high concentrations of long-acting, pegylated IL-10 have also shown anti-tumor activity.³ Here we investigated IL-10 and IL-10 receptor- α (IL-10RA) expression profiles in normal and tumor tissues as well as the immunological effects of modulating the IL-10 pathway via antibody-mediated blockade of IL-10RA.

Methods IL-10 and IL-10RA mRNA are expressed by several tumors, including renal, lung, breast, and colon cancers. Fluorescent in-situ hybridization revealed that the majority of IL-10RA was expressed by CD3-negative tumor-infiltrating cells, localized in close proximity to T cells in the tumor microenvironment (TME). Immunohistochemistry studies confirmed expression of IL-10RA in the TME, while no expression was detected in healthy tissues. Furthermore, dissociated tumor cells produced biologically active levels of IL-10 in culture.

Results Monoclonal antibodies (mAbs) against IL-10RA prevented IL-10 signaling and enhanced release of IL-12 by monocyte-derived dendritic cells activated with suboptimal LPS concentrations. The effect of IL-10RA blockade was greater than that observed with IL-10 neutralizing mAbs. In mixed lymphocyte reactions and superantigen-driven T-cell activation, IL-10RA blockade enhanced IL-2 secretion by T lymphocytes. Consistent with earlier observations in mouse models,⁴ the effect of IL-10RA blockade was nonredundant with blockade of the PD-1/PD-L1 axis, resulting in enhanced IL-2 and interferon- γ secretion by T cells when both pathways were inhibited. Blockade of IL-10RA during CD3-redirection in vitro killing of tumor cells by PBMC induced IL-12 release as well as upregulation of CD86 and HLA-DR by CD3-negative cells. In in vitro dissociated tumor cells, IL-10RA blockade induced release of IL-2, interferon- γ and other proinflammatory cytokines; additional PD-1/PD-L1 axis blockade further enhanced cytokine release.

Conclusions In summary, antibody-mediated IL-10RA blockade can potentiate immune activation in the dissociated tumor cells and may be a valuable addition to cancer immunotherapies, including redirected T-cell killing and checkpoint blockade.

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A NOVEL MECHANISM OF NEUROPILIN-1 INHIBITION RESULTS IN IMPROVED TUMOR GROWTH INHIBITION IN VIVO

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Background Neuropilin-1 (NRP1) is a co-receptor that complexes with diverse ligands and their cognate receptors. As such, it plays a role in multiple different biological processes, including axon guidance and angiogenesis. NRP1 contains two CUB domains (a1 and a2) involved in binding the ligand Semaphorin3A (SEMA3A), two Factor V/VIII domains (b1 and b2) involved in VEGF ligand binding and one MAM domain (c domain). While functional antibodies with anti-tumor activity have been generated against the SEMA3A and VEGF binding

domains, little attention has been paid to the c domain of NRP1, which has been implicated in the dimerization of NRP1, a prerequisite for functionality. We therefore hypothesized that c domain-binding antibodies would offer an opportunity to generate functional inhibitors of both SEMA3A and VEGF signaling and therefore improved anti-tumor activity.

Methods Recombinant human NRP1 comprising all subdomains was used to identify fully human anti-NRP1 antibodies. Specific antibodies were tested for their ability to block NRP1 interactions with recombinant SEMA3A and VEGF protein in vitro. Blocking antibodies were subsequently assessed for their functional effects, such as inhibition of SEMA3A-mediated growth cone collapse. Antibodies with diverse binding characteristics were then tested for in vivo anti-tumor activity in multiple cancer models of interest.

Results Recombinant NRP1 containing the a1, a2, b1, b2 and c subdomains was used to successfully identify a series of specific monoclonal antibodies that cross-reacted with Cynomolgus monkey and mouse NRP1, but not human NRP2. Except for the a2 domain, epitope mapping showed an even distribution of mAbs for binding to each of the NRP1 subdomains, including the c domain that has been proposed to play a role in dimerization. Using biolayer interferometry, we identified antibody classes with direct SEMA3A and/or VEGF blocking properties. Further optimization of these antibodies yielded mAbs with subnanomolar affinities that showed significant tumor growth inhibition in multiple mouse models, including anti-PD1 non-responsive models.

Conclusions Here we demonstrate the identification of fully human monoclonal antibodies that specifically bind to the c domain of human NRP1. A subset of these c domain binders do not block either SEMA3A or VEGF binding to NRP1 but do show in vivo efficacy, suggesting a role for the c domain of NRP1 in the formation of functional (dimeric) complexes. Thus, c domain binding antibodies show remarkable inhibition of tumor growth in mouse cancer models and offer a novel means of therapeutic intervention in patients who are refractory to immune checkpoint inhibition.

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A LOW AFFINITY BIVALENT MESOTHELIN-BINDING MATCH4 MULTISPECIFIC T CELL ENGAGER INCREASES CYTOTOXIC SELECTIVITY FOR HIGH MESOTHELIN EXPRESSING CELLS

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Background The effective treatment of solid tumors remains an unmet medical need. Several concepts exist to treat malignancies, including antibody-drug or -immunotoxin conjugates, immune checkpoint inhibition, CAR- T cells, as well as bispecific T cell engagers. CD3-based T cell engagers are highly potent therapeutic molecules with T cell cytotoxicity activities in the picomolar range. Alongside this highly potent anti-tumor activity is the risk of on-target off-tumor effects due to low levels of expression of the target antigen in normal tissue, as has been observed for the tumor-associated antigen mesothelin (MSLN).

Methods Low-affinity antibody fragments to the tumor-associated antigen MSLN were generated, and a multispecific