Methods To activate antigen-specific CD4+ T cells in vivo, we utilized our nucleic acid platform, UNITE (UNiversal Intracellular Targeted Expression), which fuses a tumor-associated antigen with lysosomal-associated membrane protein 1 (LAMP1). This lysosomal targeting technology results in enhanced antigen presentation and a balanced T cell response. LTS220A, encoding a mutated form of MCPyV-LT that abrogates its pro-oncogenic properties, was introduced into the UNITE platform. LTS220A-UNITE, known as ITI-3000, was administered to female C57BL/6 mice intradermally in the ear with electroporation.

Results ITI-3000 promoted a potent, antigen-specific CD4+ T cell response to MCPyV-LT. Vaccination with ITI-3000 significantly delayed and slowed growth of B16F10 tumors expressing LTS220A in prophylactic and therapeutic settings, respectively. ITI-3000 induced a favorable tumor microenvironment (TME), including significantly enhanced numbers of CD4+ T cells, CD8+ T cells, NK cells, and NKT cells. Tumor-infiltrating myeloid cells were reduced in frequency in vaccinated mice and polarized towards an anti-tumor phenotype. Cytokine analysis of the TME showed significantly enhanced levels of cytokines associated with anti-tumor immune responses in ITI-3000-vaccinated mice, including IFNγ, TNFα, IL-2, and IL-1β. Additionally, ITI-3000 synergized with PD-1 blockade, further reducing tumor burden and enhancing survival in mice receiving combination therapy.

Conclusions We find that DNA vaccination with ITI-3000 using the UNITE platform enhances CD4+ T cell responses to MCPyV-LT and results in anti-tumor immune responses in a mouse model of Merkel cell carcinoma.

Ethics Approval This study was approved by Immunomic Therapeutics’ Institutional Animal Care and Use Committee, protocol number 16-11-002.

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A BISPECIFIC ANTIBODY TARGETING CD40 AND EPCAM INDUCES SUPERIOR ANTI-TUMOR EFFECTS COMPARED TO THE COMBINATION OF THE MONOSPECIFIC ANTIBODIES

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Background Alligator has developed a new concept, Neo-X', to enable antigen presenting cells to efficiently enhance priming of neoantigen-specific T cells, which may be the missing aspect in tumors that lack T cell infiltration. We hypothesize that binding of the CD40 x EpCAM bsAb (4224) to CD40 on DCs and EpCAM on tumor exosomes or tumor debris leads to i) activation of the DC, ii) uptake of the tumor material, iii) cross-presentation of tumor-derived neoantigen (present in exosomes or debris) and iii) priming of tumor neoantigen-specific T cells, resulting in an increased quantity and/or quality of the tumor-targeting T cell pool. CD40 cross-linking by engagement with a tumor antigen on a tumor cell is required to achieve a functional agonistic effect, and subsequent DC activation will therefore only be achieved in the presence of tumor antigens.

Methods 4224 evaluated in vitro using human monocyte-derived DC, co-cultured with cells expressing EpCAM. In addition the functional effects were evaluated using tumor cell lines and B-cell lines expressing CD40. In vivo, the anti-tumor efficacy of the CD40 x EpCAM bsAb was determined in human CD40 transgenic mice bearing MB49 bladder carcinoma tumors transplanted with human EpCAM or controls.

Results In vitro, we have demonstrated that the CD40 x EpCAM bsAb induces tumor target dependent activation of dendritic cells, as analyzed by flow cytometry measuring HLA-DR and CD86 expression on the DC and by measuring IL-12p40 levels in the supernatant. Further, the ability of bsAbs within the Neo-X' concept to mediate co-localization of tumor debris and CD40 expressing antigen presenting cells depends on the receptor density of the tumor target. In vivo, 4224 displayed a potent, EpCAM-dependent anti-tumor effect with significantly reduced tumor growth and improved survival compared to an equivalent dose of the combination of the monospecific CD40 Ab and EpCAM targeting antibody. The tumor-localizing property of 4224 also showed potential for improved safety compared to CD40 monospecific antibodies. A biodistribution analysis demonstrated that the bispecific 4224 in the RUBY-format displayed similar half-life as the monospecific CD40 mAb in mice.

Conclusions In conclusion, the Neo-X’ concept, by targeting CD40 and a tumor specific antigen, has the potential to mediate an expansion of the tumor-specific T cell repertoire, resulting in increased T cell infiltration and potent anti-tumor effects.

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INHIBITION OF THE KINASE ACTIVITY OF HEMATOPOIETIC PROGENITOR KINASE 1 ENHANCES ANTI-PD-1-INDUCED REINVIGORATION OF HUMAN TUMOR-INFILTRATING CD8+ T CELLS

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Background Immune checkpoint inhibitors (ICIs) including anti-CTLA-4, anti-PD-1, and anti-PD-L1 have been clinically used for the treatment of various types of cancer. However, ICIs have a limited efficacy, and it is required to develop a strategy to enhance the efficacy of ICIs. Hematopoietic progenitor kinase 1 (HPK1) was recently known to inhibit T cell receptor (TCR) signaling by targeting SLP76 thus suppress T-cell effector functions.

Methods In the present study, we examined the expression of HPK1 and SLP76 in tumor-infiltrating lymphocytes (TILs) obtained from renal cell carcinoma tissues, in relation with the expression of PD-1 and other immune checkpoint receptors by performing flow cytometry analysis. In addition, we examined if inhibition of the kinase activity of HPK1 by CMPD0914, that is a potent, selective and orally available HPK1 inhibitor, enhanced effector functions of tumor-infiltrating CD8+ T cells in the presence of anti-PD-1 blocking antibodies.

Results First, we found that HPK1 and SLP76 are expressed in both CD8+ and CD4+ T cells including Foxp3+ regulatory T cells irrespective of PD-1 expression. Intriguingly, the expression levels of HPK1 and SLP76 were significantly higher in the PD-1bright population compared to the PD-1- or PD-1dim populations. Further characterization revealed that HPK1 and SLP76 were highly expressed in CD8+ T-cell populations expressing TOX, a transcription regulator of T-cell exhaustion,
or TCF-1, a transcription factor related to progenitor-like exhausted T cells. In ex vivo functional assays, anti-PD-1 treatment increased the production of IFN-γ and TNF, and the expression of a proliferation marker, Ki-67 among tumor-infiltrating CD8+ T cells. Interestingly, the effects of anti-PD-1 treatment were further enhanced by the combination treatment with CMPD0914.

Conclusions In summary, we demonstrated that HPK1 and with CMPD0914.

In summary, we demonstrated that HPK1 and with CMPD0914. The clinical success of PD-1- and CTLA-4-immune checkpoint inhibitors highlights the key contribution of immunosuppression to limiting effective anti-tumor responses. However, as many patients do not respond to anti-PD1 or CTLA4 therapy1-3 novel therapeutics that target additional immunosuppressive mechanisms are needed. Regulatory T cells (Tregs) inhibit immune responses in the tumor microenvironment via multiple suppressive mechanisms.1-5 Existing Treg-targeting agents lack specificity for intratumoral Tregs and can also deplete effector cells, a property that has likely contributed to the lack of clinical activity observed to date. CCR8 (C-C chemokine receptor 8) is selectively expressed on highly activated intratumoral Tregs, its high expression correlates with poor prognosis in multiple human tumor types6-7 and depletion of CCR8+ Tregs in preclinical models elicited potent anti-tumor activity. These observations provided rationale for the development of a CCR8-specific human depleting antibody.

Methods Human FOXP3 and CCR8 expression was correlated across multiple tumor types using TCGA datasets and expression of CCR8 evaluated in primary tumor explants and PBMCs by flow cytometry. The efficacy of anti-CCR8 antibody treatment was evaluated in the M38 and CT26 murine tumor models. The depletion of Tregs following anti-CCR8 treatment was assessed by flow cytometry. Flow cytometric-based binding assays were performed using cell lines expressing human or cynomolgus CCR8. Purified human NK cells were co-cultured with CCR8+ target cells and flow cytometry was used to evaluate antibody-dependent killing activity.

Results CCR8 expression was highly correlated with FoxP3 across multiple cancer subtypes and was low to absent on effector T cells. Importantly, CCR8 was not detected on any peripheral human leukocyte subset. In murine tumor models, anti-CCR8 antibody treatment reduced tumor growth in a dose- and Fc-receptor-dependent manner and resulted in complete regressions and the development of memory. Tumor shrinkage was associated with a reduction in intratumoral Tregs and increased representation of intratumoral CD8+ T cells. FPA157 is a highly specific human and cynomolgus crossreactive CCR8 antibody that does not bind closely related chemokine receptors. FPA157 was engineered to enhance antibody-dependent cell-mediated cytotoxicity (eADCC) and elicited potent NK-mediated killing of target cells expressing CCR8 at levels observed on human intratumoral Tregs.

Conclusions FPA157 is a CCR8-specific monoclonal antibody with eADCC activity that is being developed for the treatment of cancer. Depletion of CCR8+ Tregs induced substantial anti-tumor activity in pre-clinical models, thus supporting the clinical evaluation of FPA157 as a novel approach to alleviate immune suppression in the microenvironment of human solid tumors.

REFERENCES