572 PYX-102, AN ANTI-KLRG1 ANTIBODY, ENHANCES CYTOTOXIC ACTIVITY OF CD8-T-CELLS FROM PBMC AND HUMAN TUMOR SAMPLES BY BLOCKING THE INTERACTION BETWEEN KLRG1 AND CADHERINS

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Background KLRG1 is an inhibitory receptor expressed on T and NK-cells. On CD8-T-cells, KLRG1 is expressed on highly differentiated antigen-specific effector memory T and CD45RA-effector memory T cells. These cells display strong anti-tumor cytotoxicity by releasing IFNg and TNFa, however this process is inhibited when KLRG1 is engaged by various tumor-expressed cadherins. The percentage of KLRG1+CD8-T-cells increases with age, suggesting why age is a risk factor for cancer. We hypothesized that patients with KLRG1+CD8-T-cells may benefit from an antagonistic antibody blocking KLRG1-cadherin interactions, thereby restoring the function of cytotoxic T and NK-cells, resulting in broad tumor-killing activity.

Methods Antibodies were generated by immunization of mice followed by humanization. Binding was measured by Octet and FACS using KLRG1-overexpressing cells. Blocking was studied using labelled KLRG1-tetramers. Assays measuring IFNg or TNFa used artificial antigen-presenting cells (aAPC) mixed with KLRG1+CD8-T-cells isolated from PBMCs from healthy donors or cancer patients or from dissociated tumors. A human IgG4-antibody was used as negative control. Cytotoxic assays employed BiTE molecules binding to CD3-expressing T-cells and CD19- or HER2-expressing target cells.

Results PYX-102, an IgG4-antibody which targets human KLRG1, was isolated from a wild-type mouse hybridoma after immunization with hKLRG1-Fc. Binding affinity (EC_{50}) to human KLRG1 expressed in ExpiCHO cells was 1.0nM. Binding to cynomolgus monkey or mouse KLRG1 was weak or not detected. PYX-102 blocked a hKLRG1-tetramer from binding to E-cadherin-overexpressing K562 cells (EC_{50}=1.1nM). Furthermore, PYX-102 demonstrated binding to CD8-T-cells (2.3nM) and CD56+NK-cells (1.4nM) isolated from human PBMCs.

Functionally, we utilized E-cadherin-expressing aAPC which also expressed TCR-activating ligands (TCR-CHO-K1-cells). When co-culturing these cells with KLRG1+CD8-T-cells from healthy donors, the addition of PYX-102 induced pro-inflammatory cytokines IFNg (1,243pg/mL with an EC_{50}=0.29nM) and TNFa (206pg/mL, EC_{50}=0.32 nM). Importantly, IFNg was also induced by PYX-102 when utilizing CCR7-CD8+T-cells from cancer patient PBMCs (1 prostate, 2 kidney) mixed with TCR-CHO-K1-cells. In the same assay but using CD8 +tumor-infiltrating lymphocytes (TIL) isolated from 2 dissociated kidney cancers, IFNg-producing CD8+TIL cells were increased by PYX-102. Lastly, using BiTE molecules (CD3xCD19 or CD3xHER2) to bring KLRG1-enriched CD8-T-cells in proximity to CD19- or HER2-expressing target cells, PYX-102 reduced viability of HEK-CD19-E-cadherin or HCC2935-HER2-E-cadherin target cells. Tumor antigen-specific KLRG1+CD8-T-cells were observed in breast, lung, kidney, ovarian, esophageal and colorectal tumors which are known to express cadherins, strongly supporting the rational for targeting KLRG1+CD8-T-cells.

Conclusions Our data on PYX-102, which blocks the KLRG1-cadherin interaction and leads to activation of CD8-T-cells, supports further development of this promising and innovative anti-cancer experimental therapeutic.

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