AKT Inhibition During *Ex Vivo* TIL Expansion Enhances Cytokine Production and Function While Increasing the Population of Less Differentiated (CD39$^-$CD69$^-$) CD8$^+$ T-Cells

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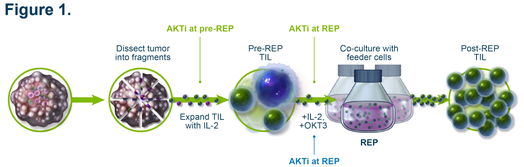
Background

- Adoptive cell therapy using autologous tumor-infiltrating lymphocytes (TIL) has shown durable responses in patients with metastatic melanoma[1] and other epithelial malignancies
- Recently, a memory-progenitor stem-like (CD39$^-$CD69$^-$) phenotype was associated with complete regression and TIL persistence in a cohort of patients with metastatic melanoma[2]
- Strategies to expand TIL with less differentiated and more stem-like attributes may result in improved persistence, functionality, and better anti-tumor activity
- Pharmacologic inhibition of protein kinase B (AKT) in TIL has been shown to induce transcriptional, metabolic, and functional properties characteristic of memory T cells[3]
- In this study, we investigated whether AKT inhibition during *ex vivo* TIL expansion could increase the proportion of less-differentiated, more stem-like cells with improved cytokine output and functionality

Methods

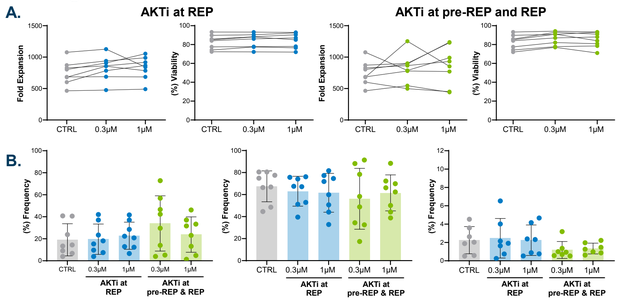
- Patient tumors (N=8) from different indications (melanoma, non-small cell lung cancer [NSCLC], head & neck, ovarian, and breast) were received, fragmented, and subjected to a 22-day expansion protocol for TIL generation
- Two doses (0.3 μM and 1 μM) of the pan-AKT inhibitor (AKTi) ipatasertib were added to the culture during *ex vivo* expansion
- The expansion potential, as well as the phenotypic and functional characteristics of TIL were evaluated on the final TIL product

Figure 1.



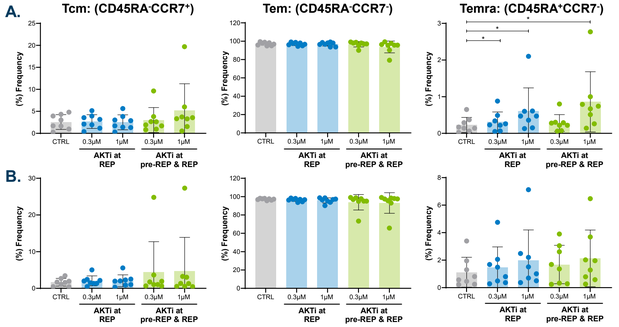
Results

Figure 2. AKTi treatment maintains TIL expansion and viability without affecting the T-cell ratio



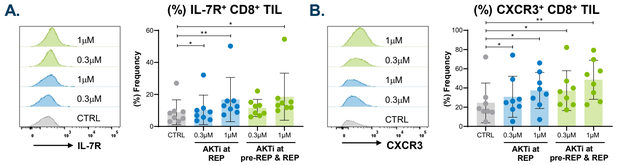
Expansion, viability and T-cell distribution in control and AKTi-treated TIL. TIL were left untreated (CTRL, gray bars) or treated with increasing concentrations of the pan-AKTi ipatasertib. Treatment was added either during the REP stage only (blue bars) or during pre-REP and REP (green bars). **A.** Fold expansion and viability of TIL at the end of the 22-day expansion process. **B.** Frequency of CD8$^+$, CD4$^+$, and CD4$^+$ (Foxp3$^+$) cells after the expansion process on cryopreserved cells.

Figure 3. AKT inhibition increases the frequency of CD8$^+$ Temra cells



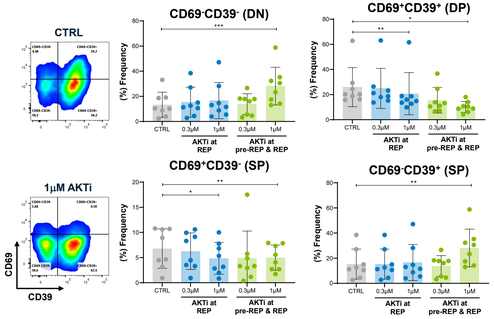
T-cell subsets in control and AKTi-treated TIL. Frequency of Tcm (CD45RA$^-$CCR7$^+$), Tem (CD45RA$^-$CCR7$^-$), and Temra (CD45$^+$CCR7$^-$) cells in **A.** CD8$^+$ and **B.** CD4$^+$ TIL after treatment. *P < 0.05

Figure 4. AKTi treatment increases IL-7R and CXCR3 expression on CD8$^+$ TIL



Cytokine and chemokine receptor expression on control and AKTi-treated TIL. Cryopreserved control or AKTi-treated TIL were analyzed by flow cytometry. Representative histogram and frequencies of **A.** IL-7R$^+$ and **B.** CXCR3$^+$ CD8$^+$ TIL. *P < 0.05, **P < 0.01

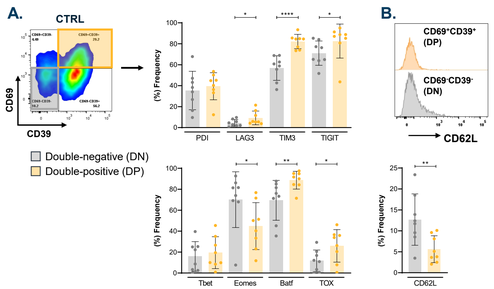
Figure 5. AKT inhibition increases the frequency of CD69$^-$CD39$^-$ CD8$^+$ TIL



Frequency of CD69 and CD39 subsets on CD8$^+$ TIL. Distribution of CD69 and CD39 single- and double-positive populations in control and AKTi-treated CD8$^+$ TIL as assessed by flow cytometry.

*P < 0.05
**P < 0.01
***P < 0.001

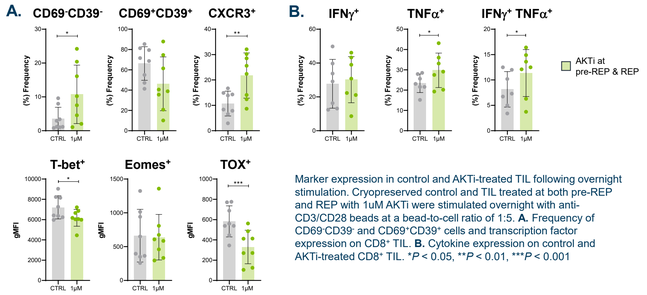
Figure 6. CD69$^-$CD39$^-$ CD8$^+$ TIL are less differentiated



Expression of inhibitory receptors and transcription factors on CD69$^-$CD39$^-$ and CD69$^+$CD39$^+$ CD8$^+$ TIL. **A.** Frequency of PD1, LAG3, TIM3, and TIGIT as well as Tbet, Eomes, Batf and TOX on CD69$^-$CD39$^-$ and CD69$^+$CD39$^+$ cells. **B.** Representative histogram and frequency of CD62L expression on CD69$^-$CD39$^-$ and CD69$^+$CD39$^+$ CD8$^+$ TIL.

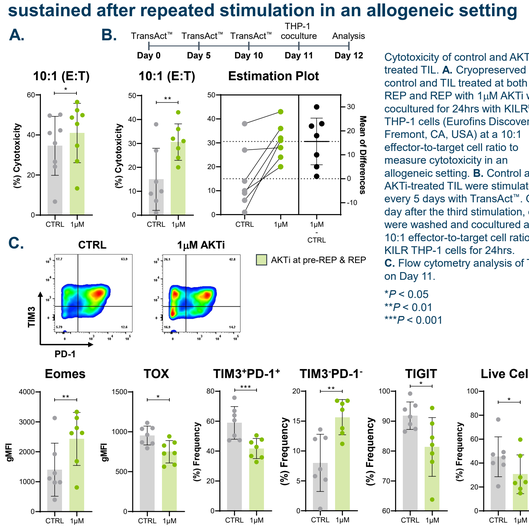
*P < 0.05
**P < 0.01
****P < 0.0001

Figure 7. AKTi-treated TIL maintain a higher frequency of CD69$^-$CD39$^-$ cells, lower TOX expression, and higher cytokine output following stimulation



Marker expression in control and AKTi-treated TIL following overnight stimulation. Cryopreserved control and TIL treated at both pre-REP and REP with 1uM AKTi were stimulated overnight with anti-CD3/CD28 beads at a bead-to-cell ratio of 1:5. **A.** Frequency of CD69$^-$CD39$^-$ and CD69$^+$CD39$^+$ cells and transcription factor expression on CD8$^+$ TIL. **B.** Cytokine expression on control and AKTi-treated CD8$^+$ TIL. *P < 0.05, **P < 0.01, ***P < 0.001

Figure 8. AKTi-treated TIL show increased cytotoxicity that is sustained after repeated stimulation in an allogeneic setting



Cytotoxicity of control and AKTi-treated TIL. **A.** Cryopreserved control and TIL treated at both pre-REP and REP with 1μM AKTi were cocultured for 24hrs with KILR® THP-1 cells (Eurofins DiscoverX, Fremont, CA, USA) at a 10:1 effector-to-target cell ratio to measure cytotoxicity in an allogeneic setting. **B.** Control and AKTi-treated TIL were stimulated every 5 days with TransAct™. One day after the third stimulation, cells were washed and cocultured at a 10:1 effector-to-target cell ratio with KILR THP-1 cells for 24hrs. **C.** Flow cytometry analysis of TIL on Day 11.

*P < 0.05
**P < 0.01
***P < 0.001

Conclusions

- AKTi treatment increased the frequency of IL-7R and CXCR3 expressing CD8$^+$ TIL without affecting expansion and viability, while maintaining T-cell ratios
- Treatment of TIL with ipatasertib, particularly when given at both the pre-REP and REP stages of *ex vivo* TIL expansion at a concentration of 1μM, augmented the proportion of less-differentiated and more memory-like CD69$^-$CD39$^-$ CD8$^+$ T cells
- AKTi-treated TIL maintained higher frequencies of CD69$^-$CD39$^-$ cells with reduced TOX levels and increased cytokine output following stimulation
- Increased cytotoxic capacity was observed with AKTi-treated TIL in an allogeneic setting, which was sustained even after repeated TIL stimulation
- Temporally inhibiting AKT signaling during TIL expansion could represent an approach for improving the quality of TIL and augment therapeutic efficacy in the clinical setting

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Abbreviations

AKT, protein kinase B; AKTi, AKT inhibitor; CTRL, control; DN, double-negative; DP, double-positive; IL-2, interleukin-2; NSCLC, non-small cell lung cancer; REP, rapid expansion protocol; SN, single-negative; SP, single-positive; TIL, tumor-infiltrating lymphocytes.

References

1. Sarnaik AA et al, *J Clin Oncol* 2021; 39(24):2656-66.
2. Krishna S et al, *Science* 2020;370(6522):1328-34.
3. Crompton et al, *Cancer Res* 2015; 75(2):296-305.

Disclosures

- All authors meet the criteria for authorship set forth by the International Committee of Medical Journal Editors
- All authors are employees of Iovance and may have stock options