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Impact of treatment with ibrutinib or venetoclax on CAR T cells generated from patients with chronic lymphocytic leukemia: interim analysis of the GIMEMA CLL2020 study

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OBJECTIVES

This project aims at assessing in CLL patients the impact of treatment with targeted agents (i.e. ibrutinib or venetoclax) on T cells and on patient-derived anti-CD19-4-1BB CAR T cells in terms of:

- manufacturing efficiency
- immunophenotypic profile
- functional properties

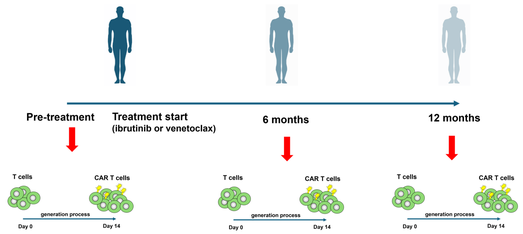
CONCLUSIONS

CAR-T cells can be effectively generated from CLL patients but display immunological features of dysfunction. From patients receiving 12-month venetoclax therapy we could generate CAR-T cells with a more favorable differentiation profile and partial restoration of exhaustion marker expression. Enrollment has now been completed, and assessments from an additional cohort of 8 patients, covering all timepoints, are currently underway. Conclusive results, including an evaluation of functional changes observed in CAR-T cells generated at different stages of targeted treatment, will be available at the time of data presentation.

INTRODUCTION

- The therapeutic effectiveness of CAR-T cells in chronic lymphocytic leukemia (CLL) remains suboptimal, primarily due to dysfunctions in patient-derived T cells. The immunological factors affecting the functionality of CAR-T cells generated from CLL patients, as well as the impact of targeted agents used to treat CLL on T-cell fitness, has yet to be fully elucidated.
- The functionality and the persistence of infused CAR-T cells are strictly connected to **the composition and the fitness** of both the starting T-cell source and the final CAR-T cell product[1].
- Main determinants of clinical response are the presence of **less differentiated T-cell subsets** and **lower expression of inhibitory molecules** on T-cell surface.

STUDY DESIGN



Samples were collected before the start of treatment and after 6 and 12 months of therapy with **ibrutinib (n=7) or venetoclax (n=8)**

METHODS

Analyses were performed on 15 CLL patients enrolled in the GIMEMA CLL2020 trial (NCT04640909). The T-cell phenotype was evaluated by multicolor flow cytometry. Anti-CD19-4-1BB CAR-T cells were produced by lentiviral transduction, and tested for generation efficiency, immunophenotype and in vitro anti-tumor activity.

REFERENCES

1. Reviewed in Vitale C et al, Hemasphere 2023

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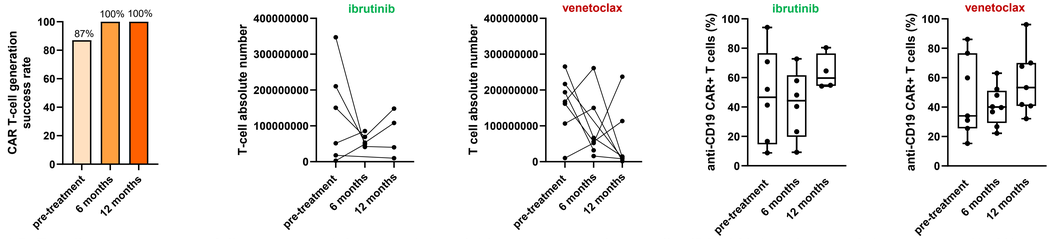
DISCLOSURES

MC: Abbvie, Astra Zeneca, Beigene, Behring, GSK, Johnson&Johnson (participation in speakers' bureau or Advisory Board); Abbvie, GSK, Johnson&Johnson (Research support); Abbvie, Beigene, Johnson&Johnson, Roche (Support for attending meetings and/or travel)

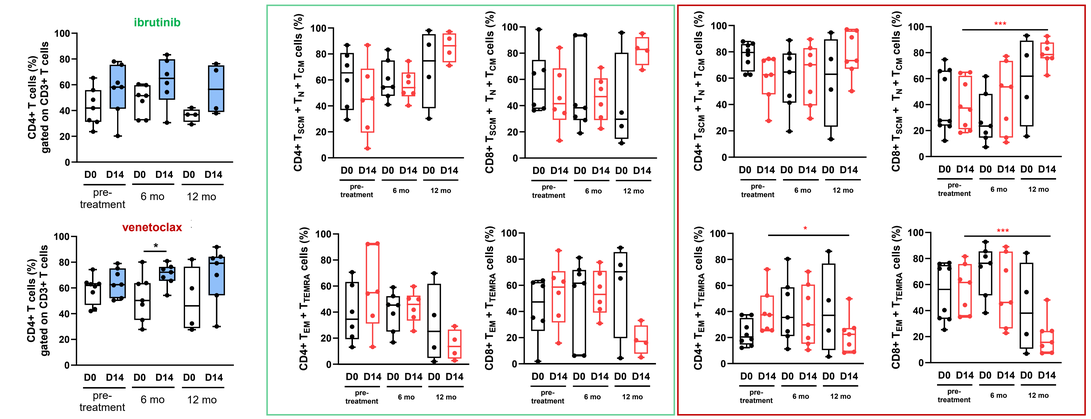
RESULTS

Manufacturing efficiency

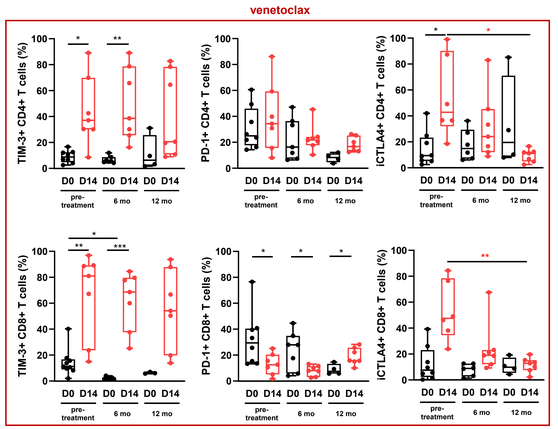


CAR-T cells were successfully generated from peripheral blood mononuclear cells (PBMC) in 13 out of 15 patients (87%) at pre-treatment and at the 6-month timepoint, and in all 11 patients (100%) with available 12-month samples. No significant impact of therapy was noted on CAR T-cell yield in both cohorts. The transduction efficiency remained consistent across the analyzed timepoints. Specifically, in the venetoclax-treated cohort, the mean CAR expression on T cells was 47% at pre-treatment, 41% at 6 months and 57% at 12 months, whereas in the ibrutinib-treated cohort, the corresponding values were 47%, 42% and 64%, respectively.

Immunophenotypic features



Immunophenotypic analysis revealed a preferential enrichment of CD4$^+$ T lymphocytes in the final CAR T-cell product compared to the starting T-cell source - a trend observed consistently across all timepoints and treatment groups. Interestingly, CAR T cells generated after 12 months of treatment with venetoclax demonstrated a more favorable distribution of the differentiation subsets - with a predominance of less mature subpopulations in both the CD4+ and CD8+ - compared to the pre-treatment timepoint.



In terms of immune checkpoints expression, after 6 months of treatment with venetoclax we observed a significant decrease in the percentage of TIM-3$^+$ CD8$^+$ T cells in the pre-manufacturing T cells. Despite this positive immunomodulatory effect, TIM-3 expression was upregulated during the generation process, across both CD4$^+$ and CD8$^+$ CAR T cells. During treatment with targeted agents, we also observed a decreasing trend in PD-1 expression in CD4+ T cells, which was detectable - though not significantly - in both the initial T-cell source and in the final CAR-T cell products. CAR-T cells produced after 12 months of treatment with venetoclax showed a reduced expression of intracellular CTLA-4 compared to pre-treatment levels.

In terms of functionality, CAR-T cells were able to proliferate and effectively induce target cell lysis when co-cultured with a CD19$^+$ tumor target (i.e. MEC1 cell line). Despite the more favorable profile in terms of exhaustion markers expression observed after 6 and even more after 12 months of treatment with venetoclax no significant differences were observed in the killing or proliferative ability of CAR-T cells compared to pre-treatment levels.