Immune Profile Of Uveal Melanoma Patients: Impact On Clinical Outcome

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Keywords: Immunotherapy, uveal melanoma, PD-1, cytokines, soluble factors, soluble checkpoint

Posted Date: July 7th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-614744/v1>

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Abstract

Background.

Immunotherapy represents a common therapeutic option for metastatic uveal melanoma, despite the low activity. Although overall survival is often dismal, long survivors can be observed. In this study, the prognostic role of the soluble isoforms of immunomodulatory receptors and cytokines/chemokines was evaluated as well as their ability to identify patients who can benefit more from anti-PD-1 therapy.

Methods.

Sera from 22 metastatic uveal melanoma patients were assayed to evaluate the levels of cytokines/chemokines and soluble immune checkpoint molecules by multiplex immunoassay analysis. The changes of these molecules during anti-PD-1 therapy were assessed. The correlation between soluble isoforms of immunomodulatory receptors/cytokines/chemokines and survival was analysed. A comparison between circulating immune profile of metastatic uveal melanoma and cutaneous melanoma during anti-PD-1 therapy was also performed.

Results.

The levels of sCD137, sCD28, sPD-1, sPD-L2 sLAG3, sCD80 and sTim3 were significantly enhanced during anti-PD-1 treatment. Similarly, the levels of IP-10, CCL2 and IDO activity were significantly increased during anti-PD-1 therapy. HVEM, IL-8 and IDO activity were higher in patients with survival < 6 months. Considering these 3 molecules, we obtained a score able to predict patients' survival. Serum CD137, sGITR and sCD27 were significantly lower in patients with survival > 30 months. Serum GITR, sCD27, sPD-1, sCD80, IFN γ and IDO activity were significantly higher in uveal melanoma patients than in cutaneous melanoma patients during anti-PD-1 therapy.

Conclusions.

The molecules detected in uveal melanoma during anti-PD-1 treatment reflect the poor activation of immune system and may justify the limited response to immune checkpoint inhibitors. Nevertheless, some patients with metastatic uveal melanoma had a long survival. These patients could be identified through a score based on circulating immune molecules such as HVEM, IDO and IL-8. The comparison of immune profile during anti-PD-1 therapy between uveal melanoma and cutaneous melanoma reflects the different efficacy of immune checkpoint inhibitors in these diseases.

Background

Uveal melanoma (UM) is a rare malignancy but represents the most common tumor with origin in the eye [1]. Despite the radical treatment of primary tumor, metastatic spread often occurs [2]. The first and the most frequent site of metastases is the liver [3]. Hepatic involvement, which accounts for about 90% of metastatic disease [4], is usually characterized by multifocal metastases. Therefore, surgical resection of hepatic metastases is not possible for the majority of the patients [5]. The survival for patients with metastatic disease is limited. The systemic treatments commonly used are the same tested in clinical trials for cutaneous melanoma (CM) [6].

Chemotherapy and target therapies have been employed with poor results [7–11]. To date, immune checkpoint inhibitors (ICIs) are used for the treatment of metastatic uveal melanoma (mUM) [6].

Ipilimumab showed a modest activity, allowing a survival of 36 weeks in pretreated patients [12]. In the DeCOG study, the survival with Ipilimumab was 6.8 months in a population including 8 naïve and 45 pretreated patients [13]. In pre-treated patients, Karydis [14] reported 8% of partial response and 24% of disease stabilization with Pembrolizumab, with a median progression-free survival (PFS) of 3 months. Algazi observed a similar outcome with Pembrolizumab, Nivolumab and Atezolizumab [15] in 48 pre-treated patients and 8 naïve patients. Pembrolizumab showed a limited efficacy also in a prospective observational study, with a PFS of 3.8 months [16]. The association of Nivolumab and Ipilimumab allowed an overall survival (OS) of 12.7 months and a PFS of 3 months [17].

The low activity of the immune checkpoint inhibitors can be explained by the ability of uveal melanoma cells to elude immunity [18]. The eye is considered an immune-privileged site with own immunosuppressive mechanisms. UM cells are able to escape from systemic immune surveillance also in the liver [19]. In addition, the low mutational burden of this type of melanoma can be responsible of the poor results obtained with immunotherapy until now [20].

The current issue is to understand if there is a rationale to use immunotherapy in uveal melanoma, eventually with agents which act through pathways different from PD-1 and CTLA-4 [21]. On the other hand, it is not well clear if a benefit can be expected also with anti-PD-1 and CTLA-4 agents in selected patients [22].

Mounting evidence demonstrates that soluble isoforms of immunomodulatory receptors play a crucial role in immune regulation [23, 24]. The origin of the soluble receptors has not been completely elucidated. It has been reported that they can be produced by a proteolytic cleavage of membrane bound, by an alternative splicing of mRNA, or released in circulation associated with exosomes or microvesicles. [25]. The soluble immune molecules may play a prognostic role in cancer and may be predictive of the treatment efficacy [26]. It was demonstrated that serum levels of CTLA-4 correlate with response to Ipilimumab in melanoma patients [27].

Cytokines also play a key role during immunotherapy. For example, an upregulation of 11 cytokines was observed in melanoma patients treated with an anti-PD-1 alone or in combination with an anti-CTLA-4 who developed high grade immune-related adverse events [28].

This study aimed to evaluate the role of circulating immune checkpoint-related proteins, cytokines and chemokines in metastatic uveal melanoma in order to investigate their prognostic role and their modulation during anti-PD-1 treatment.

Patients And Methods

Patients enrollments and samples collection

We considered patients with metastatic uveal melanoma referred to the Oncology Unit of Fondazione Policlinico Universitario Agostino Gemelli IRCCS. The patients older than 18 years, with measurable unresectable metastatic disease, received Pembrolizumab as first line therapy, administered intravenously at the dose of 2 mg/kg every 3 weeks or 200 mg flat dose every 3 weeks (when the flat dose has been introduced) until disease progression, unacceptable toxicity or consent withdrawn. Toxicity was reported according to Common Terminology Criteria for Adverse Events (CTCAE v. 4.0). Radiological and clinical assessments were performed according to good clinical practice. Progression-free survival, response rate, clinical benefit, overall survival and tolerability were evaluated. Response was assessed according with RECIST criteria 1.1. PFS was calculated from the first day of treatment to progression or death for any reason. Survival was defined as the interval from the first detection of metastases to death for any cause.

A group of BRAF wild type metastatic cutaneous melanoma (mCM) patients treated in first line setting with an anti-PD-1 agent were also considered for the comparison with mUM patients.

All the patients provided written informed consent before participating in the study.

Peripheral blood samples were drawn from all patients into a tube without anticoagulant and left at room temperature to allow blood to clot. Later, samples were centrifugated to collect serum that was stored and frozen at -80°C until use. In a group of metastatic UM patients, samples were collected before treatment with Pembrolizumab (T0) and after 3 cycles (> T0). Samples from a group of cutaneous melanoma patients during anti-PD-1 therapy were also collected. The study was conducted in accordance with the Helsinki declaration of 1975 and was approved by the local ethics committee.

Detection of soluble molecules in serum

Sera from uveal and cutaneous melanoma patients were assayed to evaluate the levels of cytokines and soluble immune checkpoint molecules (sICs) by multiplex immunoassay analysis using the ProcartaPlex Human Inflammation Panel (20 Plex, catalog number EPX200-12185-901; sE-Selectin; GM-CSF; ICAM-1/CD54; IFN alpha; IFN gamma; IL-1 alpha; IL-1 beta; IL-4; IL-6; IL-8; IL-10; IL-12p70; IL-13; IL-17A/CTLA-8; IP-10/CXCL10; MCP-1/CCL2; MIP-1alpha/CCL3; MIP-1 beta/CCL4; sP-Selectin; TNF alpha) (eBioscience) and the Human Immuno-Oncology Checkpoint 14-plex ProcartaPlex Panel 1 (catalog number EPX14A-15803-901; BTLA; GITR; HVEM; IDO; LAG-3; 47; PD-1; PD-L1; PD-L2; TIM-3; CD28; CD80; CD137; CD27; CD152) (eBioscience). Assay was conducted using 50 µl of serum for each sample and adding it in a 96-

well plate with a mixture of colour-coded magnetic beads coated with antibody that recognize specific analytes. Later biotinylated detection antibodies that bind analytes of interest were added and then bound to Phycoerythrin-conjugated streptavidin that through its signal intensity allow to detect the analyte concentration. Samples were measured using Luminex 200 platform (BioPlex, Bio-Rad) and data, expressed in pg/ml of protein, were analysed using Bio-Plex Manager Software.

Trp/kyn ratio analysis

Serum levels of tryptophan (trp) and kynurenine (kyn) were evaluated through modified liquid chromatography – tandem mass spectrometry method.

Samples were deproteinized using 50 µl of TCA 4% aqueous solution and following centrifuged at 14,000 rpm for 15 min. Supernatants were injected into chromatographic system to perform separation using an Agilent Liquid Chromatography System series 1100 (Agilent Technologies, USA), on a biphenyl column (100 × 2.1 mm, Kinetex 2.6 µm Biphenyl, 100 Å, Phenomenex, CA, USA) equipped with a security guard precolumn (Phenomenex, Torrance, CA, USA). Gradient elution was performed with a flow rate of 400 µl/min and mobile phases consisted of 0.1% aqueous formic acid (A) and 100% acetonitrile (B).

The mass spectrometry method was performed on a 3200 triple quadrupole system Applied Biosystems, Foster City, CA, USA) equipped with a Turbo Ion Spray source. The detector was set in the positive ion mode. The instrument was set in the Multiple Reaction Monitoring mode. Data were acquired and processed by the Analyst 1.5.1 Software.

Statistical analysis

PFS and OS were calculated with Kaplan-Meier method.

Summary data were expressed as average and standard error of mean.

Two-tailed no parametric statistical tests were used to compare different groups. Wilcoxon matched-pairs signed rank test was used to analyse mUM group and compare T0 to > T0.

mUM patients were divided into three groups according to the survival: fast progressors (FP), slow progressors (SP) and long survivors (LS); one-way Anova test was used to compare these populations. Comparison between mUM and mCM was performed using unpaired Mann-Witney test. All the results were considered significant when p value was < 0.05.

The level of all the studied molecules was described by calculating mean and Standard Deviation (SD), and median and interquartile range (IQR). The values were compared between patients with a survival < 6 months or ≥ 6 months using the Mann-Whitney test because the data were not normally distributed. The molecules for which the comparison showed a p-value < 0,05 or for which there was a particular clinical interest (considering a p-value not higher than 0,2) were investigated with the objective to identify the better cut-off for distinguishing long survivors or no long survivors and a Receiver Operating Characteristic (ROC) analysis was performed. A score was finally defined by assigning the value of 1 to

each molecule associated to the worst prognosis based on the cut-off. Consequently, the overall score was obtained by summing the single molecules score with the higher value corresponding to the worst prognosis.

Finally, a Spearman correlation was performed to correlate the value of the score with the survival time in months.

Results

Patients' characteristics

A total of 22 UM patients were evaluated. Patients' characteristics are summarized in Table 1. A group of 11 advanced BRAF wild type CM patients was also considered.

Table 1
Patients' characteristics

<i>Metastatic uveal melanoma</i>	22
Median age (range)	67.9 y (54–87)
M/F	11/11
Enucleation for primary tumor	16
Previous local treatment for liver metastases	2
Site of metastases	
liver	21
lung	5
bone	6
brain	0
other	7
Hepatic and extra-hepatic metastases	9
Extra-hepatic metastases only	1
BRAF mutation	0
<i>Metastatic cutaneous melanoma</i>	11
Median age (range)	67.8 (42–84)
Site of metastases	
liver	2
lung	4
bone	2
brain	2
other	10
BRAF mutation	0

Among metastatic UM patients, the median age was 67.9 years (range 54–87). Eleven subjects were male and 11 female. Liver metastases were found in 21 patients. None of the patients had BRAF mutation. Ocular enucleation was previously performed for the treatment of primary tumor in 16 patients, while 6 patients never underwent to enucleation. Local treatment of liver metastases (metastasectomy)

was previously carried out in 2 patients. One of them developed a not-resectable metastatic disease, while the other patient is free from disease recurrence at the time of data analysis.

All 20 patients with not-resectable metastatic disease underwent anti-PD-1 treatment with Pembrolizumab. A median of 8.8 cycles for patient were administered (range 1–52).

Modulation of immune molecules during anti-PD-1 treatment in metastatic Uveal Melanoma patients

We evaluated the release of circulating immune checkpoints and cytokines/chemokines before starting Pembrolizumab (T0) for 12 mUM patients. The sera were collected and analyzed also after 3 cycles of therapy (> T0) for all the patients, except for 3 patients who rapidly progressed. The PFS for all these patients was 3.7 months (Fig. 1A). The concentration of numerous sICs resulted significantly modulated during anti-PD1 treatment (Fig. 1B-D). In particular, analyzing the levels of sCD137 and sCD28 at T0 and > T0 (Fig. 1B), the molecules resulted significantly enhanced by 3.25-fold ($p = 0.007$) and 2.4-fold ($p = 0.01$), respectively. Similarly, the concentration of the soluble form of inhibitory receptors belonging to the immunoglobulin family significantly increased during anti-PD-1 therapy, including sPD-1 (1.96-fold, $p = 0.01$), sLAG3 (1.6-fold, $p = 0.007$) and sTim3 (1.54-fold, $p = 0.03$) (Fig. 1B). In addition, also the levels of sPD-L2 (1.38-fold, $p = 0.04$) and sCD80 (1.3-fold, $p = 0.01$), the soluble forms of PD-1 and CTLA-4 ligands respectively, resulted augmented at > T0 compared to baseline (T0) (Fig. 1B).

Pro and anti-inflammatory cytokines and chemokines were also evaluated. The most remarkable results were the significant increase of IP-10 (2.16-fold, $p = 0.007$) and CCL2 (1.29-fold, $p = 0.004$) after the beginning of anti-PD-1 therapy (> T0) (Fig. 1C). Furthermore, also Indoleamine 2,3-dioxygenase (IDO) activity, evaluated as Kynurenine/tryptophan ratio, resulted increased ($p = 0.046$) during anti-PD-1 treatment (Fig. 1D).

A score based on cytokine and soluble immune checkpoint inhibitor levels at baseline can select patients with better survival

Twelve mUM patients with baseline samples (T0) were divided into 2 groups, based on survival from the time of starting pembrolizumab: patients with survival < 6 months and patients with survival $\geq$ 6 months (Fig. 2A). Mean (SD and median IQR) of all the studied molecules are reported in the supplementary table.

The value of 2 molecules was significantly higher in patients with survival < 6 months: HVEM with a median value of 47 pg/ml (IQR 9–111,75) for those with survival < 6 months and 6 pg/ml (IQR 6–35) for those with survival $\geq$ 6 months IDO activity showed a median value of 0,038 (IQR 0,024–0,043) for patients with survival < 6 months and 0,019 (IQR 0,017–0,024) for those with survival $\geq$ 6 months.

A third molecule, IL8, was considered for the ROC analysis because of clinical interest. Indeed, it has been previously demonstrated that increased levels of this cytokine are predictive of poor efficacy in patients treated with an ICI [29]. In our study, IL 8 showed a median value of 289,92 pg/ml (IQR 48,30–314,38) for patients with survival < 6 months and of 7 pg/ml (IQR 2,70–57,15) for those with survival $\geq$ 6 months.

ROC curve analysis identified the better cut-off for the three selected molecules (Fig. 2B-D): HVEM ≥ 50 pg/ml (sensitivity 60%; specificity 83,3%; Accuracy 72,7%; AUC 0,817); IDO activity $\geq 0,024$ (sensitivity 100%; specificity 66,7%; Accuracy 81,8%; AUC 0,883); IL8 ≥ 50 pg/ml (sensitivity 80%; specificity 66,7%; Accuracy 72,7%; AUC 0,743).

Assigning a score of 1 to values higher than the cut-off for each of the three identified molecules and by summing single scores, an overall score ranging from 0 to 3 was obtained, where 3 corresponding to the worst prognosis.

The overall score showed significantly higher value in patients with survival < 6 months (Fig. 3A). The ROC analysis for the overall score (Fig. 3B) identified the better cut-off for worst survival prediction which was 2 (Overall score ≥ 2 ; sensitivity 60%; specificity 100%; Accuracy 81,8%; AUC 0,867). These results suggest that the presence of two or more of the identified molecules with values higher than the critical level is associated with a worst prognosis. Furthermore, higher values of the overall score correlate with lower survival (in months) with a coefficient $\rho = -0.490$ ($p = 0.126$). Figure 3C shows the survival of mUM patients according to the score.

Soluble factors are associated with different survival in mUM patients

Basing on the course of the metastatic disease, we identified 3 different groups of uveal melanoma patients (Fig. 4A):

- 1) Patients defined as “fast progressors” (FP) who rapidly progressed with a median survival < 6 months despite immunotherapy;
- 2) Patients defined as “slow progressors” (SP) who remained alive despite disease progression but with a median survival shorter than 30 months;
- 3) Patients defined as “long survivors” (LS) with a median survival > 30 months. Among them, 3 patients were under treatment with pembrolizumab at the time of inclusion in the study.

Among the molecules analyzed, sCD137, sGITR and sCD27 resulted significantly changed among the three groups of patients ($p < 0.05$) (Fig. 4B). Patients with fast-progressive disease had higher concentration of sICs compared to SP and LS patients (sCD137: FP vs SP $p = 0.03$; FP vs LS $p = 0.02$; sGITR: FP vs SP $p = 0.04$; sCD27: FP vs LS $p = 0.05$). Similarly, patients with slow progressive disease showed higher levels of sCD137 ($p = 0.01$), sGITR ($p = 0.01$) and sCD27 ($p = 0.01$) compared to long survivors, suggesting that the release in serum of these soluble ICs is related to severity of the disease. Similarly, elevated levels of IL-8 were detected in serum of FP patients compared to LP ($p = 0.01$) and LS ($p = 0.04$) patients (Fig. 4C). IDO activity (Kyn/Trp ratio) was similar between LS patients and those with slow progressive disease and significantly higher in fast progressive patients ($p = 0.02$) (Fig. 4D).

Metastatic Uveal Melanoma vs Metastatic Cutaneous Melanoma: differences in the release of soluble immune molecules

In order to define the immunological characteristics of metastatic uveal melanoma and metastatic cutaneous melanoma during anti-PD-1 therapy, we evaluated the release of sICs, cytokines/chemokines and IDO activity in CM patients and the results were compared with UM patients. For this analysis, we considered mUM patients with > T0 samples (9 patients) and a group of metastatic cutaneous melanoma patients whose samples were collected during first line anti-PD-1 therapy (11 patients). Median PFS for UM melanoma patients was 3.8 months, while median PFS for CM patients was not reached at the time of data analysis (Fig. 5A). Median OS for UM patients was 15.3 months, not reached for CM (Fig. 5A). Despite all patients were treated with an anti-PD-1 agent, the levels of several soluble immune molecules resulted remarkably different between two groups during the treatment. The concentration of sGITR and sCD27 (Fig. 5B) were significantly higher in UM patients ($p = 0.02$ and $p = 0.03$, respectively). sPD-1 was the unique soluble form of immune checkpoint receptors whose concentration was statistically different between the two types of cancer: higher in UM patients compared to CM ($p = 0.01$) (Fig. 5C). Among the soluble form of ligands, also the levels of sCD80 resulted increased in UM ($p = 0.03$) (Fig. 5D). Moreover, we observed that UM and CM release differently IFN γ and whose concentration were higher in the serum UM patients ($p < 0.001$) (Fig. 5E). As IFN γ is a potent inducer of IDO expression, we also compared IDO activity between two tumors and the results confirmed the higher presence of this enzyme in UM patients ($p = 0.04$) (Fig. 5F).

No significant modulation was observed for the other proteins tested (data not shown).

Discussion

Uveal melanoma and cutaneous melanoma share systemic treatments despite their different clinical and biological behavior. Indeed, anti-PD-1 therapy is largely used also for metastatic uveal melanoma but it allows a limited benefit in this disease [16].

Therefore, a current issue is to understand if there is a rationale for immunotherapy in metastatic uveal melanoma [21]. A deeper knowledge of immunological features of this disease can help to answer the questions: 1) if alternative strategies involving pathways different from PD-1 and CTLA-4 can be more promising; 2) if a selection of the patients based on immunological factors can allows a better outcome also for patients treated with anti-PD-1 agents.

Our study aimed to investigate the circulating immune profile of metastatic uveal melanoma in order to find an answer to these issues.

Levels of circulating soluble immune checkpoint molecules (sICs) and inflammatory cytokines/chemokines were determined in serum of 12 metastatic uveal melanoma patients collected at

baseline (T0) and during anti-PD-1 therapy. Our data demonstrated that after 3 cycles of Pembrolizumab the concentration of several sICs is significantly changed compared to T0. In particular, the levels of sCD137 and sCD28 during anti-PD-1 treatment resulted significantly enhanced. CD137 and CD28 are known as costimulatory receptors but previous studies have shown that their soluble forms have an inhibitory role in immune response [30, 31].

Similarly, the concentration of the soluble form of inhibitory receptors including sPD-1, sLAG3 and sTim3 significantly increased during anti-PD-1 therapy.

The chemokines IP-10 and CCL2, also increased after the beginning of anti-PD-1 therapy (> T0). Considering that these chemokines, as well as the soluble immune checkpoint molecules, are associated with poor prognosis in many tumors [32, 33], these results suggest that immunosuppression appears to be predominant in this setting of patients despite anti-PD-1 therapy.

Furthermore, the IDO activity increased during the systemic treatment. IDO acts as immune checkpoint involved in peripheral immune tolerance due to its ability to inhibit T-cells proliferation by depleting them from tryptophan to sensitize T-cells to apoptosis [34–39], reducing immune activation. Moreover, it was demonstrated that IDO can predict primary resistance to anti-PD1 treatment in solid tumors, such as non-small cell lung cancer [40, 41]. This data supports the observation that an immunosuppressive profile can be found in patients with metastatic uveal melanoma under anti-PD-1 treatment.

In this study, we also investigated the predictive role for survival of sICs, cytokines and chemokines at baseline in the group of 12 patients treated with Pembrolizumab.

Among all the factors evaluated, we found that HVEM, IDO activity and IL-8 were correlated with survival, with higher values in patients with poor survival (< 6 months). Similarly to IDO, HVEM seems to promote Treg functions [42]. Thus, in metastatic uveal melanoma an immunosuppressive environment detectable in patients' blood samples is associated with poor prognosis despite anti-PD-1 treatment. IL-8 is a proinflammatory cytokine: high plasmatic levels of IL-8 are associated with decreased efficacy of checkpoint inhibitors in an inflamed tumor [29, 43]. Considering IDO, HVEM and IL-8, we obtained a score based on their serum basal levels, able to predict patients' survival. A prospective validation in a larger patients' population is advisable to avoid ineffective treatments.

Considering all the metastatic uveal melanoma patients included in the study, we found three different course of disease with specific profiles of circulating immune checkpoints and cytokines. Therefore, all the patients with metastatic uveal melanoma were divided into three groups considering the survival: fast progressors (FP), slow progressors (SP) and long survivors (LS). Serum levels of CD137, GITR and CD27 resulted significantly different among the three groups of the patients, with fast-progressive disease group that had higher concentration of sICs compared to SP and LS.

Similarly, IDO activity was significantly higher in fast progressive patients, as well as IL-8.

In contrast to cutaneous melanoma, UM is usually poorly responsive to checkpoint inhibitors [44]. In order to define immunological differences between metastatic uveal melanoma and metastatic cutaneous melanoma during anti-PD-1 therapy, we evaluated the release of sICs, cytokines/chemokines and IDO activity in CM patients and the results were compared with UM patients. Despite all the patients were treated with an anti-PD-1 agent, the levels of several soluble immune molecules were remarkably different between the two groups during the treatment. In fact, the concentration of sGITR and sCD27 were significantly higher in UM patients. It has been demonstrated that sGITR promotes Helios expression and enhances function of regulatory T cells [45]. On the other hand, the immunological function of sCD27 has not yet been clarified. Among the soluble form of inhibitory receptors, only sPD-1 concentration was statistically different between the two types of cancer: higher in UM patients compared to CM. Regarding the soluble form of ligands, the levels of sCD80 resulted increased in UM. It is well known that CD80 on APC cells is required for rejection of immunogenic tumor in animal models [46]. Recently, it has been demonstrated that CD80 on APC can bind PD-L1 avoiding PD-1/PD-L1 interaction and consequently blocking an inhibitory signal for T cell activation [47]. High serum level of CD80 could reflect the shedding of CD80 and contribute to maintain an immunosuppressive microenvironment.

Furthermore, we observed a higher concentration of both IFN γ in the serum of UM patients. IFN γ is a potent inducer of IDO expression, indeed a higher presence of this enzyme in UM patients was confirmed.

The different expression of circulating factors in the serum of patients with metastatic uveal melanoma and metastatic cutaneous melanoma during anti-PD-1 therapy can offer a possible explanation of the different clinical efficacy obtained with checkpoint inhibitors in these melanoma subtypes. Indeed, our findings show immunosuppressive features of uveal melanoma compared to cutaneous melanoma.

Conclusions

This study provides preliminary data in a limited population of patients with a rare disease as uveal melanoma. The unavailability of a baseline sample for all the patients with uveal melanoma limits the interpretation of the data. Other studies with a largest number of patients are necessary to confirm these observations. Nevertheless, this is the first study that evaluates the correlation between sICs, cytokines and chemokines with clinical outcome in metastatic uveal melanoma.

The limited response to anti-PD-1 therapy in uveal melanoma may be justified by a poor activation of immune system. However, some patients with metastatic uveal melanoma had a long survival. These patients could be identified by a score based on the circulating immune molecules such as HVEM, IDO and IL-8. These data support the role of immunity on the course of the advance disease, suggesting that immunotherapy should not be abandoned for metastatic uveal melanoma. Some of the studied immunological molecules could also offer new therapeutic targets.

Moreover, the comparison of circulating immune profile during anti-PD-1 therapy between uveal melanoma and cutaneous melanoma reflects the different efficacy of immune checkpoint inhibitors in these diseases.

List Of Abbreviations

UM
uveal melanoma
CM
cutaneous melanoma
ICIs
immune checkpoint inhibitors
mUM
metastatic uveal melanoma
PSF
progression-free survival
OS
overall survival
CTCAE
common terminology criteria for adverse events
mCM
metastatic cutaneous melanoma
sICs
soluble immune checkpoint molecules
trp
tryptophan
Kyn
kynurenine
FS
fast progressors
SP
slow progressors
LS
long survivors
SD
standard deviation
IQR
interquartile range
IDO
indoleamine 2,3-dioxygenase
ROC
receiver operating characteristic

Declarations

Ethics approval and consent to participate

The study has been approved by Ethics Committee of Fondazione Policlinico Universitario Agostino Gemelli IRCCS (ID3709). All the patients provided written informed consent before participating in the study.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

Competing interests

The authors declare that they have no competing interests. ER had a role as consultant for MSD. EB reported speakers' and travel fees from MSD, Astra-Zeneca, Celgene, Pfizer, Helsinn, Eli-Lilly, BMS, Novartis, and Roche; consultant's fees from Roche and Pfizer; and institutional research grants from AstraZeneca and Roche. PM has/had a role as consultant/advisory for BMS, Roche Genentech, MSD, Novartis, Amgen, Merk Serono, Pierre Fabre and Incyte. GT has role as consultant for BMS and MSD.

Funding

The study was funded by institutional resources by Università Cattolica del Sacro Cuore.

Authors' contributions

ER, IGZ, GS, MN designed the study, acquired, analyzed and interpreted the data and drafted the work. ADF and AC acquired, analyzed and interpreted the data and drafted the work. MS and LL analyzed and interpreted the data. MMP, MGS and MAB acquired the data. AB, EB, PM and GT revised the work. All the authors approved the submitted version.

Acknowledgements

EB is currently supported by the Associazione Italiana per la Ricerca sul Cancro (AIRC) under Investigator Grant (IG) No. IG20583 and by Institutional funds of Università Cattolica del Sacro Cuore (UCSC-project D1-2018/2019). GT is supported by AIRC, IG18599, AIRC 5x1000 21052. GS is currently supported by Institutional funds of Università Cattolica del Sacro Cuore (UCSC-project D1 2018/2019). MN reports research grant from Incyte and IPSEN.

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Figures

Fig. 1

Figure 1

Modulation of soluble immune molecules in mUM patients during anti-Pd1 treatment. A. PFS of mUM patients treated with Pembrolizumab. B-D. Levels of soluble immune checkpoint proteins and inflammatory cytokines/chemokines were measured in sera of metastatic uveal melanoma patients at baseline (T0) and during anti-PD1 treatment (>T0). Proteins were analyzed by Luminex multiplex beads and results are reported as concentration (pg/ml). B: sCD137, sCD28, sPD1, sLAG3, sTim3, sPD-L2 and sCD80; C: chemokines IP10 and CCL2 ; D: IDO activity measured as KYN/trp ratio by modified liquid chromatography –tandem mass spectrometry (LC-MS/MS) method. The kyn/trp ratio was reported as average value $\pm$ SEM (T0: 0.033 $\pm$ 0.015; >T0: 0.063 $\pm$ 0.032). Wilcoxon matched-pairs signed rank test was used and a p value < 0.05 was considered statistically significant. (PFS: progression free survival. m: months)

Fig. 2

Figure 2

Baseline levels of cytokines and soluble immune checkpoint inhibitors can select patients with better survival. A. Course of disease for 12 uveal melanoma patients treated with Pembrolizumab: Solid line: patients with OS < 6 months. Dotted line: patients with OS ≥ 6 months. B-D. ROC curve analysis for the identified molecules: B: HVEM; C: IDO ratio; D: IL-8. The values were compared between patients with a survival < 6 months or ≥ 6 months using the Mann-Whitney test. (OS: overall survival. m: months.)

Fig. 3

Figure 3

A score based on cytokines and soluble immune checkpoint inhibitors can predict patients' survival. A. Overall score for total sample and by patients' survival. B. ROC curve analysis for the overall score. A score of 1 was assigned to values higher than the cut-off for each of the three identified molecules; an overall score ranging from 0 to 3 was obtained by summing single scores. C. Patients survival based on the score: solid line: patients with score 2-3; dotted line: patients with score 0-1. (OS: overall survival. SD: standard deviation. IQR: interquartile range)

Fig. 4

Figure 4

Profiling of soluble immune molecules in UM patients stratified according to the course of metastatic disease. A. UM patients were classified in fast progressor (FP), slow progressor (SP) and long survivor (LS) according to their response to pembrolizumab treatment. B-D. In the histograms, the serum levels of each protein were reported as average value $\pm$ SEM: B: sCD137 (FP: 361,8 pg/ml $\pm$ 123,1; SP: 140,1 pg/ml $\pm$ 16,77; LS: 65,5 pg/ml $\pm$ 20,75), sGITR (FP: 70,2 pg/ml $\pm$ 29,81 ; SP: 25,33 $\pm$ 6,644 ; LS: 11,4 $\pm$ 0,4) and sCD27 (FP: 10258 $\pm$ 3758; SP: 6027 $\pm$ 870,5; LS: 2610 $\pm$ 460,5); C: levels of IL-8 (FP: 212,31 pg/ml $\pm$ 114,3; SP: 12,33 $\pm$ 4,017; LS: 6,333 $\pm$ 2,348); D: IDO activity (FP: 0.047 $\pm$ 0.021; SP: 0.02 $\pm$ 0.007; LS: 0.023 $\pm$ 0.006). (OS: overall survival. FP: fast progressors, with a survival <6 months. SP: slow progressors, with a survival >6 months and <30 months. LS: long survivors, with a survival >30 months).

Fig. 5

Figure 5

Immune profile of mUM patients compared to mCM A. PFS and OS of patients treated with an anti-PD-1 as first line treatment for metastatic disease. Solid line: uveal melanoma. Dotted line: cutaneous melanoma. B-E. mUM patients were compared to CM patients, both treated with anti-PD-1 therapy. In the scatter plot the longest bars represent the average value, the shortest ones indicate the error bar ($\pm$ SEM). B: sGITR (mUM: 52,49 pg/ml $\pm$ 20,56; mCM: 12,88 $\pm$ 2,065) and sCD27 (mUM: 8204 $\pm$ 2190, mCM: 3025 $\pm$ 641,4B); C: sPD1 (mUM: 129,6 $\pm$ 34,79 ; mCM: 40,18 $\pm$ 13,84; D: levels of sCD80 (mUM: 630,9 $\pm$ 185,6 ; mCM: 240,1 $\pm$ 113,1); E: IFN γ (mUM: 258,1 $\pm$ 92,4 ; mCM: 4,778 $\pm$ 1,665). F. The histogram shows the IDO activity (Kyn/trp ratio) in mUM and CM (UM: 0.069 $\pm$ 0.01 ; CM: 0.035 $\pm$ 0.0036). (PFS: progression free survival. OS: overall survival. UM: uveal melanoma. CM: cutaneous melanoma. NR: not reached)

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