T-cell Transcriptional and Mitochondrial Fitness Reprogramming in CLL Using PI3Kδ Inhibitors

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Introduction

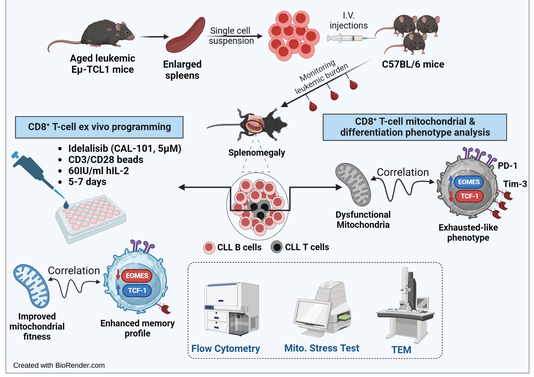
T-cell dysfunction associated with CLL presents a major barrier for successful adoptive cell therapy in patients. This problem is attributed to the phenotypic skewness of CLL T cells towards terminal differentiation phenotypes that, in most cases, cannot tolerate the ex vivo stimulation and expansion protocols. Importantly, a handful of recent reports have demonstrated metabolic defects in T cells from CLL patients which represent a new facet for the T-cell dysfunction problem in this disease. **Nevertheless, the field is still lacking sufficient studies to validate these metabolic abnormalities, in order to correlate with the differentiation and transcriptional changes of T cells in the preclinical Eμ-TCL1 murine model.**

PI3K signaling pathway is a main regulator of T-cell differentiation and metabolic programming following antigen recognition. Previous evidence has shown that T cells can maintain proliferative capacity following PI3K/AKT inhibition resulting in durable T-cell phenotypes with enhanced functional capacity. **Despite these observations, specific application of this knowledge in CLL is still required to improve adoptive cell therapies**.

Aims

1) Use the Eμ-TCL1 preclinical model to uncover the potential correlation between mitochondrial/metabolic defects and the exhausted-like features of T cells in CLL.
2) Investigate the potential benefits of ex vivo priming of Eμ-TCL1 T cells with the PI3Kδ inhibitor, idelalisib, to improve the metabolic fitness and transcriptional reprogramming of T cells.

Methods



1. Eμ-TCL1 CD8⁺ T-cell mitochondrial abnormalities

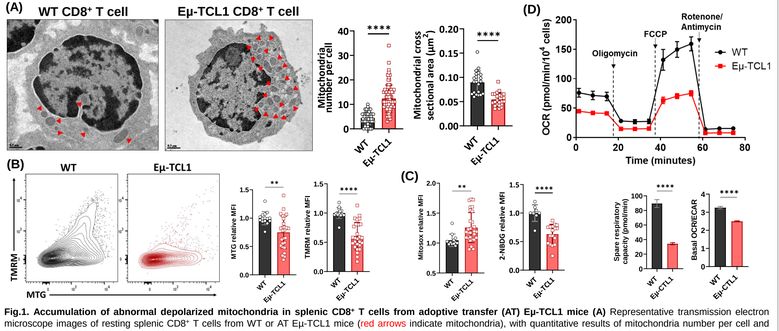


Fig.1. Accumulation of abnormal depolarized mitochondria in splenic CD8⁺ T cells from adoptive transfer (AT) Eμ-TCL1 mice (A) Representative transmission electron microscope images of resting splenic CD8⁺ T cells from WT or AT Eμ-TCL1 mice (red arrows indicate mitochondria), with quantitative results of mitochondria number per cell and mitochondrial cross sectional area (right panel, each dot represents a separate replicate image analyzed via FIJI software) **(B)** Representative flow cytometry contour plots (left panel) and quantitative results (right panel) of mitochondrial mass (MTG, mitotracker green) and membrane potential (TMRM) measurement in WT or AT Eμ-TCL1 CD8⁺ T cells (each dot represents an individual animal) **(C)** Quantitative results of mitochondrial ROS (mitosox) and cellular glucose uptake (2-NBDG) measurement **(D)** Mitochondrial stress test result of CD8⁺ T cells with quantitative analysis of mitochondrial functional parameters shown below. Flow cytometry gating was done on cells of interest, singlets, viable cells and CD8⁺. Data presented as mean ± SD. *P* values were calculated by two-tailed, unpaired Student's t-test. *$p<0.05$; **$p<0.01$; ***$p<0.001$; ****$p<0.0001$

2. Effect of CLL progression on T-cell exhaustion/metabolic defects

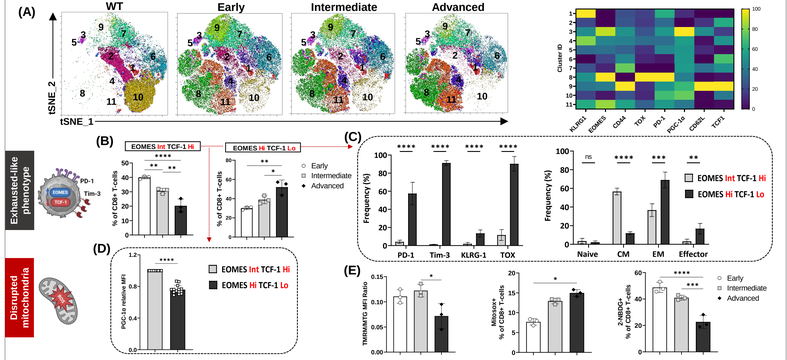


Fig.2. Leukemia progression drives gradual CD8⁺ T-cell exhaustion and metabolic disturbance (A) Flow cytometry t-distributed stochastic neighborhood embedding (tSNE) and Xshift clustering analyses of splenic CD8⁺ T cells from WT or adoptive transfer (AT) Eμ-TCL1 mice at different disease stages based on the expression of surface markers and transcription factors shown in the heat map (right panel) **(B)** Quantitative analysis of Eμ-TCL1 CD8⁺ T-cell populations based on the expression levels of EOMES and TCF-1 transcription factors **(C)** Flow cytometry quantitative results of exhaustion-related markers expression (left panel), memory phenotypes (right panel, **naive:** CD44⁻ CD62L⁺, **central memory (CM):** CD44⁺ CD62L⁺, **effector memory (EM):** CD44⁺ CD62L⁻ and **effector:** CD44⁻ CD62L⁻) and **(D)** relative intracellular PGC-1α expression inside the EOMES/TCF-1 gates mentioned in (B) **(E)** Flow cytometry analysis of membrane potential (TMRM) to mitochondrial mass (MTG) ratio, ROS production (mitosox) and glucose uptake (2-NBDG) of CD8⁺ T cells at different disease stages. Flow cytometry gating was done on cells of interest, singlets, viable cells and CD8⁺. Data presented as mean ± SD. *P* values were calculated by two-tailed, unpaired Student's t-test or one-way ANOVA with Tukey's multiple comparisons post-test. *$p<0.05$; **$p<0.01$; ***$p<0.001$; ****$p<0.0001$

3. Loss of metabolic and stress signaling control in terminally differentiated Eμ-TCL1 T cells

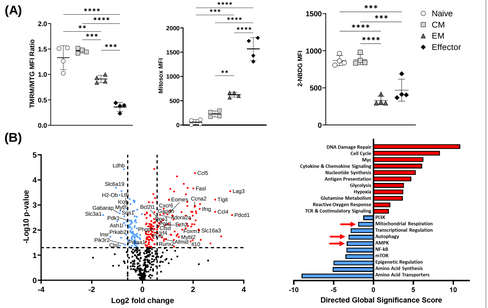


Fig.3. Eμ-TCL1 CD8⁺ T-cell metabolic abnormalities are associated with the expansion of terminally differentiated/effector phenotypes (A) Quantitative flow cytometry analysis of mitochondrial membrane polarization (TMRM/MTG), ROS production (mitosox) and glucose uptake (2-NBDG) in the different memory populations of AT Eμ-TCL1 CD8⁺ T cells **(B)** Nanostring gene expression analysis showing significantly **up-regulated** or **down-regulated** genes in **late versus early-stage** Eμ-TCL1 CD8⁺ T cells (left panel) and differentially regulated signaling pathways (right panel) based on the directed global significance score provided by ROSALIND cloud-based software platform. Labelled genes on the volcano plot are related to either glycolysis, mitochondrial respiration, amino acid transporters, autophagy, AMPK, cytokine and chemokine signaling or TCR and costimulatory signaling. Data presented as mean ± SD. *P* values were calculated by one-way ANOVA with Tukey's multiple comparisons post-test. *$p<0.05$; **$p<0.01$; ***$p<0.001$; ****$p<0.0001$

4. Ex vivo idelalisib treatment reprograms Eμ-TCL1 CD8⁺ T cells

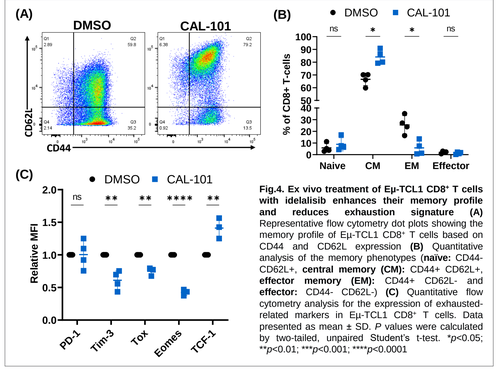


Fig.4. Ex vivo treatment of Eμ-TCL1 CD8⁺ T cells with idelalisib enhances their memory profile and reduces exhaustion signature (A) Representative flow cytometry dot plots showing the memory profile of Eμ-TCL1 CD8⁺ T cells based on CD44 and CD62L expression **(B)** Quantitative analysis of the memory phenotypes (**naive:** CD44-CD62L+, **central memory (CM):** CD44+ CD62L+, **effector memory (EM):** CD44+ CD62L- and **effector:** CD44- CD62L-) **(C)** Quantitative flow cytometry analysis for the expression of exhausted-related markers in Eμ-TCL1 CD8⁺ T cells. Data presented as mean ± SD. *P* values were calculated by two-tailed, unpaired Student's t-test. *$p<0.05$; **$p<0.01$; ***$p<0.001$; ****$p<0.0001$

5. Idelalisib improves mitochondrial activity of Eμ-TCL1 CD8⁺ T cells

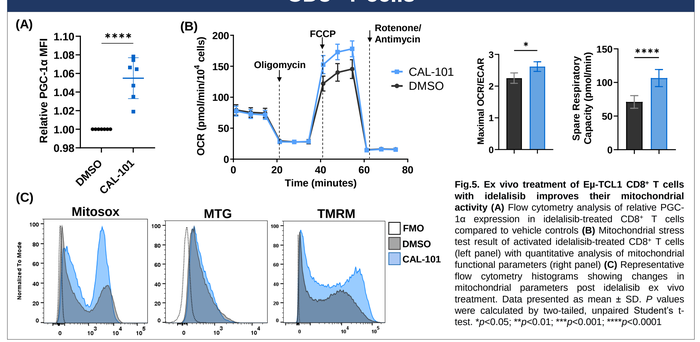


Fig.5. Ex vivo treatment of Eμ-TCL1 CD8⁺ T cells with idelalisib improves their mitochondrial activity (A) Flow cytometry analysis of relative PGC-1α expression in idelalisib-treated CD8⁺ T cells compared to vehicle controls **(B)** Mitochondrial stress test result of activated idelalisib-treated CD8⁺ T cells (left panel) with quantitative analysis of mitochondrial functional parameters (right panel) **(C)** Representative flow cytometry histograms showing changes in mitochondrial parameters post idelalisib ex vivo treatment. Data presented as mean ± SD. *P* values were calculated by two-tailed, unpaired Student's t-test. *$p<0.05$; **$p<0.01$; ***$p<0.001$; ****$p<0.0001$

6. Idelalisib enhances cytotoxic activity of Eμ-TCL1 CD8⁺ T cells against CLL cells

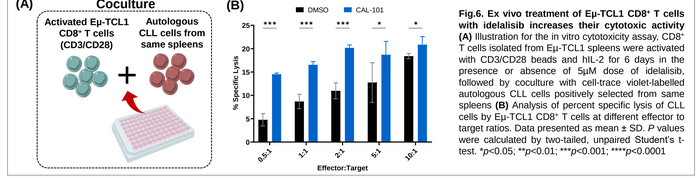


Fig.6. Ex vivo treatment of Eμ-TCL1 CD8⁺ T cells with idelalisib increases their cytotoxic activity (A) Illustration for the in vitro cytotoxicity assay, CD8⁺ T cells isolated from Eμ-TCL1 spleens were activated with CD3/CD28 beads and hIL-2 for 6 days in the presence or absence of 5μM dose of idelalisib, followed by coculture with cell-trace violet-labelled autologous CLL cells positively selected from same spleens **(B)** Analysis of percent specific lysis of CLL cells by Eμ-TCL1 CD8⁺ T cells at different effector to target ratios. Data presented as mean ± SD. *P* values were calculated by two-tailed, unpaired Student's t-test. *$p<0.05$; **$p<0.01$; ***$p<0.001$; ****$p<0.0001$

Conclusions

1. Eμ-TCL1 CD8⁺ T cells exhibit significant abnormalities in mitochondrial function and key properties including biogenesis, ROS production and membrane potential as demonstrated by acquiring a depolarized mitochondrial phenotype.
2. The changes in mitochondrial properties and glucose uptake are dependent on the leukemic burden and the associated accumulation of exhausted-like or terminally differentiated T-cell phenotypes.
3. CD8⁺ T-cell subsets with upregulated EOMES and downregulated TCF-1 expression are characterized by marked reduction in PGC-1α, master regulator of mitochondrial function.
4. Gene expression analysis of advanced versus early-stage Eμ-TCL1 CD8⁺ T cells highlights the upregulation of glycolysis-associated genes and downregulation of autophagy and stress signaling genes which might have a role in the accumulation of dysfunctional mitochondria.
5. Ex vivo treatment with the PI3Kδ inhibitor, idelalisib, reprograms Eμ-TCL1 T cells into less-exhausted memory cells with improved mitochondrial activity and enhanced cytotoxic effect.

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