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

by enhancing adhesion and transmigration of T cells to endothelial (HUVEC) cells. AT1412 was most potently enhancing transendothelial T-cell migration, in contrast to a high affinity version of AT1412 or other high affinity anti-CD9 reference antibodies (e.g. ALB6). Enhanced immune cell infiltration is also observed in immunodeficient mice harbouring a human immune system (HIS). AT1412 strongly enhanced CD8 T-cell and macrophage infiltration resulting in tumor rejection (A375 melanoma). PD-1 checkpoint blockade is further sustaining this effect. In a second melanoma model carrying a PD-1 resistant and highly aggressive tumor (SK-MEL5) AT1412 together with nivolumab was inducing full tumor rejection, while either one of the antibodies alone did not.

Conclusions The safety of AT1412 has been assessed in preclinical development and is well tolerated up to 10 mg/kg (highest dose tested) by non human primates. AT1412 demonstrated a half-life of 8.5 days, supporting 2–3 weekly administration in humans. Besides transient thrombocytopenia no other pathological deviations were observed. No effect on coagulation parameters, bruising or bleeding were observed macro- or microscopically. The thrombocytopenia is reversible, and its recovery accelerated in those animals developing anti-drug antibodies. First in Human clinical study is planned to start early 2021.

Ethics Approval Study protocols were approved by the Medical Ethical Committee of the Leiden University Medical Center (Leiden, Netherlands).

Consent Blood was obtained after written informed consent by the patient.

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722 INHIBITION OF INTEGRIN AVß8-MEDIATED TGF-ß ACTIVATION WITH C6D4 PROVIDES IMPROVED POTENCY AND SELECTIVITY VS GENERAL TGF-ß INHIBITORS FOR CANCER IMMUNOTHERAPY

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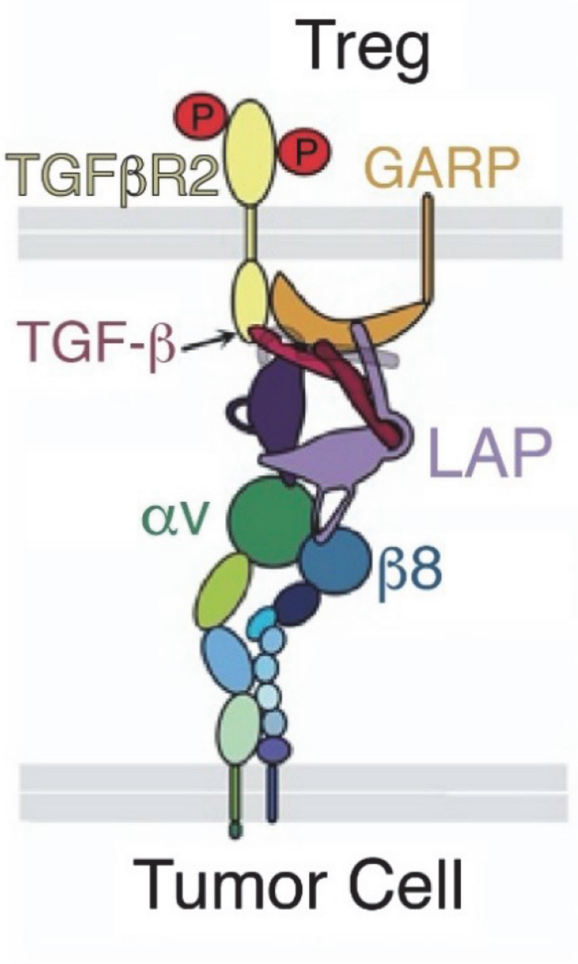
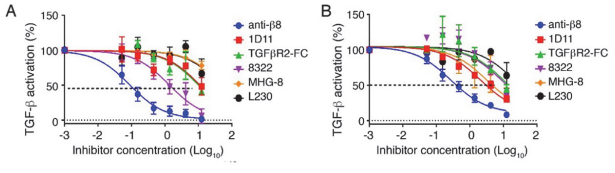
Background TGF-ß plays a key role in immune evasion as a critical regulator of both innate and adaptive tumor immunity and promotes broad immunosuppressive effects on numerous inflammatory cell subpopulations ultimately resulting in tumor immune tolerance and evasion.[1] It has also been implicated in resistance to immune checkpoint therapies, and additive or synergistic effects of dual TGF-ß and PD-1 inhibition has been reported.[2 3] A number of TGF-ß inhibitors are in clinical development with different modes of action. Most protein-based inhibitors are designed to block diffusible TGF-ß from interacting with its proximal signaling receptor TGF-ßR2 and includes monoclonal antibodies (Mabs) and receptor traps. This investigation compares inhibition of TGF-ß by a number of inhibitors and the integrin avß8 (C6D4) to assess their relative potential as cancer therapeutics.

Methods No reporter system currently exists to investigate the mechanistic basis of cell-intrinsic TGF-ß activation, whereby the L-TGF-ß presenting cell is also the cell that responds toTGF-ß signaling (figure 1). To build a cell-intrinsic TGF-ß activation system, TMLC cells were stably transfected with wild-type (WT) TGF-ß. Without co-transfecting GARP, TMLC do not present L-TGF-ß on their cell surface. When co-transfected with TGF-ß and GARP, high levels of cell surface expression of L-TGF-ß are detected. Additionally, to build a cell-intrinsic TGF-ß activation system which express a non-releasable form of TGF-ß, we mutated the L-TGF-ß furin cleavage site (R249A) and similarly expressed the L-TGF-ß(R249A)/GARP complex on the surface of TGF-ß reporter cells (TMLC). These cell-intrinsic TGF-ß activation systems were used to assess the relative abilities of Mabs avß8, TGF-ß, TGF-ßR2, GARP or TGF-ßR2 receptor trap to inhibit avß8-mediated TGF-ß activation.

Results avß8 exhibited superior inhibitory activity compared with other TGF-ß inhibitors, which was similar in both diffusible and non-diffusible models (figure 2). The biologic relevance of these finding was confirmed using CD4+ T-cells in place of the reporter cells where TGF-ß-dependent Treg generation was almost completely blocked by avß8 but was poorly inhibited by the other TGF-ß inhibitors.



Abstract 722 Figure 1 Novel cell-intrinsic TGF-β reporter system



Abstract 722 Figure 2 Inhibition curves showing inhibition of αvβ8-mediated TGF-β activation by various inhibitors including anti-β8 (C6D4) in a model of diffusible (A) or non-diffusible ((R249A mutant) L-TGF-β (B)