1034

Venetoclax resistance induced by activated T cells can be counteracted by sphingosine kinase inhibitors in chronic lymphocytic leukemia

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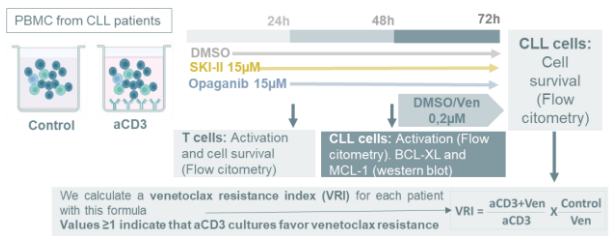
BACKGROUND

The treatment of CLL patients with venetoclax-based regimens has demonstrated efficacy and a safety profile (Roberts et al. 2016; Mato et al. 2018), but the emergence of resistant cells and disease progression is a current complication (Leverson and Cojocari 2018; Jayappa et al. 2021). We previously reported that the sphingosine kinase 1 and 2 (SPHK1/2) inhibitor, SKI-II enhanced the in vitro cell death triggered by fludarabine, bendamustine or ibrutinib and reduced the activation and proliferation of CLL cells (Almejún et al. 2017**). Since we previously showed that autologous activated T cells (aaT) from CLL patients favor the generation of venetoclax resistance** (Elias et al. 2022;Elías et al. 2018)**, we aimed to determine whether SPHK inhibitors could overcome this negative effect.**

METHODS

Peripheral blood mononuclear cells (PBMC) were obtained from twenty-eight unrelated CLL patients that were free from clinically relevant infectious complications and were treatment naïve or without treatment for ≥3 months before the investigation began.

- **Model for the evaluation of SPHKs inhibitors on the generation of venetoclax (Ven) resistance**



- **Model for the evaluation of SPHKs inhibitors on already venetoclax resistant cells**

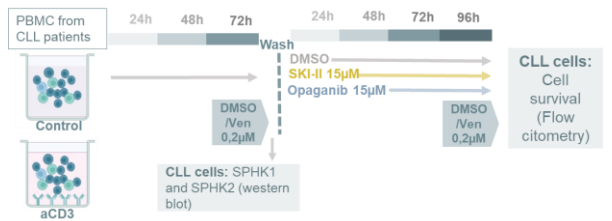


Fig. 1: SKI-II and opaganib reduced the upregulation of the activation markers CD86, PD-1 and PDL-1 on CLL cells induced by aaT cells.

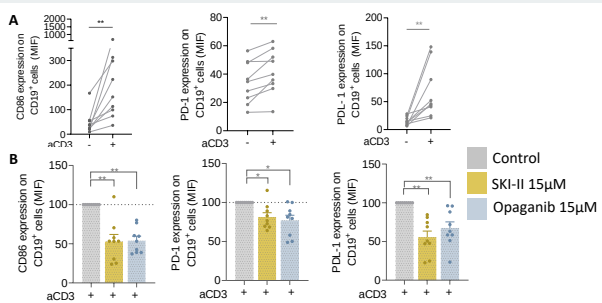


Fig. 1: A) Wilcoxon paired test. B)Wilcoxon Signed Rank Test, * p< 0.05, ** p<0,01 *** p<0.001, n=9.

Fig. 2: SKI-II and opaganib reduced the upregulation of proliferation marker Ki67 on CLL cells induced by aaT cells.

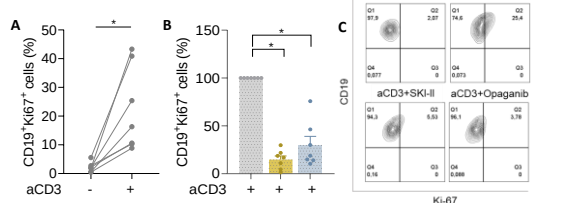


Fig. 2: A) Wilcoxon paired test. B)Wilcoxon Signed Rank Test, * p< 0.05, n=7. C) Results obtained with one representative CLL sample.

Fig. 3: SPHK inhibitors reduced the expression of CD40L induced on CD4⁺ cells upon CD3 crosslinking without affecting their survival.

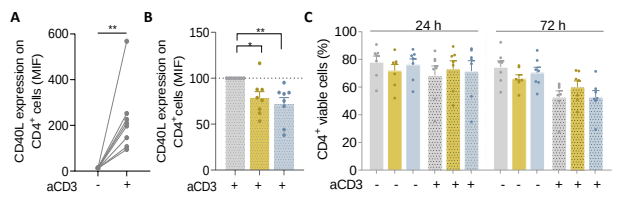


Fig. 3: A) Wilcoxon paired test. B)Wilcoxon Signed Rank Test, * p< 0.05, **p<0.01 n=8.

RESULTS

Fig. 4: SKI-II and opaganib prevented the generation of venetoclax-resistance induced by aaT cells

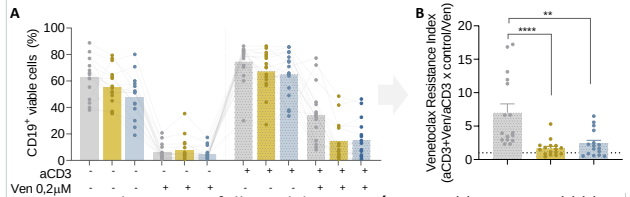


Fig. 4. Friedman test followed by Dunn´s test, ** p<0.01, **** p<0.0001, n=16

Fig. 5: Both inhibitors reduced the upregulation of BCL-XL on malignant cells induced by aaT cells while MCL-1 expression was only reduced by SKI-II under these culture conditions.

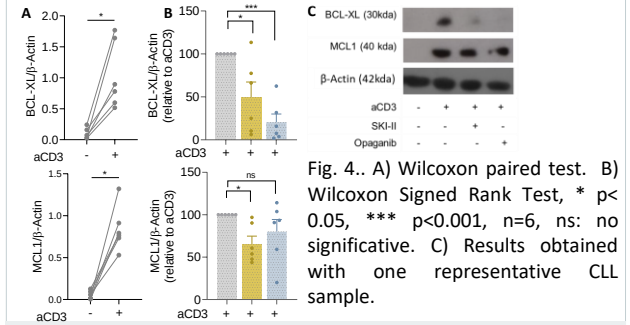


Fig. 4.. A) Wilcoxon paired test. B) Wilcoxon Signed Rank Test, * p< 0.05, *** p<0.001, n=6, ns: no significative. C) Results obtained with one representative CLL sample.

Fig. 6: The presence of aaT cells enhanced SPHK2 expression in CLL cells, which was higher in those resistant to venetoclax.

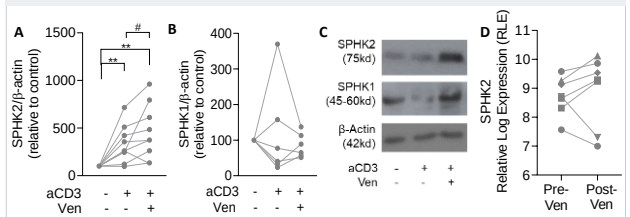


Fig. 6:. A)Wilcoxon Signed Rank Test, ** p<0.01. Wilcoxon matched-pairs signed rank test, # p<0.05 (n=9) D) SPHK2 expression on CLL cells of 7 CLL patients at progression on venetoclax therapy compared to prior-treatment (public data set RNA-Seq GSE192685)

Fig. 7: SPHK inhibitors were able to re-sensitize resistant CLL cells to a second venetoclax treatment

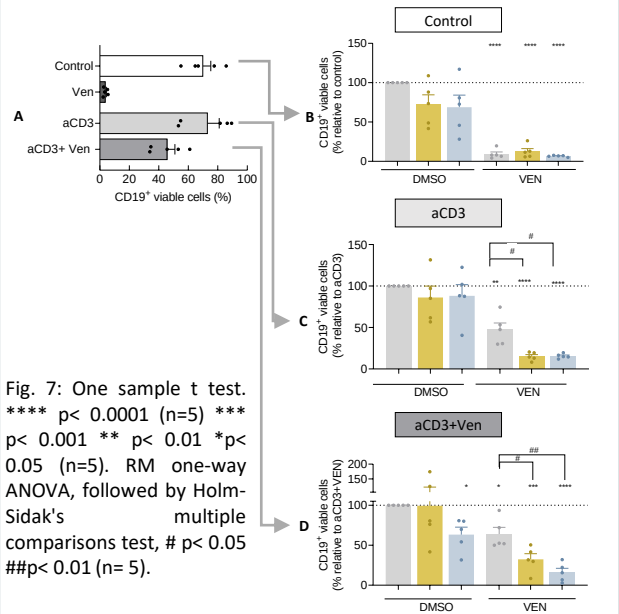


Fig. 7: One sample t test. **** p< 0.0001 (n=5) *** p< 0.001 ** p< 0.01 *p< 0.05 (n=5). RM one-way ANOVA, followed by Holm-Sidak's multiple comparisons test, # p< 0.05 ##p< 0.01 (n= 5).

CONCLUSIONS

- Venetoclax-resistant CLL cells express high levels of SPHK2.
- Both SPHK inhibitors reduce the activation and the expression of BCL-XL induced by aaT cells on CLL cells.
- SKI-II and opaganib diminish the generation of venetoclax-resistance and re-sensitize already venetoclax-resistant CLL cells to the drug, suggesting that the inhibition of SHPK2 may be involved in this process.
- Our results highlight the therapeutic potential of SPHK inhibitors in combination with venetoclax as a promising treatment option for the patients.

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