

determine irAE type-specific incidence, the incidence of multiple irAEs in a single patient, and response to corticosteroid therapy.

**Acknowledgements** We would like to thank the Department of Hematology/Oncology of the University of Cincinnati, the University of Cincinnati Cancer Center, and the Department of Internal Medicine of the University of Cincinnati for their support.

**Ethics Approval** Considered and Approved by the University IRB Approval #2019-0610.

## REFERENCES

- Weber JS, Hodi FS, Wolchok JD, et al. Safety Profile of Nivolumab Monotherapy: A Pooled Analysis of Patients With Advanced Melanoma. *J Clin Oncol* 2017;**35**(7):785–792.
- Yamauchi I, Yasoda A, Matsumoto S, et al. Incidence, features, and prognosis of immune-related adverse events involving the thyroid gland induced by nivolumab. *PLoS ONE*. 2019;**14**(5):e0216954.

<http://dx.doi.org/10.1136/jitc-2020-SITC2020.0252>

253

### ANTI-TIGIT ANTIBODIES REQUIRE ENHANCED FcγR CO-ENGAGEMENT FOR OPTIMAL T AND NK CELL-DEPENDENT ANTI-TUMOR IMMUNITY

<sup>1</sup>Rebecca Ward\*, <sup>2</sup>Elena Paltrinieri, <sup>1</sup>Marilyn Marques, <sup>1</sup>Priyadarshini Iyer, <sup>3</sup>Sylvia Dietrich, <sup>4</sup>Jeremy Waight, <sup>1</sup>Mark Bushell, <sup>5</sup>Nicholas Wilson, <sup>1</sup>Jennifer Buell, <sup>1</sup>David Savitsky, <sup>1</sup>Dhan Chand. <sup>1</sup>Agenus Inc, Lexington, MA, USA; <sup>2</sup>Pyxis Oncology, Cambridge, MA, USA; <sup>3</sup>Dragonfly Therapeutics Inc, Waltham, MA, USA; <sup>4</sup>GlaxoSmithKline, King of Prussia, Pennsylvania, USA; <sup>5</sup>Gilead Sciences, San Carlos, CA, USA

**Background** T-cell immunoreceptor with Ig and ITIM domains (TIGIT) is an important negative regulator of the immune response to cancer that contributes to resistance/relapse to anti-PD-1 therapy.<sup>1</sup> In clinical trials, anti-human (h) TIGIT antibodies have shown promising activity in combination with anti-PD-1/PD-L1 antibodies for the treatment of various solid tumors.<sup>2</sup> However, the optimal format for anti-TIGIT antibodies remains controversial. Here we describe a novel Fcγ receptor (FcγR)-dependent mechanism of action that is critical for enhancing T and NK cell anti-tumor immunity, and, further informs on the optimal design of anti-TIGIT antibodies.

**Methods** We investigated a panel of Fc-silent, Fc-competent, and Fc-engineered anti-mouse (m) TIGIT antibody variants in syngeneic murine CT26 tumor-bearing or B16F10 pseudo-metastases models. To further elucidate the relative contribution of T and NK cells in controlling tumor growth, we assessed the activity of Fc-engineered anti-TIGIT antibodies in NK cell-depleted or T cell-deficient (Nu-Foxn1nu) CT26 tumor-bearing mice. Immune-related pharmacodynamic changes in the tumor microenvironment were assessed by flow cytometry. We further validated these findings in primary human T and NK cell activation assays using Fc-engineered anti-human TIGIT antibodies.

**Results** The Fc-engineered anti-mTIGIT antibody, which demonstrates enhanced binding to mouse FcγRIV, was the only variant to deliver single agent anti-tumor activity. The Fc-enhanced variant outperformed the Fc-competent variant while the Fc-inert variant had no anti-tumor activity. Tumor control by anti-mTIGIT antibodies was not dependent on Treg depletion, but rather on increased frequency of CD8+ T cells and activated NK cells (Ki67, IFNγ, CD107a and TRAIL) in the tumor microenvironment. Concordant with observations in the mouse, Fc-engineered anti-hTIGIT antibodies with improved binding to FcγRIIIA demonstrate superior T and NK cell

activation in PBMC-based assays compared to a standard hIgG1 variant. Notably, superior activity of the Fc-engineered anti-hTIGIT antibody was observed from PBMC donors that express either high or low affinity FcγRIIIA. Blockade of FcγRIIIA or depletion of CD14+ and CD56+ cells reduced the functional activity of the Fc-enhanced anti-TIGIT antibody, confirming the requirement for FcγR co-engagement to maximize T cell responses.

**Conclusions** Our data demonstrate the importance of FcγR co-engagement by anti-TIGIT antibodies to promote immune activation and tumor control. First generation anti-TIGIT antibodies are not optimally designed to co-engage all FcγRIIIA variants. However, Fc-enhanced anti-TIGIT antibodies unlock a novel FcγR-dependent mechanism of action to enhance T and NK cell-dependent anti-tumor immunity and further improve therapeutic outcomes.

## REFERENCES

- Johnston RJ, et al., The immunoreceptor TIGIT regulates antitumor and antiviral CD8(+) T cell effector function. *Cancer Cell* 2014; **26**:923–37.
- Rodriguez-Abreu D, et al., Primary analysis of a randomized, double-blind, phase II study of the anti-TIGIT antibody tiragolumab (tira) plus atezolizumab (atezo) versus placebo plus atezo as first-line (1L) treatment in patients with PD-L1-selected NSCLC (CITYSCAPE). *Journal of Clinical Oncology* 2020; **38**:15\_suppl, 9503–9503.

<http://dx.doi.org/10.1136/jitc-2020-SITC2020.0253>

254

### CTLA-4 BLOCKADE PROMOTES TREG GLUCOSE METABOLISM AND REDUCES TREG FUNCTIONAL STABILITY IN GLYCOLYSIS-DEFECTIVE TUMORS

<sup>1</sup>Roberta Zappasodi\*, <sup>1</sup>Inna Serganova, <sup>1</sup>Ivan Cohen, <sup>1</sup>Masatomo Maeda, <sup>1</sup>Yasin Senbabaoglu, <sup>1</sup>Masahiro Shindo, <sup>1</sup>Rachana Maniyar, <sup>1</sup>Mayuresh Mane, <sup>1</sup>Avigdor Leftin, <sup>2</sup>McLane Watson, <sup>1</sup>Svena Verma, <sup>1</sup>Matthew Lubin, <sup>1</sup>Myat Kyaw Ko, <sup>1</sup>Arnab Ghosh, <sup>1</sup>Mohsen Abu-Akeel, <sup>1</sup>Ellen Ackerstaff, <sup>1</sup>Jason Koutcher, <sup>3</sup>Ping-Chih Ho, <sup>2</sup>Greg Delgoffe, <sup>1</sup>Ronald Blasberg, <sup>1</sup>Jedd Wolchok, <sup>1</sup>Taha Merghoub. <sup>1</sup>Memorial Sloan Kettering Cancer Center, New York, USA; <sup>2</sup>University of Pittsburgh, Pittsburgh, PA, USA; <sup>3</sup>University of Lausanne, Lausanne, Switzerland

**Background** Durable clinical responses to immune checkpoint blockade (ICB) occur in a limited fraction of patients. We thus hypothesized that the characteristic tumor metabolic switch towards aerobic glycolysis could contribute to ICB resistance. High glucose consumption and lactate production by tumor cells can indeed restrict nutrient availability for tumor-infiltrating T cells, which also rely on glycolysis to proliferate and function. Therefore, we investigated whether targeting tumor glucose metabolism potentiates ICB anti-tumor activity.

**Methods** We modeled tumor-selective glycolysis inhibition by knocking down the critical glycolytic enzyme lactate dehydrogenase A (LDHA-KD) in the murine metastatic breast carcinoma 4T1 and melanoma B16, which are known immune-refractory tumor models. Anti-CTLA-4 and anti-PD-1 were tested in immunocompetent mice orthotopically implanted with control vs. LDHA-KD tumor cells. Changes in glucose metabolism were assessed by Seahorse and fluorescent-glucose flow-cytometry staining. Changes in immune cells were measured by multiparameter flow cytometry. Glucose-dependent effects of anti-CTLA-4 in regulatory T cells (Tregs) were tested in standard suppression assays with increasing glucose concentration (0.5–10 mM). Pearson correlations between glycolysis and intra-tumor immune-cell infiltration by CIBERSORT immune-deconvolution method were analyzed in bulk RNA-