are resistant to PSMA-targeted radioligand therapy with ^{177}Lu-PSMA-617. These data provide a rationale for evaluating AMG 160 in patients with mCRPC who have progressed on ^{177}Lu-PSMA-617. AMG 160 is currently being evaluated in a phase 1 study in patients with mCRPC (NCT03792841).

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Ethics Approval All animal experimental protocols were approved by the UCLA Animal Research Committee (# 2005-090).

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718 AMG 509, A STEAP1 X CD3 BISPECIFIC XMAB® 2+1 IMMUNE THERAPY, EXHIBITS AVIDITY-DRIVEN BINDING AND PREFERENTIAL KILLING OF HIGH STEAP1-EXPRESSING PROSTATE AND EWING SARCOMA CANCER CELLS

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Background Metastatic castration-resistant prostate cancer (mCRPC) and Ewing sarcoma (EWS) are diseases for which immune therapies could potentially provide benefit. STEAP1 (Six Transmembrane Epithelial Antigen of the Prostate 1) is a cell surface protein with elevated expression in mCRPC [1] and EWS.[2]

Methods We designed AMG 509, a novel, half-life extended, STEAP1 x CD3 XmAb® 2+1 bispecific antibody to induce T cell-mediated cytotoxicity against STEAP1-expressing cancer cells. AMG 509 contains two identical anti-STEAP1 Fab domains, an anti-CD3 scFv domain, and an effectorless Fc domain that extends serum half-life. We characterized STEAP1 expression in normal and tumor tissues by immunohistochemistry, and we assessed the pharmacological properties of AMG 509 including binding, T cell-mediated redirected lysis, and in vivo antitumor activity.

Results We detected high STEAP1 surface expression on 80% of primary prostate tumors (n=88), 89% of mCRPC lesions (n=114), including 84% of mCRPC bone metastases (n=31), and 63% of EWS samples (n=35). In contrast, in normal tissues (n=72), low STEAP1 expression was detected in only six other tissues, including the normal prostate. AMG 509 bound to recombinant human CD3ϵ with a K_D of 27.6 nM, and it bound specifically to 293T cells transfected with human STEAP1 with an EC_{50} of 3.8 nM. AMG 509 triggered potent T cell-redirected lysis of STEAP1-positive cancer cells, with a median EC_{50} of 37 pM across 19 cancer cell lines that endogenously express various levels of STEAP1. AMG 509-mediated cytotoxicity was specific, as it showed no activity against prostate cancer cells in which STEAP1 was knocked out. AMG 509 was 65-fold more potent in inducing the redirected lysis of prostate cancer cells in vitro than an XmAb® molecule with a single anti-STEAP1 Fab domain. AMG 509 had greater cytotoxic activity against high STEAP1-expressing cancer cells than against low STEAP1-expressing cancer cells, and it had minimal activity against normal cells. This preferential killing of high STEAP1-expressing cells is likely driven by the avidity conferred by the dual STEAP1-binding domains, a feature that may help reduce off-target effects in the clinic. In vivo, AMG 509 induced robust anti-tumor activity in prostate cancer and EWS mouse xenograft models, with concomitant CD8+ T-cell activation and expansion in tumors.

Conclusions AMG 509 is a specific, first-in-class T cell-recruiting antibody with avidity-driven activity against STEAP1-positive malignancies. AMG 509 is currently being evaluated for safety, pharmacokinetics, and efficacy in a phase 1, first-in-human study in patients with mCRPC (NCT04221542).

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Trial Registration ClinicalTrials. gov Identifier: NCT04221542

Ethics Approval All animal experimental protocols were approved by an Institutional Animal Care and Use Committee (IACUC protocol number 2015-01243) and were conducted in accordance with the guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) (Amgen) or the standards of the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals (IACUC protocol number 15015x) in a facility certified with an Office of Laboratory Animal Welfare (OLAW) (UTHSA).

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719 CD122-DIRECTED INTERLEUKIN-2 COMPLEXES AND αPD-L1 DIFFERENTIALLY REQUIRE INNATE AND ADAPTIVE IMMUNITY TO TREAT LOCAL AND METASTATIC BLADDER CANCER

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Background αPD-L1 bladder cancer (BC) immunotherapy is effective in <30% of cases.[1] To address the large αPD-L1-unresponsive subset of patients, we tested αIL-2/IL-2 complexes (IL-2c) that block IL-2 from binding high-affinity IL-2Rα (CD25) for preferential IL-2Rβ (CD122) binding.[2] Immunosuppressive regulatory T cells capture IL-2 by CD25 whereas antitumor CD8+ T, γδ T, and NK cells use CD122. We hypothesized that the tumor microenvironment, including local immune cells in primary versus metastatic BC, differentially affects immunotherapy responses and that IL-2c effects could differ from, and thus complement αPD-L1.

Methods We used PD-L1+ mouse BC cell lines MB49 and MBT-2, for orthotopic, intravesical (i.e., in bladder) and intravenous challenge studies of local versus lung metastatic BC.

Results αPD-L1 or IL-2c alone reduced tumor burden and extended survival in local MB49 and MBT-2. Using in vivo cell depletions, we found that γδ T cells and NK cells, but strikingly not CD8+ T cells, were necessary for IL-2c efficacy in bladder. We confirmed γδ T cell requirements for IL-2c, but not αPD-L1 efficacy in γδ T cell-null TCRδKO mice. TCRβKO conventional T cell-null mice exhibited IL-2c, but

not αPD-L1 responsiveness for orthotopic BC treatment. Neither agent alone treated lung metastatic MB49 or MBT-2 but the drug combination improved survival in both tumor models. Combination treatment effects in lungs were distinct from bladder, requiring CD8+ T and NK cells, but not γδ T cells.

Conclusions BC immunotherapy effects differ by anatomic compartment and use distinct mechanisms to treat primary and metastatic BC. CD122-directed IL-2 is a promising BC immunotherapy strategy, and IL-2c is a candidate mediator through innate immune effects. αPD-L1 could improve IL-2c efficacy by engagement of adaptive immune responses including to improve metastatic disease treatment efficacy.

Ethics Approval All procedures involving animals in this study were approved by the UT Health San Antonio Institutional Animal Care and Use Committee (IACUC) and conducted in accordance with UT Health San Antonio Department of Laboratory Animal Resources standards.

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720 TARGETING MARCO AND IL-37R ON ANTI-INFLAMMATORY MACROPHAGES IN LUNG CANCER BLOCKS REGULATORY T CELLS AND SHIFT BALANCE TO SUPPORT CYTOTOXIC LYMPHOCYTE FUNCTION

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Background The progression and metastatic capacity of solid tumors are strongly influenced by immune cells in the tumor microenvironment (TME). In non-small cell lung cancer (NSCLC) accumulation of anti-inflammatory tumor-associated macrophages (TAMs) is associated with worse clinical outcome and resistance to therapy. Numerous clinical trials aiming to recover T cell anti-tumor activity have been failing due to the persistence immune suppression in TME. Thus, there is a clinical need for alternative treatments targeting the suppressive function of the TME. We have previously shown that antibodies targeting the scavenger receptor MARCO reprograms the pro-tumoral TAMs in murine cancer models. Here, we investigated the immune landscape of NSCLC in the presence of MARCO expressing TAMs. We tested targeting MARCO or the tumor mechanisms inducing MARCO on human TAMs and hypothesized that targeting these mechanisms will remodel the suppressive environment and relive the anti-tumor responses to increase the efficacy of immunotherapy.

Methods To test our hypothesis, we first investigated the immune landscape of NSCLC in the presence of pro-tumoral MARCO+TAMs compared with tumors infiltrated by MARCO-TAMs. We next used RNAseq to analyze differential gene expression in NSCLC tumors infiltrated by MARCO positive or negative macrophages. In vitro, cytokine differentiated macrophages alternatively cultured with lung cancer cell lines were co-cultured with Natural Killer (NK) cells and T cells to mimic their interaction in the TME. Later, macrophages were treated with targeting antibodies and their phenotype and function were examined prior and following interaction with other immune cells.

Results We found that MARCO expressing TAM numbers correlated with increased occurrence of regulatory T cell and effector T cells and decreased NK cells in NSCLC infiltrated by MARCO+TAMs. Furthermore, transcriptomic data from the tumors uncovered a correlation between MARCO expression and the anti-inflammatory cytokine IL-37. Studies in vitro subsequently showed that lung cancer cells polarized macrophages to express MARCO and gain an anti-inflammatory phenotype through the release of IL-37. These human MARCO expressing TAMs blocked cytotoxic T cell and NK cell activation, inhibiting their proliferation, cytokine production and tumor killing capacity. Mechanistically, MARCO+ macrophages enhanced regulatory T (Treg) cell proliferation and IL-10 production and diminished CD8 T cell activities. Targeting MARCO or IL-37 receptor (IL-37R) repolarized TAMs resulted in recovered cytolytic activity and anti-tumoral capacity of NK cells and T cells.

Conclusions In summary, our data demonstrate a novel immune therapeutic approach targeting human TAM immune suppression of NK and T cell anti-tumor activities and remodel immune suppression.

Ethics Approval The study was approved by Institutional Ethics Board, approval number Dnr 2013.977-31.1.

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721 AT1412, A PATIENT-DERIVED CD9 ANTIBODY IN PRECLINICAL DEVELOPMENT PROMOTING TUMOR IMMUNE INFILTRATION AND INDUCING TUMOR REJECTION

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Background Adaptive immunity to cancer cells forms a crucial part of cancer immunotherapy. Recently, the importance of tumor B-cell signatures were shown to correlate with melanoma survival. We investigated whether tumor-targeting antibodies could be isolated from a patient that cured (now 13 years tumor-free) metastatic melanoma following adoptive transfer of ex vivo expanded autologous T cells.

Methods Patient's peripheral blood B cells were isolated and tested for the presence of tumor-reactive B cells using AIMM's immmortalisation technology. Antibody AT1412 was identified by virtue of its differential binding to melanoma cells as compared to healthy melanocytes. AT1412 binds the tetraspanin CD9, a broadly expressed protein involved in multiple cellular activities in cancer and induces ADCC and ADCP by effector cells.

Results Spontaneous immune rejection of tumors was observed in human immune system (HIS) mouse models implanted with CD9 genetically-disrupted A375 melanoma (A375-CD9KO) tumor cells, while A375wt cells were not cleared. Most notably, no tumor rejection of A375-CD9KO tumors was observed in NSG mice, indicating that blockade of CD9 makes tumor cells susceptible to immune rejection.CD9 has been described to regulate integrin signaling, e.g. LFA-1, VLA-4, VCAM-1 and ICAM-1. AT1412 was shown to modulate CD9 function