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 IFN-γ and TNF-α, however, this process is inhibited when KLRG1 is engaged by various tumor-expressed cadherins (E, N and R). The percentage of KLRG1+CD8+T cells in blood increases with age, suggesting why age is a risk factor for cancer.

We hypothesized that patients with KLRG1+CD8+ T cells might 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 labeled KLRG1-tetramers.
- Assays measuring IFN-γ or TNF-α used artificial antigen-presenting cells (aAPC) mixed with KLRG1+CD8+T cells isolated from PBMCs from healthy donors, cancer patients or from dissociated tumors. A human IgG4 antibody was used as a negative control.
- Cytotoxic assays employed bispecific T cell engager (BiTE) molecules binding to CD3-expressing T cells and CD19- or HER2-expressing target cells.

RESULTS

Figure 1. Binding affinity to human KLRG1 and cyno KLRG1 expressed in ExpiCHO cells. Binding to mouse KLRG1 was not detected

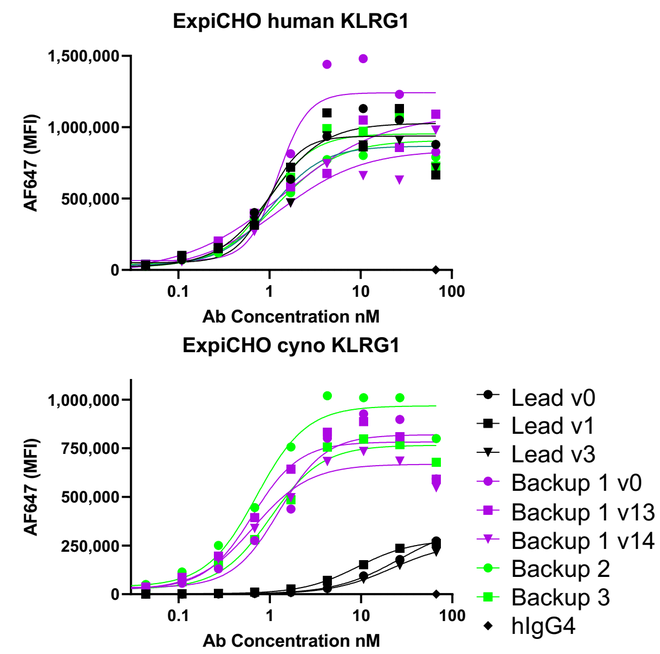


Figure 2. PYX-102 blocked a hKLRG1-tetramer from binding to E-cadherin-overexpressing K562 cells

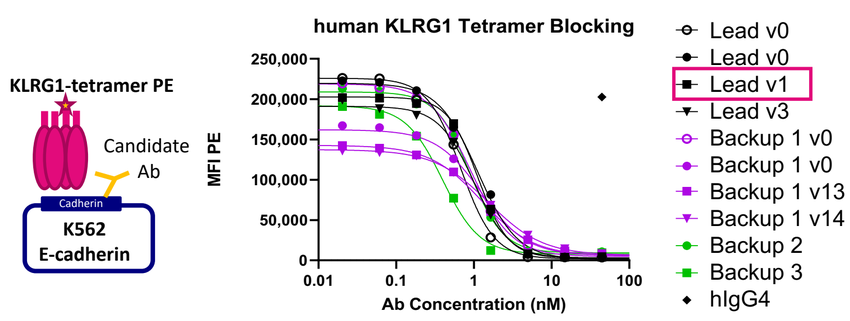


Figure 3. PYX-102 demonstrated binding to CD8+T cells and CD56+NK-cells isolated from human PBMCs

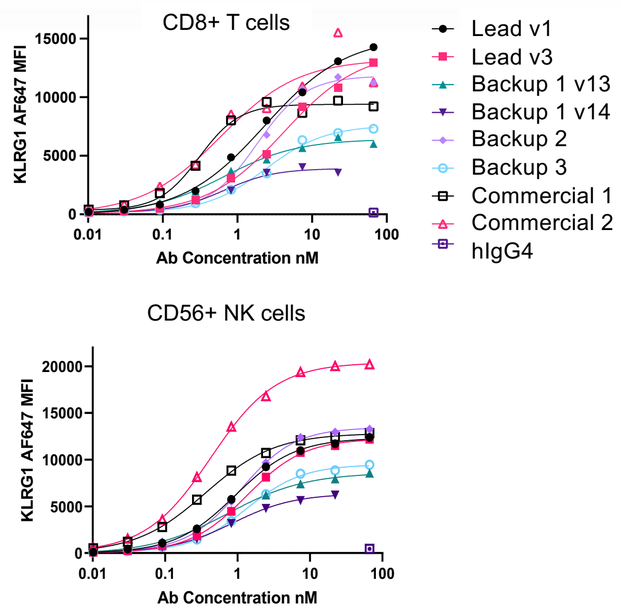


Figure 4. PYX-102 blocked KLRG1-cadherin interaction and enhanced cytokine production from CD8+T cells

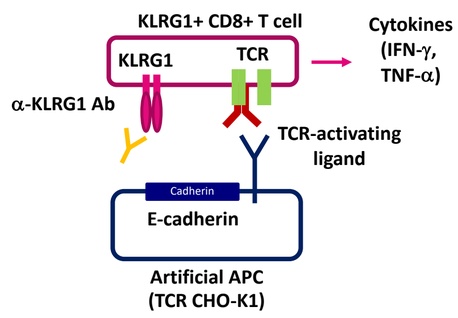


Fig 4A. E-cadherin expressing artificial antigen presenting cells (aAPC) were co-cultured with CD8+T cells in the presence of PYX-102

Fig 4B. PYX-102 enhanced IFN-γ and TNF-α production from KLRG1-enriched CD8+T cells from healthy donor PBMC, dose response

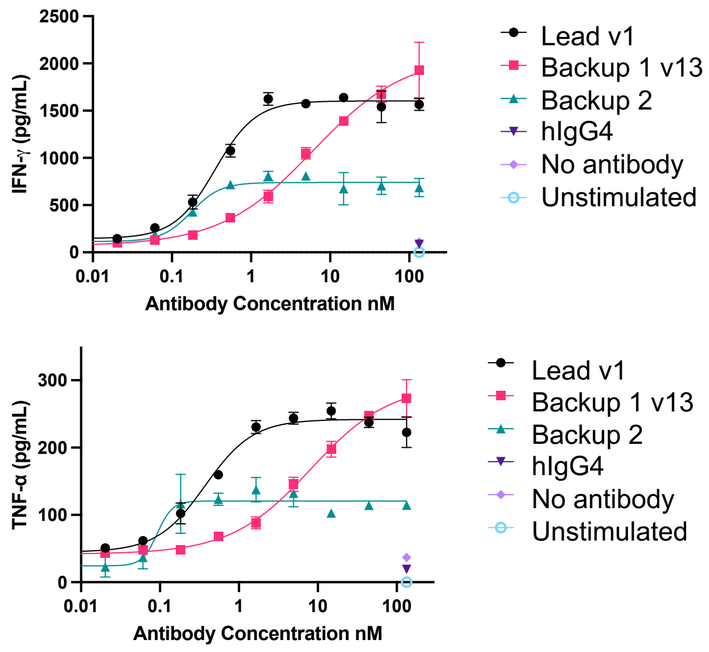


Fig 4C. PYX-102 enhanced IFN-γ production from KLRG1-enriched CD8+T cells from cancer patient PBMC (1 prostate and 2 kidney) , fixed dose

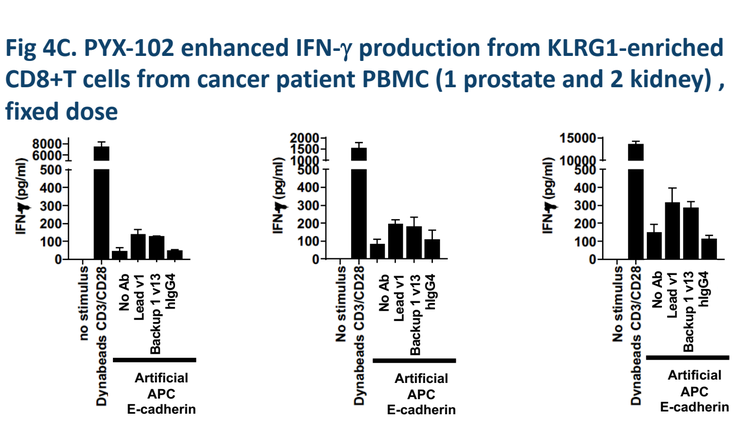


Fig 4D. PYX-102 enhanced percentages of IFN-γ+ CD8+ TILs from eight dissociated tumors (3 kidney, 2 gastric, 1 melanoma, 1 lung and 1 ovarian), fixed dose

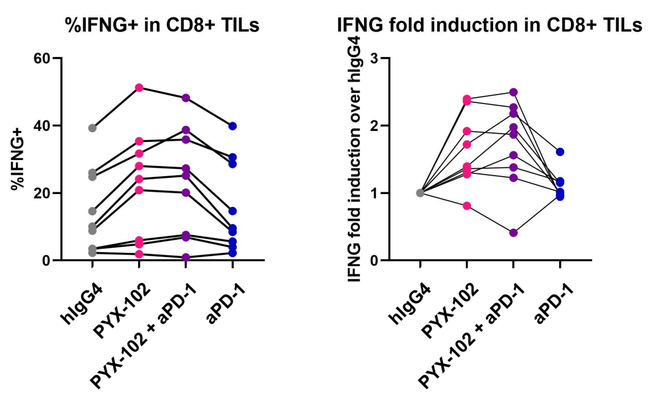


Figure 6. Bispecific T cell engager (BiTE) molecules (CD3xCD19 or CD3xHER2) to bring KLRG1-enriched CD8+T cells in proximity to CD19- or HER2-expressing target cells. PYX-102 reduced viability of HEK-CD19- E-cadherin or HCC2935-HER2-E-cadherin target cells

Fig 6A. HEK cell (CD19+E-cadherin+) killing with CD8+T cells, CD3xCD19 and PYX-102

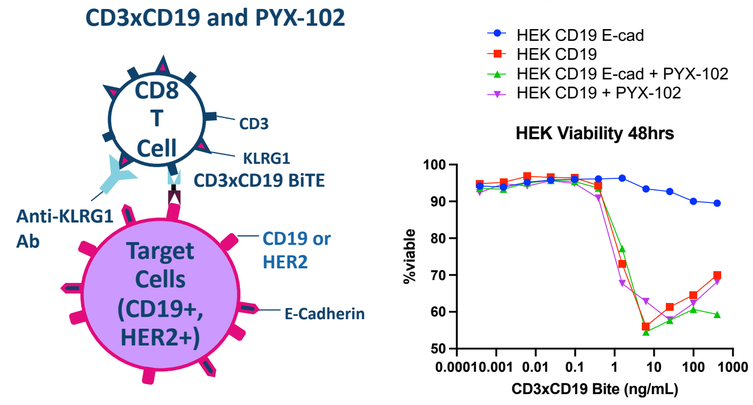


Fig 6B. HCC2935 (HER2+E-cadherin+) killing with CD8+T cells, CD3xHER2 and PYX-102 from 3 donors

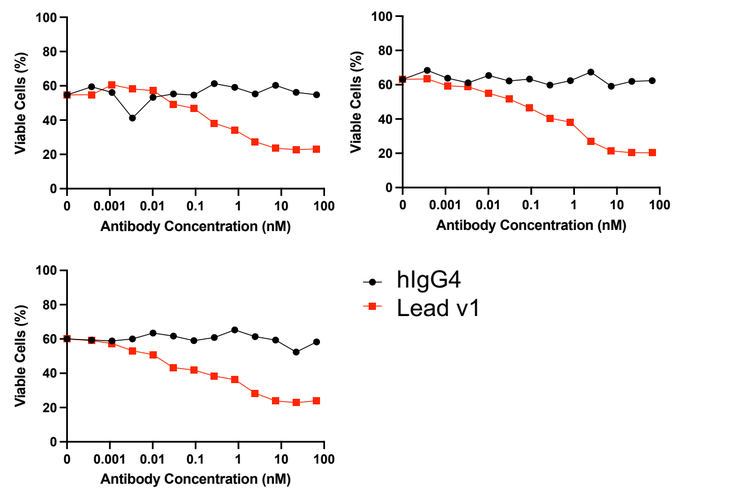
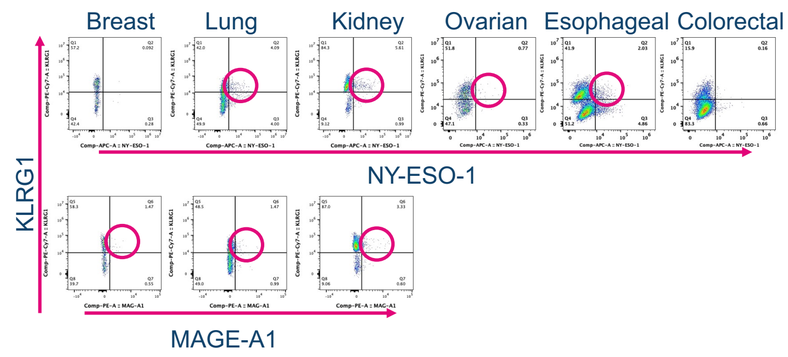


Figure 7. Dissociated tumor cells were stained with KLRG1 and tumor-antigen HLA- tetramers (NY-ESO-1 and MAGE-A1). Tumor antigen-specific KLRG1+CD8+ T cells were observed in multiple tumors



PYX-102 SUMMARY

ExpiCHO Human KLRG1 binding (EC50, nM)	ExpiCHO Cyno KLRG1 binding (EC50, nM)	Human KLRG1 tetramer blocking (IC50, nM)	Human KLRF1 binding
0.96	9.18	1.13	No

Human PBMC Binding		Cytokine production induced by artificial APC E-cadherin			
CD8+ (EC50, nM)	CD56+ (EC50, nM)	IFN-γ (EC50, nM)	TNF-α (EC50, nM)	IFN-γ Max Activity (pg/ml)	TNF-α Max Activity (pg/ml)
2.3	1.4	0.29	0.32	1,243	206

CONCLUSION

PYX-102 blocks the KLRG1-cadherin interaction and leads to activation of CD8+T cells from healthy PBMCs, cancer PBMCs and dissociated tumors.

Tumor antigen-specific KLRG1+CD8+T cells were observed in multiple tumors.

These data support the continued development of PYX-102 as a promising and innovative experimental therapeutic.