the tumor-reactive T cells. In solid tumors, high levels of circulating CD137$^+$ T cells are associated with the clinical response and the disease-free status. Here, we examined the role of the CD137$^+$ T cells in the improvement of patients' selection for immunotherapy treatment.

Experimental Design: Peripheral blood mononuclear cells derived from 109 patients with metastatic cancer (66 patients for the identification cohort and 43 for the validation cohort) were analyzed for the expression of CD3, CD4, CD8, CD137, and PD1 molecules before the beginning of anti-PD1 therapy. Twenty healthy donors were used as control. The soluble form of CD137 (sCD137) was also analyzed. The CD137$^+$ T cell subsets and the sCD137 were correlated with the clinicopathologic characteristics. The distribution of CD137$^+$ T cells was also examined in different tumor settings.

Results: The percentage of CD137$^+$ T cells was higher in healthy donors and in those patients with a better clinical status (performance status = 0–1, n°metastasis≤2) and these high levels were ascribed to the CD8$^+$CD137$^+$ T cell population. The high frequency of CD137$^+$ and CD8$^+$CD137$^+$ T cells resulted as a prognostic factor of overall survival (OS) and progression-free survival (PFS), respectively, and were confirmed in the validation cohort. High levels of CD3$^+$CD137$^+$PD1$^+$ lymphocytes were associated with a low number of metastasis and longer survival. Instead, the high concentration of the immunosuppressive sCD137 in the serum is associated with a lower PFS and OS. In tumor bed, patients with a complete response showed a high percentage of CD137$^+$ and CD8$^+$ T cells.

Conclusions: We propose the CD137$^+$ T subset as an immune biomarker to define the wellness status of the immune system for successful anticancer immunotherapy.

Introduction

In recent years, cancer treatments have been revolutionized by immunotherapy, due to the ability of immune checkpoint inhibitors (ICI) to steer the patients' immune system toward immune activation. In particular, the anti-PD1 treatments aim to reinvigorate and amplify a preexisting tumor-specific T cell subset by blocking the PD1/PDL1 axes (1). However, although these treatments, administered alone or in combination, have obtained encouraging results in several cancer types, most of the patients show limited benefits (2–4).

Besides the tumor biological parameters that have been extensively studied, it is becoming clear that the fitness of the immune system is strictly correlated to the success of immunotherapy. Indeed, the identification of specific biomarkers able to define the wellness of the immune system seems to significantly contribute to the patients' selection.

CD137 receptor (4-1BB, TNFRSF) is a TNFR-family member with costimulatory function expressed by CD8$^+$ and CD4$^+$ T cells upon activation, by natural killer (NK) cells, dendritic cells (DC), eosinophils, and vascular endothelium cells (5–7). Its counter receptor is the CD137L expressed by activated antigen-presenting cells (APC). CD137 induces a bidirectional stimulus that activates both interacting cell types (8). The triggering of the CD137-CD137L pathway induces T cell division and survival, enhances the effector function of lymphocytes (9), protects against activation-induced T cell apoptosis (10), enhances the T cell mitochondrial metabolism (11), and promotes the DNA demethylation of CD8 main genes (12). In APCs, it induces maturation and survival, and enhances the capacity for antigen presentation (13).

CD137 molecule is also released as a soluble form (sCD137) as result of alternative splicing variants (14), but its function completely differs from the membrane-bound molecule. Indeed, its interaction with CD137L on DCs prevents their maturation and the activation of T cells (15).

[1]Laboratory of Tumor Immunology and Cell Therapies, Department of Experimental Medicine, "Sapienza" University of Rome, Rome, Italy. [2]Division of Oncology, Department of Radiological, Oncological and Pathological Science, Policlinico Umberto I, "Sapienza" University of Rome, Rome, Italy. [3]Medical Physics Unit, "S. Orsola-Malpighi" Hospital, Bologna, Italy. [4]Department of Radiology, Oncology and Pathology, "Sapienza" University of Rome, Rome, Italy. [5]Department of Immunology, H. Lee Moffitt Cancer Center, Tampa, Florida. [6]Oncology Unit, Department of Clinical and Molecular Medicine, Sant'Andrea Hospital, Sapienza University of Rome, Rome, Italy. [7]Medical Oncology, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy. [8]Medical Oncology, Università Cattolica del Sacro Cuore, Rome, Italy. [9]Department of Clinical and Molecular Medicine, Sapienza University of Rome, Rome, Italy. [10]AOU Policlinico Umberto I, Rome, Italy.

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

M. Nuti and C. Napoletano contributed equally to this article.

Corresponding Author: Chiara Napoletano, Department of Experimental Medicine, "Sapienza" University of Rome, Viale Regina Elena 324, Rome 00161, Italy. Phone: 3906-4997-3025; E-mail: chiara.napoletano@uniroma1.it

Clin Cancer Res 2022;28:1027–37

doi: 10.1158/1078-0432.CCR-21-2918

Translational Relevance

The introduction of immunotherapy in the treatment of patients with cancer highlighted the critical role of patients' immune fitness for successful immunotherapy. Patients with a better or less dysregulated immunity appeared to have a better clinical outcome after the immunologic treatments. We proposed the $CD137^+$ T cell subset as a marker to define the "quality" of the immune activation of patients with cancer able to predict the clinical outcome independently of tumor histotype and previous therapies. We believe that the frequency of this cellular subset, evaluated before the beginning of immunotherapy, could be used by the oncologists as immunologic biomarker able to define the wellness of the immune system. This parameter seemed to significantly contribute to the patients' selection monitoring the response to immunologic treatments and predicting the clinical outcome of patients with cancer.

In the field of tumor immunology, the CD137 molecule has acquired great interest when this receptor was shown to identify the naturally occurring antigen-specific T cells able to kill tumor cells upon expansion (16). Evidence had demonstrated that the activation of CD137 was MHC dependent and that its engagement induced the activation of T cells only in presence of a consistent antigen stimulation (17). Experiments carried out in the mouse model showed that only the $CD137^+$ T cell subset was able to inhibit tumor growth compared with $CD137^-$ T cell population (16).

Recently, the role of $CD137^+$ T cells in eliciting an antitumor immune response has been highlighted in patients with cancer. Ye and colleagues (16) demonstrated that $CD137^+$ T cells were present at different levels in the tumor microenvironment, peripheral blood, and ascites of patients with ovarian cancer. Moreover, $CD137^+$ lymphocytes located in the tumor bed identified the tumor-reactive T cells, independently by the expression of PD1 molecule, which usually is used as a marker of the exhausted lymphocytes (16, 18).

Several studies also demonstrated the positive correlation between the frequency of circulating $CD137^+$ lymphocytes and the clinical outcome. In metastatic renal clear cell carcinoma (mRCC), high levels of $CD137^+$ T cells positively correlated to response to tyrosine kinase inhibitors (19, 20). In the non–small cell lung cancer (NSCLC) setting, patients positive for the autoantibody IgM-rheumatoid factors showed decreased levels of circulating $CD137^+$ T cells. These patients showed early progression or a reduced overall survival (OS; ref. 21).

All this evidence suggests a strong association between the presence of high levels of $CD137^+$ T cells and the prolonged survival of patients with cancer highlighting the role of $CD137^+$ lymphocytes in the induction of an efficacious immune response against tumors.

In this work, we analyzed the circulating levels of $CD137^+$ T cells in a cohort of patients belonging to different metastatic cancer settings [NSCLC, RCC, uveal melanoma, and head and neck squamous cell carcinoma (HNSCC)] before the beginning of anti-PD1 treatment. The frequency of $CD137^+$ T cells was correlated with several clinical parameters, such as gender, performance status (PS), response to immunotherapy, progression-free survival (PFS), and OS. The results, also confirmed in a validation cohort, demonstrate that the $CD137^+$ cells can be used as a parameter to define the immunologic fitness of patients with cancer and highlight the potential role of the $CD137^+$ T cell subset as a biomarker to determine the success of immunotherapy.

Materials and Methods

Patients

This study enrolled patients with a confirmed diagnosis of metastatic NSCLC, RCC, HNSCC, and uveal melanoma who underwent treatment with single-agent anti-PD1 as the first or second line of treatment at the Medical Oncology Department of Policlinico Umberto I Hospital, Azienda Ospedaliera S. Andrea and Fondazione Policlinico Universitario Agostino Gemelli IRCCS between April 2017 and August 2021. The patients were divided into two cohorts: identification (66 patients) and validation (43 patients). Patients belonging to the validation cohort were mainly prospective enrolled in the last 2 years. Patients were treated according to the tumor type with nivolumab or pembrolizumab with standard dose and schedule until disease progression or unacceptable toxicity. Toxicity was reported according to Common Terminology Criteria for Adverse Events (version 4.0) and was evaluated on day 1 of every cycle until the end of treatment. Criteria of inclusion were: age > 18 years; histologically documented diagnosis of NSCLS, RCC, HNSCC, or uveal melanoma; adequate cardiac, pulmonary, renal, liver, and bone marrow function; and Eastern Cooperative Oncology Group PS scored between 0–2. Criteria of exclusion were: autoimmune disease, systemic immunosuppression, and any significant comorbidity. PS defines the functional status of a patient. Patients scored as PS = 0 are fully active, able to carry on all pre-disease performance without restriction. Patients with PS = 1 are restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature. Patients scored as PS = 2 are ambulatory and capable of all self-care, but are unable to carry out any work activities; they are up and about more than 50% of waking hours (22). PFS, OS, and clinical response rate were evaluated. PFS was defined as the time from anti-PD1 therapy starts until the first documented tumor progression or death from any cause. OS was defined as the interval between the beginning of immunotherapy (OS) or tumor diagnosis (OStot) to death for any case. The response was assessed every 4 weeks until disease progression using immune-related RECIST and classified as an incomplete and partial response, stable disease, and progressive disease. The clinical response rate was used to classify patients as responders (R; patients with a complete, partial response and stable disease) and non-responders (NR; progressors) after 6 months of therapy. The study was conducted in accordance with the Declaration of Helsinki and with good clinical practice guidelines. All patients signed informed consent. The Institutional Ethics Committee of the three involved institutions agreed to the final version of the protocol (RIF.CE: 4181).

Peripheral blood mononuclear cells and serum collection

Peripheral blood mononuclear cells (PBMCs) derived from blood samples of 109 patients with cancer before the beginning of anti-PD1 treatment and from 20 healthy donors (Policlinico Umberto I Ethics Committee Protocol, RIF.CE: 4214) were isolated by Ficoll-Hypaque gradient (Lympholite-H). Cancer patients' sera were collected using BD Vacutainer Plus Plastic Serum tubes (Becton Dickinson) after centrifugation at 1,800 rpm for 10 minutes. PBMCs and patients' sera were cryopreserved until use.

Immunophenotyping

Cell immunophenotype was carried out by cytofluorimetry using a multi-parametric analysis combining the following conjugated anti-human mAbs: anti-CD3 BV510 (clone HIT3a), anti-CD8 APC-H7 (clone SK1), anti-CD137 APC (clone 4B4-1), anti-PD1 BB700 (clone EH12.1), and anti-Ki67 BV421 (clone B56), all

purchased by Becton Dickinson. The autofluorescence of the cells and the fluorescence minus one were used as negative controls for the expression of CD137, PD1, and Ki67 molecules. Samples were analyzed by FACSCanto II flow cytometer and analyzed by FACSDiva (version 8.0.2, BD Biosciences) and FlowJo (version 10.8.8, Becton Dickinson) analysis software. The percentage of CD137$^+$, CD4$^+$CD137$^+$, CD8$^+$CD137$^+$, and CD3$^+$CD137$^+$PD1$^+$ cells was evaluated gating on CD3$^+$ cells and PBMCs. The values of CD137$^+$PD1$^+$ cells were calculated gating on CD3$^+$PD1$^+$ cells and on PBMCs. The percentage of Ki67$^+$ cells was evaluated considering the CD8$^+$CD137$^+$PD1$^+$ and CD8$^+$CD137$^-$PD1$^+$ as 100%.

IHC

IHC was performed on paraffin slides representative of each tumor with the Leica Bond 3 auto Stainer, using the primary antibodies to CD4 (4B12), CD8 (4B11), CD20 (L26), CD21 (2G9), CD23 (1B12), and CD3 (LN10), all purchased by Leica Biosystems, and CD137 (ab197942, Abcam). The signal was obtained with Bond Polymer Refine detection that contains peroxide block, post primary, polymer reagent, DAB chromogen (brown signal) and hematoxylin counterstain. The section was dehydrated and mounted. Tertiary lymphoid structures (TLS) characterization was determined on the basis of cellular marker composition (CD20, CD21, CD23, and CD3; refs. 23, 24).

Measurement of sCD137 in the serum

sCD137 was evaluated in the serum of patients with cancer belonging to the identification cohort before the beginning of immunotherapy using the CD137 (4-1BB; Soluble) Human ELISA kit (Thermo Fisher Scientific) according to the manufacturer' instructions. The concentration of sCD137 was evaluated by Multiskan FC (Thermo Fisher Scientific) at 450 nm of absorbance.

Statistical analysis

Descriptive statistics (median, range, and percentages) of clinical and biological characteristics of patients with cancer were calculated. Student *t* test was used for comparing continuous variables between cases and controls, whereas Fisher exact test or χ^2 test was used for categorical variables. The impact of clinicopathologic variables on OS and PFS was analyzed by both the univariate and multivariate analyses (UVA and MVA, respectively). With regards to UVA, patients' OStot or OS (from diagnosis or therapy, respectively) and PFS were analyzed using the Kaplan–Meier method and log-rank tests. Prognostic clinicopathologic variables deemed of potential relevance in the UVA (corresponding to a cutoff of $P < 0.10$) were included in the multivariate Cox proportional hazards regression analysis. A nomogram to predict 1- or 2-year OS probability was developed on the basis of covariates retaining a statistically significant power ($P < 0.05$) in MVA. Discrimination of nomogram was tested by Kaplan–Meier curves. A $P < 0.05$ was considered statistically significant. Statistical analyses were performed using R-package software. The calculation of the sample size was performed assuming an α-level of 0.05 and a β-level of 0.20 (power 80%). With this assumption, the required sample size is 28 cases in each group (i.e., a total of 56 cases) to detect a percentage difference of at least 1.5% between groups having a SD in the two study groups of 2%. The total number was increased to 66 patients to take into consideration patients' loss at follow-up.

Data availability statement

Data were generated by the authors and included in the article.

Results

Patients' characteristics

In this study were enrolled 109 patients with cancer; 66 patients were included in the identification cohort, while 43 were included in the validation cohort (**Table 1**). Patients belonging to the identification cohort were affected by metastatic NSCLC, RCC, HNSCC, and uveal melanoma. Half of the patients were scored as PS = 0 before the treatment with ICIs, 38% were classified as PS = 1, and only 12% of patients were defined as PS = 2. All patients were treated with an anti-PD1 agent as first-line (27%) or second-line treatment (73%). Forty-four percent of patients experienced G1 or G2 grade of toxicity and only 1 patient interrupted the anti-PD1 treatment for unacceptable toxicity. Clinical response rate was used to classify R (51%) and NR (49%) patients to anti-PD1 treatment. Thirty-two patients suffered from progressive disease, 17 patients showed stable disease, and 17 patients had a partial response after 6 months of the beginning of therapy. Patients belonging to the validation cohort suffered from metastatic NSCLC (40%) and HNSCC (60%). Eighty-one percent of the patients were scored as PS = 0,1 and most of them (88%) were

Table 1. Clinical and biological characteristics of patients with cancer.

Clinical parameters	Identification cohort (no. of patients/%)	Validation cohort (no. of patients/%)
Total	66	43
Tumor:		
NSCLC	34 (52)	17 (40)
RCC	8 (12)	0 (0)
HNSCC	10 (15)	26 (60)
UM	14 (21)	0 (0)
Age:		
<64 years	32 (48)	17 (40)
≥64 years	34 (52)	26 (60)
Gender:		
Female	27 (41)	8 (19)
Male	39 (59)	35 (61)
Performance status before ICIs:		
0	33 (50)	21 (49)
1	25 (38)	14 (32)
2	8 (12)	8 (19)
n° metastasis before ICIs:		
≤2	46 (70)	34 (21)
>2	20 (30)	9 (79)
Toxicity:		
No	37 (56)	14 (32)
Yes	29 (44)	29 (68)
Previous therapies:		
No	18 (27)	38 (88)
Yes	48 (73)	5 (12)
Response to ICIs:		
No	32 (48)	18 (41)
Yes	34 (52)	15 (35)
ND	0 (0)	10 (24)
Biological parameters	**Median (range)**	
%CD3CD137 cells	1.2 (0.1–7.4)	1.07 (0.15–3.47)
%CD3CD8CD137 cells	0.8 (0.1–2.9)	0.53 (0.076- 1.91)
%CD3CD4CD137 cells	0.2 (0.04–6.1)	0.51 (0.04–2.03)
%PD1CD137 cells	1.85 (0.2–9.9)	0.69 (0.1–2)
Soluble CD137	158 pg/mL (6–16,636)	ND

Abbreviations: ND, not determined; UM, uveal melanoma.

treated with an anti-PD1 agent as first-line treatment. Forty-one percent and 35% of the patients were classified as NR and R, respectively. The response of 24% of the patients was not evaluated because these patients did not reach the 6-month follow-up at the time of the analysis. In addition to the patients' characteristics, **Table 1** reports all the biological parameters determined in the two cohorts of patients with cancer. In particular, the median values of circulating $CD3^+CD137^+$ cells and the corresponding medians of CD4 and CD8 subpopulations are shown, such as the median value of $CD3^+CD137^+PD1^+$ cells and the median concentration of the soluble form of CD137 released in the serum.

High circulating levels of $CD137^+$ T cells are associated with a better clinical status

CD137 molecule is expressed by activated lymphocytes and by antigen-specific T cells both against viral and tumor antigens (25, 26). To investigate whether healthy donors and patients with cancer showed differences in the percentage of $CD3^+CD137^+$ cells, the levels of circulating $CD137^+$ lymphocytes were evaluated in 20 healthy donors and 66 patients with cancer belonging to the identification cohort. Results demonstrated that healthy donors expressed a significantly higher percentage of $CD3^+CD137^+$ cells (3.2% ± 1.2%, corresponding to 1.64% ± 0.8% of the PBMC fraction) than tumor patients (1.8% ± 1.6% corresponding to 0.74% ± 0.8% of the PBMC fraction; $P < 0.0007$; **Fig. 1A**). A significant difference was also found analyzing the frequency of $CD3^+$ cells that resulted higher in healthy donors compared with patients with cancer (52.2 ± 11.96 vs. 42 ± 18; $P = 0.01$; Supplementary Fig. S1A) suggesting that the level of $CD3^+$ T cells and in particular of $CD137^+$ T cells could be considered as potential parameters to monitor the wellness status of the immune system. However, when these cellular subsets were analyzed according to clinical response, R showed a percentage of $CD137^+$ T cells (2.2% ± 1.8%) significantly higher compared with the NR group (1.3 ± 1.1; $P < 0.03$; **Fig. 1B**), although the frequency of the total $CD3^+$ cells did not differ between the two groups (R vs. NR: 45.4% ± 18% vs. 37.6 ± 19.7; $P = 0.1$; Supplementary Fig. S1B). This difference resulted to be mainly ascribed to $CD8^+CD137^+$ lymphocytes, that resulted strictly correlated with $CD137^+$ T cells [$r = 0.68$; 95% confidence interval (CI) = 0.52–0.79; $P = 0.0001$], and that represented more than the 50% of the $CD137^+$ T cells (corresponding to 0.41% ± 0.39% of the total PBMC fraction). No association was found between the levels of $CD4^+CD137^+$ cell subset and the clinical response (**Fig. 1C**).

The frequency of $CD137^+$ T cells was further investigated in patients with cancer in regard to several clinical parameters, that is, PS, number of metastasis, toxicity, and previous therapies. **Figure 1D** shows the significant results obtained from this analysis. The percentage of $CD137^+$ T cells was inversely associated with PS and the number of metastasis. Patients with PS = 0–1 and with the number

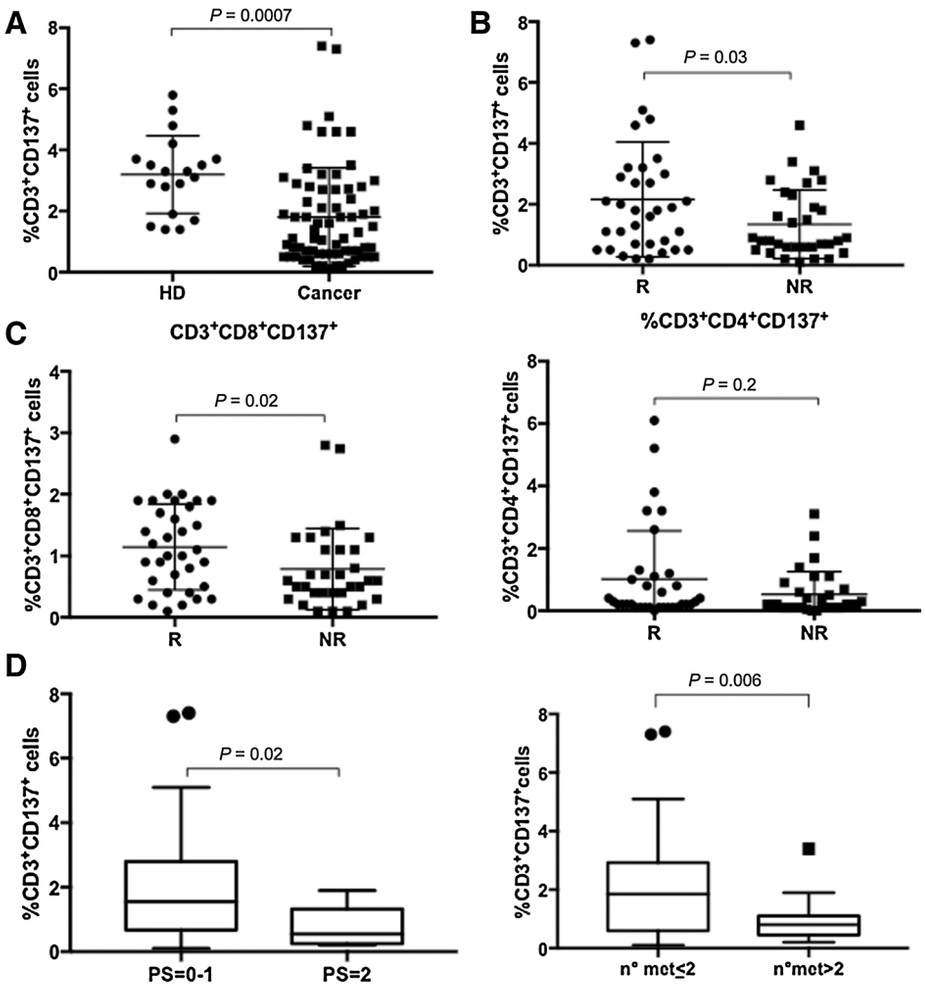

Figure 1.
The high frequency of $CD137^+$ T cells correlates with the response to treatments and with clinical parameters. **A,** The scattered dot plot represents the percentage values of circulating $CD3^+CD137^+$ cells evaluated by cytofluorimetry in 20 healthy donors (HD) and 66 patients with cancer (Cancer) ± SD. The scattered plots show the percentage of circulating $CD3^+CD137^+$ cells (**B**), $CD3^+CD8^+CD137^+$ cells and $CD3^+CD4^+CD137^+$ cells (**C**) in R and NR patients to anti-PD1 treatment ± SD. **D,** Tukey box plots represent the median distribution of $CD3^+CD137^+$ cells according to PS and number of metastasis (n°met) ± the lowest and the largest data point excluding any outlier. Unpaired Student *t* test was used to compare the different groups. $P < 0.05$ was considered significant.

of metastasis ≤ 2 had a significantly higher level of circulating CD137$^+$ T cells, suggesting that a better clinical status is correlated with high levels of CD137$^+$ T cells. The presence of toxicity and the administration of previous therapy seemed not to be associated with the frequency of CD137$^+$ T cells (Supplementary Fig. S1C and S1D).

The presence of CD137$^+$ T cells in the tumor microenvironment appears to be associated with a complete pathologic response to immunotherapy

To evaluate the expression pattern of CD137$^+$ T cells in the tumor microenvironment, we analyzed the tumor samples from 3 patients with oligometastatic NSCLC (**Fig. 2**) who underwent radical surgery to achieve the complete local control after immunotherapy treatment. **Figure 2A** shows the tumor area and surrounding lung parenchyma analyzed in these 3 patients with a detailed histologic examination of a TLS characterized as shown in the Supplementary Fig. S2. In **Fig. 2B**, the distribution of CD137$^+$, CD8$^+$, and CD4$^+$ cells in these representative TLSs is shown. Results demonstrated that the distribution of CD137$^+$ T cells in the tumor microenvironment appeared to differ among patients in relation to the response to immunologic treatment. Indeed, patient 1 showed a complete pathologic response with a high tumor regression grade. The tumor bed was characterized by proliferative fibrosis, neovascularization, and high numbers of tumor-infiltrating lymphocytes and TLSs. The representative TLS found around the tumor showed a high number of CD137$^+$ and CD8$^+$ cells suggesting the involvement of cytotoxic CD137$^+$ cells in the elimination of tumor cells. Patient 2 showed a pathologic partial response with a small nest of residual cancer associated with proliferative fibrosis, necrosis, inflammatory infiltrates with foamy macrophages, and occasional tertiary lymphoid follicles. TLS had a low infiltration of CD137$^+$ and CD8$^+$ T cells compared with patient 1. Patient 3 showed a very limited pathologic response, with extensive residual neoplasia, classified as non-major pathologic response (non-MPR). The residual tumor was characterized by the presence of sparse inflammatory infiltrates with rare TLSs. The number of both CD137$^+$ and CD8$^+$ cells in the TLS was scarce. The amount of CD4$^+$ T lymphocytes was similar in the 3 patients. Interestingly, the number of lymphocytes positive for both CD137 and CD8 within the inflammatory infiltrates was inversely related to the extent of pathologic response, while CD4$^+$ T cells seemed to not correlate with the response. Similar results were obtained analysing the tumor-infiltrating lymphocytes derived from 2 patients who suffered from cutaneous melanoma and HNSCC (Supplementary Fig. S3) who were treated with immunotherapy and chemotherapy, respectively, before

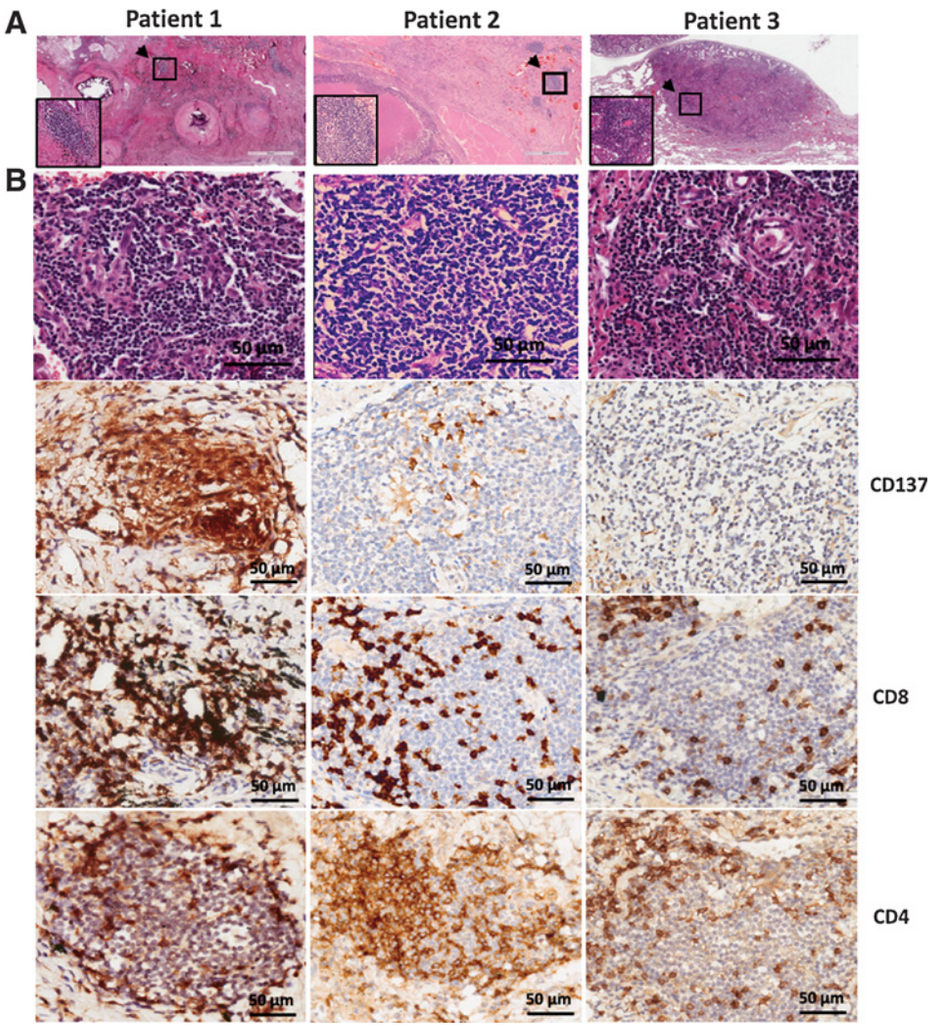

Figure 2.
Paraffine tumor slides derived from 3 patients with NSCLC with different responses to anti-PD1 treatment. **A,** Patient 1: Pathologic complete response with diffuse proliferative fibrosis, calcifications, cholesterol clefts, and intense inflammatory infiltrates with several tertiary lymphoid follicles (arrows, insert); Patient 2: pathologic partial response with a small nest of residual cancer (left) associated with proliferative fibrosis, necrosis, inflammatory infiltrates with foamy macrophages, and occasional tertiary lymphoid follicles (arrows, insert); Patient 3: absence of pathologic response: scanty interstitial inflammatory infiltrates and rare tertiary lymphoid follicles within neoplasia (arrows, insert). Original magnification 1×. **B,** Patient 1: tertiary lymphoid follicles from a lung cancer sample with pathologic complete response show a high number of lymphocytes positive for both CD137 and CD8. A small number of CD4$^+$ T lymphocytes is also present; Patient 2: tertiary lymphoid follicles from a lung cancer sample with pathologic partial response show a lower number of lymphocytes positive for both CD137 and CD8 as compared with case 1 while the amount of CD4$^+$ T lymphocytes is higher; Patient 3: tertiary lymphoid follicles from a lung cancer sample with absence of pathologic response (non-MPR) show only scanty lymphocytes positive for both CD137 and CD8 while the amount of CD4$^+$ T lymphocytes is similar to case 1. Original magnification 4×.

surgery. The patient with melanoma showed a complete response and exhibited high levels of infiltrating CD137$^+$ and CD8$^+$ T cells in the tumor microenvironment. The patient with HNSCC, with a pathologic incomplete response (non-MPR), showed a low level of CD137$^+$ and CD8$^+$ T cells in the tumor bed. These data further suggested that the presence of CD137$^+$ cells is strongly associated with tumor regression and with response to therapy independently by the type of treatment.

CD137$^+$ T cells as a predictive and prognostic biomarker for PFS and OS in anti-PD1 treated patients

Clinical and biological parameters of patients with cancer were further examined by UVA (**Fig. 3**; Supplementary Tables S1 and S2). This analysis showed that PS was a clinical parameter associated with an increased PFS (Supplementary Table S1) and OS (Supplementary Table S2). In particular, patients with PS = 0–1 had a longer PFS (PS = 0–1 vs. PS = 2: median survival 8 vs. 1.5 months) and OS (PS = 0–1 vs. PS = 2: median survival 15 vs. 3 months) than patients scored as PS = 2. Another clinical characteristic that showed a correlation with the OS was the patients' gender (females vs. males; Supplementary Table S2). Females appeared to have longer survival compared with males (females vs. males: median survival 26 vs. 7 months) after the beginning of the anti-PD1 treatment. Moreover, patients with a percentage of circulating CD3$^+$CD137$^+$ and CD3$^+$CD8$^+$CD137$^+$ cells higher than the median values (1.2% and 0.8%, respectively) showed a prolonged PFS (**Fig. 3A**) and OS (**Fig. 3B**). Also, CD3$^+$CD4$^+$CD137$^+$ cells were associated with a prolonged PFS ($P = 0.02$), but not with OS ($P = 0.05$). It is interesting to note that the overall percentage of CD8$^+$ T cells is not associated with an increase of the PFS (HR: 0.63; 95% CI: 0.32–1.09; $P = 0.093$; Supplementary Fig. S4A), demonstrating that this correlation is unique for CD3$^+$CD8$^+$CD137$^+$ cells. On the contrary, high levels of total CD8$^+$ T cells improved the OS of patients with cancer (HR: 0.53; 95% CI: 0.25–0.9; $P = 0.02$; Supplementary Fig. S4B).

The MVA revealed that a high level of CD3$^+$CD8$^+$CD137$^+$ cells was an independent prognostic factor of PFS (Supplementary Table S2) and that the female gender, PS = 0–1, and high levels of CD3$^+$CD137$^+$ cells were significantly associated with longer OS evaluated after the beginning of immunotherapy (Supplementary Table S3). These three prognostic factors were integrated into the dynamic prediction nomogram to evaluate the survival probability of

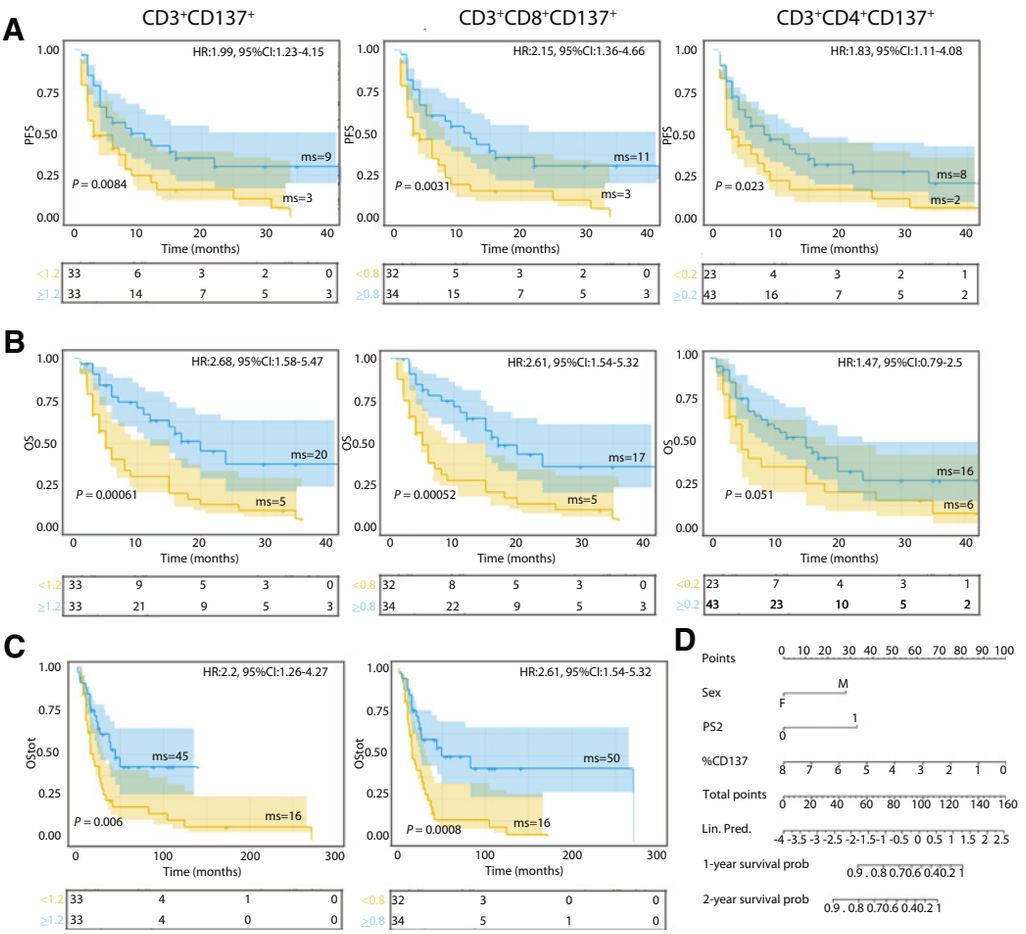

Figure 3.

The percentage of the different CD3$^+$CD137$^+$ subsets correlates with patients' survival. **A,** Kaplan–Meier curves of PFS after anti-PD1 treatment. **B,** Kaplan–Meier curves of OS evaluated at the beginning of anti-PD1 treatment. **C,** Kaplan–Meier curves of OS evaluated at diagnosis (OStot). All these curves were obtained considering the median values of the percentage of circulating CD3$^+$CD137$^+$ (1.2%), CD3$^+$CD8$^+$CD137$^+$ (0.8%), and CD3$^+$CD4$^+$CD137$^+$ (0.2%), cells. Log-rank test was used to compare survival between the two groups. ms, months. **D,** Prognostic nomogram of OS probability at 1 and 2 years in metastatic cancer patients treated with anti-PD1. $P < 0.05$ was considered significant.

patients with cancer treated with immunotherapy at 1 and 2 years (**Fig. 3D**). To use the nomogram, a vertical line needed to be delineated to the point raw to assign point values for each variable. Thereafter, the corresponding points were to be summed to obtain the total points. Finally, from the total points, a vertical line needed to be drawn to get the value of 1- or 2-year OS probability. The female sex corresponded to 0 points and PS = 2 corresponded to 36 points, while the levels of %CD137$^+$ T cells of 5 corresponded to 37.5 points. The total point of 73.5 corresponded to a 1- and 2-year OS of about 0.8 (80%) and 0.6 (60%), respectively. It is interesting to note that a poor OS was observed when patients showed lower values of CD137$^+$ cells.

The UVA of clinical and biological parameters was also performed analyzing the OS of patients with cancer from tumor diagnosis (**Fig. 3C**; Supplementary Table S3). Confirming the results described above, PS = 0–1, and high levels of CD3$^+$CD137$^+$ and CD3$^+$CD8$^+$CD137$^+$ cells characterized patients with longer survival. Moreover, at the MVA, the high levels of CD3$^+$CD137$^+$ cells became an independent protective factor for patients' survival.

Patients with cancer with high levels of CD137$^+$PD1$^+$ T cells showed a longer OS

PD1 molecule is a marker of activated T cells, but in the last years has also been identified as a marker for exhausted lymphocytes (27). Recently, it has been demonstrated that the expression of CD137 molecule plays a critical role in the discrimination of the activated and exhausted T cells identifying the CD137$^+$PD1$^+$ subset as the most functionally active T cell population (16). The PD1$^+$ T cells were therefore analyzed for the expression of CD137 molecule in our cohort of patients with cancer and in healthy donors and were correlated with clinical parameters as described previously (**Fig. 4**). The frequency of CD137$^+$PD1$^+$ T cells in healthy donors was significantly higher (7.7% ± 3.3%) compared with patients with cancer (2.7% ± 2.3%; **Fig. 4A**). Moreover, the levels of CD137$^+$PD1$^+$ T cells were inversely associated with the number of metastases: patients with a low number of metastases (<2) showed high levels of CD3$^+$CD137$^+$PD1$^+$ (**Fig. 4B**). However, when this population was analyzed in regard to clinical response to anti-PD1 treatment, no significant differences between Rs and NRs were observed (**Fig. 4C**).

Analyzing the frequency of CD3$^+$CD137$^+$PD1$^+$ in regard to PFS and OS, high levels of CD3$^+$CD137$^+$PD1$^+$ cells (>1.85%) were associated with a longer OS evaluated at tumor diagnosis (**Fig. 4D**), but not with PFS (Supplementary Fig. S4C). OS calculated from the beginning of immunotherapy showed a trend between patients with a CD3$^+$CD137$^+$PD1$^+$ frequency < 1.85% and > 1.85% (P = 0.055); however, this difference was not statistically significant (**Fig. 4E**).

Moreover, to understand the abundance of CD137 expression in the different PD1$^+$ T cell subsets and their correlation with clinical parameters, the frequency of CD8$^+$CD137$^+$PD1$^+$ and CD4$^+$CD137$^+$PD1$^+$ T cells was analyzed. The levels of CD3$^+$CD8$^+$CD137$^+$PD1$^+$ (0.43% ± 0.3%, corresponding to 0.16% ± 0.1% of the PBMC fraction) and CD3$^+$CD4$^+$CD137$^+$PD1$^+$ (0.49% ± 0.9%, corresponding to 0.2% ± 0.4% of the PBMC fraction) cells represent 44% and 63% of the total CD3$^+$CD8$^+$CD137$^+$ and CD3$^+$CD4$^+$CD137$^+$, respectively. When these cellular subsets were analyzed according to the response to anti-PD1 treatment, we observed that R patients showed increased levels of CD3$^+$CD8$^+$CD137$^+$PD1$^+$ (0.59% ± 0.4%) compared with NRs (0.34 ± 0.28; P = 0.01), while no significant differences were obtained for CD3$^+$CD4$^+$CD137$^+$PD1$^+$ T cell subpopulation (R vs. NR: 0.68% ± 1% vs. 0.34% ± 0.73%; P = 0.09; **Fig. 4F**). These two populations were also analyzed in regard to PFS and OS; however, no significant correlation with the survival was obtained (CD3$^+$CD8$^+$CD137$^+$PD1$^+$cells, PFS: HR: 1.52; 95% CI: 0.87–3.1; P = 0.12; OS: HR: 1.32; 95% CI: 0.72–2.56; P = 0.3; CD3$^+$CD4$^+$CD137$^+$PD1$^+$cells, PFS: HR: 1.3; 95% CI: 0.69–2.56; P = 0.3; OS: HR: 1.2; 95% CI: 0.68–2.38; P = 0.6).

Finally, to evaluate whether the CD137 expression was enriched in activated CD8$^+$ T cells, the activation status of this cellular subset was analyzed by marking the CD8$^+$CD137$^-$PD1$^+$ and CD8$^+$CD137$^+$PD1$^+$ T cells with the proliferation marker Ki67. As shown in **Fig. 4G**, the expression of Ki67 was significantly higher in CD8$^+$CD137$^+$PD1$^+$ T cell population compared with CD8$^+$CD137$^-$PD1$^+$(49.65% ± 16.86 vs. 38% ± 19.08, respectively; P = 0.004), demonstrating that the CD137 marker mainly identified those lymphocytes with the higher proliferation capacity.

sCD137 is associated with poor survival

Because of the negative role of sCD137 in the modulation of the immune response (15), the levels of this molecule were also analyzed in the serum of patients with cancer before the beginning of immunotherapy and were associated with survival. As expected, the sCD137 did not correlate with CD137 expressed on the plasma membrane of T cells. Indeed, no correlation of sCD137 with CD137$^+$ (r = −0.26; 95% CI: −0.53 to 0.05; P = 0.09), CD8$^+$CD137$^+$ (r = −0.2; 95% CI: −0.47–0.41; P = 0.09), and also with overall CD8$^+$ (r = 0.1; 95% CI: −0.19 to 0.41; P = 0.4) T cells was found. Patients with a serum concentration of sCD137 >158 pg/mL showed a shorter PFS and OS calculated both before the beginning of immunotherapy and at tumor diagnosis (**Fig. 5A**, **B**, and **C**, respectively), confirming the negative impact of this molecule on the clinical outcome.

Validation of the prognostic role of CD137 in a prospectively collected cohort of patients with cancer

To validate the percentage of CD137$^+$ T cells as a prognostic factor of survival, we analyzed the blood samples derived from 43 patients with cancer prospectively collected from an independent cohort of patients with metastatic cancer (see **Table 1** for the characteristics of the patients). The UVA confirmed the association of CD137$^+$ T cells (cut-off = 1.2%) with the OS (HR: 8.26; 95% CI: 1.34–12.8; P = 0.001; **Fig. 6A**), and with the OStot (HR: 6.87; 95% CI: 1.13–11.24; P = 0.02; Supplementary Fig. S5), revealing a favorable survival outcome for those patients with a percentage of CD137$^+$ T cell >1.2 before the beginning of immunotherapy. Moreover, also the frequency of CD8$^+$CD137$^+$ T cells (cut-off = 0.8%) found in the identification cohort was confirmed as prognostic factor of PFS (HR: 5.2; 95% CI: 1.02–10.59; P = 0.04; **Fig. 6B**).

Finally, the nomogram obtained in the identification cohort was used to evaluate the survival probability of cancer patients treated with immunotherapy of the validation group. The points assigned to each factor were the same used in the identification cohort (female/male: 0/28 points; PS = 0–1/PS = 2: 0/36 points; the levels of %CD137$^+$ T cells of 5 corresponded to 37.5 points). The nomogram score was assigned to each patient and plotted as a Kaplan–Meier curve (**Fig. 6C**). Patients with a score lower than the median value showed a poor survival (P = 0.01), confirming the analysis carried out in the identification cohort.

Discussion

The wellness of the immune system we believe represents a novel key point to define the success of anticancer immunotherapy. The immune profile of each individual is the result of their own immune history such as lifestyle, the encounter with pathogens, age, gender, microbiota composition, and several other factors including

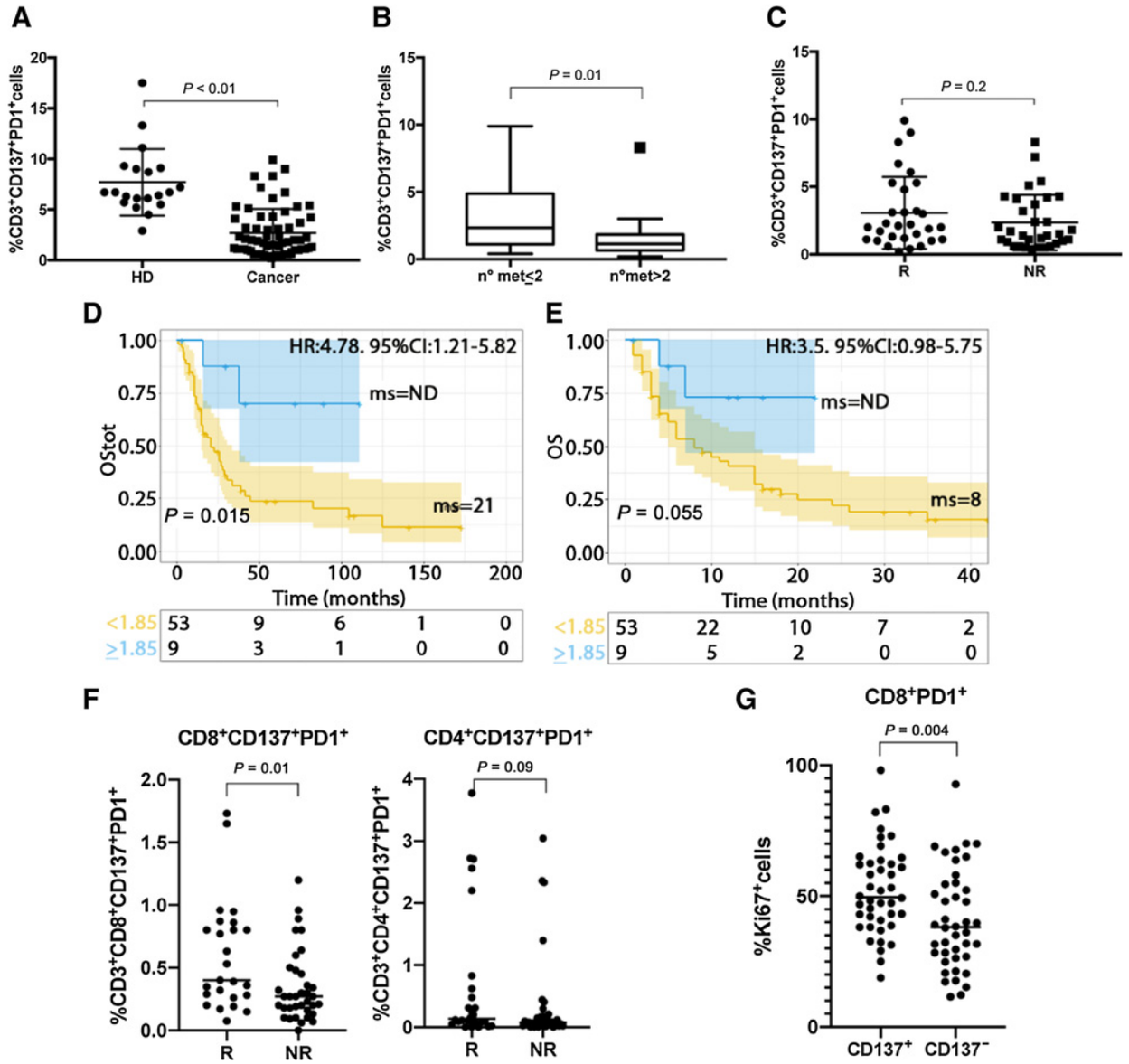

Figure 4.

High levels of CD137$^+$PD1$^+$ T cells correlate with a better clinical status and survival**. A,** The scattered dot plot represents the percentage values of circulating CD137$^+$PD1$^+$ T cells evaluated in 20 HDs and 66 patients with cancer (Cancer) ± SD by cytofluorimetry. The values of CD137$^+$PD1$^+$ cells were calculated gating on CD3$^+$PD1$^+$ cells. **B,** The Tukey box plots represent the median distribution of CD137$^+$PD1$^+$ T cells according to the number of metastasis (n°met) ± the lowest and the largest data point excluding any outliers. **C,** The scattered dot plot shows the percentage of CD137$^+$PD1$^+$ T cells in R and NR patients. Unpaired Student *t* test was used to compare the different groups. **D,** Kaplan–Meier curves of OS evaluated at diagnosis (OStot) considering the median value of the percentage of CD137$^+$PD1$^+$ T cells (1.85%). **E,** Kaplan–Meier curves of OS evaluated from the beginning of immunotherapy (OS). **F,** The scattered plots show the percentage of circulating CD3$^+$CD8$^+$CD137$^+$PD1$^+$ and CD3$^+$CD4$^+$CD137$^+$PD1$^+$ cells in R and NR patients to anti-PD1 treatment. **G,** Percentage of Ki67 expression on CD8$^+$CD137$^-$PD1$^+$ T cells and CD8$^+$CD137$^+$PD1$^+$ T cells. $P < 0.05$ was considered significant. ms, months; ND, not yet defined.

predisposing and heritable conditions (28). This complexity further increases in cancer patients. Each patient favors the expansion of specific cellular subsets concerning their disease mechanisms which influence the response to treatments. Moreover, the use of therapies that specifically target the immune system contributes to further modify the activation state of the immune cells, making the delicate balance between tumor and immune system even more complex (29). In this scenario, the identification of specific biomarkers able to define the wellness and activation state of cancer patients' immunity could be a key point to better identify those patients more prone to receive beneficial effects from immunologic treatments.

In this work, we propose the CD137$^+$ T cell subset as a driver of successful antitumor immunotherapy, demonstrating that this population could define the "quality" of the immune activation thus predicting the patients' clinical outcome independently of tumor histotype, previous therapies, as well as toxicity. Indeed, we show that

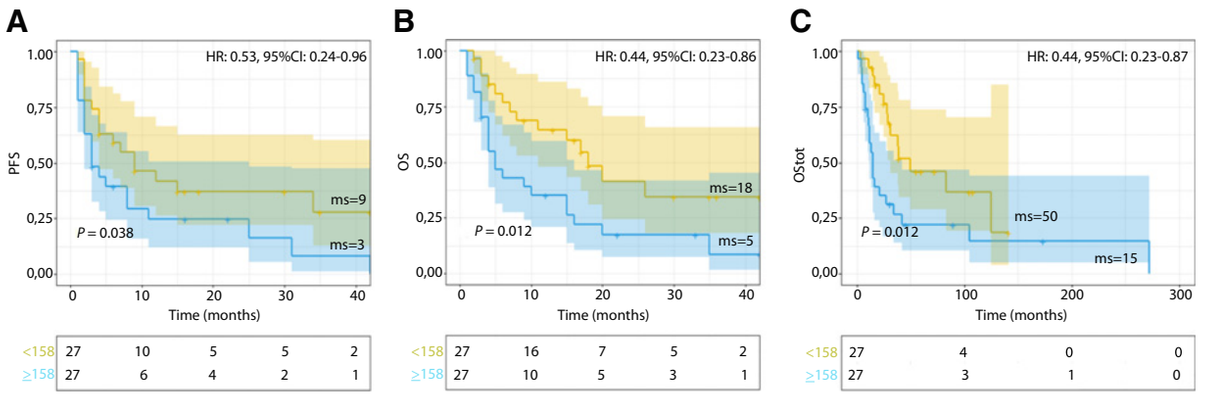

Figure 5.

A low concentration of sCD137 is correlated with a better survival. **A,** Kaplan–Meier curves of PFS after anti-PD1 treatment. **B,** Kaplan–Meier curves of OS evaluated at the beginning of anti-PD1 treatment. **C,** Kaplan–Meier curves of OS evaluated at diagnosis (OStot). All the curves were calculated considering the concentration median values of sCD137 (158 pg/mL). Log-rank tests were used to compare survival between two groups. $P < 0.05$ was considered significant. ms, months.

the frequency of CD137$^+$ T cells, but also of CD3$^+$ cells, is higher in healthy donors, and in those patients with a better clinical status, who presumably have a fully or less dysregulated active immune system. However, the CD137$^+$ T cells seem to have a critical role in the response to anti-PD1 therapy compared with CD3$^+$ cells because no differences in the frequency of CD3$^+$ cells were observed between R and NR patients. These data are consistent with patients' survival: high levels of CD137$^+$ T cells have been identified as an independent prognostic factor of survival, confirming that the expansion of this cellular subset represents a crucial point to obtain beneficial effects from immunotherapy. Interestingly, combining the levels of CD137$^+$ T cells with PS and patients' gender in a nomogram analysis, it is possible to identify the profile of the patients who will benefit from immunotherapy in terms of survival. In particular, poor OS is observed when patients show low values of CD137$^+$ cells. Moreover, the CD8$^+$CD137$^+$ subset seems to be the main T-cell population involved in this balance resulting as an independent prognostic factor of PFS. The importance of this cellular subset is further highlighted by the analysis carried out on the frequency of the overall CD8 population. Indeed, no association between the overall CD8$^+$ T cells and the PFS was found, demonstrating the unique involvement of CD8$^+$CD137$^+$ cells in the induction of an effective antitumor immune response. In this scenario, also the CD4$^+$CD137$^+$ cells influence the patients' survival, suggesting the critical role of this cellular subset in maintaining the cytotoxic response against tumor. However, despite their impact on the patients' survival, the levels of CD4$^+$CD137$^+$ T cells were not associated with the response.

In line with our results, several studies underlined the main involvement of the CD8$^+$CD137$^+$ population compared with the CD4$^+$CD137$^+$ subset. The circulating CD137$^+$ T cell subset has been identified as a biomarker to predict early relapse and clinical response in patients with mRCC (19, 30), and the maintenance of high levels of CD8$^+$CD137$^+$ T cells were also associated with the duration of the response in the same setting of patients (19). In stage III metastatic melanoma, CD137 on circulating CD8$^+$ lymphocytes correlated with the disease-free status (31). These results are supported by *in vivo* administration of anti-CD137 agonistic antibodies that promote the expansion of CD8$^+$ T cells in several diseases including cancer (32, 33).

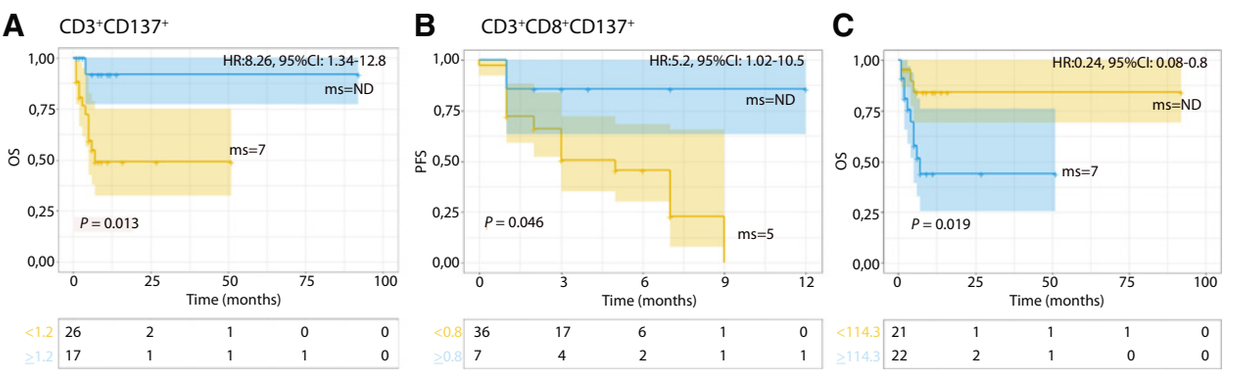

Figure 6.

CD137 was validated as prognostic factor to survival. **A,** Kaplan–Meier curve of OS after anti-PD1 treatment calculated in the validation cohort considering the median percentage of CD137 (1.2%). **B,** Kaplan–Meier curve of PFS after anti-PD1 treatment calculated in the validation cohort considering the median percentage of CD8$^+$CD137$^+$ T cells (0.8%). **C,** Kaplan–Meier curve of OS evaluated in the validation cohort using the median score of 114.3 obtained assigning to each patient belonging to the validation group the nomogram score used in the identification cohort. Log-rank tests were used to compare survival between two groups. $P < 0.05$ was considered significant. ms, months; ND, not determined.

In the tumor setting, these therapies positively correlated to clinical outcomes and have shown beneficial effects on the tumor, increasing the frequency of the antitumor specific memory T cells that confer a long-lasting antitumor immune response and protection (34). Interestingly, these therapies enhance the recruitment of specific lymphocytes in the tumor microenvironment and positively act on the immunosuppression by reducing the proliferation of regulatory T cell (Treg) and myeloid-derived suppressor cells (35, 36), making patients with cancer more prone to receive immunotherapy. All these studies confirm the hypothesis that the frequency of CD137 represents a key point to obtain an efficacious antitumor immune response. The combination of CD137 targeting drugs with other strategies targeting the immunosuppression (that also dampen the $CD137^+$ T cells) may represent innovative and successful approaches for future immunotherapies.

We then analyzed the frequency of these cells also in the tumor microenvironment, observing the distribution of $CD137^+$ T cells in the TLSs that surrounded the tumors of 3 patients with NSCLC who reached a different clinical outcome. The TLSs represent the first sites of T cell priming for the induction of an antitumor response and in patients with cancer are often associated with a good prognosis (37). Our results show that only the patient with a complete tumor response has high levels of $CD137^+$ and $CD8^+$ T cells in TLS, confirming the hypothesis that the intensity of the antitumor immune response is strictly correlated with the amount of $CD137^+$ T cells in the tumor bed. The 3 patients with NSCLC described in this work represent a *proof of concept* and strongly sustain the idea that CD137 molecule identifies the cells with a real antitumor reactivity. Several studies have analyzed the distribution of CD137 in the tumor *milieu* (18) and have identified the CD137 molecule as a biomarker to detect and isolate the full *repertoire* of tumor-specific CD8 T cells distributed in the tumor site. These infiltrating cells have become the ideal candidates for adoptive T cell therapies because they can be easily isolated and expanded, opening novel prospectives for the optimization of these therapeutic and innovative interventions (25, 38).

$CD137^+$ T cells have been also analyzed for the expression of PD1 molecule. The $CD137^+PD1^+$ and $CD8^+CD137^+PD1^+$ subsets were associated with the response to anti-PD1 therapy, and with a longer OS. Moreover, $CD8^+CD137^+PD1^+$ showed a higher proliferation capacity when compared with $CD8^+CD137^-PD1^+$ cells, demonstrating the existence of several T cell populations with different levels of activation based on the expression of CD137 and PD1 markers. Several studies demonstrated that the coexpression of these two molecules identifies those lymphocytes that exhibit a higher tumor reactivity (18) suggesting the critical role of CD137 markers in determining the activation state of T cells. In particular, in hepatocarcinoma, $CD137^+PD1^{high}$ T cells show a transcriptomic profile correlated with a T cell activation and a high proliferative capacity. In ovarian cancer, $CD137^+$-infiltrating T cells can secrete high levels of IFNγ upon stimulation independently by the expression of PD1 molecule. It is conceivable to believe that treatment with anti-PD1 drugs relies on this subset of T cells for their maximal efficacy, opening new possible combinatory strategies based on the degree of preexisting T cell activation state.

Another important parameter evaluated in this study is the concentration of the soluble form of CD137. High levels of this circulating released molecule was associated with a short PFS and OS in our cohort of patients with cancer, but no correlation was found between sCD137 and several $CD137^+$ T cell subsets. This is an immunoinhibitory molecule produced by overactivated immune cells, including Tregs, and transformed cells that allow a faster tumor progression *in vivo* (15). Under hypoxia conditions, several cancer cell lines release high levels of this molecule independently by the expression of the membrane-bound CD137, suggesting its beneficial effect for cancer survival. The cross-linking between sCD137 and CD137 agonists neutralizes the activities of both molecules decreasing the levels of sCD137 and dampening the activation capacity of the anti-CD137 antibody (39). Both mechanisms contribute to the success of CD137 agonist treatment in mouse models and should be considered for designing more effective treatments.

This study identified and validated the role of $CD137^+$ T cells as a biomarker of immune wellness able to predict the success of anticancer immunotherapy. These results can be considered of particular interest because they can be used by the oncologists to predict the clinical outcome of patients with cancer and to monitor the response to immunologic treatments. Considering the off-target effects of several drugs used in this setting of patients, further studies need to be considered to evaluate the role of $CD137^+$ T cells as a prognostic factor in those patients undergoing non-immunologic-based therapies.

Authors' Disclosures

E. Rossi reports personal fees from Novartis and MSD outside the submitted work. G. D'Amati reports personal fees from Roche outside the submitted work. P. Marchetti reports grants and personal fees from Roche, MSD, BMS, Novartis, and Pfizer; grants from AstraZeneca and Boehringer Ingelheim; and personal fees from Lilly outside the submitted work. No disclosures were reported by the other authors.

Authors' Contributions

I.G. Zizzari: Conceptualization, resources, formal analysis, supervision, funding acquisition, validation, writing–original draft, writing–review and editing. **A. Di Filippo:** Data curation, methodology, writing–review and editing. **A. Botticelli:** Data curation, supervision, methodology, writing–review and editing. **L. Strigari:** Formal analysis, writing–review and editing. **A. Pernazza:** Data curation, methodology, writing–review and editing. **E. Rullo:** Data curation, methodology, writing–review and editing. **M.G. Pignataro:** Data curation, methodology, writing–review and editing. **A. Ugolini:** Data curation, methodology, writing–review and editing. **F. Scirocchi:** Data curation, methodology, writing–review and editing. **F.R. Di Pietro:** Data curation, methodology, writing–review and editing. **E. Rossi:** Data curation, methodology, writing–review and editing. **A. Gelibter:** Data curation, methodology, writing–review and editing. **G. Schinzari:** Data curation, methodology, writing–review and editing. **G. D'Amati:** Data curation, formal analysis, methodology, writing–review and editing. **A. Rughetti:** Resources, data curation, funding acquisition, methodology, writing–review and editing. **P. Marchetti:** Supervision, writing–review and editing. **M. Nuti:** Conceptualization, resources, supervision, funding acquisition, writing–review and editing. **C. Napoletano:** Conceptualization, data curation, formal analysis, supervision, validation, methodology, writing–original draft, writing–review and editing.

Acknowledgments

This study was supported by Sapienza University of Rome (RM120172B803DB14, A. Rughetti and RP120172A7DF1B76, I.G. Zizzari) and AIRC (IG 2015 no.17432, M. Nuti). F. Scirocchi and A. Ugolini are fellows of the PhD Course "Network Oncology and Precision Medicine," Department of Experimental Medicine, Sapienza University of Rome (Rome, Italy).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received August 15, 2021; revised November 9, 2021; accepted December 28, 2021; published first January 3, 2022.

References

1. Bruni D, Angell HK, Galon J. The immune contexture and Immunoscore in cancer prognosis and therapeutic efficacy. Nat Rev Cancer 2020;20:662–80.
2. Rossi E, Schinzari G, Zizzari IG, Maiorano BA, Pagliara MM, Sammarco MG, et al. Immunological backbone of uveal melanoma: is there a rationale for immunotherapy? Cancers 2019;11:1055.
3. Ravi P, Mantia C, Su C, Sorenson K, Elhag D, Rathi N, et al. Evaluation of the safety and efficacy of immunotherapy rechallenge in patients with renal cell carcinoma. JAMA Oncol 2020;6:1606–10.
4. Brahmer JR, Reckamp KL, Baas P, Crinò L, Eberhardt WE, Poddubskaya E, et al. Nivolumab versus docetaxel in advanced squamous-cell non–small-cell lung cancer. N Engl J Med 2015;373:123–35.
5. Vinay DS, Kwon BS. 4-1BB signaling beyond T cells. Cell Mol Immunol 2011;8: 281–4.
6. Wilcox RA, Chapoval AI, Gorski KS, Otsuji M, Shin T, Flies DB, et al. Cutting edge: expression of functional CD137 receptor by dendritic cells. J Immunol 2002;168:4262–7.
7. Broll K, Richter G, Pauly S, Hofstaedter F, Schwarz H. CD137 expression in tumor vessel walls. High correlation with malignant tumors. Am J Clin Pathol 2001;115:543–9.
8. Lippert U, Zachmann K, Ferrari DM, Schwarz H, Brunner E, Mahbub-Ul Latif AHM, et al. CD137 ligand reverse signaling has multiple functions in human dendritic cells during an adaptive immune response. Eur J Immunol 2008;38: 1024–32.
9. Cannons JL, Lau P, Ghumman B, DeBenedette MA, Yagita H, Okumura K, et al. 4–1BB ligand induces cell division, sustains survival, and enhances effector function of CD4 and CD8 T cells with similar efficacy. J Immunol 2001;167: 1313–24.
10. Vinay DS, Kwon BS. Genes, transcripts, and proteins of CD137 receptor and ligand. In: Chen L, editor. CD137 pathway: immunology and diseases. New York: Springer; p.20061–14.
11. Menk AV, Scharping NE, Rivadeneira DB, Calderon MJ, Watson MJ, Dunstane D, et al. 4-1BB costimulation induces T cell mitochondrial function and biogenesis enabling cancer immunotherapeutic responses. J Exp Med 2018; 215:1091–100.
12. Aznar MA, Labiano S, Diaz-Lagares A, Molina C, Garasa S, Azpilikueta A, et al. CD137 (4-1BB) costimulation modifies DNA methylation in CD8(+) T cell-relevant genes. Cancer Immunol Res 2018;6:69–78.
13. Shao Z, Schwarz H. CD137 ligand, a member of the tumor necrosis factor family, regulates immune responses via reverse signal transduction. J Leukoc Biol 2011; 89:21–9.
14. Kim JD, Kim CH, Kwon BS. Regulation of mouse 4-1BB expression:multiple promoter usages and a splice variant. Mol Cell 2011;31:141–9;.
15. Luu K, Shao Z, Schwarz HJ. The relevance of soluble CD137 in the regulation of immune responses and for immunotherapeutic intervention. J Leukoc Biol 2020; 107:731–8.
16. Ye Q, Song DG, Poussin M, Yamamoto T, Best A, Chunsheng L, et al. CD137 accurately identifies and enriches for naturally occurring tumor reactive T cells in tumor. Clin Cancer Res 2014;20:44–55.
17. Saoulli K, Lee SY, Cannons JL, Yeh WC, Santana A, Goldstein MD, et al. CD28-independent, TRAF2-dependent costimulation of resting T cells by 4-1BB ligand. J Exp Med 1998;187:1849–62.
18. Kim HD, Park S, Jeong S, Lee YJ, Lee H, Kim CG, et al. Delineates distinct activation status of exhausted tumor-infiltrating CD8 + T cells in hepatocellular carcinoma. Hepatology 2020;71:955–71.
19. Zizzari IG, Napoletano C, Botticelli A, Caponnetto S, Calabrò F, Gelibter A, et al. TK inhibitor pazopanib primes DCs by downregulation of the β-catenin pathway. Cancer Immunol Res 2018;6:711–22.
20. Zizzari IG, Napoletano C, Di Filippo A, Botticelli A, Gelibter A, Calabrò F, et al. Exploratory pilot study of circulating biomarkers in metastatic renal cell carcinoma. Cancers 2020;12:2620.
21. Ugolini A, Zizzari IG, Ceccarelli F, Botticelli A, Colasanti T, Strigari L, et al. IgM-Rheumatoid factor confers primary resistance to anti-PD-1 immunotherapies in NSCLC patients by reducing CD137+T-cells. EBioMedicine 2020;62:103098.
22. Oken M, Creech R, Tormey D, Horton J, Davis TE, McFadden ET, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 1982;5:649–55.
23. Werner F, Wagner C, Simon M, Glatz K, Mertz KD, Laubli H, et al. A standardized analysis of tertiary lymphoid structures in human melanoma: disease progression- and tumor site-associated changes with germinal center alteration. Front Immunol 2021;12:675146.
24. Goc J, Germain C, Vo-Bourgais TK, Lupo A, Klein C, Knockaert S, et al. Dendritic cells in tumor-associated tertiary lymphoid structures signal a Th1 cytotoxic immune contexture and license the positive prognostic value of infiltrating CD8+ T cells. Cancer Res 2014;74:705–15.
25. Wehler TC, Karg M, Distler E, Konur A, Nonn M, Meyer RG, et al. Rapid identification and sorting of viable virus-reactive CD4(+) and CD8(+) T cells based on antigen-triggered CD137 expression. J Immunol Methods 2008;339: 23–37.
26. Wolfl M, Kuball J, Ho WY, Nguyen H, Manley TJ, Bleakley M, et al. Activation-induced expression of CD137 permits detection, isolation, and expansion of the full repertoire of CD8+ T cells responding to antigen without requiring knowledge of epitope specificities. Blood 2007;110:201–10.
27. Sharpe AH, Pauken KE. The diverse functions of the PD1 inhibitory pathway. Nat Rev Immunol 2018;18:153–67.
28. Brodin P, Davis MM. Human immune system variation. Nat Rev Immunol 2017; 17:21–9.
29. Araujo B, de Lima V, Hansen M, Spanggaard I, Rohrberg K, Hadrup SR, et al. Immune cell profiling of peripheral blood as signature for response during checkpoint inhibition across cancer types. Front Oncol 2021;11:558248.
30. Giraldo NA, Becht E, Vano Y, Petitprez F, Lacroix L, Validire P, et al. Tumor-infiltrating and peripheral blood T-cell immunophenotypes predict early relapse in localized clear cell renal cell carcinoma. Clin Cancer Res 2017;23:4416–28.
31. Jacquelot N, Roberti MP, Enot DP, Rusakiewicz S, Ternes N, Jegou S, et al. Predictors of responses to immune checkpoint blockade in advanced melanoma. Nat Commun 2017;8:592.
32. Agarwal A, Newell KA. The role of positive costimulatory molecules in transplantation and tolerance. Curr Opin Organ Transplant 2008;13:366–72.
33. Vinay DS, Kwon BS. 4-1BB (CD137), an inducible costimulatory receptor, as a specific target for cancer therapy. BMB Rep 2014;47:122–9.
34. Narazaki H, Zhu Y, Luo L, Zhu G, Chen L. CD137 agonist antibody prevents cancer recurrence: contribution of CD137 on both hematopoietic and non-hematopoietic cells. Blood 2010;115:1941–8.
35. Houot R, Goldstein MJ, Kohrt HE, Myklebust JH, Alizadeh AA, Lin JT, et al. Therapeutic effect of CD137 immunomodulation in lymphoma and its enhancement by Treg depletion. Blood 2009;114:3431–8.
36. Gauttier V, Judor JP, Le Guen V, Cany J, Ferry N, Conchon S. Agonistic anti-CD137 antibody treatment leads to antitumor response in mice with liver cancer. Int J Cancer 2014;135:2857–67.
37. Di Caro G, Castino GF, Bergomas F, Cortese N, Chiriva-Internati M, Grizzi F, et al. Tertiary lymphoid tissue in the tumor microenvironment: from its occurrence to immunotherapeutic implications. Int Rev Immunol 2015;34: 123–33.
38. Seliktar-Ofir S, Merhavi-Shoham E, Itzhaki O, Yunger S, Markel G, Schachter J, et al. Selection of shared and neoantigen-reactive T cells for adoptive cell therapy based on CD137 separation. Front Immunol 2017;8:1211.
39. Palazón A, Martínez-Forero I, Teijeira A, Morales-Kastresana A, Alfaro C, Sanmamed MF, et al. The HIF-1α hypoxia response in tumor-infiltrating T lymphocytes induces functional CD137 (4-1BB) for immunotherapy. Cancer Discov 2012;2:608–23.