Combining Nivolumab and Ipilimumab with Infliximab or Certolizumab in Patients with Advanced Melanoma: First Results of a Phase Ib Clinical Trial

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ABSTRACT

Purpose: TNF blockers can be used to manage gastrointestinal inflammatory side effects following nivolumab and/or ipilimumab treatment in patients with advanced melanoma. Our preclinical data showed that anti-TNF could promote the efficacy of immune checkpoint inhibitors.

Patients and Methods: TICIMEL (NTC03293784) is an open-label, two-arm phase Ib clinical trial. Fourteen patients with advanced and/or metastatic melanoma (stage IIIc/IV) were enrolled. Patients were treated with nivolumab (1 mg/kg) and ipilimumab (3 mg/kg) combined to infliximab (5 mg/kg, $N = 6$) or certolizumab (400/200 mg, $N = 8$). The primary endpoint was safety and the secondary endpoint was antitumor activity. Adverse events (AEs) were graded according to the NCI Common Terminology Criteria for Adverse Events and response was assessed following RECIST 1.1.

Results: Only one dose-limiting toxicity was observed in the infliximab cohort. The two different combinations were found to be safe. We observed lower treatment-related AEs with infliximab as compared with certolizumab. In the certolizumab cohort, one patient was not evaluable for response. In this cohort, four of eight patients exhibited hepatobiliary disorders and seven of seven evaluable patients achieved objective response including four complete responses (CRs) and three partial responses (PRs). In the infliximab cohort, we observed one CR, two PRs, and three progressive diseases. Signs of activation and maturation of systemic T-cell responses were seen in patients from both cohorts.

Conclusions: Our results show that both combinations are safe in human and provide clinical and biological activities. The high response rate in the certolizumab-treated patient cohort deserves further investigations.

Introduction

Immune checkpoint inhibitors (ICIs) such as anti–CTLA-4 (ipilimumab) and anti–PD-1 (nivolumab), given as single agents or in combination, improve progression-free survival (PFS) and overall survival (OS) in patients with unresectable locally advanced and/or metastatic melanoma (1–4). The median PFS with the combination of anti–PD-1 and anti–CTLA-4 is 11.5 months while the 5-year PFS rate is 36% and the 5-year OS rate is 52% (2). Although this treatment has achieved unprecedented therapeutic effects, depending on the treatment regimen, almost 40%–60% of patients are nonprimary responders and 20% of responders relapse within 3 years following treatment initiation. This highlights the need to improve therapeutic efficacy in patients with unresectable locally advanced and/or metastatic melanoma.

ICIs have a specific tolerability profile, as illustrated by the very complex adverse event (AE) pattern of the anti–PD-1/anti–CTLA-4 combined therapy. Indeed, 96% of patients in the CheckMate 067 study and treated with ipilimumab (3 mg/kg) plus nivolumab (1 mg/kg) experienced at least one AE. Among those, 59% developed treatment-related grade 3–4 AEs (2). A large majority of AEs are of immune nature and mimic autoimmune and/or autoinflammatory disorders. Management of AEs is now codified and requires corticosteroids and immune suppressors including anti-TNF blocking antibodies (5).

TNF is produced by cancer and/or stromal cells and has been extensively described to favor the establishment of a proinflammatory microenvironment thus promoting immune regulatory responses, enhancing cancer cell proliferation, tumor angiogenesis, and metastasis (6, 7). We have previously shown in preclinical studies that TNF deficiency/blockade promotes tumor regressions and prevents anti–PD-1–induced tumor-infiltrating T-lymphocyte death (8–10). Another study also showed that treating mice with TNF inhibitors concomitantly with anti–CTLA-4 and anti–PD-1 not only improves ICI efficacy, but also reduces colitis and hepatitis in melanoma and colon cancer models (11).

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

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Translational Relevance

Immune checkpoint inhibitors (ICIs) are currently administered as a first-line treatment in patients with advanced melanoma. Anti-TNFα blocking antibodies are used for the management of colitis in the context of ICI-based therapy. Whereas the consequences of anti-TNF on antitumor immune responses have not been evaluated, the impact on clinical outcome in retrospective studies is a matter of debate. In a phase Ib clinical trial, we assessed the safety of combining nivolumab and ipilimumab to anti-TNF in patients with melanoma. Anti-TNF, certolizumab or infliximab, were administered in two different cohorts. Both triple combinations were safe with the occurrence of manageable immune-related adverse events and associated with increased systemic Th1-related immune responses. The certolizumab cohort displayed more adverse events and better response rates than the infliximab cohort. Our study lays the ground for further trials aiming at assessing the clinical efficacy of the combination.

In patients with melanoma, the use of infliximab for the management of ICI-induced colitis and its impact on therapeutic responses is a matter of debate (12–15). Three studies suggest that, while efficiently resolving ICI-induced colitis, infliximab does not seem to impede response to treatment (12, 13, 15). However, the retrospective analysis of a large cohort of patients with unresectable locally advanced melanoma and treated with ICIs suggests that management of AEs using a combination of infliximab and corticosteroid is associated with shorter OS as compared with corticosteroids alone (14).

We conducted a phase Ib clinical trial to evaluate for the first time the safety of combining anti–CTLA-4 (ipilimumab) and anti–PD-1 (nivolumab) to anti-TNF (infliximab or certolizumab) for the treatment of patients with unresectable locally advanced and/or metastatic melanoma. We here report the results collected on 14 patients enrolled during the first part of this study on safety, tolerability, efficacy, and pharmacodynamics of both triple combinations.

Patients and Methods

Study design and participants

TICIMEL is an open-label, nonrandomized, phase Ib clinical trial to assess the safety, tolerability, and antitumor activity of anti-TNF (certolizumab or infliximab) in combination with ipilimumab and nivolumab in patients with unresectable locally advanced and/or metastatic melanoma. The study, conducted at the Institut Universitaire du Cancer de Toulouse - Oncopole (IUCT-O; Toulouse, France), was composed of a safety phase with two parallel cohorts (ipilimumab/nivolumab/certolizumab and ipilimumab/nivolumab/infliximab) and an expansion phase. We report here the results collected during the first part of the study.

Eligible patients had histologically proven stage IIIc–IV unresectable locally advanced and/or metastatic melanoma with documented BRAFV600 status; were ages > 18 years and had an Eastern Oncology Group Performance status (ECOG) of 0 or 1. Key exclusion criteria were uveal melanoma and active intracranial disease. Additional inclusion and exclusion criteria are summarized in the TICIMEL protocol p22. All patients provided written, informed consent before study entry. The trial protocol is available online and was reviewed and approved by the French committee for the protection of persons and the French drug agency (Agence Nationale de Sécurité du Médicament; date of approval August 4, 2017 EUDRACT 2016-005139-34). All study procedures were carried out in accordance to the International Council for Harmonization tripartite guideline on good clinical practice (Helsinki declaration) and approved by the French committee for the protection of persons (Comité de protection des personnes Nord Ouest III). An independent data monitoring committee (IDMC) monitored and evaluated data from the study. This study is registered under ClinicalTrials.gov, number NCT03293784.

Procedures

Patients and treatments

After giving their signed and informed consent, patients were alternatively assigned to one of two parallel cohorts: ipilimumab+nivolumab+certolizumab (certolizumab cohort) or ipilimumab+nivolumab+infliximab (infliximab cohort). All patients were naïve of any previous line of treatment (including adjuvant therapy with anti–PD-1 or with BRAFV600+MEK inhibitor; adjuvant therapy with IFN was allowed). In each cohort, new enrolments were allowed once a given patient had completed a 4-week observation period spanning over the first cycle of treatment. Treatment schedule was published previously (16). Briefly, all patients received 1 mg/kg of nivolumab every 3 weeks plus 3 mg/kg of ipilimumab every 3 weeks for four doses, followed by 3 mg/kg of nivolumab every 2 weeks for cycle 5 and beyond. Patients enrolled in the certolizumab cohort, received 400 mg subcutaneous certolizumab every 3 weeks for three doses; then 200 mg every 2 weeks for cycle 4 and beyond. Patients assigned to the infliximab cohort received 5 mg/kg of infliximab every 3 weeks for three doses, followed by 5 mg/kg of infliximab every 8 weeks.

Tumor responses were assessed at baseline and every 12 weeks for 2 years or until disease progression, whatever occurred first, by following the RECIST 1.1 using CT scan or MRI (17). AEs were graded according to the NCI Common Terminology Criteria for AE version V4.03 and assigned as related or unrelated to study treatment by the investigator. Complete methods are available in the study protocol.

Ancillary analyses

Flow cytometry on fresh blood: A total of 200 μL of fresh blood (collected on EDTA at week 0 and week 6) were incubated with antibodies directed against markers of T-cell maturation as well as markers allowing for the identification of Th-related subpopulations for 30 minutes (see Supplementary Table S12). Red blood cells were then lysed using the BD Pharm Lyse 10× buffer (BD Biosciences). Remaining immune cells were stained for 30 minutes in a PBS solution containing a fixable viability dye (Zombie NIR, BioLegend) and fixed for 10 minutes in a 2% formal saline solution. Samples were acquired using an LSR-Fortessa X-20 cytometer (BD Biosciences). Data have been analyzed using the FlowJo 10 software. For one patient from the infliximab cohort, staining for the T-cell maturation panel had to be made on peripheral blood mononuclear cells (PBMCs) for the W6 time point.

Flow cytometry on PBMCs: Analyses have been performed on frozen PBMCs prepared from fresh blood of patients enrolled in this clinical trial and obtained at week 0 and week 6 following treatment initiation. Both time points (W0 and W6) for each patient were assessed during the same experiment. Cells were stained for 30 minutes at 4°C with viability dye and antibodies directed against membrane markers for T-cell subpopulations and activation or monocyte subpopulations (see Supplementary Table S12). For Ki67 staining of T-cell subpopulations, cells were fixed and permeabilized using the "FoxP3 fixation/

permeabilization buffer" (eBioscience, Thermo Fisher Scientific) for 30 minutes at 4°C. Cells were then washed twice in 1× permeabilization buffer (eBioscience, Thermo Fisher Scientific) and incubated for 30 minutes at 4°C with antibodies directed against human Ki67. For the analysis of monocytes, following antibody staining, cells were washed and fixed for 10 minutes in 2% formal saline solution before acquisition. Samples were acquired using an LSR-Fortessa X-20 cytometer (BD Biosciences). Data have been analysed using the FlowJo 10 software.

Plasma cytokine assessment: Plasmas were prepared within 1 hour following collection of patients' blood and frozen at −80°C. IFNγ, TNF, IL2, and IL6 levels were assessed on undiluted plasma by Evotec using the Mesoscale technology according to manufacturer's recommendations.

Ex vivo *assessment of cytokine production:* This assay has been performed on frozen PBMCs prepared from fresh blood of patients enrolled in this clinical trial and obtained at week 0 and week 6 following treatment initiation. CD4 and CD8 T cells were sequentially purified from PBMCs using CD4 and CD8 microbeads allowing for positive selection as well as MS separation columns from Miltenyi Biotec. Purification was performed according to manufacturer's recommendations. CD4 and CD8 T cells were then allowed for recovery overnight in RPMI (Gibco) culture medium containing 10% decomplemented A/B serum (Institut de Biotechnologies Jacques Boy, Reims, France), 1 mmol/L sodium pyruvate (Sigma-Aldrich), 100 U penicillin, 100 μg/mL streptomycin (Sigma-Aldrich), 100 U/mL human recombinant IL2, 10 μmol/L HEPES solution (Gibco), and 1× nonessential amino acid (MEM, Gibco). The next morning cells were restimulated with 1× "Cell stimulation cocktail" (eBioscience) for 4 hours at 37°C. After the first hour of incubation 1× of "protein transport inhibitor cocktail" (eBioscience) was added to the culture medium for the remaining 3 hours. Appropriate controls with cells incubated in the presence of the "Protein transport inhibitor" solution alone, were included to each experiment. Both time points (W0 and W6) for each patient were assessed during the same experiment. Cells were then stained for 30 minutes at 4°C with a viability dye and antibodies directed against membrane markers for T-cell subpopulations and activation (see Supplementary Table S12). Cells were fixed and permeabilised using the "FoxP3 fixation/permeabilisation buffer" (eBioscience, Thermo Fisher Scientific) for 30 minutes at 4°C. Cells were then washed twice in 1× permeabilization buffer (eBioscience, Thermo Fisher Scientific) and incubated for 30 minutes at 4°C with antibodies directed against human IFNγ and TNF diluted in 1× permeabilization buffer (see Supplementary Table S12). Samples were acquired using an LSR-Fortessa X-20 cytometer (BD Biosciences). Data have been analyzed using the FlowJo 10 software.

Outcomes

The primary endpoint was the occurrence during the first 12 weeks of dose-limiting toxicities (DLTs; study protocol p32). The initial DLT evaluable population was defined by patients who received at least three doses of nivolumab and ipilimumab and at least two doses of anti-TNF. On the basis of recommendations from the IDMC and in agreement with previous studies (18–20), the required number of doses for DLT evaluation was amended to at least two doses of nivolumab, ipilimumab, and anti-TNF (as formally approved on March 2, 2019).

Secondary endpoints were antitumor activities, including the proportion of patients achieving an objective response (a complete response or partial response, according to RECIST version 1.1) and PFS.

Statistical analyses

During the safety phase, a similar methodology was used to evaluate safety of the association of nivolumab and ipilimumab and each anti-TNF. For a given anti-TNF, an initial cohort of three patients was planned to receive the association. If one DLT or less was observed, an additional cohort of three patients was allowed. The given association was considered as safe if one patient or less among six presented with a DLT.

Analyses were done in the following populations: (i) the safety set, which included all patients who received at least one dose of infliximab or certolizumab; with the exception of DLT, which were analyzed in the DLT analysis set; (ii) the activity evaluable set, which included all patients in the safety analysis set who had measurable disease at baseline and had at least one postbaseline tumor assessment.

Descriptive statistics were used to summarize demographics, baseline characteristics, AEs, safety parameters, and response data, including the primary and secondary outcomes. Paired continuous data were compared using the Wilcoxon matched-pairs signed-rank test. All statistical tests were two sided and $P < 0.05$ were considered significant. All statistical analyses were done with STATA software version 16.

Role of the funding source

Claudius Regaud Institute (ICR) and Cancer Research Center of Toulouse (CRCT) designed the TICIMEL clinical trial. ICR and CRCT wrote the TICIMEL protocol, which has been reviewed and approved by BMS. BMS has funded the clinical trial and part of the ancillary study. Cancéropôle Grand Sud-Ouest (GSO), IUCT-O, and Association pour la Recherche sur le Cancer (ARC) have funded part of the ancillary study. Fondation Toulouse Cancer Santé (FTCS) has funded the salary support of M. Virazels and S. Brayer. Fondation pour la Recherche Médicale (FRM) has funded the salary support of J. Milhès. Collection, analysis, and interpretation of data have been jointly assessed by ICR and CRCT. Decision to submit the paper for publication has been jointly taken by ICR, CRCT, and the co-senior authors. The corresponding authors confirm that they had full access to all the data in the study and share the final responsibility for the decision to submit for publication.

Protocol

The full protocol of this trial is available.

Data sharing

The anonymized derived data from this study that underlie the results reported in this article will be made available beginning 12 months and ending 5 years after this article's publication, to any investigators who sign a data access agreement and provide a methodologically sound proposal.

Results

Between January 2018 and September 2019, 14 patients with metastatic melanoma were enrolled, eight in the certolizumab cohort and six in the infliximab cohort. Patients' demographics and baseline characteristics are provided in **Table 1**. The median duration follow-up was 13.3 months [95% confidence interval (CI), 2.9–19.9].

Patients and treatments

At the cut-off date (December 6, 2019), one patient from the infliximab cohort was still receiving the study treatment. In this latter cohort, treatment discontinuations were related to disease progression [$N = 3$ (50%)] and toxicity [$N = 2$ (33.3%)]. In the certolizumab cohort, one patient (12.5%) was taken off treatment due to disease

Table 1. Patients demographic and baseline characteristics.

	Certolizumab *N* (%)	Infliximab *N* (%)
Sex ($N = 14$)		
Male	5 (62.5)	5 (83.3)
Female	3 (37.5)	1 (16.7)
Age at inclusion ($N = 14$)		
Median	49.5	61
Range	(33:73)	(50:75)
ECOG Performance status ($N = 14$)		
ECOG 0	7 (87.5)	4 (66.7)
ECOG 1	1 (12.5)	2 (33.3)
LDH (U/L) ($N = 14$)		
Normal	2 (25)	3 (50)
ANCS	6 (75)	3 (50)
AJCC stage at screening ($N = 14$)		
Stage IIIc	2 (25)	2 (33)
Stage IV	6 (75)	4 (66.7)
Number of metastatic sites (if stage IV at screening) ($N = 10$)		
1 site	1 (16.7)	0 (0)
2 sites	2 (33.3)	2 (50)
3 sites	2 (33.3)	2 (50)
5 sites	1 (16.7)	0 (0)
BRAF V600 ($N = 14$)		
Mutated	5 (62.5)	1 (16.7)
NRAS ($N = 13$)		
Mutated	1 (14.3)	1 (16.7)
Missing	1	0
Prior adjuvant therapy ($N = 14$)		
No	8 (100)	5 (83.3)
Yes[a]	0 (0)	1 (16.7)

Abbreviations: AJCC, American Joint Committee on Cancer; ANCS, abnormal not clinically significant; LDH, lactate dehydrogenase.
[a]Infliximab cohort: One patient received IFNα 10 years prior to inclusion.

progression; however, the main cause for therapy discontinuation was the occurrence of AEs [$N = 6$ (75%)].

In the certolizumab cohort, the median duration of treatment was 33 days (range, 21–113), with patients receiving a median number of 2.5 cycles of treatment [nivolumab (range, 2–6), ipilimumab (range, 2–4), certolizumab (range, 2–5)]. In the infliximab cohort, the median duration of treatment was 64 days (range, 45–155) with patients receiving a median number of four cycles of nivolumab [range (3–10+); + ongoing treatment] and ipilimumab [range (3–4+)] as well as three cycles of infliximab [range (3–5+); Supplementary Table S1].

Safety assessment

In the certolizumab cohort, one patient, who developed a clinically threatening course of melanoma, received BRAF and MEK inhibitors before the end of the DLT evaluation period. This patient was considered not to be evaluable for DLT and antitumor activity. No DLT was observed in seven other patients enrolled the certolizumab cohort. In the infliximab cohort, only one patient developed a DLT defined as an alteration of the left ventricular ejection fraction during the course of a lung infection.

Treatment-related AEs of any grade occurred in 13 (92.9%) of 14 patients, representing seven (87.5%) and six (100%) patients from the certolizumab and infliximab cohorts, respectively. The median number of treatment-related AEs per patient was 11 (range, 9–18) and three (range, 2–14) in the certolizumab and infliximab cohorts, respectively. Moreover, nine (64.3%) patients experienced at least one grade 3–4 treatment-related AE [certolizumab cohort $N = 6$ (75%), infliximab cohort $N = 3$ (50%)], mainly hepatobiliary [$N = 4$ (28.6%)] and gastrointestinal disorders [$N = 4$ (28.6%)'; **Table 2**]. Of note, two patients from the certolizumab cohort (hepatic cytolysis and macrophage activation syndrome) and one patient from the infliximab cohort (hepatic cytolysis) experienced grade 4 AEs, with the two hepatic cytolysis considered as related to treatment (Supplementary Tables S2 and S3). Moreover, nine (64.3%) patients experienced serious AEs related to treatment. Both these phenomena were observed more frequently upon certolizumab as compared with infliximab treatment (**Table 2**; Supplementary Table S2). Regardless of their grade and severity, the main treatment-related toxicities observed in

Table 2. Treatment-related AEs.

	Total $N = 14$		Certolizumab cohort $N = 8$		Infliximab cohort $N = 6$	
	All *N* (%)	Grade 3–4 *N* (%)	All *N* (%)	Grade 3–4 *N* (%)	All *N* (%)	Grade 3–4 *N* (%)
At least one treatment-related AE	13 (92.9)	9 (64.3)	7 (87.5)	6 (75)	6 (100)	3 (50)
General disorders and administration site conditions	10 (71.4)	0 (0)	7 (87.5)	0 (0)	3 (50)	0 (0)
Gastrointestinal disorders	9 (64.3)	4 (28.6)	4 (50)	3 (37.5)	5 (83.3)	1 (16.7)
Skin and subcutaneous tissue disorders	9 (64.3)	1 (7.1)	6 (75)	1 (12.5)	3 (50)	0 (0)
Hepatobiliary disorders	6 (42.9)	4 (28.6)	4 (50)	3 (37.5)	2 (33)	1 (16.7)
ALAT	2 (14.3)	1 (7.1)	2 (25)	1 (12.5)	0 (0)	0 (0)
ASAT	2 (14.3)	2 (14.3)	1 (12.5)	1 (12.5)	1 (16.7)	1 (16.7)
Endocrine disorders	4 (28.6)	0 (0)	3 (37.5)	0 (0)	1 (16.7)	0 (0)
Nervous system disorders	4 (28.6)	0 (0)	3 (37.5)	0 (0)	1 (16.7)	0 (0)
Metabolism and nutrition disorders	3 (21.4)	1 (7.1)	3 (37.5)	1 (12.5)	0 (0)	0 (0)
Respiratory, thoracic, and mediastinal disorders	3 (21.4)	0 (0)	2 (25)	0 (0)	1 (16.7)	0 (0)
Musculoskeletal and connective tissue disorders	2 (14.3)	0 (0)	2 (25)	0 (0)	0 (0)	0 (0)
Vascular disorders	2 (14.3)	0 (0)	1 (12.5)	0 (0)	1 (16.7)	0 (0)
Blood and lymphatic system disorders	1 (7.1)	0 (0)	1 (12.5)	0 (0)	0 (0)	0 (0)
Cardiac disorders	1 (7.1)	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)
Psychiatric disorders	1 (7.1)	0 (0)	1 (12.5)	0 (0)	0 (0)	0 (0)

Abbreviations: ALAT, alanine aminotransferases; ASAT, aspartate aminotransferases.

both cohorts included general disorders [fatigue $N = 5$ (35.7%), injection site reaction $N = 5$ (35.7%)], skin and cutaneous disorders [pruritus $N = 6$ (42.9%), maculo-papular rash $N = 5$ (35.7%)] as well as AEs affecting the gastrointestinal system [diarrhea $N = 6$ (42.9%), nausea $N = 4$ (28.6%), colitis $N = 2$ (14.3%; Supplementary Table S2]. A high proportion of patients [$N = 4$ (50%)] in the certolizumab cohort experienced AEs affecting the hepatobiliary system ($N = 10$ events, nine events related to treatment; **Table 2**; Supplementary Tables S2 and S3). Overall, the number of patients displaying at least one treatment-related AE was lower in the infliximab cohort than in the certolizumab cohort with the exception of gastrointestinal disorders (**Fig. 1**). No death was reported during this first study period.

Efficacy assessment

Figure 2A–C illustrate the pattern and duration of responses in each cohort. In the certolizumab cohort, six patients achieved an objective response (OR) according to RECIST (complete response: $N = 3$, partial response: $N = 3$) and one additional patient not evaluable according to RECIST exhibited a complete response. Four patients were still responders after 1 year of follow-up. One patient, who depicted an OR at week 12 progressed at week 36 with development of active intracranial disease. In the infliximab cohort, three of six (50%) patients exhibited an OR according to RECIST, including one patient still responder after 1 year of follow-up. Three patients displayed a PD at week 12 (**Fig. 2A–D;** Supplementary Tables S4 and S5).

Assessment of systemic T-cell responses

We then assessed how certolizumab and infliximab affect systemic immune responses in patients. Half of the patients received systemic corticosteroids after week 6, we hence performed our analyses on patients' fresh blood, PBMCs, and plasma at baseline (W0) and at week 6 (W6) posttreatment induction. Six of seven and three of six patients from the certolizumab and infliximab cohorts, respectively, displayed an increase in total lymphocyte numbers at W6 (defined as an increase above 20% from baseline; **Fig. 3A**; Supplementary Table S6). Whereas five (71.4%) patients from the certolizumab cohort displayed an increase in both $CD4^+$ and $CD8^+$ T-cell numbers, this phenomenon was observed in 2 (33.3%) patients from the infliximab cohort (**Fig. 3B** and **C**). When combining both cohorts, we observed a significant increase in the numbers of total circulating lymphocytes as well as $CD4^+$ and $CD8^+$ T cells at W6 as compared with baseline (**Fig. 3A–C**). When assessing for maturation of T-cell responses, we observed that all (100%) and four (66.7%) patients from the certolizumab and infliximab cohorts, respectively, displayed an increase in central memory (CM) $CD4^+$ T-lymphocyte numbers at W6. All patients from both cohorts displayed an increase in effector memory (EM) $CD4^+$ T-lymphocyte numbers at W6 (Supplementary Fig. S1). In terms of $CD8^+$ T cells, five (71.4%) and two (33.3%) patients from the certolizumab and infliximab cohorts, respectively, displayed an increase in CM $CD8^+$ T cells. Similarly, five (71.4%) and four (66.7%) patients from the certolizumab and infliximab cohorts, respectively, displayed an increase in EM $CD8^+$ T cells. When combining both cohorts, patients had increased numbers of $CD4^+$ ($P = 0.0015$) and $CD8^+$ ($P = 0.0037$) EM T cells as well as $CD4^+$ ($P = 0.0071$) but not $CD8^+$ ($P = 0.2489$) CM T cells. Moreover, 5 (71.4%) and 2 (33.3%) patients from the certolizumab and infliximab cohorts, respectively, displayed an increase in $CD4^+$ terminally differentiated $CD45RA^+$ (TEMRA) T cells at W6. Six (85.7%) and four (66.7%) patients from the

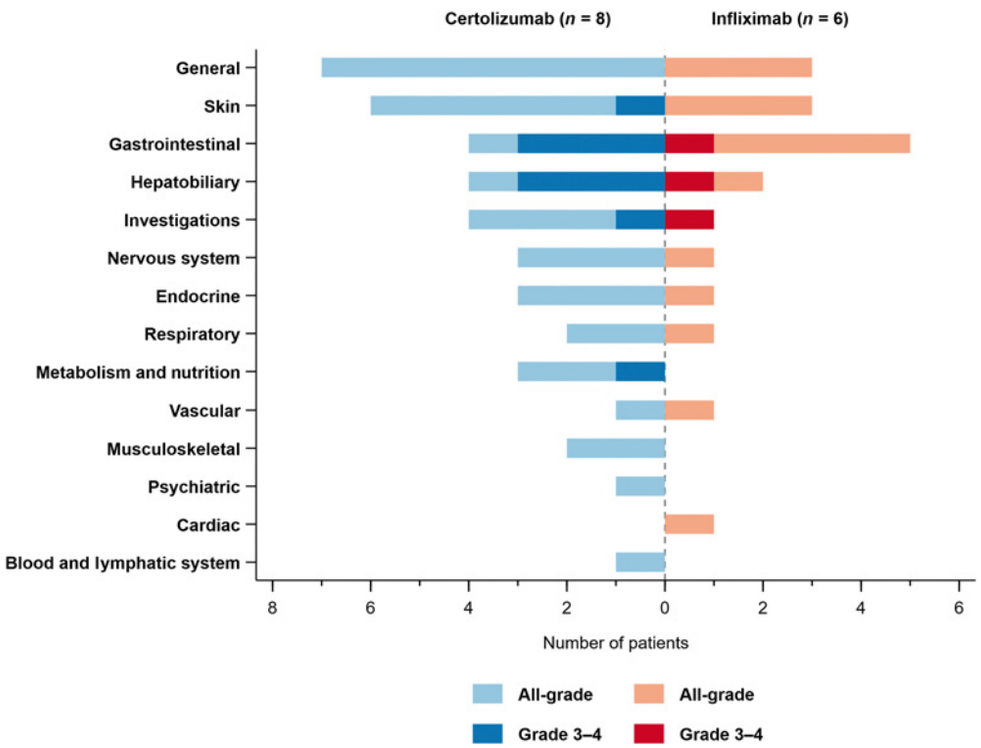

Figure 1.

Treatment-related AEs. Number of patients exhibiting treatment-related AEs are represented by system organ in patients with advanced melanoma treated with nivolumab and ipilimumab in combination with certolizumab or infliximab.

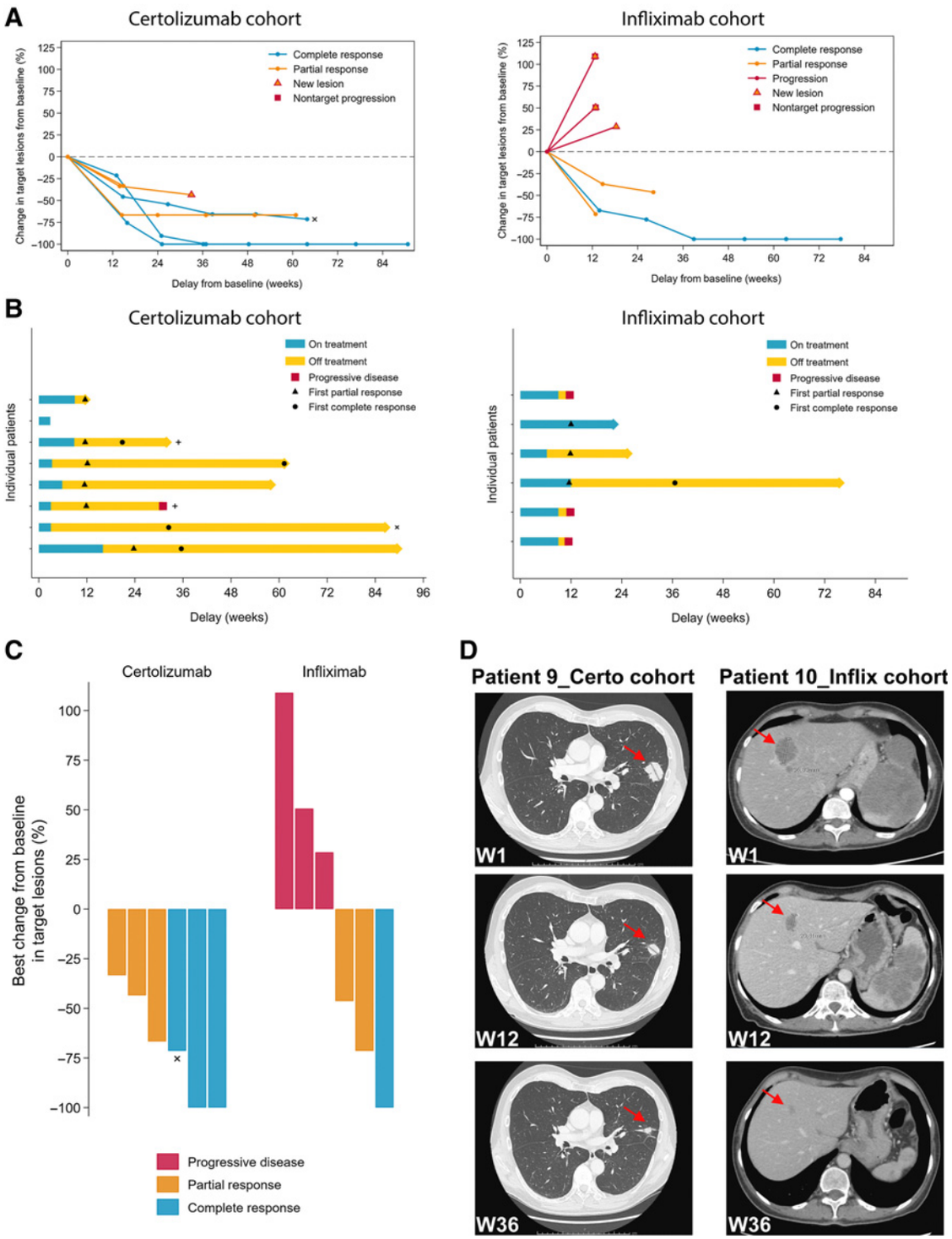

Figure 2.

Clear signs of efficacy in patients treated with nivolumab, ipilimumab, and TNF blockers. **A,** Evolution of tumor burden in patients from the certolizumab (left) and infliximab (right) cohorts over time. One patient in complete response from the certolizumab cohort, who was not evaluable as per RECIST at the first two tumor assessments, is not included in this graph. **x:** complete response despite two persistent lymph node lesions (<10 mm). **B,** Evolution of the response in patients from both cohorts over time. +**:** patients still on nivolumab after discontinuation of study treatment; **x:** patient evaluable as per RECIST only at the third assessment. **C,** Best response in all patients. **x:** patient considered in complete response despite two persistent lymph nodes (<10 mm). **D,** CT scan images showing examples of target tumor regression in a patient from the certolizumab cohort with a lung metastasis (patient 9, left) as well as in a patient from the infliximab cohort with a liver metastasis (patient 10, right).

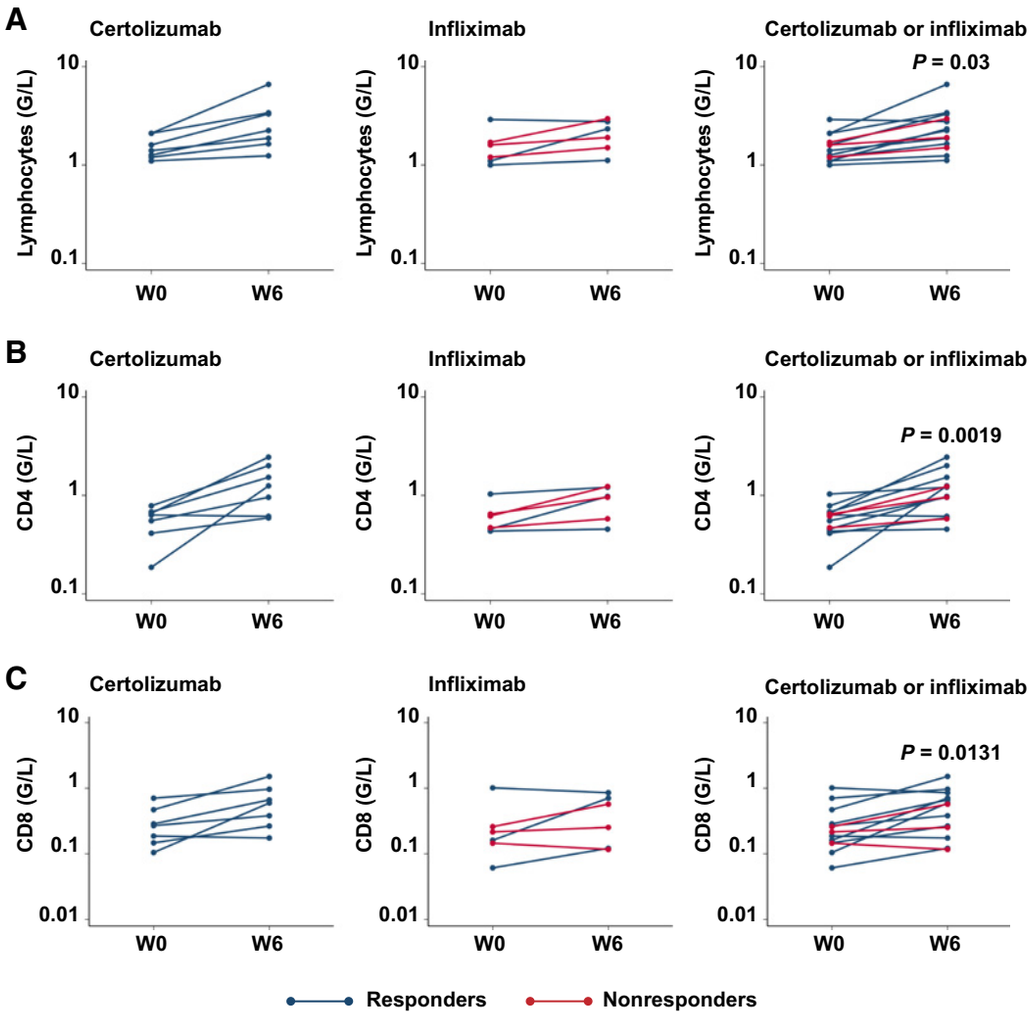

Figure 3.

Increased T-cell numbers in the blood of patients with advanced melanoma treated with nivolumab and ipilimumab in combination with certolizumab or infliximab. **A–C,** Total numbers of lymphocytes, CD4 T cells and CD8 T cells were assessed at baseline (W0) and 6 weeks (W6) following initiation of the treatment protocol. The total number of lymphocytes was assessed by blood count (**A**) and the levels of CD4 (**B**) and CD8 (**C**) T cells in patients' blood were assessed by flow cytometry. Numbers of CD4 and CD8 T cells were extrapolated from the total number of lymphocytes per patient at each time point. Because of the low number of patients, statistical analyses were only performed on data pooled from both cohorts. Highlighted clinical response was evaluated at W12.

certolizumab and infliximab cohorts, respectively, displayed an increase in $CD8^+$ TEMRA at W6. Finally, higher numbers of circulating $CD8^+$ TEMRA were detected at W6 as compared with W0 when combining results from both cohorts ($P = 0.0071$; Supplementary Figs. S1 and S2).

In addition to the remodeling of systemic T-cell subpopulations, we observed an increase in the proportion and number of proliferating $Ki67^+$ $CD4^+$ and $CD8^+$ T cells in the blood of patients treated with certolizumab and infliximab (Supplementary Tables S7 and S8). These were mainly related to a significant increase in the proportion of proliferating EM T cells and, to a lesser extent, CM T cells when combining both cohorts (Supplementary Tables S7 and S8; Supplementary Figs. S3 and S4).

We next monitored variations in levels of Th1-related cells in the blood of patients from both cohorts. Treatment with ICI therapy combined to either anti-TNF significantly increased both the proportion and numbers ($P = 0.0022$) of systemic Th1-related cells in all patients (**Fig. 4A** and **B**; Supplementary Table S9). We also observed a significant increase in numbers but not percentages of Th1/Th17-, Th2-, and Th17-related cells between W0 and W6 in both cohorts (Supplementary Table S9; Supplementary Figs. S5 and S6). To confirm that ICI therapy combined to anti-TNF promoted Th1 systemic responses in patients we assessed the *ex vivo* production of IFNγ and TNF by $CD4^+$ T cells following phorbol 12-myristate 13-acetate/ionomycin stimulation. Most patients from both cohorts [$N = 10$ (76.9%); six (85.7%) patients from the certolizumab cohort and four (66.7%) patients from the infliximab cohort] exhibited increased systemic numbers of $PD1^+IFN\gamma^+TNF^+$ CD4 T cells at W6 ($P = 0.0015$; **Fig. 4C** and **D**). Conversely, numbers of circulating $PD1^+IFN\gamma^+TNF^+$ $CD8^+$ T cells increased in 4 patients from each cohort (**Fig. 4C** and **D**).

Finally, IFNγ and TNF plasma concentrations were monitored. IFNγ levels significantly increased in both cohorts ($P = 0.0024$), 11

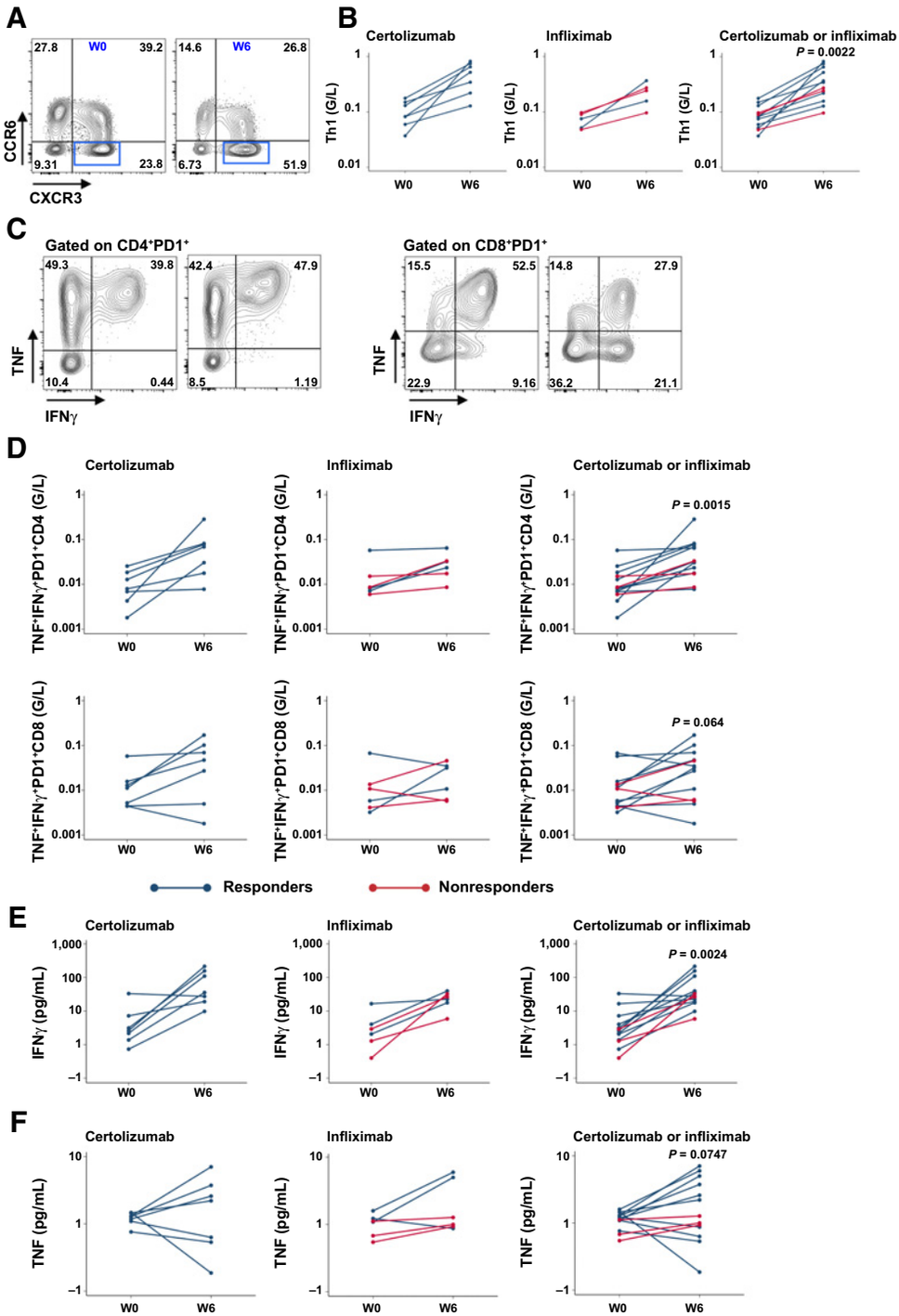

Figure 4.

Increased numbers of Th1-related cells in the blood of patients treated with nivolumab and ipilimumab combined to certolizumab or infliximab. **A** and **B,** The presence of Th1-related cells ($CD4^+CXCR3^+CCR6^-$) was assessed on whole blood from patients at baseline (W0) and 6 weeks (W6) following treatment initiation. Representative example of flow cytometry staining (**A**) and graphs showing the numbers of circulating Th1 cells at W0 and W6 in both cohorts (**B**). Numbers of Th1 cells were extrapolated from the total number of circulating $CD4^+$ T cells at each time point. **C** and **D,** The production of IFNγ and TNF by T cells was assessed following polyclonal *ex vivo* restimulation of purified CD4 and CD8 T cells from PBMCs at W0 and W6 by flow cytometry. Representative flow cytometry images (**C**) and summary graphs (**D**) are depicted. IFNγ (**E**) and TNF (**F**) cytokine levels assessed in the plasma of patients at W0 and W6 by mesoscale. Because of the low number of patients, statistical analyses were only performed on data pooled from both cohorts. Highlighted clinical response was evaluated at W12.

(84.6%) patients showing an increase of 2-fold or more at W6, including six (46.2%) patients with an increase of 10-fold or more. In sharp contrast, TNF levels increased slightly, five (38.5%) patients showed an increase of 2-fold or more (**Fig. 4E** and **F**). We also observed increased plasma levels of IL2 when combining both patient groups ($P = 0.0071$; Supplementary Table S10). No difference in IL6 levels was observed in both cohorts following the two first treatment cycles (Supplementary Table S10).

Analysis of monocyte subpopulations

Certolizumab as well as infliximab-treated patients displayed no significant variation in the proportion and number of both classical ($CD14^+CD16^-$) and nonclassical ($CD14^{-/low}CD16^+$) circulating monocytes between W0 and W6 (Supplementary Table S11). However, five (71.4%) and six (100%) patients from the certolizumab and infliximab cohorts, respectively, showed decreased proportions of HLA-DR^{low} $CD14^+CD16^-$ monocytes, previously defined as circulating monocytic myeloid-derived suppressor cells (monocytic MDSC). When combining patients from both cohorts, this decrease was found significant as was the increase in the proportion and number of HLA-DR^{high} $CD14^+CD16^-$ monocytes (Supplementary Table S11; Supplementary Figs. S7 and S8).

Discussion

For the first time and despite the low number of patients enrolled in each cohort, we provide evidence for the safety and potential benefits of simultaneously combining ipilimumab and nivolumab to anti-TNF in patients with advanced immune checkpoint blockade–naïve melanoma. Indeed, only one patient from the infliximab cohort exhibited a DLT. In the latest update of the CheckMate 067 study, the combination of nivolumab (1 mg/kg) and ipilimumab (3 mg/kg; Nivo1/Ipi3) to treat patients with metastatic melanoma was reported to induce treatment-related grade 3–4 AEs in 59% of patients (2). With 50% of the infliximab-treated patients developing grade 3–4 treatment-related AEs, our data so far indicate that the tritherapy associating nivolumab and ipilimumab to infliximab is at least as safe as the Nivo1/Ipi3 therapy. With 75% of patients from the certolizumab cohort developing grade 3–4 treatment-related AEs, our results suggest that this combination might display a higher toxicity profile. Importantly, all treatment-related AEs were easily manageable.

Of interest is the high incidence of treatment-related hepatobiliary disorders in patients treated with certolizumab (50%). As a comparison, 33% of patients treated with Nivo1/Ipi3 were previously reported to develop hepatotoxicity (2). Similarly, 33% of patients from the infliximab cohort developed hepatobiliary disorders. Chronic infusion of anti-TNF to patients affected with autoimmune diseases has been associated, in rare cases, with liver toxicity (21, 22). However, these phenomena were mainly observed upon infliximab treatment and are now suggested to also rely on the patient's own genetics. Another potential factor facilitating the occurrence of hepatotoxicity might be the high levels of plasmatic IFNγ found at W6 in patients, especially in the certolizumab cohort. Indeed, IFNγ is known to promote apoptosis of hepatocytes during inflammatory conditions (23). This observation reinforces the hypothesis related to the existence of synergistic signals triggered by the three molecules. Whether and how combining certolizumab to Nivo1/Ipi3 favors the development of such AEs warrants further studies.

Infliximab is currently used for the management of persistent forms of immunotherapy-induced gastrointestinal disorders in patients with cancer (15). This drug can be administered concomitantly with corticosteroids following ICI interruption. Although anti-TNF are well known anti-inflammatory molecules, four retrospective studies suggested that anti-TNF do not negatively impact patients' survival upon ICI therapy (12, 13, 15, 24). However, a retrospective study associated infliximab to a decreased OS of patients treated with ICIs (14). Although data should be interpreted with caution due to the low number of patients enrolled in the first part of TICIMEL, combining Nivo1/Ipi3 to either certolizumab or infliximab did not impair the response to ICIs. Interestingly, certolizumab seems to provide more clinical and biological evidence of synergistic pharmacodynamic activity than infliximab. Indeed, all evaluable patients from the certolizumab cohort displayed an OR, whereas three of six patients from the infliximab cohort did not respond. So far, four (57%) and one (16.7%) patients from the certolizumab and the infliximab cohorts, respectively, achieved sustained CR. This indicates that TNF is unlikely a critical effector cytotoxic cytokine toward melanoma cells upon ICI therapy.

Interestingly, certolizumab and infliximab combined with ICIs increased the proportion of HLA-$DR^{high}CD14^+CD16^-$ monocytes in the blood of patients, a cell population previously positively associated with response to anti–PD-1 in patients with melanoma (25). Conversely, we observed a decrease in the proportion of HLA-$DR^{low}CD14^+CD16^-$ monocytes previously describe as monocytic MDSCs (26). In addition to decrease the proportion of one immunosuppressive cell population, both tritherapies increased the percentage and numbers of systemic $Ki67^+$ proliferating CD4 and CD8 T cells, with the majority of proliferating T cells exhibiting an EM or CM phenotype. Moreover, we observed an increase in peripheral blood Th1-related cells as well as increased numbers of PD-$1^+TNF^+IFN\gamma^+CD4^+$ T cells, both of which likely promote antitumor immune responses and favor the occurrence of immune-related AEs (23, 27, 28). Strikingly, all patients from both cohorts exhibited a Th1 orientation at W6. We also detected increased numbers of Th2, Th17, and Th1/Th17 cells in patients' blood. However, the proportions of these cells among total T cells did not significantly increase as opposed to what was seen with Th1 cells. These observations, together with the significant increase in plasmatic IFNγ levels following both tritherapies, suggest a reorientation of the systemic immune response toward Th1 responses. Considering that long-term TNF blockade increases Th1 cell levels in the blood of patients affected with autoimmune diseases (29–31), it is tempting to speculate that anti-TNF favor Th1-dependent immune responses, which could be potentiated by ipilimumab and nivolumab combination. Accordingly, our preclinical studies demonstrated that TNF deficiency (i) enhanced IFNγ production from leukocytes of tumor-draining lymph nodes upon restimulation by irradiated melanoma cells (8) and (ii) elicited IFNγ expression in murine melanoma tumors, a phenomenon further increased upon anti–PD-1 therapy (10).

Further analyses of the circulating T cells indicate that most patients displayed an increase in EM T cells at W6. Interestingly, the proportion of patients exhibiting an increase of CM T cells and TEMRA was higher in the certolizumab cohort. Thus, both anti-TNF do not identically impact antimelanoma immune responses. Whereas certolizumab and infliximab are potent TNF blockers, the differences we observed on the clinical and biological data may reflect various differences between both molecules. First, certolizumab and infliximab bind to similar but not identical epitopes, which may differentially modulate reverse signalling pathways triggered by membrane TNF engagement. Second, whereas certolizumab is a humanized pegylated Fab', infliximab is a chimeric IgG1, the Fc fragment of which likely modulates immune responses. Finally, while infliximab is

administered intravenously, certolizumab is injected subcutaneously, which may differently impact immune responses (32).

Considering the results obtained in the first part of TICIMEL, our observations suggest that both tri-therapies are safe under our therapeutic regimens and that certolizumab may increase the rate of responders. To further investigate the above-mentioned hypotheses, the second part of our clinical trial will enrol 12 additional patients in the certolizumab cohort and six more patients in the infliximab cohort.

Authors' Disclosures

A. Montfort reports grants from Bristol Myers Squibb, Cancéropôle Grand Sud Ouest, Institut Universitaire du cancer (IUCT-O), Association pour la recherche sur le cancer (ARC), Fondation Toulouse Cancer Santé, and Fondation pour la Recherche médicale during the conduct of the study; grants from Fondation de France and Ligue Régionale contre le cancer (Midi-Pyrénées) outside the submitted work. M. Virazels reports grants from Bristol-Myers Squibb, Cancéropôle Grand Sud-Ouest (GSO), Institut Universitaire du Cancer de Toulouse (IUCT-O), Association pour la Recherche sur le Cancer, Fondation Toulouse Cancer Santé, and Fondation pour la Recherche Médicale during the conduct of the study. C. Dufau reports grants from Bristol-Myers Squibb, Cancéropôle Grand Sud-Ouest, Institut Universitaire du Cancer de Toulouse, and Association pour la Recherche sur le Cancer during the conduct of the study. C. Pages reports grants from BMS during the conduct of the study; personal fees from BMS, MSD, and Pierre Fabre outside the submitted work. M. Ayyoub reports grants from Roche/Genentech (imCORE), personal fees from AstraZeneca and Bristol-Myers Squibb outside the submitted work. S. Brayer reports personal fees from Fondation Toulouse Cancer Santé during the conduct of the study. J.-P. Delord reports grants from BMS during the conduct of the study; grants from Genentech, MSD, and AstraZeneca outside the submitted work. N. Andrieu-Abadie reports a patent for US10144772B2 issued, WO2015173259A1 pending, EP3142685B1 issued, ES2748380T3 issued, EP3407911A1 pending, JP2019503384A pending, US20190038763A1 pending, and WO2017129790A1 pending. C. Colacios reports a patent for US10144772B2 issued, WO2015173259A1 issued, EP3142685B1 issued, ES2748380T3 issued, EP3407911A1 pending, JP2019503384A pending, US20190038763A1 pending, and WO2017129790A1 pending. B. Ségui reports grants and personal fees from Bristol-Myers Squibb; grants from Cancéropôle Grand Sud-Ouest (GSO), Institut Universitaire du Cancer de Toulouse (IUCT-O), Association pour la Recherche sur le Cancer; personal fees from Fondation Toulouse Cancer Santé and Fondation pour la Recherche Médicale during the conduct of the study; grants and personal fees from Fondation de France and INmune Bio outside the submitted work; in addition, B. Ségui has a patent for US10144772B2 issued, WO2015173259A1 pending, EP3142685B1 issued, ES2748380T3 issued, EP3407911A1 pending, JP2019503384A pending, US20190038763A1 pending, and WO2017129790A1 pending; and published two original papers indicating the putative benefit of TNF blockade in mouse melanoma models: the first paper showed that anti-TNF and host TNF deficiency enhance the CD8 T cell–dependent immune response (Bertrand F, Rochotte J, Colacios C, Montfort A, Tilkin-Mariame AF, Touriol C, et al. Blocking tumor necrosis factor alpha enhances CD8 T-cell-dependent immunity in experimental melanoma. Cancer Res 2015;75:2619–28) and the second paper showed that anti-TNF and host TNF deficiency enhance the efficacy of anti–PD-1 therapy (Bertrand F, Montfort A, Marcheteau E, Imbert C, Gilhodes J, Filleron T, et al. TNFalpha blockade overcomes resistance to anti–PD-1 in experimental melanoma. Nat Commun 2017;8:2256) and both papers constitute the scientific rationale of the TICIMEL phase Ib clinical trial in patients with advanced melanoma. N. Meyer reports grants from BMS during the conduct of the study; personal fees from BMS, Roche, Novartis, Merck GmBh, and Sun Pharma; grants and personal fees from MSD, Pierre Fabre outside the submitted work; in addition, N. Meyer has a patent for EP3407911A1 pending, JP2019503384A pending, US20190038763A1 pending, and WO2017129790A1 pending. No disclosures were reported by the other authors.

Authors' Contributions

A. Montfort: Formal analysis, investigation, visualization, methodology, writing-original draft, writing-review and editing. **T. Filleron:** Formal analysis, validation, visualization, methodology, writing-review and editing. **M. Virazels:** Investigation. **C. Dufau:** Investigation. **J. Milhès:** Investigation. **C. Pagès:** Resources. **P. Olivier:** Formal analysis, validation, writing-review and editing. **M. Ayyoub:** Validation, methodology, writing-review and editing. **M. Mounier:** Validation, project administration. **A. Lusque:** Formal analysis, validation, visualization, methodology, writing-review and editing. **S. Brayer:** Investigation. **J.-P. Delord:** Conceptualization, writing-review and editing. **N. Andrieu-Abadie:** Validation, writing-review and editing. **T. Levade:** Validation, writing-review and editing. **C. Colacios:** Validation, writing-review and editing. **B. Sègui:** Conceptualization, supervision, funding acquisition, validation, visualization, writing-original draft, project administration. **N. Meyer:** Conceptualization, resources, supervision, funding acquisition, validation, investigation, project administration, writing-review and editing.

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