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

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

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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.[1] 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.[2] 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 pseudometastases 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.

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

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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-

sequencing data sets from human and murine tumors treated with ICB.

Results Comparison of ICB activity in LDHA-KD vs. control tumor-bearing mice revealed improved anti-tumor effects and overall survival in the setting of glycolysis-defective tumors specifically upon CTLA-4 blockade. Anti-tumor CD8+ T-cell responses correlated with Treg phenotypic and functional destabilization in anti-CTLA-4-treated LDHA-KD tumors. CTLA-4 blockade led to CTLA-4 and CD25 downregulation associated with increased IFN-gamma and TNF-alpha production in Tregs from glycolysis-defective vs. control tumors. We next mimicked high- vs. low-glycolysis tumor microenvironment (TME) in vitro using control vs. LDHA-KD tumor co-cultures with Tregs, control vs. LDHA-KD tumor-conditioned media or directly modulating glucose concentrations. In these assays, we observed that CTLA-4 blockade promotes IFN-gamma±TNF-alpha production and glucose uptake by Tregs and more efficiently counteracts Treg suppression and enhances CD28 co-stimulation at higher glucose concentrations. Lastly, by interrogating transcriptomic data from human melanoma and murine 4T1 tumors, we found that CTLA-4 blockade promotes immune-cell infiltration and metabolic fitness especially in glycolysis-defective tumors.

Conclusions Our findings indicate that increasing glucose availability in the TME may improve anti-CTLA-4 therapeutic activity and reveal a new mechanism through which CTLA-4 blockade interferes with Treg immunosuppression in a glucose-dependent manner. These results suggest that CTLA-4 blockade can be more effective in tumors with low glycolysis and/or can be best exploited in combination with inhibitors of tumor glycolysis.

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255 EFFICACY OF SEQUENTIAL IMMUNE CHECKPOINT INHIBITION (ICI) IN PATIENTS WITH GENITOURINARY MALIGNANCIES

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Background Immune checkpoint inhibitors (ICI) have become a standard of care for treatment of both metastatic renal cell carcinoma (mRCC) and metastatic urothelial carcinoma (mUC). Additional treatment with ICI following disease progression on first-line therapy has become increasingly common for patients with severe disease, but the clinical outcomes of sequential therapy have not been well studied. We report here the clinical outcomes in a cohort of patients with mRCC and mUC who received two regimens of ICI-based therapy.

Methods We performed a retrospective review of 31 mRCC patients and 11 mUC with follow-up data available who received at least 1 dose of a 2nd ICI-based regimen at the Winship Cancer Institute of Emory University from 2015–2020. Radiographic responses were determined using response evaluation criteria in solid tumors version 1.1 (RECISTv1.1). An objective response (OR) was defined as a complete response (CR) or partial response (PR). Clinical benefit (CB) was defined as an objective response or stable disease (SD) > 6 months.

Results Most patients were white (81%) and male (69%). 31 had mRCC (table 1) and 11 had mUC (table 2). Overall most patients (58%) received anti-PD-1 (Programmed cell death protein 1) monotherapy as first line, with anti-PD-L1 (Programmed death-ligand 1) monotherapy (33%) and anti-PD-1/CTLA-4 (Cytotoxic T-lymphocyte-associated protein 4) combination therapy (9%) being less prevalent. Patients spent an average of 27.1 weeks on first ICI therapy. Second ICI-based treatment was most commonly anti-PD-1/CTLA-4 (62%), followed by anti-PD-1 monotherapy (38%). A subset of patients (33%) had clinical benefit with combination anti-PD-1/CTLA-

Abstract 255 Table 1 Demographics and treatment data for patients with metastatic renal cell carcinoma

Variable		n (%)
Gender	Male	22 (71)
	Female	9 (29)
Race	White/Asian	27 (87)
	Black	5 (13)
Clear-cell histology	Yes	24 (77)
	No	7 (23)
Prior Lines of Therapy	1	13 (42)
	2	7 (23)
	3+	11 (35)
Number of distant metastatic sites	1-2	9 (29)
	3	10 (32)
	4+	12 (39)
First ICI regimen	PD-1 monotherapy	23 (74)
	PDL-1 monotherapy	4 (13)
	PD-1/CTLA4 combination	4 (13)
Reason for d/c 1st ICI Regimen	Progression	26 (84)
	Toxicity	3 (10)
	Completion	2 (6)
Second ICI Regimen	PD-1 monotherapy	6 (19)
	PD-1/CTLA4 combination	25 (81)
Best Radiographic Response to 2nd ICI Regimen	PD	18 (60)
	SD	7 (23)
	PR	4 (13)
Response Rate: 16.7%	CR	1 (3)
CB Rate: 40.0%	NE	1

Abstract 255 Table 2 Demographics and treatment data for patients with urothelial cell carcinoma

Variable		n (%)
Gender	Male	7 (64)
	Female	4 (36)
Race	White/Asian	9 (81)
	Black	2 (19)
Prior Lines of Therapy	0-2	4 (36)
	3-4	6 (55)
	5+	1 (9)
Number of distant metastatic sites	1-2	6 (54)
	3	2 (19)
	4+	3 (27)
First ICI regimen	PD-1 monotherapy	1 (9)
	PDL-1 monotherapy	10 (91)
Reason for d/c 1st ICI Regimen	Progression	10 (91)
	Toxicity	0
	Completion	1 (9)
Second ICI Regimen	PD-1 monotherapy	10 (91)
	PD-1/CTLA4 combination	1 (9)
Best Radiographic Response to 2nd ICI Regimen	PD	6 (60)
	SD	4 (40)
	PR	0
Response Rate: 0%	CR	0
CB Rate 40%	NE	1