# T Cells from CLL Patients on Venetoclax Mount Potent Cytotoxic Responses in Combination with Epcoritamab, a CD20/CD3 Bispecific Antibody


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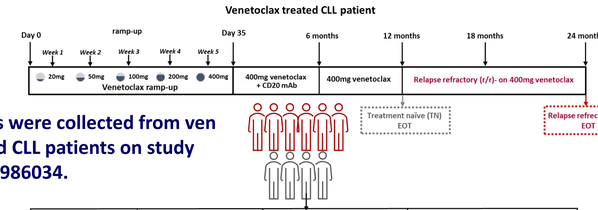
## Background

In patients with chronic lymphocytic leukemia (CLL), venetoclax (ven), achieves deep clinical responses with high rates of undetectable minimal residual disease (uMRD). Epcoritamab (epcor), a bispecific CD3xC20 mAb approved for 3rd line therapy of diffuse large B cell lymphoma (DLBCL) and follicular lymphoma (FL), is in clinical development for CLL. Treatment with Bruton tyrosine kinase inhibitors (BTKi) can enhance cytotoxic T-cell responses, and the combination of ven with epco in vitro increased killing of CLL cells over single agents.

## Aim

- To examined the effect of ven treatment on T-cell differentiation, activation state, and cytotoxicity in combination with epcor.
- Furthermore, to compare effects of ven and BTKi treatment on T-cell function in patients with CLL.

## Study group & Methods

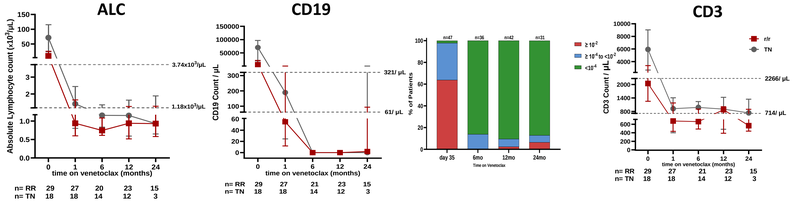



PBMCs were collected from ven treated CLL patients on study NCT03986034.

| Cell Count | Phenotypic Characterization | Total seq single cell sequencing 10x | Invitro combination with epcoritamab | Total seq 10x single cell sequencing after invitro combination with epcoritamab |
|---|---|---|---|---|
| (ALC, CD19, CD3, CD4, CD8, NK) | ven baseline n=10 | BTKi response n=7 | BTKi progression/preven CLL cells n=10 | BTKi progression/preven CLL cells n=3 |
| n=47 (TN=18, r/r=29) | ven response (Day-35) n=8 | BTKi progression/ preven n=7 | ven response T cells (D-35) n=4 | BTKi response T cells n=3 |
| | ven response (6 mo) n=10 | ven response (6 mo) n=7 | ven response T cells (6 mo) n=10 | ven response T cells (6 mo) n=3 |
| | Healthy donors n=4 | Healthy donors n=3 | Healthy donors n=3 | Healthy donors n=1 |

## Results

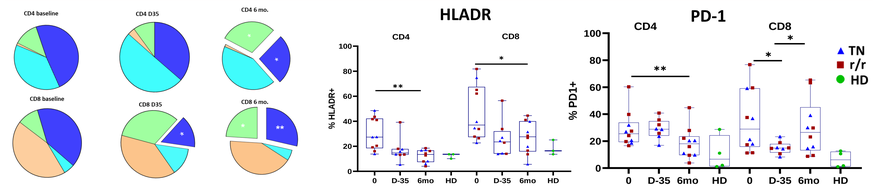
### I. Peripheral blood cell count changes on venetoclax treatment




ALC and CLL cell counts decreased rapidly and after 12 months, the rate of uMRD at $10^{-4}$ was 90%.

At 12 months, over 1 /3 of patients had CD3 counts below reference-range.

### II. Flow cytometric profiling of T cells on venetoclax treatment.

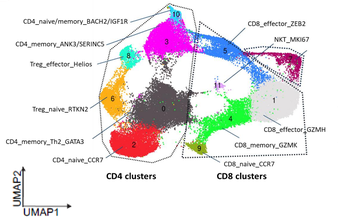



Shift of T cell differentiation into relative increase in naive, a decrease in effector-memory CD4 and CD 8 T cells.

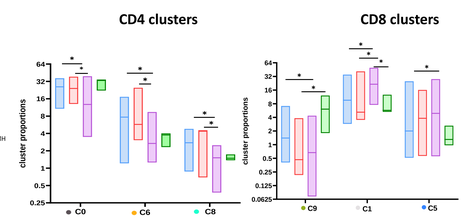
Significant decreases in expression of activation markers and checkpoint inhibitors (HLA-DR, PD-1, CTLA4).

### III. Single-cell transcriptomics changes in BTKi and venetoclax treated CLL patients.

UMAP of single-cell integrated T cell subsets and NK clusters.

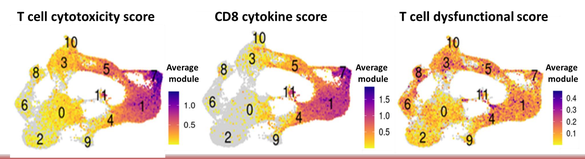



Two CD8+ effector T-cell clusters were expanded on ven, and 2 T-reg clusters were significantly smaller on ven than on BTKi.

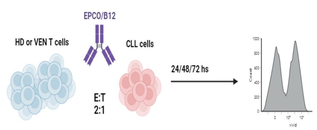



CD4:CD8 ratios were 0.8 on ven compared to 2.4 at BTKi response and progression timepoints.

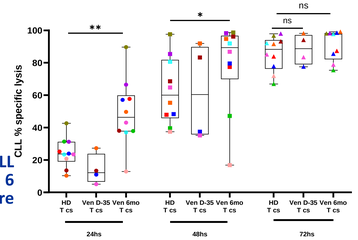
The largest CD8+ cluster on ven strongly expressed cytotoxic effector and cytokine gene signatures, while being relatively low for T-cell dysfunction gene sets.

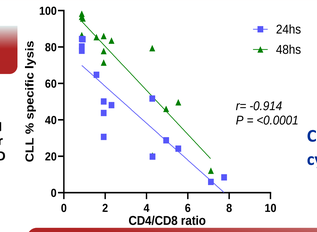



### IV. Epcoritamab induces autologous venetoclax T cells to lyse CLL tumor cells in vitro.




CLL cells specific lysis induced by epcoritamab, mixing CLL cells in vitro with either autologous T cells on ven (day-35, 6 months) OR allogenic T cells from healthy donor were equally cytotoxic.



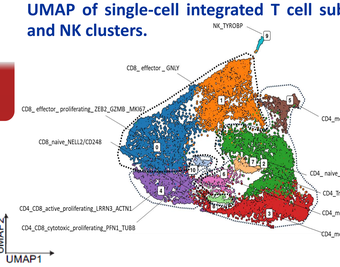




CD4:CD8 ratios were inversely correlated with the degree of CLL cell lysis.

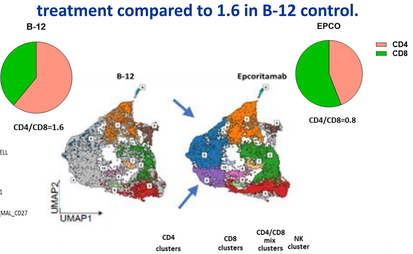
CD8 T cells are mainly responsible for epcor-mediated cytotoxicity and structuring interindividual differences.

### IV. Single-cell transcriptomics changes of samples (BTKi and ven response) reacted with epcor.

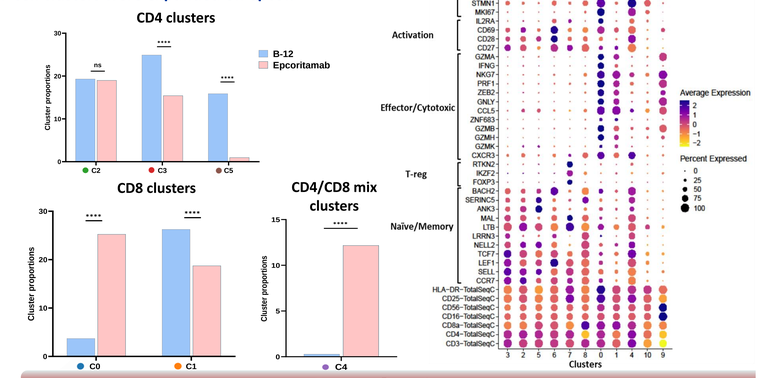
UMAP of single-cell integrated T cell subsets and NK clusters.




CD4:CD8 ratios were 0.8 on epcor treatment compared to 1.6 in B-12 control.




One CD8+_effector/cytotoxic_proliferating and 1 mix CD4/CD8 active_ proliferating T-cell clusters were expanded on epcor.




## Conclusion

- Beneficial effects of ven on the cytotoxicity of autologous T cells in response to epcor, including improved effector:target ratios, transcriptional programs enhancing cytotoxic T-cell responses, expansion of tumor-reactive TCR clonotypes, and reduced exhaustion of effector T cells.
- Thus, combination of epcoritamab with ven is promising and worthy of clinical investigation for CLL patients.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