Investigating the Roles of Bim and CX3CR1/Granzyme B in Predicting Response to PD-1 Blockade Therapy

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Abstract

Background: Immunotherapy targeting immune checkpoints has become a common approach for many types of cancer but currently there are no reliable biomarkers to predict and monitor treatment response. Immunohistochemical staining of PD-L1 could be problematic due to tumor heterogeneity, variations in specimen preparation and multiple scoring schemes. There is, therefore, a critical need for a better biomarker to guide the use of immunotherapy to improve clinical outcomes.

Objective: In this study, we investigated the roles of Bim and CX3CR1/granzyme B in patients receiving PD-1 blockade therapy (*i.e.* pembrolizumab) for small bowel adenocarcinoma (SBA) as part of planned correlative analysis of the ACCRU clinical trial, NCT02949219.

Methods: Patients with unresectable or metastatic biopsy-proven SBA (excluding ampulla of Vater and appendix) who had at least one prior line of systemic chemotherapy were eligible for the trial. Pembrolizumab (200 mg) was administered over 30 minutes intravenously every 3 weeks until unacceptable toxicity, disease progression, or patient refusal. All patients underwent peripheral blood collection at baseline prior to initiation of treatment and then after 3 cycles of treatment. Levels of Bim and CX3CR1/granzyme B in circulating T cells were quantified by flow cytometry using patients' peripheral blood mononuclear cells (PBMCs). Data was analyzed via Cox proportional hazards model, Wilcoxon Rank-Sum test, and Kaplan Meier curves using SAS 9.4.

Results: A total of 35 eligible patients were included in the analysis. Three patients had confirmed response (all partial responses) and 10 had stable disease as their best overall response. Seven patients had disease progression around the second blood draw. The median time from baseline to the second blood draw was 9 weeks. Lower levels of Bim in CD8+CD11a^{hi} T cells at baseline on the percentage scale were associated with treatment response (median 54.5 % vs. 79.5 %; p=0.0087) and disease control (partial response plus stable disease; median 71.2 % vs. 81.3 %; p=0.0104) and higher levels led to worse progression-free survival (hazard ratio=1.05, 95 % confidence interval 1.01-1.08; p=0.0051). In addition, positive changes in CX3CR1/granzyme B in CD8+CD11a^{hi} T cells from baseline were associated with better overall survival (median 20.2 months vs. 3.7 months, p=0.0086). The levels of PD-1 and Ki-67 in CD8+CD11a^{hi} T cells were not associated with treatment response or survival.

Introduction

- Pembrolizumab is a humanized monoclonal antibody against PD-1 that blocks the interactions between PD-1 receptors and their ligands. We conducted a multicenter, single arm, phase II trial to assess the single-agent efficacy of pembrolizumab in patients who had received ≥1 prior line of systemic chemotherapy for unresectable or metastatic SBA.
- As part of the planned correlative analysis, we investigated the expression of Bim and CX3CR1/granzyme B in peripheral CD8+ T cells before and after treatment with pembrolizumab by flow cytometry.
  - Bim (BCL-2 like protein 11) is a PD-1 downstream signaling molecule. Upregulation of Bim in CD8+ T cells was observed with increased PD-1/PD-L1 interactions and found to have a negative impact on survival of patients with metastatic melanoma.[1]
  - CX3CR1 is a chemokine receptor that binds CX3CL1, which is highly expressed by tumor tissues. Granzyme B, a cytotoxic serum protease, is secreted by cytotoxic T cells during T cell-mediated apoptosis. Increased expressions of CX3CR1 and granzyme B in CD8+ T cells were observed in responders after treatment with immunotherapy.[2]

Eligibility

- Biopsy-proven SBA excluding ampullary and appendiceal tumors
- Tumor cell PD-L1 expression or microsatellite instability are not required
- Had at least one prior line of systemic chemotherapy
- Prior treatment with immune checkpoint inhibitors was not allowed
- ECOG performance status of 0 or 1
- Adequate bone marrow, renal and liver function
- Patients with active CNS metastases, additional malignancies, autoimmune diseases, and infections requiring systemic therapy are excluded

Results

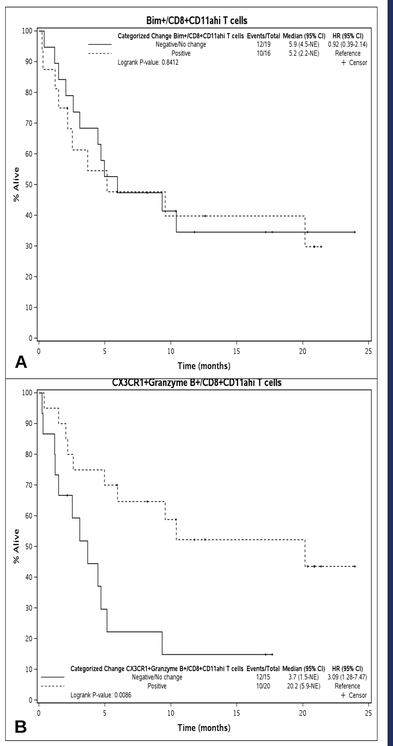
- Of the 41 patients enrolled to the study, we had baseline and post-baseline biomarker data for 35 eligible patients.
- For these 35 patients, the median time from baseline to the posttreatment blood draw was 9 weeks (range, 1.6 to 22.3 weeks).
- Of these 35 patients, 28 were progression free at the second blood draw after 3 cycles of treatment.

❖ Higher levels of Bim in CD8+CD11a^{hi} cells at baseline (pre-treatment) were associated with shorter progression-free survival.

Table 1. Univariate associations between baseline biomarkers and survival.

Biomarker	Hazard Ratio	95% CI	P-value
Progression-free survival (events/total = 31/35)			
CD8+CD11a^{hi}	0.96	0.90-1.03	0.2578
Bim+/CD8+CD11a^{hi}	1.05	1.01-1.08	**0.0051**
CX3CR1+Granzyme B+/ CD8+CD11a^{hi}	1.00	0.93-1.06	0.8859
PD-1+/CD8+CD11a^{hi}	1.00	0.98-1.01	0.9057
Ki-67+/CD8+CD11a^{hi}	1.01	0.96-1.07	0.6756
Overall survival (events/total = 22/35)			
CD8+CD11a^{hi}	0.99	0.92-1.07	0.7999
Bim+/CD8+CD11a^{hi}	1.02	0.98-1.05	0.3447
CX3CR1+Granzyme B+/ CD8+CD11a^{hi}	1.03	0.95-1.12	0.4470
PD-1+/CD8+CD11a^{hi}	0.99	0.98-1.01	0.5596
Ki-67+/CD8+CD11a^{hi}	1.02	0.95-1.09	0.6597

❖ Positive changes in CX3CR1/granzyme B in CD8+CD11a^{hi} cells from baseline were associated with better overall survival (median 20.2 months vs. 3.7 months, p=0.0086; Figure 1B).



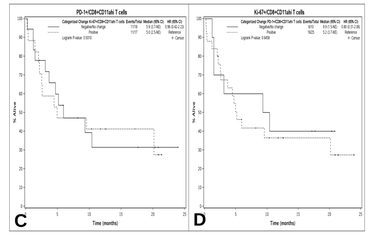


Figure 1. Kaplan Meier curves comparing overall survival in patients who had a positive change from baseline in Bim (A), CX3CR1/granzyme B (B), PD-1 (C) and Ki-67 (D) versus those who did not.

❖ Lower levels of Bim in CD8+CD11a^{hi} T cells at baseline were associated with treatment response (median 54.5 % vs. 79.5 %; p=0.0087) and disease control (partial response plus stable disease; median 71.2 % vs. 81.3 %; p=0.0104).

Table 2. Associations between baseline biomarkers and treatment response.

Biomarker	Responders (median, range)	Nonresponders (median, range)	P-value
CD8+CD11a^{hi}	10.6, 1.7-24.1	6.2, 0.5-22.5	0.4263
Bim+/CD8+CD11a^{hi}	54.5 45.0-64.8	79.5, 54.8-96.6	**0.0087**
CX3CR1+Granzyme B+/ CD8+CD11a^{hi}	2.1, 1.7-2.9	2.6, 0.3-18.7	0.7909
PD-1+/ CD8+CD11a^{hi}	26.9, 4.9-56.1	27.4, 4.0-75.8	0.7459
Ki-67+/ CD8+CD11a^{hi}	5.2, 1.5-6.9	5.6, 1.2-29.1	0.4614

Table 3. Associations between baseline biomarkers and disease control.

Biomarker	Patients with at least stable disease (median, range)	Patients with progressive disease (median, range)	P-value
CD8+CD11a^{hi}	8.7, 1.7-24.1	6.0, 0.5-22.5	0.4224
Bim+/CD8+CD11a^{hi}	71.2, 45.0-88.0	81.3, 55.1-96.6	**0.0104**
CX3CR1+Granzyme B+/ CD8+CD11a^{hi}	2.9, 0.3-18.7	2.4, 0.5-12.7	0.4224
PD-1+/CD8+CD11a^{hi}	26.9, 4.9-75.8	26.4, 4.0-74.5	0.7717
Ki-67+/CD8+CD11a^{hi}	6.9, 1.2-13.2	4.6, 1.6-29.1	0.7847

Conclusions

- Our findings are consistent with the results of previously reported studies.[1,2]
- Lower baseline Bim levels in circulating CD8+ T cells were associated with favorable treatment response to pembrolizumab.
- Upregulation of CX3CR1+/Granzyme B+/CD8+ T cells after treatment with pembrolizumab was associated with improved overall survival.

References

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