K^+ Channel Activation Promotes Tumor Infiltrating Lymphocyte (TIL) Expansion and Enhances Expression of CCR7

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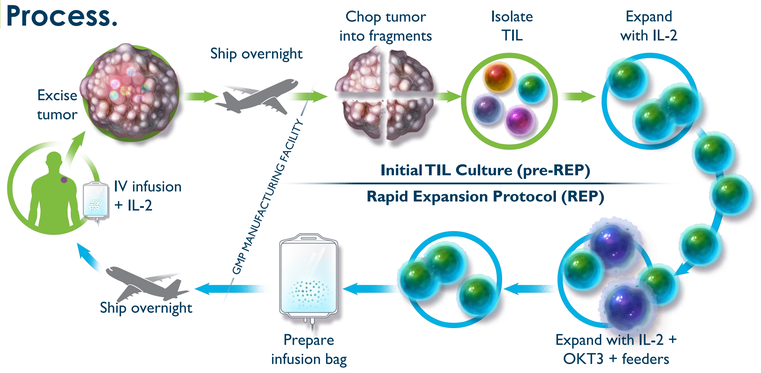
ABSTRACT

BACKGROUND: Only two K^+ channels are known to be expressed by human T-cells. Activated effector T cells express high levels of $K_v1.3$; activated naïve and central memory T-cell subsets express high levels of $K_{Ca}3.1$. Overexpression of $K_v1.3$ in murine T-cells increases K^+ efflux, improves effector function (IFNγ production) and promotes anti-tumor activity.[1] Inhibition of $K_{Ca}3.1$ suppresses murine T-cell proliferation and cytokine production. Thus, activation of K^+ channels enhances TIL growth and could promote T-cell effector function resulting in mediating tumor regression.

RESULTS: In human PBMCs and TIL, $K_{Ca}3.1$ expression was relatively low with upregulation observed within 24 h following stimulation with anti-CD3 and anti-CD28. TIL propagated in the Rapid Expansion Protocol (REP) had a 1.42-fold greater expansion (*p=0.002*) in the presence of SKA-31 and significant increases in the $CD8^+CD28^+$ (*p=0.04*), $CD8^+CD27^+$(*p=0.04*), and $CD8^+CD27^+CD28^+$subsets (*p=0.002*), consistent with a less differentiated phenotype. Significantly increased $K_{Ca}3.1$ was found in pre-REP TIL samples (n=8) as compared to normal donor PBMCs (n=6) in both CD4 and CD8 subsets (*p=0.0016*). Addition of $K_{Ca}3.1$ agonist SKA-31 in pre-REP TIL notably heightened CD25 and CCR7 expression.

CONCLUSION: We demonstrate that SKA-31 treatment enhances CCR7 expression associated with memory cells, promotes TIL expansion, and attenuates T-cell differentiation. Targeting the $K_{Ca}3.1$ channel is a novel strategy to expand and sustain less differentiated TILs and may improve the clinical application of adoptive T-cell therapy with TIL.

LION's TIL Expansion Process.



The tumor is excised from the patient and transported to the GMP Manufacturing Facility. Upon arrival the tumor is fragmented and placed in G-Rex flasks with IL-2 for TIL expansion (pre-REP expansion).

METHOD

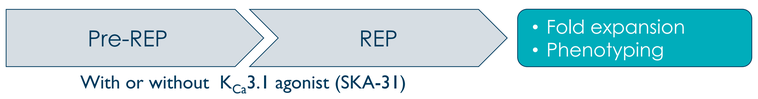


Figure 1. pre-REP TIL were propagated with REP using irradiated PBMCs, anti-CD3 (30 ng/mL), IL-2 (6000 IU/mL) alone or with $K_{Ca}3.1$ agonist SKA-31 treatment. The impact of SKA-31 treatment was assessed as a function of fold expansion and phenotypic analysis.

References
1. Eil R, Vodnala SK, Clever D, Klebanoff CA, Sukumar M, Pan JH, Palmer DC, Gros A, Yamamoto TN, Patel SJ, Guittard GC, Yu Z, Carbonaro V, Okkenhaug K, Schrump DS, Linehan WM, Roychoudhuri R, Restifo NP. Ionic immune suppression within the tumour microenvironment limits T cell effector function. *Nature*. 2016;537):539-543.

RESULTS

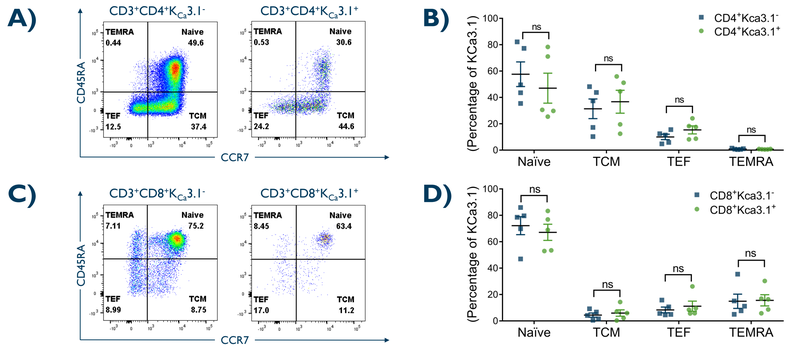


Figure 2. $K_{Ca}3.1$ is widely expressed by all T-cell subsets in normal donor PBMCs T-cell subset is defined using CD45RA and CCR7, namely naïve, central memory (TCM), effector memory (TEF), and effector memory RA^+(TEMRA) cells. Normal donor PBMCs were stained with anti-CD3, anti-CD4, anti-CD8, anti-$K_{Ca}3.1$, anti-CD45RA, and anti-CCR7 and analyzed by flow cytometry (n=6). Percentage of $K_{Ca}3.1$ expression is demonstrated in each T-cell subset of $CD3^+CD4^+$ **(A, B)** and $CD3^+CD8^+$ **(C,D)**. No statistical difference in $K_{Ca}3.1$ expression in each T-cell subset is found using student's unpaired T test. p values <0.05 are considered statistically significant.

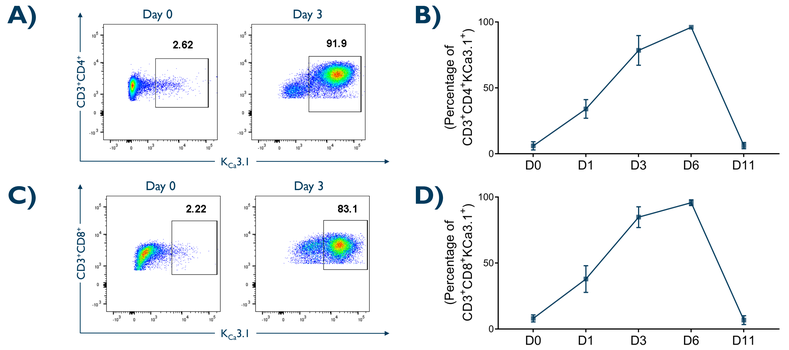


Figure 3. $K_{Ca}3.1$ expression is up-regulated following T-cell activation. Normal donor PBMCs were activated with anti-CD3 (1000 ng/ml) and anti-CD28 (500 ng/ml) (n=6). Pseudocolor plots demonstrate the percentage of $K_{Ca}3.1$ in $CD3^+CD4^+$subset **(A)** and $CD3^+CD8^+$subset **(C)** on day 0 and day 3 following TCR activation. Kinetic expression of $K_{Ca}3.1$ within 11 day time course is demonstrated in $CD3^+CD4^+$ **(B)** and $CD3^+CD8^+$subsets **(D)**.

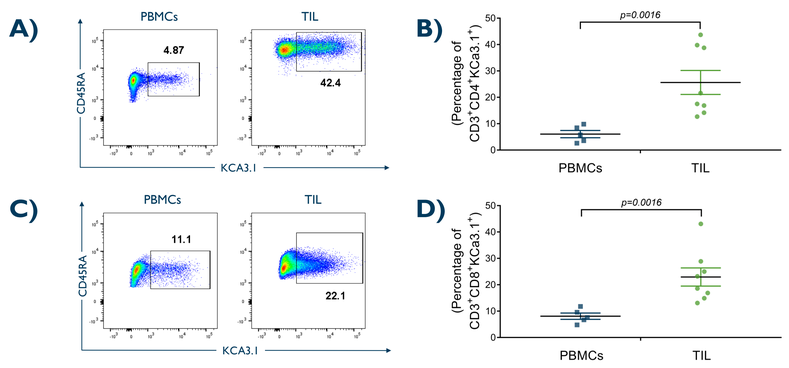


Figure 4. Heightened expression of $K_{Ca}3.1$ in pre-REP TIL. $K_{Ca}3.1$ expression was assessed by flow cytometry in normal donor PBMCs (n=6) and pre-REP TIL (n=8). Pseudocolor plots and dotplots represent the percentage of $K_{Ca}3.1$ expression demonstrated in $CD3^+CD4^+$**(A, B)** and $CD3^+CD8^+$subsets **(C, D)** of both normal donor PBMCs and TIL. p values represent the difference between normal PBMCs and pre REP-TIL using student's unpaired T test. p values <0.05 are considered statistically significant.

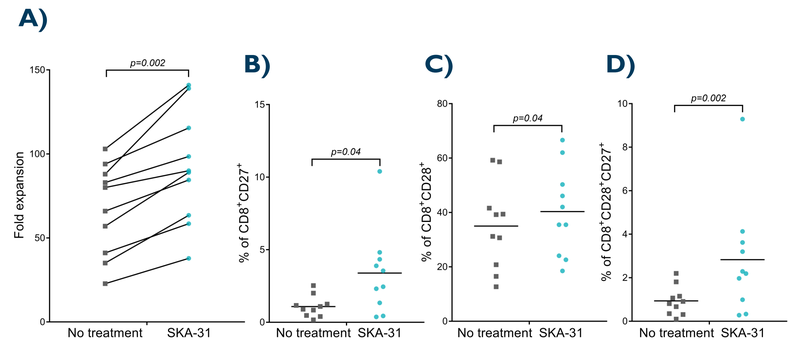


Figure 5. SKA-31 enhances TIL expansion with more sustained expression of CD27 and CD28 suggesting a less differentiated phenotype. Pre-REP TIL were propagated with Rapid Expansion Protocol (REP) using irradiated PBMCs, anti-CD3 (30 ng/mL), IL-2 (6000 IU/mL) alone or with SKA-31 for 14 days. Comparison of TIL expansion between no treatment and SKA-31 is demonstrated as fold expansion (n=10). **(A)** $CD3^+CD8^+CD27^+$subset, **(B)** $CD3^+CD8^+CD28^+$subset, **(C)** $CD3^+CD8^+CD27^+CD28^+$subset **(D)** were assessed in post-REP TIL in both no treatment and SKA-31 treatment groups (n=10). p values represent the difference between no treatment and SKA-31 using student's T test. p values <0.05 are considered statistically significant.

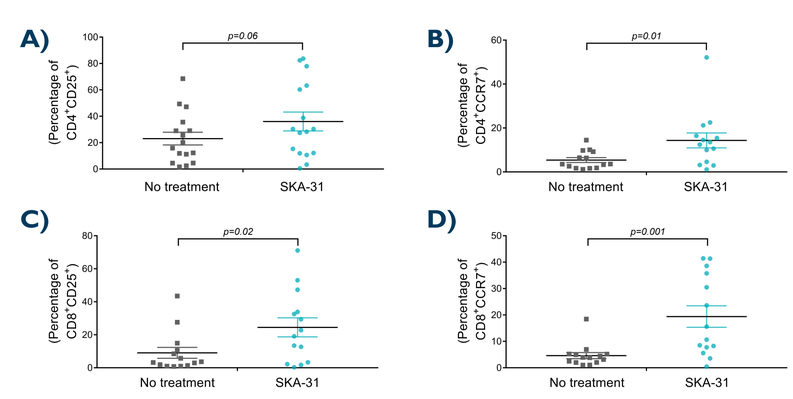


Figure 6. SKA-31 Enhances Expression of CD25 and CCR7. Pre-REP TIL were grown with either IL-2 (6000 IU/mL) alone or with SKA-31(n =14). CD25 and CCR7 expressions were assessed by flow cytometry in $CD4^+$ **(A, B)** and $CD8^+$ **(C, D)**. p values represent the difference between no treatment and SKA-31 using student's unpaired T test. p values <0.05 are considered as statistically significant.

SUMMARY

- $K_{Ca}3.1$ was expressed by all peripheral blood T-cell subsets including naïve, central memory (TCM), effector memory (TEF), and effector memory RA^+ (TEMRA) cells.
- Profound up-regulation of $K_{Ca}3.1$ was identified within 24 hours following T-cell activation.
- TIL have significantly higher level of $K_{Ca}3.1$ as compared to normal T-cells in the peripheral blood which suggests that TIL are activated T-lymphocytes.
- Activation of the $K_{Ca}3.1$ channel with the $K_{Ca}3.1$ agonist (SKA-31) promotes TIL expansion; this is potentially due to increased CD25 expression.
- SKA-31 helps sustain CD27 and CD28 expression during TIL expansion.
- Heightened CD25 and CCR7 expression was observed in pre-REP TIL grown with IL-2 in combination with SKA-31.
- Activation of the K^+ channel could be a novel strategy to promote TIL expansion and sustain a less differentiated phenotype, promoting long term engraftment.