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

Kei Yasuda*, Liyang Diao, Jianwen Feng, Matthew Iovino, Sara Lewandowski, Anthony Rodriguez, Philipp Steiner, Dan Felitsky, Ronald Herbst, Ben Keller, Ona Miller, Alyssa Quigley, Jan Pinkas.
Pyxis Oncology, Boston, MA, USA

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 (EC50) 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 (EC50=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 EC50=0.29nM) and TNFa (206pg/mL, EC50=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 CD3xCD8) 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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