Effects of Pirtobrutinib, a non-covalent BTK inhibitor, on T-cell function in chronic lymphocytic leukemia (CLL)

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INTRODUCTION / OBJECTIVES

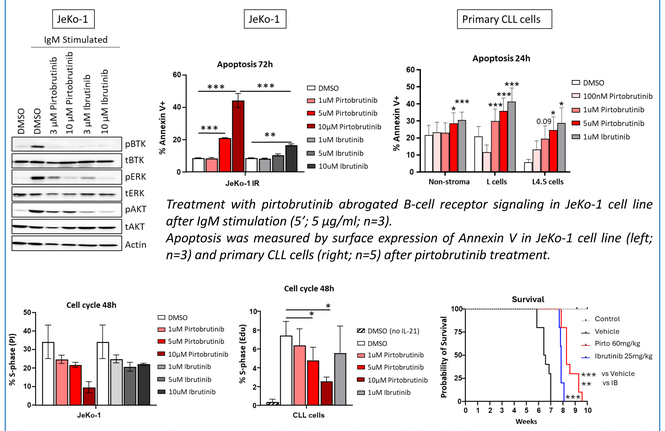
- Covalent Bruton tyrosine kinase inhibitors (cBTKi), transformed the paradigm in CLL and MCL treatment.
- cBTKi e.g. ibrutinib, have direct anti-neoplastic effects and immunomodulatory effects, such as changes in T-cell diversity, reduction in T-cell exhaustion markers and a more pro-inflammatory Th1 polarization[1-4]. Unfortunately, resistance occurs, mostly due to mutations in the C481 residue[5].
- Pirtobrutinib is a high selective, reversible, non-covalent BTKi, that interacts with BTK and water molecules in the ATP-binding region (but not with C481 residue)[6].
- Pirtobrutinib is FDA approved for treatment of CLL patients after 2 lines of therapy which must include a BTKi and a BCL2 inhibitor and for treatment of MCL patients after 2 lines of therapy which must include a BTKi.

OBJECTIVES

1) *To study the direct effects of pirtobrutinib in CLL/MCL.*
2) *To study the effects of pirtobrutinib on T-cell functionality*

RESULTS

PIRTOBRUTINIB INDUCES APOPTOSIS of MCL and CLL cells and IMPROVES SURVIVAL OF MCL PDX MICE

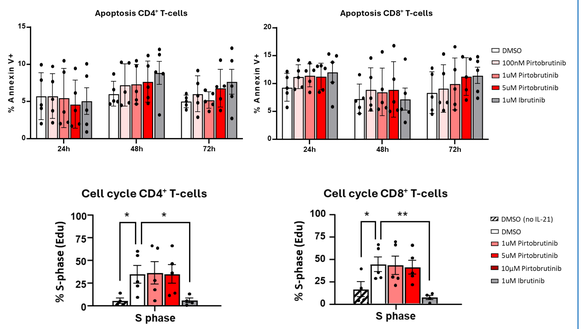


Treatment with pirtobrutinib abrogated B-cell receptor signaling in JeKo-1 cell line after IgM stimulation (5'; 5 μg/ml; n=3).
Apoptosis was measured by surface expression of Annexin V in JeKo-1 cell line (left; n=3) and primary CLL cells (right; n=5) after pirtobrutinib treatment.

S-phase was measured by flow cytometry as PI staining (JeKo-1; n=2-3) or EdU incorporation (primary CLL cells; n=7). 3x10⁶ MCL PDX cells were xenografted into NSG mice, pirtobrutinib or ibrutinib treatment started 3 weeks post-transplant (n=5-10).

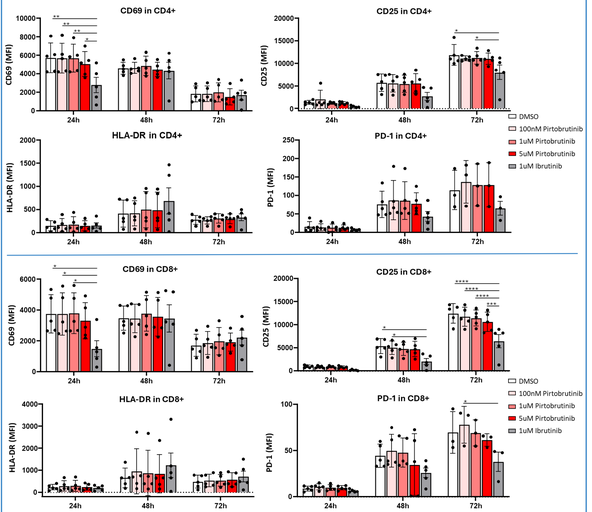
RESULTS

PIRTOBRUTINIB DOES NOT IMPAIR T CELL SURVIVAL OR CELL CYCLE PROGRESSION



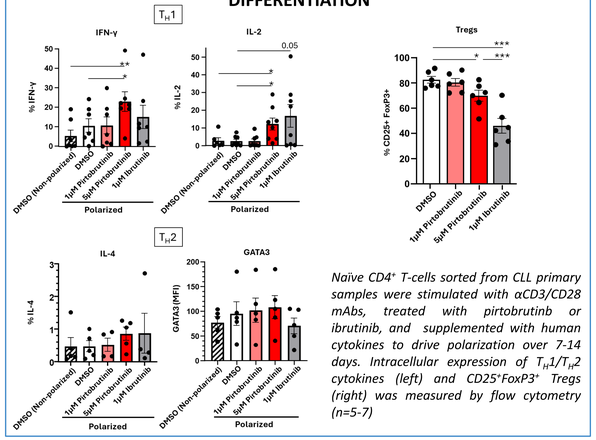
T-cells were isolated from primary CLL samples, stimulated with αCD3/CD28 mAbs and treated with pirtobrutinib or ibrutinib. Top: apoptosis was measured by surface expression of Annexin V over 72h. Bottom: S-phase was measured by flow cytometry as EdU incorporation at 48h (n=4-5).

EFFECTS OF PIRTOBRUTINIB ON T-CELL ACTIVATION



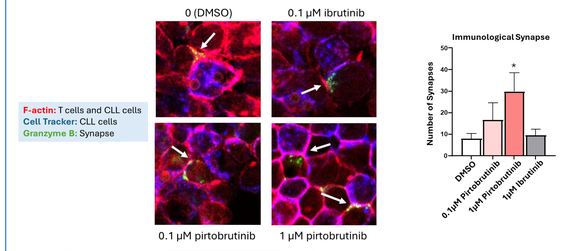
T-cells were isolated from primary CLL samples, stimulated with αCD3/CD28 mAbs and treated with pirtobrutinib or ibrutinib over 72h. Different surface activation markers (CD69, CD25, HLA-DR and PD-1) were measured by flow cytometry in CD4⁺ (top) and CD8⁺ (bottom) human T-cells (n=3-5).

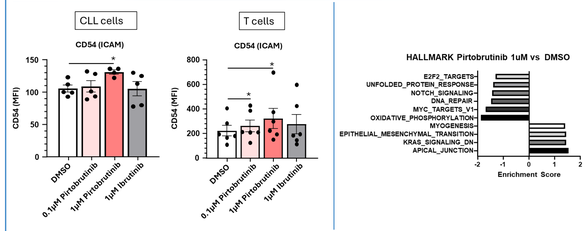
PIRTOBRUTINIB PROMOTES T_H1 POLARIZATION AND REDUCES T_{REG} DIFFERENTIATION



Naïve CD4⁺ T-cells sorted from CLL primary samples were stimulated with αCD3/CD28 mAbs, treated with pirtobrutinb or ibrutinib, and supplemented with human cytokines to drive polarization over 7-14 days. Intracellular expression of T_H1/T_H2 cytokines (left) and CD25⁺FoxP3⁺ Tregs (right) was measured by flow cytometry (n=5-7)

PIRTOBRUTINIB ENHANCES IMMUNOLOGICAL SYNAPSE

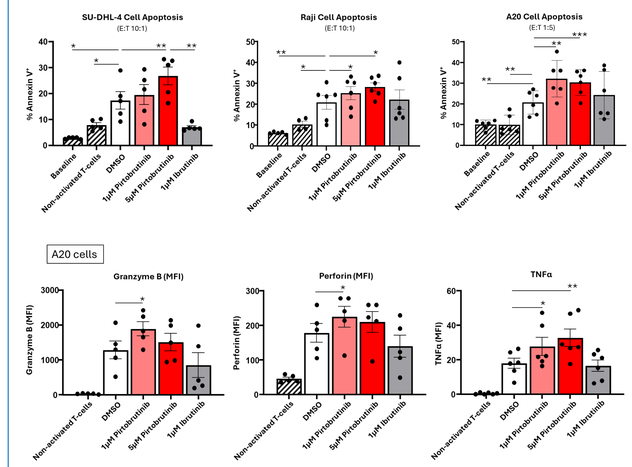




Autologous CLL cells (stimulated with CD40L from stroma cells) and T cells (stimulated with αCD3/CD28 mAbs) from primary CLL samples were treated separately with pirtobrutinib or ibrutinib for 24 hours. Cells were then co-cultured at a ratio of 1:1 for 10 minutes to detect synapses. Top: Immunofluorescent confocal images with synapses marked with an arrow. Bottom left: Surface expression of CD54 was measured by flow cytometry in CLL cells (left) and T cells (right). (n=5-6)

RESULTS

PIRTOBRUTINIB ENHANCES T CELL CYTOTOXICITY



T cells from primary CLL samples were stimulated with αCD3/CD28 mAbs and treated with pirtobrutinib or ibrutinib for 24 hours. B cell lines (SU-DHL-4, Raji or A20) were labeled with CFSE and added at the indicated ratios for an additional 48h. Top: Apoptosis was measured by surface expression of Annexin V. Bottom: Intracellular expression of Granzyme B, Perforin and TNFα was measured by flow cytometry in A20 cells (n=5-6)

SUMMARY / CONCLUSION

Pirtobrutinib exerted anti-tumor and immunomodulatory effects in vitro and in vivo:

- Induced apoptosis and cell cycle arrest in MCL cell lines and in primary CLL cells
- Promoted Th1 polarization and reduced Treg differentiation, partially rescuing the CLL immunosuppressive phenotype
- Facilitated immunological synapse, correlating with upregulation of ICAM-1
- Enhanced T-cell cytotoxicity against B-cell lymphoma cell lines

REFERENCES / ACKNOWLEDGEMENTS

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*In all figures Bars represent mean ± SEM. *p<0.05, *p<0.01, ***p<0.005*

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