Poster # 5529

Differentiated Activity Profile for the PD-1 Inhibitor Balstilimab

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Background

Targeting the Programmed Death 1 (PD-1) immune checkpoint pathway has provided an important advance for the treatment of patients with advanced cervical cancer.[1]

Balstilimab (AGEN2034), a novel anti-PD-1, has demonstrated meaningful and durable single-agent activity (ORR, 15%; DOR, 15.4 months) in the largest clinical trial to date in patients with recurrent/metastatic cervical cancer who have failed prior platinum chemotherapy (R/M CC, 140 pts; NCT03104699).[1] Notably, balstilimab was active in PD-L1$^+$ and PD-L1$^-$ tumors (ORR 20%, DOR NR and 8% DOR 15.4, respectively) and across histology (ORR SCC 18% and ACC 13%) with meaningful durability. Bal is currently in clinical trials as monotherapy and in combination with Agenus' first- and next-generation (Fc-enhanced) anti-CTLA-4 antibodies.

These data compare favorably to FDA-approved and/or standard chemotherapy in this setting and suggest a broader benefit beyond PD-L1-positive tumors which define the current indication for pembrolizumab in cervical cancer.[2]

A Method to Interrogate the Activity of PD-1 Antibodies on Exhausted T Cells *in vitro*

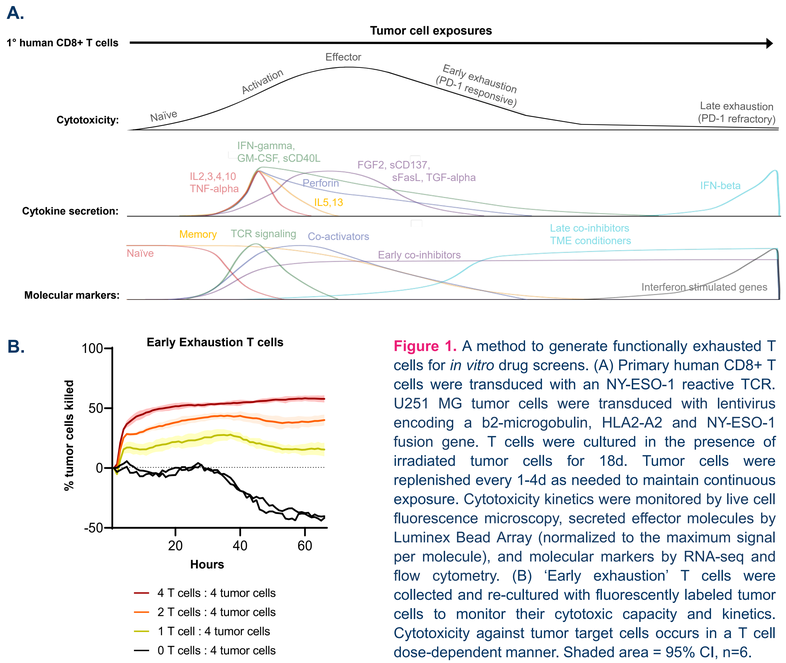

Figure 1. A method to generate functionally exhausted T cells for *in vitro* drug screens. (A) Primary human CD8+ T cells were transduced with an NY-ESO-1 reactive TCR. U251 MG tumor cells were transduced with lentivirus encoding a b2-microgobulin, HLA2-A2 and NY-ESO-1 fusion gene. T cells were cultured in the presence of irradiated tumor cells for 18d. Tumor cells were replenished every 1-4d as needed to maintain continuous exposure. Cytotoxicity kinetics were monitored by live cell fluorescence microscopy, secreted effector molecules by Luminex Bead Array (normalized to the maximum signal per molecule), and molecular markers by RNA-seq and flow cytometry. (B) 'Early exhaustion' T cells were collected and re-cultured with fluorescently labeled tumor cells to monitor their cytotoxic capacity and kinetics. Cytotoxicity against tumor target cells occurs in a T cell dose-dependent manner. Shaded area = 95% CI, n=6.

A Comparative Preclinical Study of PD-1 Antibodies Corroborates Clinical Signals

Objective

Variable response rates have been observed across clinical trials for PD-1 antibodies in R/M CC. PD-L1 positivity is an incomplete biomarker in this setting, with both balstilimab and other PD-1 antibodies demonstrating the potential to elicit responses in patients with PD-L1 negative tumors.[3] We developed a human primary cell-based assay to compare the activity of PD-1 antibodies on functionally exhausted T cells in PD-L1 positive and negative settings.

Balstilimab Demonstrates Superior Killing of PD-L1 Negative Tumor Models Compared to Nivolumab and Pembrolizumab

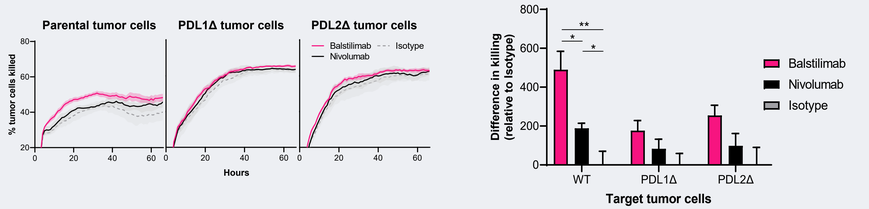

Figure 2 (Donor 1). The ability of PD-1 antibodies to improve cytotoxicity of early exhausted T cells was quantified as the difference in area under the tumor cell killing curve relative to isotype control. Balstilimab enhanced T cell cytotoxicity and outperformed nivolumab against parental tumor cell; similar trends were observed against tumor cells where PD-L1 or PD-L2 were genetically deleted. Left panels: Error bars = SEM, n=6. *p<0.05, **p<0.01, Wilcox Test. Right panels: Shaded area = 95% CI, n=6.

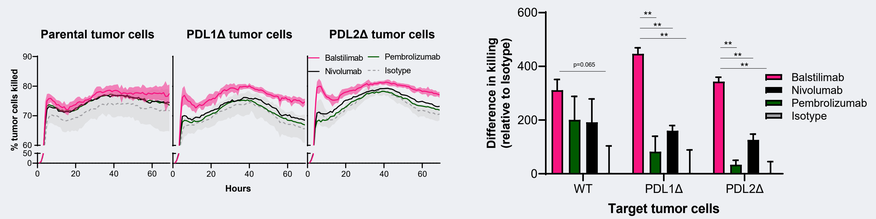

Figure 3 (Donor 2). The ability of PD-1 antibodies to improve cytotoxicity of early exhausted T cells was quantified in an independent experiment using primary T cells from a different donor. Here, balstilimab enhanced T cell cytotoxicity and outperformed nivolumab and pembrolizumab against tumor cells where PD-L1 or PD-L2 were genetically deleted; similar trends were observed against parental tumor cells. Left panels: Error bars = SEM, n=6. **p<0.01, Wilcox Test. Right panels: Shaded area = 95% CI, n=6.

Deeper Science, Better Medicine

This study illuminates subtle differences among PD-1 antibodies. Our preclinical method enables us to interrogate the molecular and cellular features linked to these functional differences. These findings may help us predict the tumor types, subtypes, and immune contexts for which balstilimab would deliver the maximal clinical benefit. Moreover, the method can be adapted for *in vitro* drug screening to identify and optimize balstilimab combination therapies.

A Phase 2 Study of Balstilimab in Patients With Recurrent or Metastatic Cervical Cancer (NCT03104699)

Balstilimab Responses Across Molecular and Histological Subgroups

Subgroup	*N*	Confirmed ORR (%) [95% CI]	DOR (mo.) Median (range)
All patients	140	**15.0** [10.0, 21.8]	**15.4** [2.8 - 17.8+]
PD-L1+	85	**20.0** [12.9, 29.7]	**NR**
PD-L1-	38	**7.9** [2.7, 20.8]	**15.4** [4.2 – 15.4]
Squamous cell carcinoma (SCC)	85	**17.6** [11.0, 27.1]	**15.4** [4.1+ - 17.8+]
Adenocarcinoma	48	**12.5** [5.9, 24.7]	**8.5** [2.8 – 15.8+]
SCC/PD-L1+	62	**21.0** [12.7, 32.6]	**NR**
Adenocarcinoma/PD-L1+	20	**20.0** [8.1, 41.6]	**7.1** [2.8 – 15.8+]

Table 1. Subset analyses of efficacy outcomes for balstilimab monotherapy in patients with recurrent/metastatic cervical cancer who had relapsed after one prior line of platinum-based chemotherapy for advanced disease. In this single-arm, phase 2 trial, balstilimab was administered intravenously at a dose of 3 mg/kg once every two weeks (Q2W), for up to 24 months. ORR, objective response rate; DOR, duration of response; NR, not reached.

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Conclusions

- Balstilimab exhibits durable responses in R/M CC subgroups that are less likely to respond to treatment, including patients with the poorest prognosis, those with PD-L1 negative and/or adenocarcinoma histology.
- Balstilimab may exhibit functional differentiation from pembrolizumab and nivolumab, with an opportunity to provide clinical benefit to a greater proportion of patients
- Preclinical studies demonstrate the potential for superior balstilimab activity in PD-L1 negative tumor models. Relative activity on PD-L1 positive versus negative tumor models varied between individual T cell donors, indicating that immune context is a critical determinant.
- Better predictive biomarkers are needed to improve clinical responses to PD-1 inhibitors in R/M CC. Systematic preclinical approaches can be used to identify biomarker signatures that capture the network-level biology of PD-1 and other immunotherapies.

References:

1. O'Malley et al. 2020 *Ann Oncol* 31: S1164-65
2. Chung et al. 2019 *J Clin Oncol* 37: 1470-1478
3. Grossman et al. 2021 *Oncogene* 40: 1393-1395

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