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Phase I study of Androgen deprivation therapy in combination with anti-PD-1 in melanoma patients pre-treated with anti-PD-1.

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CONFLICT OF INTEREST

CR is a shareholder and founder of Ribonexus Therapeutics, and an occasional consultant with fee (advisory board participation) for Roche, BMS, MSD, Merck, Sanofi, Pierre Fabre, Pfizer and Novartis; CL declares to be on advisory boards for Amgen, Bristol-Myers Squibb, Merck Serono, MSD, Novartis, Pierre Fabre, Roche, and Sanofi, to receive honoraria and funding from Bristol-Myers Squibb and Roche, to receive honoraria from Amgen, Incyte, MSD, Novartis, Pfizer, and Pierre Fabre; TL receives honoraria for consulting for BMS, MSD, Novartis, and Pierre Fabre Oncology; FJL receives honoraria for consulting for Debiopharm International SA and acts as Editor-in-Chief of Melanoma Research; EL receives honoraria for consulting for Debiopharm International SA; PC, VN, BG, GV, and JN are employees of Debiopharm International SA.

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RUNNING TITLE

Androgen deprivation and immune checkpoints inhibitors.

STATEMENT OF TRANSLATIONAL RELEVANCE

Almost half of the patients become resistant and there is a high medical need to identify an effective treatment for patients who have progressed under PD1-based regimen. Most patients who do not respond to immunotherapy have tumours devoid of tumour infiltrating lymphocytes (TILs). Melanoma cells harbour androgen receptors. Both in human and animal models, androgen deprivation regenerates the thymus in adults. Combining androgen deprivation to nivolumab in 14 male melanoma patients presenting resistance to anti-PD-1 provided disease control in 42.8% (RECIST) and 50% (iRECIST), thymus rejuvenation in two patients including one with PR together with augmentation of TILS. These findings suggest that blocking AR in combination with ICI is a promising therapeutic strategy that should be further explored in both male and female patients.

ABSTRACT

Background

Androgen deprivation regenerates the thymus in adults, expanding of T-cell receptor V beta repertoire in blood and lymphoid organs and tumour-infiltrating lymphocytes in human prostate tumours. In melanoma murine models, androgen receptor promotes metastases and androgen blockade potentiates antitumor vaccine efficacy.

This phase I study evaluated the safety, efficacy, and pharmocodynamics of androgen deprivation with the gonadotropin releasing hormone (GnRH) agonist triptorelin combined with nivolumab in male melanoma patients resistant to anti-PD-1.

Patients and methods

Adult male patients with advanced melanoma who progressed under anti-PD-1 containing regimens received triptorelin 3.75 mg every 4 weeks, nivolumab 3 mg/kg every 2 weeks, and bicalutamide 50 mg once daily during the first 28 days. Tumour response was first assessed after 3 months; adverse events (AEs) were monitored throughout the study. T-cell Receptor Excision Circles (TRECs), a biomarker of thymus activity, were explored throughout the study.

Results

Of 14 patients, 4 were locally advanced and 10 had distant metastases.

There were no grade 4 or 5 AEs. Five grade 3 AEs were reported in 4 patients.

According to RECIST v1.1, best overall response was partial response (PR) in one patient with a pancreas metastasis, stable disease (SD) in 5 patients, and progressive disease in 8 patients. According to iRECIST, a second PR occurred after an initial pseudoprogression, TRECs increased in two patients, one with PR who also had an increase in TILs, and the second with SD.

Conclusion

This combination was well tolerated. Disease control was obtained in 42.8% (RECIST) and 50% (iRECIST). The evidence for thymus rejuvenation was limited.

KEYWORDS

Advanced melanoma; immune checkpoint inhibitor; resistance; androgen deprivation therapy; triptorelin; nivolumab.

INTRODUCTION

In spite of the major advances in the field of melanoma treatment using checkpoint inhibitors, almost half of the patients become resistant and there is a high medical need to identify an effective treatment for patients who have progressed under PD1-based regimen [1]. Most patients who do not respond to immunotherapy have tumours devoid of tumour infiltrating lymphocytes (TILs), called “cold tumours”, as a consequence of immunotolerance or “immune-ignorance”[2]. One of the goals of treatments aiming at reversing resistance to PD1 blockade is thus to boost anti-cancer immune response by increasing the number of TILs.

For many years, several epidemiological studies suggested that gender is an independent determinant of melanoma outcome with females having a significantly better prognosis than males among melanoma patients across melanoma stages and treatment types [3-7]. The presence of androgen receptors (AR) in human melanoma samples is associated with a poor prognosis compared to AR-negative samples[7,8]. Blockade of AR in experimental melanoma improved response to BRAF/MEK inhibitors[7]. In a murine melanoma model, it was shown that AR can promote melanoma metastasis via altering the miRNA-539-3p signal[8].

Besides the stimulation of melanoma through AR receptors, an immunosuppressive effect of androgens was further demonstrated by showing that activation of T-cell androgen receptors with testosterone leads to the upregulation of the protein phosphatase PTPN1 that blocks T-cell differentiation.[9] In addition, testosterone blocks the differentiation of T helper (Th) 1 and Th17 cells [9] while stimulating Th2 cells[10,11] , upregulating the expression of Foxp3[12] and expanding T regulatory cells[13].

Androgen deprivation can be obtained by desensitisation of pituitary gonadotropin-releasing hormone (GnRH) receptors with continuous administration of GnRH agonist (GnRH-A) that, by suppressing the luteinizing hormone and follicle stimulating hormone, reduces testosterone to castrate levels[14].

Interestingly, androgen suppression by GnRH-A was shown to quickly restore the thymus and its function in adult male rats and mice[15,16]. In male mice, treatment with GnRH-A increased the number of $CD4^+$ and $CD8^+$ T-cells in blood and lymph nodes[17]. In prostate cancer patients, GnRH-A treatment resulted in thymus rejuvenation, as shown by the increase in the molecular recombination marker T-cell Receptor Excision Circles (TRECs), a reliable marker of newly formed T cells in the circulation[16]. In prostate cancer, AR blockade enhanced CD8+T cells activity and produced improved response to ICI[18]. In addition, GnRH-A treatment decreased the immunosuppressive phosphatase PTPN1[9] prevented

radiotherapy-induced lymphopenia[19], increased circulating $CD4^+$ and $CD8^+$ T-cells with expansion of naïve and memory T-cells as well as natural killer cells[19]. GnRH-A treatment also increased TILs in prostate cancer samples with induction of an oligoclonal response[20]. After allogenic or autologous stem cell transplantation, temporary GnRH-A treatment resulted in enhanced immune system regeneration in both males and females as illustrated by a long-term increase in TRECs levels in both sexes[21].

These effects might be in part due to a direct action of GnRH-A on immune cells. Indeed, by binding to GnRH receptors on the surface of human, porcine, and rat lymphocytes, GnRH-A increases lymphocyte proliferation upon mitogen or cytokine stimulation, and up-regulates the expression of interleukin-2 receptors in human and mouse spleen lymphocytes and thymocytes[22-25]. In addition, administration of GnRH-A to orchiectomized rats could efficiently restore age-linked decrease of thymus weight, as well as thymocyte proliferative capacity with a two-fold increase in thymocyte proliferation compared with orchidectomy alone[16], suggesting a direct effect on T-cells.

Altogether, the considerations above are strong incentive to evaluate the addition of androgen blockade using a GnRH-A to immune checkpoint blockade in patients with melanoma. We hypothesized that the GnRH-A triptorelin could overcome resistance to anti-PD1 inhibitors by inducing thymus regeneration and production of naïve T cells with large T-cell receptor repertoire that could potentially recognize tumour antigens. We further hypothesized that the addition of triptorelin to nivolumab would not significantly increase immune-related toxicity.

Here we report the results of a Phase I study aimed at evaluating the safety of triptorelin in combination with nivolumab and bicalutamide, and the potential of this combination to reverse resistance to PD-1 inhibitors in male melanoma patients pre-treated with anti-PD1.

PATIENTS AND METHODS

Study design

Debio 8200-IMM-101 was a Phase I, single-arm, open-label study performed at 6 centres in France. The study protocol and all amendments were approved by the French ethics committee (Comité pour la Protection des Personnes) and the National Agency for the Safety of Medicines and Health Products. The protocol was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines. All patients provided written informed consent before screening.

The primary objective of the study was to assess the safety and tolerability of a fixed dose of triptorelin when given in combination with the approved standard dose of nivolumab and

bicalutamide in male patients with refractory/relapsing advanced/metastatic melanoma. Secondary objectives included the evaluation of anti-tumour activity and pharmacodynamic effects of triptorelin when combined with nivolumab and bicalutamide, and the assessment of the pharmacokinetics of triptorelin when combined with nivolumab and bicalutamide.

Patients

The study population consisted of male patients aged ≥18 years with refractory/relapsing locally advanced or metastatic histologically confirmed melanoma, progressing under anti-PD-1/PD-L1 containing regimens (with or without anti-CTLA-4). Patients had to agree to collection of paired tumour biopsies, including one at screening. Other key inclusion criteria included measurable lesions according to Response Evaluation Criteria in Solid Tumours (RECIST) v1.1[26], ≤2 previous therapy lines with an anti-PD-1/PD-L1 containing regimen; Eastern Cooperative Oncology Group performance status of 0-1; serum lactate dehydrogenase <2x upper limit of normal; adequate haematological, renal, hepatic and pulmonary functions; serum testosterone concentrations within the normal reference for the age of the patient. Notably, patients with completed primary treatment of brain metastases were to be clinically stable, asymptomatic and off steroids for at least 28 days.

Key exclusion criteria included history of immune-related toxicity from a previous anti-PD-1 containing regimen leading to permanent treatment interruption; history of organ transplant; history of primary immunodeficiency; previous systemic corticosteroid therapy >10 mg/day within 14 days prior to first drug administration; history of autoimmune disease (with a few exceptions); vaccination within the 4 weeks prior to the first study drug administration; evidence of active, non-infectious pneumonitis or history of interstitial lung disease.

Treatment

Treatment consisted of triptorelin embonate 3.75 mg sustained-release formulation intramuscularly every 4 weeks and nivolumab (Opdivo®) 3 mg/kg intravenously every 2 weeks. Bicalutamide (Casodex®) 50 mg was taken orally daily for the first 28 days in order to counteract the initial testosterone peak (“flare”) expected after the first triptorelin administration. Dose reductions were not permitted for any study drugs; interruption of nivolumab treatment was permitted for a maximum of 2 weeks in case of toxicity. The planned treatment duration was 48 weeks (i.e., 12 triptorelin cycles of 4 weeks). In patients deriving benefit, treatment could continue for up to 12 cycles until disease progression, unacceptable toxicity, withdrawal of consent, or premature termination of the study, whichever came first. Patients with disease progression after 3 cycles (at Week 11 or 12) had to be reassessed 7-8 weeks later (at Week 19 or 20). If progression was confirmed,

study treatment was to be stopped, unless it was considered beneficial to the patient to continue the therapy up to 12 cycles.

Treatment extension was offered to patients who had completed Cycle 12 and might have benefited from a continuation of the combination treatment.

Study assessments and statistical analysis

Adverse events (AEs) and serious AEs (SAEs) were monitored throughout the study until 30 days after end of treatment (EOT). AEs were coded according to the Medical Dictionary for Regulatory Activities version 21.1 and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03.

Safety haematology measurements were performed at screening and every 2 weeks thereafter pre-dose from Day 1 until EOT and were analysed locally.

Evaluation of tumour response was performed after 3 triptorelin cycles, at Week 11 or 12, according to RECIST v1.1[26].

Blood samples for triptorelin pharmacokinetic assessments were collected on Days 1 and 57, pre-dose and at 1 h (i.e., just before nivolumab infusion), 2 h, and 4 h post-dose; at pre-dose of any study drugs on Days 15, 29, 71, 85, 169, 253; and at EOT (Day 337). Serum triptorelin levels were measured by liquid chromatography with tandem mass spectrometry (LC-MS/MS).

Blood samples were collected on Days 1, 15, 29, 57, 71, 85, 169, 253, and at EOT (Day 337) to evaluate the effect of treatment on testosterone and TRECs. Serum testosterone levels were measured by LC-MS/MS. TRECs in peripheral blood mononuclear cells (PBMCs) were measured by quantitative polymerase chain reaction (PCR) using the MyTREC Sensi Duplex TREC/Beta Actin Real-Time qPCR Assay kit (GenenPlus, GP-D3012096).

Tumour biopsies, were collected at baseline during screening and at cycle 3 (Week 11 or 12) from the same lesion or nearby lesions of the same tissue type prior to computed tomography scan/magnetic resonance imaging. Density of TILs ($CD3^+$, $CD8^+$, $CD163^+$, $FoxP3^+$, $CD68^+$ and $PD\text{-}L1^+$ cells) was assessed by immunohistochemistry. Only patients with assessments performed on biopsies collected from the same lesion or nearby lesions of the same tissue type at both baseline and at Cycle 3 were assessed for changes from baseline.

All statistical analyses were descriptive due to the small number of patients. Statistical analysis populations comprised the safety population (patients who received at least one dose of any study drug), which was also used for the analysis of pharmacodynamic data,

efficacy population (patients with tumour assessment according to RECIST v1.1 at baseline and at least once during treatment) and pharmacokinetics population (patients who received at least one dose of triptorelin and who had at least one triptorelin serum concentration value).

DATA AVAILABILITY

The data generated in this study are available on request to the corresponding author.

RESULTS

Patient characteristics

Between January 5 and October 30, 2018, 14 male patients with a mean (range) age of 65 (45-82) years were enrolled and treated. (**Table 1 ; Supplementary Tables 1 and 2**). All patients had been treated with anti-PD-1 as last therapy before entering the study. Eight patients were primary refractory to anti-PD-1 therapy and 6 were secondary refractory after initial response to anti-PD-1 therapy. One patient had been previously treated with anti BRAF/MEK therapy. Twelve patients had cutaneous melanomas (2 of which with unknown primary location) and 2 patients had sinonasal mucosal melanomas. Among the patients with cutaneous primaries, 4 had advanced stage III, while 9 had stage IV distant metastases. One mucosal melanoma patient had a locally advanced non-resectable disease and one also had visceral metastases (stage IV).

Median (range) duration of previous immunotherapy was 6 (1-38) months.

Safety

The majority of patients (78.6%) completed at least 3 treatment cycles, and 3 (21.4%) patients completed 12 cycles.

The most commonly reported TEAEs (**Table 2; Supplementary Table 3)** were hot flushes (in 6 [42.9%] patients), asthenia and constipation (each in 4 [28.6%] patients). The severity and frequency of these adverse events were in line with those found in prostate cancer patients during ADT due to changes in testosterone levels.

However, fatigue and constipation are also commonly reported with nivolumab monotherapy. There were no unexpected drug-related serious or Grade 3 TEAEs. No adverse events at the injection site of triptorelin were reported. The combination of triptorelin, bicalutamide (first 4 weeks) and nivolumab did not increase the immune-related toxicity compared to that expected of nivolumab alone.

There were no grade 4 or 5 AEs. Five grade 3 AEs were reported in 4 patients: neutropenia, asthenia, back pain, abdominal pain and bone pain. Grade 3 neutropenia was attributed to nivolumab and resolved following a treatment interruption of 14 days. The event of abdominal pain was attributed to triptorelin, classified as SAE, and resolved after 39 days without causing treatment interruption (as it started on the day the treatment was discontinued due to disease progression). Of note, both treatment-related events were expected as they are described on the labels of the two products. Five SAEs were reported in 4 patients: the aforementioned event of abdominal pain and an event of back pain (both of grade 3) reported in the same patient, and events of sinusitis, neoplasm progression, and epistaxis.

Efficacy:

According to RECIST v.1.1, best overall response (BOR) was assessed as one partial response (PR) in a patient with pancreas metastasis (reduction of 76% from Baseline), 5 stable diseases (SD) and 8 progressive diseases (PD). One patient with extensive sino-nasal melanoma was not evaluable for efficacy as protocol therapy was discontinued after one month due to major progression. According to a post-hoc analysis based on iRECIST criteria[27], a second PR occurred after an initial pseudoprogression in a patient with two inguinal lymph node metastases (reduction of 32% from Baseline) (**Figure 1 A and B; Supplementary Table 3**) as well as a complete disappearance of new cutaneous metastasis. Following withdrawal due to premature termination of the main study by the Sponsor on Study Day 239 (Cycle 9), this patient received 11 additional treatment cycles because the investigator considered that the combination treatment was beneficial. Among the patients with objective response, one with PR had a previous PR to pembrolizumab and one with SD had a previous PR to pembrolizumab randomized with or without epacadostat (**Supplementary Table 2**).

TRECs

TRECs levels at baseline and EOT by age category **(Figure 2 A:<70 and fig 2 B >70)** showed high variability across patients. Increases in TRECs levels from baseline were observed in 2 patients **(Figure 2 B)**: in a 71-year-old patient with PR as BOR, at all timepoints except EOT, with the highest value (630.3 µg/DNA) at Day 57; in a 72-year-old patient with SD as BOR, at Days 15, 57, 71, 85 and 169, with the highest value (464.5 µg/DNA) at Day 85.

TILs

Increases in TILs were observed for 2 patients out of 4 with paired biopsies: one patient with pancreas metastasis and PR as BOR (increase in $CD3^+$, $CD8^+$, $Foxp3^+$, $CD163^+$ and $CD68^+$ cells), and the other with PD as BOR (increase in $CD3^+$ and $CD8^+$ cells, and slight increase in $Foxp3^+$and $CD68^+$ cells) (**Figure 3; Supplementary Table 4**). The number of TILs was stable in 2 patients with SD.

Blood lymphocytes

For all patients regardless of their BOR, blood lymphocytes were close to the lower limit of normal range at baseline and during the study with no significant change.

Triptorelin Pharmacokinetics

After the first intramuscular injection, triptorelin serum levels increased rapidly, reaching mean geometric maximum serum concentration (C_{max}) of 13892 ng/L with a median (range) time to reach maximum serum concentration (t_{max}) of 1.1 (1-4) h. Similar C_{max} and t_{max} values were observed after the third triptorelin injection, indicating no accumulation after repeated dosing. Triptorelin exposure was similar in presence (Days 1-28 in Cycle 1 only) and absence (all subsequent cycles) of bicalutamide treatment. Overall, the pharmacokinetic profile of triptorelin was consistent with data obtained previously in healthy male volunteers with triptorelin 1-month formulation alone (data not shown).

Testosterone

Serum testosterone decreased after treatment initiation (**Figure 4**). Mean (standard deviation) testosterone concentrations decreased from 12.2 (5.6) nmol/L at pre-dose Baseline to 0.7 (0.7) nmol/L at EOT.

DISCUSSION

This is the first study on the combination of androgen deprivation therapy with immune checkpoint inhibition in ICI-resistant melanoma patients. Patients were treated with the monthly dose of triptorelin approved to induce and maintain chemical castration in patients with prostate cancer combined with nivolumab and bicalutamide (only for the first treatment cycle) at the recommended dosage regimens.

The combination of triptorelin, bicalutamide and nivolumab was well tolerated and did not increase the expected immune-related toxicity of nivolumab. No grade 4 or 5 AEs were reported. Only 2 grade 3 AEs (14%) were considered related to study drugs: abdominal pain and neutropenia, attributed to triptorelin and nivolumab respectively. Of note, both events were expected as described on the labels of the respective products, and both resolved.

Our study hypothesis was that androgen deprivation would result in thymus rejuvenation and increase in TILs. We monitored T cell receptor excision circles (TRECs) that are known to be released upon rearrangement of the T cell receptor and to constitute reliable markers of newly formed circulating T cells that are associated with thymus regeneration. we found that 2 of 14 patients had increased levels of TRECs in PBMCs: one patient who experienced PR of pancreas metastasis and one with SD. Unfortunately, in the patient with PR according to iRECIST, TRECs levels were not assessed during either the pseudoprogression period, or the PR period.

Three months after the first triptorelin injection, TILs were increased in 2 patients. The aforementioned patient with PR of pancreas metastasis with increased TRECs, had increased TILs of different phenotypes (CD4+ cells, CD8+ cells, T regulatory cells) as well as macrophages. Another patient who experienced fast progression and no increase in TRECs, exhibited increase in $CD4^+$ and $CD8^+$ cells. No change in TILs were observed in 2 patients with SD. Due to the small number of patients with data at both Baseline and Cycle 3 (3 or 4 patients, depending on the marker tested), the TILs data are difficult to interpret.

Previous studies in various settings including melanoma have clearly reported immune effects of GnRH-A on TRECs and TILs. In a study of treatment-naïve advanced prostate cancer patients, GnRH-A treatment resulted in a >25% increase in TRECs levels in PBMCs in 6 out of 10 patients[16]. In another study, patients with localized prostate cancer treated with GnRH-A at different times before surgery exhibited a progressive increase in TILs with a plateau at Week 3[20]. After the implementation of our study protocol, it was reported that 3 out of 10 patients with prostate cancer resistant to the anti-androgen enzalutamide, had responses after adding pembrolizumab[28]. . These observation are consistent with recent striking results demonstrating a direct immunostimulating action of AR blockade with increase of $CD8^+$ IFN-γ secretion and a synergistic effect between AR blockade and PD1 blockade in a murine prostate cancer. (notre ref 18: https://www.nature.com/articles/s41586-022-04522-6)

However, prostate cancer is androgen-dependent and a synergistic effect between PD1 blockade and shedding of tumour-associated antigens due to androgen-deprivation could be

involved. But imelanoma cells can also harbour AR andIt was even shown that mice of both sexes receiving BRAF/MEK inhibitors had improved response upon AR blockade[7]

We questioned whether the clinical benefit observed in half of our patients could be due to a anti-PD1 rechallenge effect. Indeed, in two small series of 8 melanoma patients who had been retreated after interruptions of various ICI therapies were found to respond to a rechallenge with anti-PD1 therapy[29,30]. It is important to note that for those patients, reintroduction of a PD-1 inhibitor occurred after a various time interval (range 0.3-17.3 months) during which most patients received other therapies such as chemotherapy or radiotherapy, compared with our study, during which no other anti-cancer therapies were given (range 30-180 days). Therefore, although we cannot totally rule it out, it is very unlikely that the clinical benefit of the combination triptorelin and nivolumab in our patients could be related to nivolumab only.

In addition, our patients had undergone several prior treatments with various ICIs to which they had become resistant after relatively long treatment durations (median of 6 months) (supplementary Table 2).

One additional reason why we did not observe a higher level of thymus regeneration and better clinical outcome is that our patients had low lymphocyte counts that could not be significantly improved by the combination of triptorelin and nivolumab. Even in the 2 patients with signs of thymus function upregulation, the peripheral lymphocyte counts did not increase.

The representativeness of Study Participants is shown in **Supplementary Table 5.**

Our study did not include females. However, it was shown that melanoma cells upregulate AR and could undergo better response to targeted therapy after AR blocking[7]. This finding would suggest to include females in future trials of AR blockade.

Conclusion

The combination of nivolumab and androgen deprivation with triptorelin is well tolerated in male patients with advanced melanoma. It was associated with a disease control in 6 of 13 evaluable patients (46.1%) but objective responses were seen only in one and two patients according to RECIST or iRECIST criteria respectively. Thymus regeneration was found in two patients including one responding patient who also had increased TILs , in line with our working hypothesis that triptorelin and/or chemical castration could restore the thymic function and increase TILs.

A randomized controlled study evaluating anti-PD1+ triptorelin versus anti-PD1 alone would provide the opportunity to determine the relative importance of triptorelin and nivolumab and to investigate more response biomarkers.

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Legends for figures

Figure 1 A: Percentage change in tumour size over time, efficacy population (n=13). **B:** Best percentage change in tumour size during the study, efficacy population (n=13). One patient experienced PR after initial progression (pseudoprogression).

BOR, best overall response; PD, progressive disease; PR, partial response; RECIST, Response Evaluation Criteria in Solid Tumours.

Figure 2: TRECs levels according to age category (A <70; B >70) and RECIST response**.** Changes (%) in TRECs levels from Baseline by patient and BOR, safety population (n=14). BOR, best overall response.

TRECs, T cell receptor excision circle.

Figure 3. Changes in TILs' density at Baseline and after cycle 3, by patient and BOR. CD3+ cells; CD8+ cells; CD163+ cells; FOXP3+ cells; CD68+ cells; subjects with evaluable paired biopsies of safety population (n=3-4 depending on the biomarker tested).

BOR, best overall response; TILs, tumour infiltrating lymphocytes.

Figure 4. Serum testosterone levels at Baseline and during study treatment, safety population (n=14). The plus symbol + denotes the mean at each timepoint; the top and bottom lines of each box denote the 75th and 25th percentiles; the horizontal line between them denotes the median. The whiskers denote 1.5 times the interquartile range, and values outside this range are represented by circles.

Nobs, number of observations at timepoint.

Table 1. Patients characteristics, safety population

Number of patients	**14**
Age, year	
Mean (range)	64.9 (45-82)
Race	
White, n(%)	11 (78.6)
Black, n(%)	3 (21.4)
Cutaneous melanoma, n(%)	12 (85.7)
Mucosal melanoma, n(%)	2 (14.3)
Stage III, n	**4**
M0, N2c, n	2
M0, N3c, n	2
Stage IV, n	**10**
M1c, N0, n	3
M1c, N2b, n	1
M1c, N2c, n	1
M1a, N3c, n	2
M1c, N3c, n	1
M1d, N3c, n	1
M1b, N3c, n	1

Table 2 TEAEs reported in >1 patient by SOC and PT – Safety population

SOC, n (%) PT, n (%)	**Total (N=14)**
Gastrointestinal disorders	**9 (64.3)**
Constipation	4 (28.6)
Diarrhoea	3 (21.4)
Nausea	3 (21.4)
Abdominal pain	2 (14.3)
General disorders and administration site conditions	**8 (57.1)**
Asthenia	4 (28.6)
Vascular disorders	**8 (57.1)**
Hot flush	6 (42.9)
Hypertension	2 (14.3)
Skin and subcutaneous tissue disorders	**7 (50.0)**
Pruritus	3 (21.4)
Dry skin	2 (14.3)
Vitiligo	2 (14.3)
Investigations	**5 (35.7)**
Blood creatine phosphokinase increased	2 (14.3)
Weight decreased	2 (14.3)
Musculoskeletal and connective tissue disorders	**5 (35.7)**
Back pain	2 (14.3)
Respiratory, thoracic and mediastinal disorders	**5 (35.7)**
Dyspnoea	2 (14.3)
Blood and lymphatic system disorders	**4 (28.6)**
Anaemia	2 (14.3)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	**4 (28.6)**
Tumour pain	3 (21.4)
Psychiatric disorders	**4 (28.6)**
Depression	2 (14.3)
Metabolism and nutrition disorders	**3 (21.4)**
Decreased appetite	3 (21.4)

Abbreviations: AE: adverse event; CTCAE: Common Terminology Criteria for Adverse Events; MedDRA: Medical Dictionary for Regulatory Activities; PT: Preferred Term; SOC: System Organ Class; TEAE: treatment emergernt adverse event

Notes: AEs are coded according to the version 21.1 of MedDRA. A patient is counted only once at the worst CTCAE grade for multiple events within the same PT/SOC. TEAEs are all AEs starting or worsening during the on-treatment period.

Fig 1A

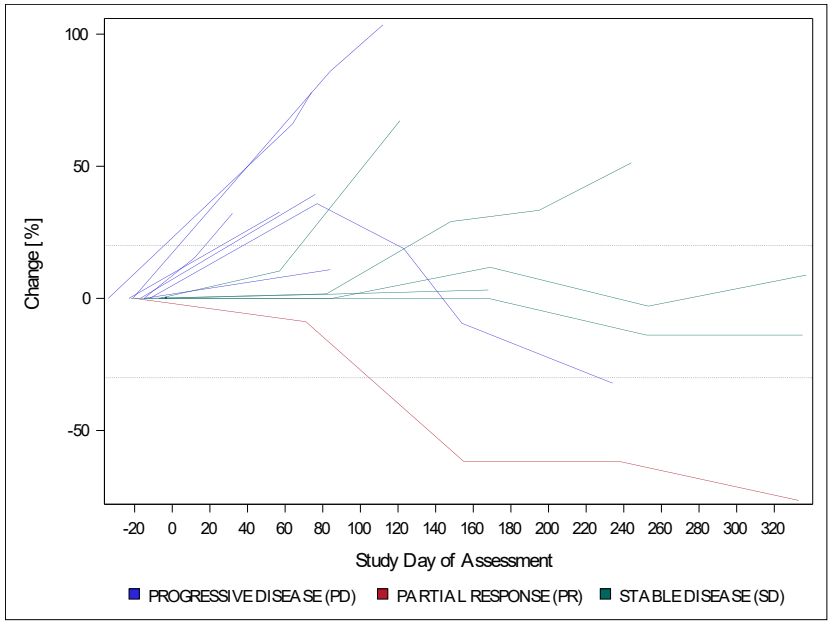

Fig 1B

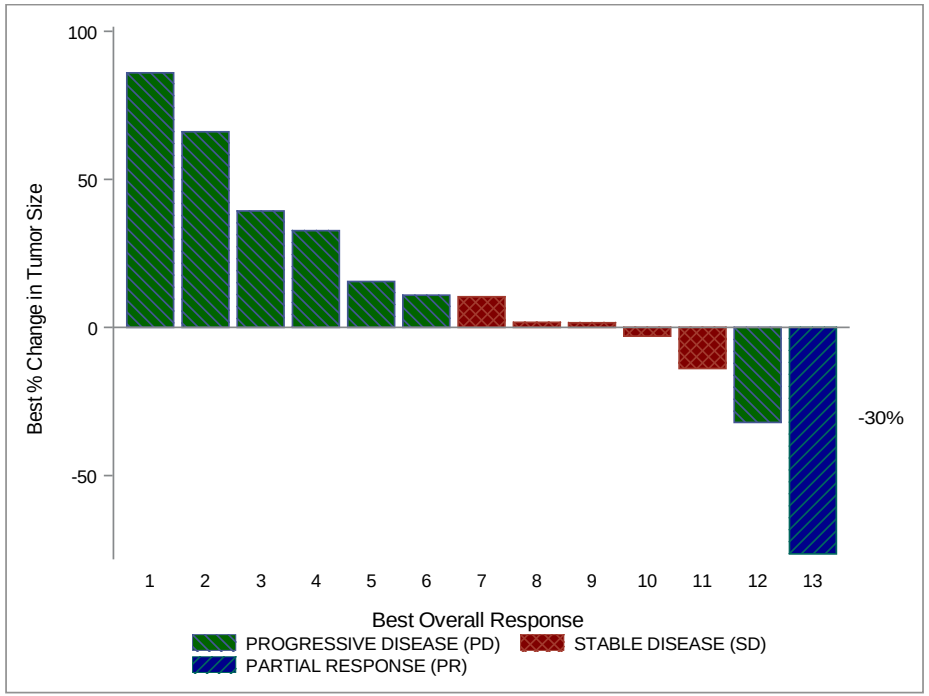

Fig 2A

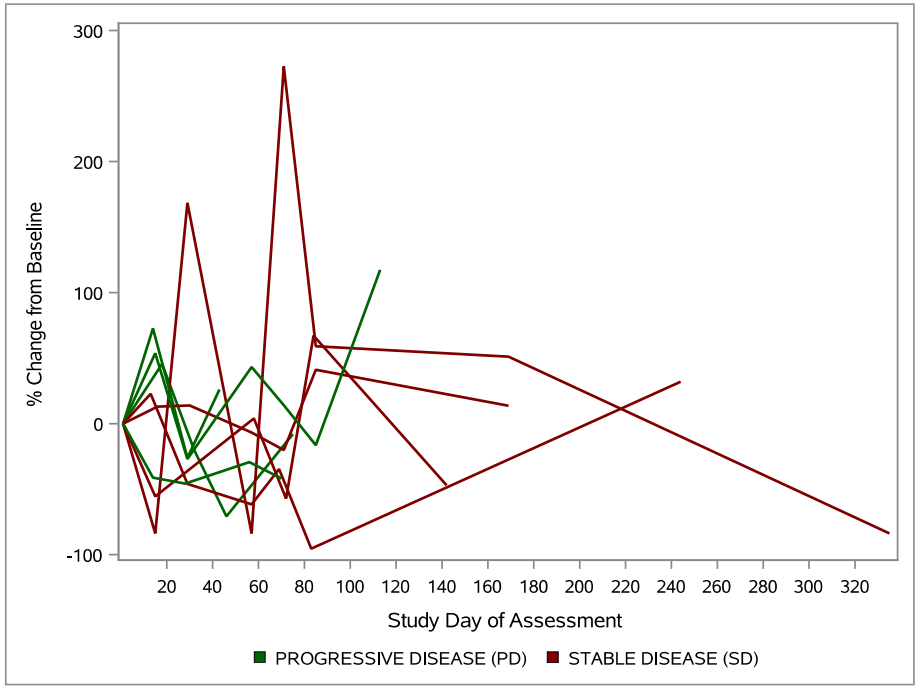

Fig 2B

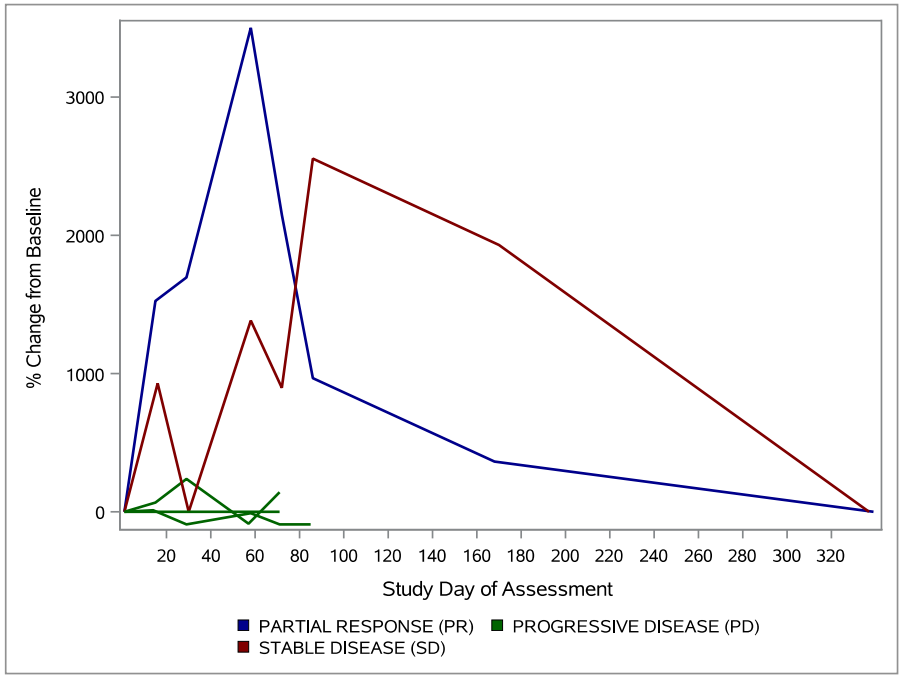

Fig 3

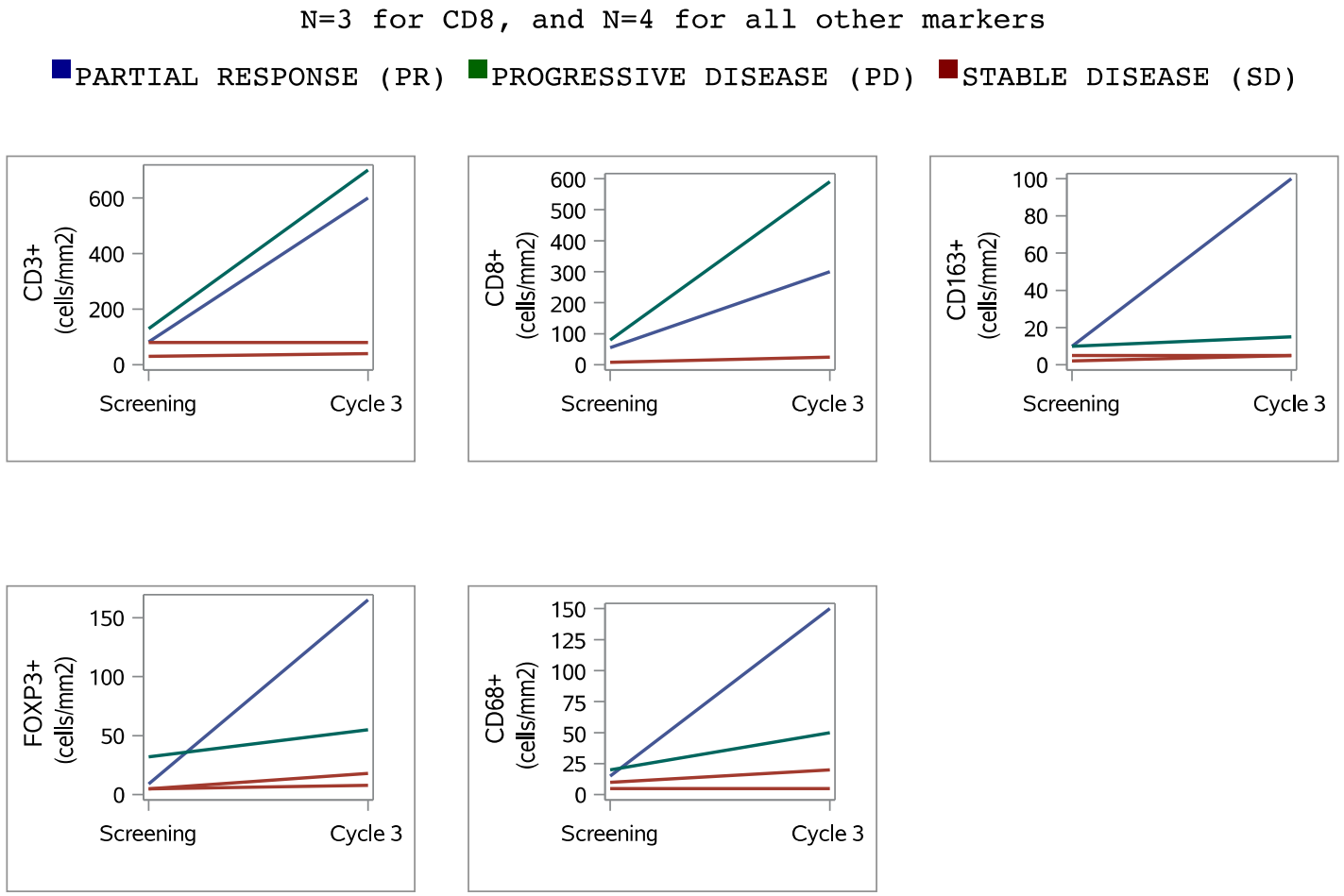

Fig 4