Background CD4 and CD8 T cells are genetically and functionally distinct cell subsets of the adaptive immune system that play pivotal roles in immune surveillance and disease control. During development in the thymus, transcription factors ThPOK and Runx3 regulate the differentiation and maturation of these two lineages into single positive T cells that enter the periphery with mutually exclusive expression of either the CD4 or CD8 co-receptor. 1–2 Despite our expectation that these two cell fates are fixed, mature CD4 +CD8+ double positive (DP) T cells have been described in the context of numerous immunological responses, including cancer, but their molecular and functional properties and therapeutic relevance remain controversial and largely unknown.3–5

Methods Our lab has identified and characterized a heterogeneous DP T cell population in murine and human melanoma tumors comprised of CD4 and CD8 T cells expressing the opposite co-receptor and a parallel uptake in the open repertoire T cells isolated from murine and human melanoma tumor bearing mice.

Results Specifically, up to 50% of transferred CD4 Trp1 T cells will re-express CD8 to become a DP T cell in the tumor microenvironment. Further, these CD4 derived DP T cells upregulate CD8 lineage regulator Runx3 and cytolytic genes Gzmb, Gzmk, and Prf1 to become potent cytotoxic T cells. Alternatively, a subset of CD8 Pmel T cells differentiate into DP T cells characterized by the increased expression of CD4, ThPOK, and regulatory marker FoxP3 (figure 1). In addition, we utilized 10x single cell and ATAC sequencing to further characterize these divergent DP T cell populations among open repertoire T cells isolated from murine and human melanoma tumors.

Conclusions Our findings highlight the capability of single positive T cells to differentiate in response to antigen and local stimuli into novel T cell subsets with polyfunctional characteristics. The resulting cell subsets will potentially affect the tumor microenvironment in distinct ways. Our studies may inform therapeutic approaches to identify antigen specific T cells as well as innovative signaling pathways to target when genetically engineering T cells to optimize cytotoxic function in the setting of adoptive cell therapy.

Ethics Approval The human biospecimen analyses were approved by Memorial Sloan Kettering Cancer Center IRB #06-107

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Immune-stimulants and immune modulators

550 AN AXL-TARGETING MONOCLONAL ANTIBODY THAT INHIBITS AXL ACTIVITY AND POOTENTLY STIMULATES THE INNATE IMMUNE RESPONSE

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Background Axl is a member of the TAM (Tyro3/Axl/MerTK) family of receptor tyrosine kinases and a negative regulator of innate immunity. Activation of Axl through its ligand Gas6 leads to suppression of myeloid cell activity, while its activation in tumor cells drives tumor growth, metastasis, and is associated with acquired resistance to targeted therapies, radiotherapy and chemotherapy.

Methods Purified monoclonal antibodies and variants thereof were tested in human cancer lines and primary human myeloid cells for effects on Axl signaling and immune activation, respectively.

Results We describe a humanized IgG1 Axl-targeting monoclonal antibody (mAb), CDX-0168, that binds to the ligand-binding domain of Axl with sub-nanomolar affinity and potently inhibits Gas6 binding. In tumor cells, CDX-0168 inhibits Gas6-dependent Axl phosphorylation and signaling and elicits tumor cell killing via ADCC in vitro and in vivo. In primary human immune cells, CDX-0168 treatment induces potent release of pro-inflammatory cytokines and chemokines from dendritic cells, monocytes and macrophages through an Fc receptor-dependent mechanism and enhanced T cell activation in mixed lymphocyte reactions. Axl inhibition may further enhance antitumor activity associated with PD-(L)1 blockade. To this end, we generated a tetravalent bispecific Axl x PD-L1 antibody combining CDX-0168 with a potent anti-PD-L1 mAb (9H9) using an IgG-scFv format. The bispecific antibody elicits greater cytokine release and T cell activation in vitro than the combination of the parental antibodies, while maintaining robust Axl and PD-L1 blockade.

Conclusions Additional studies investigating simultaneous blockade of the Axl and PD-L1 pathways with other agents may further exploit the potential for this novel anti-cancer therapeutic approach.

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552 SUMOYLATION INHIBITOR TAK-981 ACTIVATES NK CELLS AND MACROPHAGES VIA TYPE I INTERFERON SIGNALING AND SHOWS SYNERGISTIC ACTIVITY IN COMBINATION WITH RITUXIMAB AND DARATUMUMAB IN PRECLINICAL MODELS

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Background TAK-981 is a first-in-class small molecule inhibitor of the SUMO activating enzyme in Phase 1 clinical trials. SUMOylation has previously been implicated in the
regulation of innate immune responses and expression of Type I interferons, and ex vivo treatment of human and mouse immune cells with TAK-981 results in transcriptional upregulation of IFN-beta and Type I IFN receptor (IFNAR) signaling. We previously showed that TAK-981 increases NK cell activation and M1 macrophage polarization, leading to enhanced ADCC and ADCP in the presence of rituximab. In vivo, TAK-981 induces IFNAR-dependent antitumor activity and synergizes with rituximab in xenograft-bearing mice. Here we investigated the mechanism of synergistic activity with rituximab and evaluated the combination of TAK-981 with daratumumab, another therapeutic mAb.

Methods The role of effector function of rituximab in the mechanism of synergy with TAK-981 was evaluated in OCI-Ly10-bearing SCID mice treated with TAK-981 and the LALA-PG version of rituximab, in which mutations in the Fc region prevent FcγR binding. The combination of TAK-981 and rituximab was also evaluated in OCI-Ly10 tumor-bearing mice in which macrophages and/or NK cells were depleted with clodronate and anti-asialo GM1. TAK-981 in combination with daratumumab was evaluated in two CD38+ xenograft models, Daudi (Burkitt’s lymphoma) and LP-1 (multiple myeloma). To test ADCP activity, Daudi-KIRL cells were incubated with human monocyte-derived macrophages (hMDM) treated with TAK-981 in the presence or absence of rituximab or daratumumab, with or without a neutralizing antibody to IFNAR2.

Results Unlike rituximab, LALA-PG mutated rituximab did not synergize with TAK-981 in OCI-Ly10 tumor-bearing mice, indicating a requirement for Fc effector function. Depletion of macrophages with clodronate or NK cells with anti-asialo GM1 lessened the anti-tumor effect of the TAK-981 and rituximab combination, while dual depletion of macrophages and NK cells had a greater impact. TAK-981 showed synergistic activity in combination with daratumumab in two CD38+ xenograft models, Daudi and LP-1. In vitro, TAK-981-treated hMDM showed increased phagocytic activity against Daudi cells, and this effect was further enhanced in the presence of rituximab or daratumumab but prevented by a neutralizing antibody to IFNAR2.

Conclusions In preclinical models, TAK-981 synergizes with rituximab through a mechanism involving Type I IFN dependent enhancement of ADCC and ADCP, and the combination of TAK-981 with daratumumab is also synergistic.

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