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Targeting myeloid-derived suppressor cells using all-trans retinoic acid in melanoma patients treated with Ipilimumab

Richard P. Tobin[a,b,1], Kimberly R. Jordan[a,c,1], William A. Robinson[a,d,f,2], Dana Davis[a,b,1], Virginia F. Borges[a,d,e,f,2], Rene Gonzalez[a,d,f,3], Karl D. Lewis[a,d,f,3], Martin D. McCarter[a,b,f,*]

[a] University of Colorado Denver Anschutz Medical Campus, Aurora, CO, USA
[b] Division of Surgical Oncology, Department of Surgery, USA
[c] Department of Immunology and Microbiology, USA
[d] Division of Medical Oncology, Department of Medicine, USA
[e] Young Women's Breast Cancer Translational Program, USA
[f] University of Colorado Cancer Center, Aurora, CO, USA

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ABSTRACT

Background: Immune checkpoint inhibitors have improved overall survival rates for many cancers, yet the majority of patients do not respond to treatment and succumb to disease progression. One tumor-related mechanism limiting the efficacy of immunotherapies in melanoma is the recruitment and expansion of myeloid-derived suppressor cells (MDSCs). Therefore, targeting MDSCs in combination with immunotherapies is an attractive strategy to improve response rates and effectiveness.

Methods: We tested this strategy by designing a randomized phase II clinical trial treating advanced melanoma patients with either Ipilimumab monotherapy or Ipilimumab plus all-trans retinoic acid (ATRA). Clinicaltrails.gov identifier (NCT02403778). The frequency of circulating MDSCs and the activation of CD8(+) T cells was measured by flow cytometry. Expression of immunosuppressive genes was measured with quantitative real time-PCR. T cell suppressive functions were measured by mixed lymphocyte reaction.

Results: Here we show that *in vitro* treatment with ATRA decreases immunosuppressive function of MDSCs in mixed lymphocyte reactions. Additionally, ATRA reduces the expression of immunosuppressive genes including PD-L1, IL-10, and indoleamine 2,3-dioxygenase by MDSCs. Furthermore, the addition of ATRA to standard of care Ipilimumab therapy appears safe, as ATRA did not increase the frequency of grade 3 or 4 adverse events. Finally, ATRA significantly decreased the frequency of circulating MDSCs compared to Ipilimumab treatment alone in advanced-stage melanoma patients.

Conclusions: These results illustrate the importance of MDSCs in immunotherapy resistance and provide evidence that targeting MDSCs in cancer patients may augment immunotherapeutic approaches.

1. Introduction

Ipilimumab, a fully humanized anti-cytotoxic lymphocyte antigen 4 (CTLA-4) antibody, was the first U.S. Food and Drug Administration (FDA) approved therapy to significantly improve overall survival (OS) in advanced melanoma patients [1] and was the vanguard for a new generation of immunotherapies. In clinical trials, Ipilimumab increased median overall survival from 6.4 to 10.1 months in in pre-treated metastatic melanoma patients and, more importantly, demonstrated that a subpopulation of patients experience a durable response [1]. While Ipilimumab has improved the outlook for many melanoma patients, melanoma wields a vast arsenal of resistance mechanisms that decrease

* Corresponding author at: University of Colorado Denver Anschutz Medical Campus, Department of Surgery, Division of Surgical Oncology, 12631 E. 17th Avenue, MS C313, Aurora, CO 80045.
E-mail addresses: richard.tobin@ucdenver.edu (R.P. Tobin), kimberly.jordan@ucdenver.edu (K.R. Jordan), william.robinson@ucdenver.edu (W.A. Robinson), dana.davis@ucdenver.edu (D. Davis), virginia.borges@ucdenver.edu (V.F. Borges), rene.gonzalez@ucdenver.edu (R. Gonzalez), karl.lewis@ucdenver.edu (K.D. Lewis), martin.mccarter@ucdenver.edu (M.D. McCarter).
[1] 12800 E. 19th Ave., RC1N, Aurora, CO 80045, USA.
[2] 12801 E. 17th Ave., RC1S, Aurora, CO.80045, USA.
[3] 1665 Aurora Ct., Aurora, CO 80045, USA.

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the efficacy of many types of cancer therapies [2–5].

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immature immunosuppressive myeloid lineage cells [6–8]. The frequency of MDSCs is expanded in the spleen, circulation of patients with cancer, and in the tumor microenvironment of a variety of cancers [9–11]. Notably, increased numbers of circulating MDSCs are correlated with decreased responses to Ipilimumab in melanoma patients [12,13]. Accumulation of MDSCs in the tumor microenvironment and periphery is driven by tumor-derived factors including chemokines, growth factors, and cytokines [8]. MDSCs promote tumor growth by producing immunosuppressive molecules in the tumor microenvironment such as interleukin-10 (IL-10), transforming growth factor-β (TGFβ), reactive oxygen species (ROS), as well as expressing cell surface receptors that inhibit T cell proliferation and activation, and producing molecules that directly promote tumor growth and invasion such as vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs) [8,14]. This complex set of immunosuppressive and tumor promotional mechanisms pose a significant obstacle to the successful treatment of melanoma and other cancers. Therefore, targeting MDSCs represents an attractive strategy to improve the effectiveness of existing immunotherapies.

The vitamin A derivative all-trans retinoic acid (ATRA) is the current standard of care therapy for the treatment of acute promyelocytic leukemia (APL) [15]. In APL patients, ATRA terminally differentiates immature myelocytic tumor cells, resulting in death of the tumors cells [16]. Similar to its effects on immature myelocytic tumor cells, ATRA induces the differentiation of MDSCs, resulting in decreased frequencies of MDSCs in the circulation. Although the mechanism of MDSC differentiation following ATRA treatment is still under investigation, ATRA has been shown to decrease both the frequency and function of MDSCs through activation of ERK1/2, upregulation of glutathione synthase, and generation of glutathione [17]. The increased production of glutathione decreased production of ROS and resulted in subsequent terminal differentiation of MDSCs [17].

ATRA has been combined with other therapies in several clinical trials directly targeting solid tumors [18–21], and in two previous clinical trials targeting MDCSs [22,23]. In the prior MDSC trials, ATRA decreased the frequency of circulating MDSCs in advanced renal cell carcinoma and lung cancer patients when combined with either high dose IL-2 [22] or with a cancer vaccine [23]. Based on these preceding observations, we designed a clinical trial with the goal of reducing the effects of MDSCs in advanced melanoma patients. This trial builds on the success of Ipilimumab by combining it with ATRA to target MDSCs. Patient enrollment in this study was halted due to low enrollment following the approval of anti-PD1 therapies. However, these initial results show that MDSCs can be targeted with ATRA in melanoma patients during immune checkpoint inhibitor therapy.

2. Methods

2.1. Description of patient population and treatment

Patients with stage III or stage IV melanoma were enrolled in this study. The trial was registered at ClinicalTrials.gov, https://clinicaltrials.gov/ct2/show/NCT02403778, in February 2015 as NCT02403778. All patients provided a written informed consent, and the treatment protocol was approved by Colorado Multiple Institutional Review Board. Patients being considered for treatment with Ipilimumab were consented and randomized to either Ipilimumab alone or combination Ipilimumab plus ATRA. Patients under the age of 18, who received systemic therapy within four weeks prior to beginning this study, women pregnant or nursing, or patients with a contraindication for taking either ATRA or Ipilimumab were excluded from this trial. Based upon these criteria, ten patients were enrolled at the University of Colorado Cancer Center (Aurora, CO) from December 2015 to August 2016 (Table 1). None of the patients enrolled in this study were previously treated with systemic therapies for their melanoma. Ipilimumab was given according to the standard of care consisting of four adjuvant infusions of 10 mg/kg for stage III patients or four infusions of 3 mg/kg for stage IV patients every three weeks [1,24]. Of the patients with stage III disease, three received the additional maintenance doses of Ipilimumab, while four did not receive the maintenance doses due to either toxicity or personal choice to end therapy. Patients in the Ipilimumab plus ATRA arm were treated with ATRA at 150 mg/m^2 on the day before, the day of, and the day after Ipilimumab infusion (Fig. 1). Patients were assigned to treatment groups by random allocation concealment using blinded envelope draw. Progression was determined according to response evaluation criteria in solid tumors (RECIST 1.1) guidelines, as measured by standard of care imaging every three months. Safety was monitored, and adverse events were classified according to Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

Table 1
Baseline patient characteristics.

Characteristic	Ipilimumab plus ATRA	Ipilimumab	Total
	[N = 4]	[N = 6]	[N = 10]
Sex – no. (%)			
F (%)	3 (75)	3 (50)	6 (60)
M (%)	1 (25)	3 (50)	4 (40)
Disease stage at study entry - no. (%)			
III	2 (50)	6 (100)	8 (80)
IV	2 (50)	0 (0)	2 (20)
Age - years			
Mean	54.8	50.4	52.1
Range	(48.2–60.1)	(33.8–66.6)	(33.8–66.6)
BMI			
Mean	31.7	25.95	28.5
Range	(22.88–41.0)	(22.7–33.1)	(22.88–41.0)

This trial was originally designed to recruit 24 patients into each arm and was powered to test the intended initial intended primary objectives 1) determine the safety and tolerability of Ipilimumab and ATRA combination therapy in advanced melanoma patients, 2) determine if MDSC frequency in peripheral blood of advanced melanoma patients is altered by Ipilimumab and ATRA combination therapy, and 3) determine if Ipilimumab and ATRA alter the suppressive function of peripheral blood MDSCs. The trial was closed to accrual due to low enrollment following the approval of anti-PD1 therapies.

2.2. Sample collection

Peripheral blood was collected from each patient into tubes containing acid citrate dextrose anticoagulant (BD Biosciences). Research blood draws were performed at five time points; pre-treatment (0 to 30 days prior to the first Ipilimumab infusion), Draw 2 (day 21), Draw 3 (day 42), Draw 4 (day 63), and post treatment (84–130 days) as illustrated in Fig. 1A. Each research blood draw corresponded with standard of care clinical blood testing including complete blood counts (CBC) with automated differential count.

2.3. In vitro MDSC generation and RT-PCR

Human MDSCs were generated by first isolating CD14(+) cells from leukoreduction system chambers collected from normal healthy donors (Bonfils Blood Center) by positive magnetic selection (Miltenyi Biotec). Isolated CD14(+) cells were incubated with 20 ng/mL GM-CSF and 20 ng/mL IL-6 [25] (Biolegend) for five days in RPMI 1640 media (Gibco) supplemented with 10% normal human serum (Gemini Bio Products), 2 mM L-glutamine (Mediatech), 100 μg/mL Streptomycin (Mediatech), 100 IU/mL Penicillin (Mediatech), 25 mM HEPES