2022 Prolonged treatment with ibrutinib modulates phenotypic and functional features of immune cell compartments in chronic lymphocytic leukemia

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BACKGROUND

CLL is characterized by a wide range of immune alterations, responsible for the increased susceptibility to infections, the occurrence of autoimmunity and the failure to control disease progression. Besides its direct anti-tumor activity, the BTK inhibitor ibrutinib has shown to exert immunomodulatory effects. The aim of this study was to analyze immune changes occurring in CLL patients treated with ibrutinib.

METHODS

Thirty-one patients with progressive CLL and the indication to start ibrutinib therapy were included. Peripheral blood samples were collected before and after 1, 6, 12 and 24 months of ibrutinib treatment. Fifteen additional patients with early-phase disease (ES-CLL) and 14 healthy donors (HD) were analyzed. Immune cell counts, the expression of surface markers and functional assays were performed by flow cytometry. Clinical response was assessed according to the IWCLL guidelines.

Number of patients	**31**
Age at ibrutinib start, median (range)	71 (42-80)
Males, number (%)	19 (61%)
Lymphocyte count at ibrutinib start, x10^9/L, median (range)	40.850 (1.430-292.650)
IGHV unmutated, number (%)[1]	24 (80%)
Deletion 17p, number (%)[1]	13 (43%)
TP53 mutated, number (%)	8 (26%)
Treatment-naive patients, number (%)	7 (23%)
Number of prior therapies, median (range)[2]	2 (1-7)
Pantients treated with ibrutinib plus rituximab, number (%)[2]	4 (8%)
PR or CR at 12 months of treatment with ibrutinib, number (%)	23 (74%)

[1]data available in 30 patients [2]data available in 25 patients

RESULTS

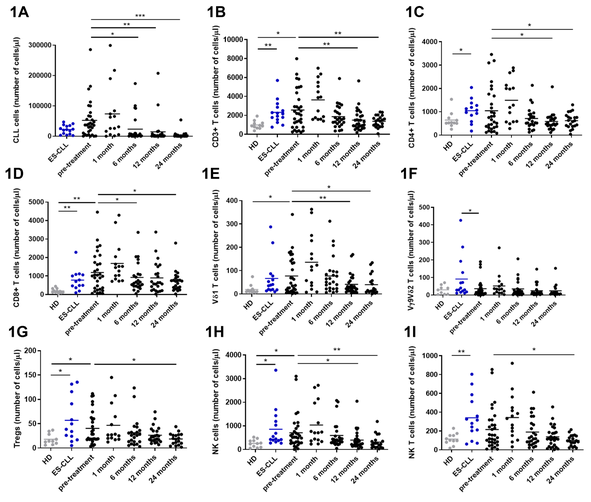

Figure 1. Ibrutinib treatment induces the normalization of the count of different immune cell populations. At the 6-month timepoint, we observed a significantly lower value in the number of CLL cells, which persisted after 12 and 24 months of ibrutinib treatment (**1A**). We detected a significant reduction of CD3+ and CD4+ T-cell counts at month 12, and a decrease of CD8+ T-cell number already at month 6 of therapy (**1B-D**). Regarding γδ T-cell compartment, the count of Vδ1 T cells – but not Vγ9Vδ2 T cells – significantly decreased by 12 months of treatment (**1E,F**). A prolonged (i.e. 24-month) therapy with ibrutinib also favored the normalization of Tregs, NK and NK T-cell counts (**1G-I**).

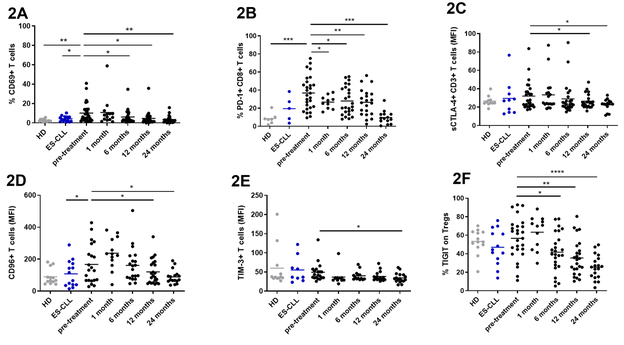

Figure 2. Ibrutinib therapy mitigates features of T-cell exhaustion. Before treatment, we found a higher expression of the early activation marker CD69 on T cells from pre-treatment compared to ES-CLL and HD, which normalized after 6 months of therapy (**2A**). Phenotypic analysis revealed that PD-1 expression on CD8+ T cells was significantly lowered by 6 and 12 months of ibrutinib treatment (**2B**). Ibrutinib also induced a significant downmodulation of other inhibitory checkpoints expressed on T cells: CTLA-4, CD96 and Tim-3 (**2C-E**). Of note, TIGIT expression was reduced on Tregs at 12 months of therapy (**2F**). Similar to T cells, CD69, CD96, TIGIT and Tim-3 were significantly decreased overtime by ibrutinib therapy on NK and NK T cells (not shown).

Correlation with clinical response to therapy. The correlation of phenotypic changes with clinical response at 12 months demonstrated that the positive immunomodulatory effects of ibrutinib were mainly observed in patients achieving a partial (n=21) or complete (n=2) response and not in patients with a stable disease (n=8).

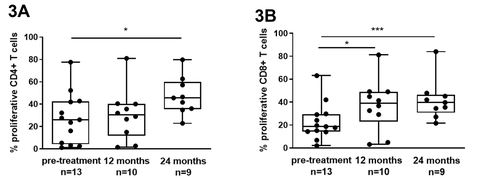

Figure 3. Ibrutinib treatment improves T-cell proliferation ability. An improvement in the % of proliferative cells in response to polyclonal stimuli of both CD4+ (**3A**) and CD8+ (**3B**) T cells was detected at month 24.

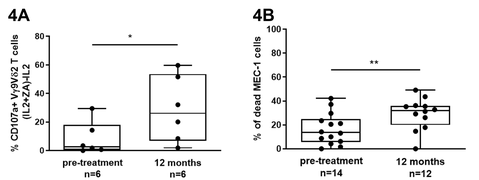

Figure 4. Functional assays. Our functional data showed that, besides a positive phenotypic modulation, 12 months of ibrutinib treatment enhanced Vγ9Vδ2 T-cell cytotoxicity following the stilmulation with zoledronic acid and IL-2 (**4A**) and NK-cell-mediated ADCC of obinutuzumab (10 µg/ml) towards MEC-1 cell line (**4B**).

CONCLUSIONS

Our results show that ibrutinib exerts a wide range of immune-modulating effects, which are mainly associated with the achievement of a clinical response to therapy.