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HPK-1 Inhibition Enhances Efficacy of Bispecific Antibodies in Chronic Lymphocytic Leukemia (CLL)

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Background

- Chronic lymphocytic leukemia (CLL) is marked by T-cell dysfunction, which results in immune suppression and inadequate antitumor immune responses.
- Hematopoietic progenitor kinase-1 (HPK-1) acts as a negative regulator of T-cell activity, and its inhibition has shown promise in improving T-cell functionality.
- While cellular therapies have proven effective in various lymphomas, initial CLL studies have been less successful, largely due to the disease's immunosuppressive characteristics.

Aims

This study aimed to explore the effectiveness of HPK-1 inhibition combined with CD20XCD3 bispecific antibodies as a treatment strategy for CLL

Methods

- Peripheral blood mononuclear cells (PBMCs) from CLL patients were treated with selective HPK-1 inhibitors, such as HY-138568 or BGB15025, and subsequently activated with anti-CD3/CD28 antibodies. Flow cytometry was used to assess the expression of activation markers, CD69 and CD25.
- The secretion of interferon-gamma (IFN-γ) and granzyme B from the treated cells was measured using an ELISA assay.
  The impact of the bispecific antibody glofitamab on T-cell morphology, immune synapse formation, and granzyme B induction was analyzed through confocal microscopy.
- Cytotoxic effects on CLL cells were evaluated using the Incucyte assay. In this assay, PBMCs were negatively selected to isolate B cells (CLL cells) and T cells, with B cells labeled using BioTracker CSFE dye. The isolated T cells underwent a 2-hour pretreatment with HPK-1 inhibitors before co-culturing with CLL cells at a 4:1 effector to target (E:T) ratio, using glofitamab or an isotype control, with Cytotox red dye to detect cell death.

Results

- PBMCs from CLL patients treated with HPK-1 inhibitors and activated with anti-CD3/CD28 beads showed a significant increase in CD69 and CD25 surface expression on CD8+ T cells, with no significant change in CD4+ T cells (Fig 1A-C). HPK-1 inhibitor pretreatment led to a notable increase in IFN-γ and granzyme B release in response to CD3/CD28 activation
  (Fig 1D-I).
- Incubation of CLL patient PBMCs with CD20xCD3 bispecific antibodies, including mosunetuzumab, epcoritamab, and glofitamab, resulted in increased IFNγ secretion, particularly with glofitamab.
  This effect was enhanced when combined with HPK-1 inhibitors (Fig 1J,K).
- Glofitamab treatment also induced morphological changes in T-cells, indicating activation and cytotoxicity, characterized by increased CD8+ T-cell size, granzyme B release, and the formation of CLL-CD8+ T-cell clusters, with HPK-1 inhibitors further promoting these clusters (Fig 2A-B).
- The combined treatment of glofitamab and HPK-1 inhibition significantly enhanced granzyme B release and anti-CLL T-cell cytotoxicity, with a considerable number of dead CLL cells observed in the clusters (Fig 2C-F).

Fig 1. HPK1 inhibition augments the activation of T cells in the presence of CD3/CD28 or bispecific antibodies. Peripheral blood CLL cells were treated with the indicated concentration of HY-138568 or BGB15025 for 2 hours. DMSO treated cells served as controls (NT). (A-C). Following treatment, the cells were stimulated with Dynabeads® Human T cells-Activator CD3/CD28 for 24 hours at 37°C. After incubation, the cells were stained with Pacific Blue Anti-Human CD8 and PE Anti-Human CD69 or CD25. Samples were acquired by FACSCanto II (BD) and analyzed using BD FACSDiva software.
A. Flow cytometric dot-plots of CD8-Pacific Blue versus CD69-PE expression on samples of one representative CLL case.
B. Quantification of CD69 mean fluorescence intensity (MFI) in CD8+ population (n=7)
C. Quantification of CD25 MFI in CD8+ population (n=5). (D-I) Following treatment, the cells were cultured in Anti-CD3/CD28-coated plates for 48 hours at 37°C in a humidified 5% CO2 atmosphere. D+G. IFN-γ secretion was measured from supernatants using ELISA-based assay platform. (n=12). E+H. IFN-γ levels in M-CLL samples (n=6). F+I. IFN-γ levels in UM-CLL samples (n=6). (J-K) Following treatment, 10 µg/mL of CD20XCD3 bispecific antibody [Epcoritamab (E) / Glofitamab (G) / Mosunetuzumab (M)] was added for 48 hours. J. IFN-γ secretion was measured from supernatants using ELISA-based assay platform (n=4). K. IFN-γ secretion following treatment with HPK inhibitors and glofitamab (n=7). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

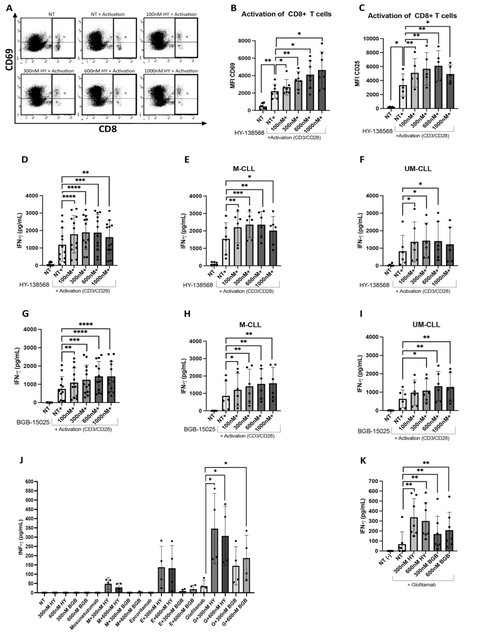
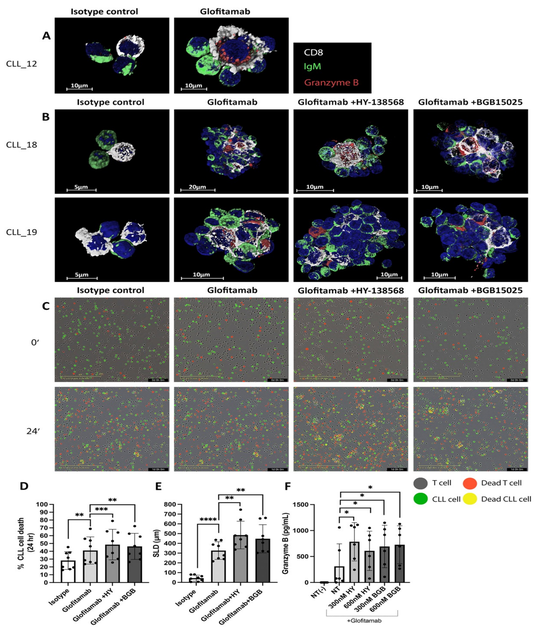


Fig 2. The effect of glofitamab and HPK1 inhibitors on immune synapse formation, granzyme B induction and CLL cell death. (A-B). CLL cells were seeded on poly-L-lysine coated glass, then treated with 300 nM HY-138568 or with 600 nM BGB15025 for 2 hours. Then the cells were incubated with 1µg/mL of the CD20XCD3 bispecific antibody glofitamab or human IgG kappa isotype control for 48 hours. The cells were fixed and permeabilized, followed by staining for CD8 (white), granzyme B (red), IgM (green) and DAPI (blue). The images presented are 3D reconstruction of representative confocal imaging of synapse formation between B (green) and CD8+ T (white) CLL cells. Patients are coded by numbers and displayed as CLL_number. A. Representative images of cells treated with isotype control or glofitamab.
B. Representative images of cells treated with isotype control, glofitamab, glofitamab and HY-138568, or glofitamab and BGB15025 (n=5). (C-E). B and T cells were isolated from CLL patients-derived PBMCs using
B-CLL and pan T isolation kits. Isolated B cells (CLL cells) were labeled with BioTracker 488 Green CSFE dye. Isolated T cells were treated with 300 nM HY-138568 or with 600 nM BGB15025 for 2 hours. Then, CLL cells and autologous T-cells were seeded at 4:1 E:T ratio and incubated with 1µg/mL of the anti-CD20Xanti-CD3 bispecific antibody glofitamab or isotype control for 24 hours. Cytotox red dye was used to detect cell killing. CLL cell killing (% green+ red cells / green cells) was analyzed using Incucyte™ system for indicated times. At least four images from distinct regions within each well were taken at intervals of six hours. The experiment was performed in triplicates. C. Representative images from 2 time points using cells from one patient. D. Quantification of % CLL cell killing using glofitamab (+/- HPK1 inhibitors) or isotype control after 24 hours of incubation (n=8).
E. Sum of longest diameter (SLD, in µm) of clusters after 24 hours of incubation.
F. CLL cells were treated with the indicated concentration of HY-138568 or BGB15025 for 2 hours. Following treatment, the cells were incubated with 1 µg/mL of the anti-CD20Xanti-CD3 bispecific antibody glofitamab or isotype control for 48 hours. Granzyme B levels were measured from supernatants using ELISA-based assay platform. (n=6). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.



Conclusions

- This study highlights the promising potential of integrating HPK-1 inhibitors with bispecific antibodies to boost antitumor T-cell cytotoxicity in patient-derived CLL samples.
- Our findings may pave the way for the development of innovative combination therapies that utilize HPK-1 inhibition to enhance cellular therapies in CLL.