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.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0857

858 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 iiii) 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 transfected 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 shows 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.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0858

859 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 HPK-1 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,