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Background Anti-checkpoint antibodies blocking T cell co-inhibitory molecules (e.g. α PD-1, α CTLA-4) allow immune effector cells to persist, expand, and maintain cytotoxic function in the tumor microenvironment (TME). Despite being effective in immune "hot" tumors that are infiltrated by effector anti-tumor cells, immune "cold" tumors fail to respond to checkpoint blockade. "Cold" tumors are populated with immune suppressive cells including regulatory T cells, M2 macrophages, and myeloid derived suppressor cells, which inhibit immune effector infiltration and function. These suppressive populations, along with tumor cells, express co-inhibitory checkpoints already targeted with current immunotherapeutics, but also some checkpoints in need of further investigation. We hypothesized that by targeting these checkpoints with cytoreductive antibodies which selectively deplete suppressive populations and tumor cells via ADCC/ADCP, we will compromise "cold" immune privilege and restore an efficient anti-tumor immune response.

Methods To identify novel targets to produce checkpoint cytoreductive antibodies we conducted in silico analysis that prioritized immune-inhibitory targets with tumor-specific or tumor-selective expression on cell surface. We cross-referenced a previously published list of transmembrane proteins against publicly sourced datasets including TCGA, HPA, GTEx, BioGPS, and SAGE.¹ We then characterized the expression profile of each selected target on tumor cells in vitro and on cell populations in the TME ex vivo via multiparameter flow cytometry. Finally, we assessed the impact of existing checkpoint-targeting cytoreductive antibodies on survival and tumor growth in murine "hot" and "cold" tumors.

Results VISTA and DLL3 were identified via in silico analysis as co-inhibitory surface proteins specifically and selectively in the TME and not in healthy tissues. DLL3 is mainly expressed on tumor cells while VISTA was described mostly on immunosuppressive myeloid cells. An anti-DLL3 antibody was produced by a previous laboratory as an IgG1 antibody, and we engineered a version in the depletive (IgG2a) isotype, which will enable us to target this checkpoint with either a blocking or a depleting antibody. Flow cytometry analysis identified VISTA on multiple myeloid cell populations in "cold" 4T1 murine mammary tumors while its expression was low in spleen. To start assessing the efficiency of depleting versus non-depleting antibodies, "hot" CT26 murine tumors and 4T1 tumors were treated with an α CTLA-4-IgG2a or α CTLA-4-IgG1. Groups treated with depleting antibodies showed increased survival compared to groups treated with non-depleting antibodies.

Conclusions Novel immune-inhibitory checkpoints can be identified and targeting them with cytoreductive antibodies leads to a higher anti-tumor immune response. This investigation opens the door to more efficient combination therapies.

Acknowledgements Supported by a training fellowship from The University of Texas Health Science Center at Houston Center for Clinical and Translational Sciences TL1 Program (Grant No. TL1 TR003169).

REFERENCES

1. Wang J, et al. Siglec-15 as an immune suppressor and potential target for normalization cancer immunotherapy. *Nat Med* **25**,(2019).