NX-2127 and NX-5948, two clinical stage cereblon-recruiting BTK degraders, facilitate T-cell functionality in CLL

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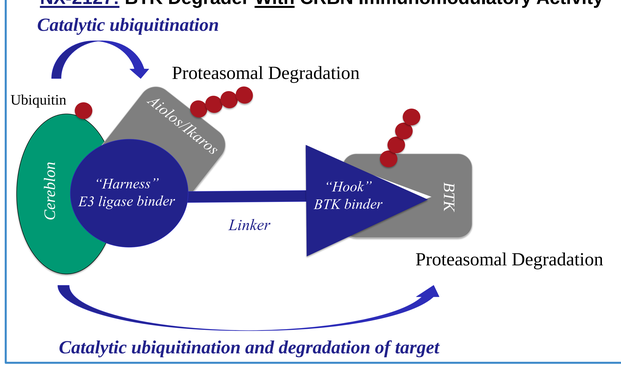
Introduction & Objectives

Chronic lymphocytic leukemia (CLL) is the most common leukemia found in adults within Western countries, with approximately five cases per 100,000 people occurring each year.[1,2]

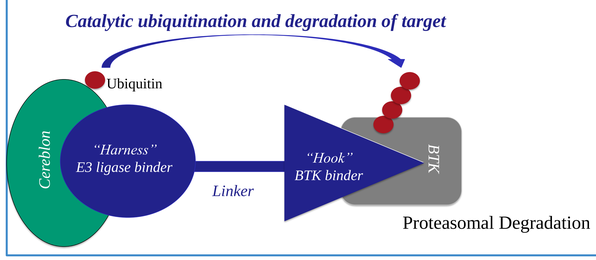
Inhibitors targeting Bruton's tyrosine kinase (BTK) have altered the treatment paradigm of CLL. In addition to their direct anti-neoplastic effects, the BTK inhibitors ibrutinib and acalabrutinib were found to favorably modulate T cell function in both pre-clinical and clinical studies of CLL. Specifically, treatment with ibrutinib downmodulated expression of T cell exhaustion markers, shifted T cell polarization towards a pro-inflammatory T_H1 phenotype, and improved overall T cell number and repertoire diversity.[3,4,5] However, resistance to BTK inhibitors eventually occurs due to the development of mutations within BTK.

Targeted Protein Degradation (TPD) has emerged as a strategy to circumvent this acquired resistance to BTK inhibition. The compounds NX-2127 and NX-5948 were designed to induce BTK degradation by recruiting the E3 ubiquitin ligase, cereblon (CRBN), which was originally recognized for its modulation of the immune system. However, only NX-2127, and not NX-5948, was designed to maintain CRBN immunomodulatory function.

NX-2127: BTK Degrader With CRBN Immunomodulatory Activity



NX-5948: BTK Degrader Without CRBN Immunomodulatory Activity



Objectives

1) Determine the effects of NX-2127 and NX-5948 on T-cell functionality.
2) Determine to what extent CRBN modulation (NX-2127) contributes to pre-clinical (and ultimately clinical) activity of BTK degraders.

Acknowledgements

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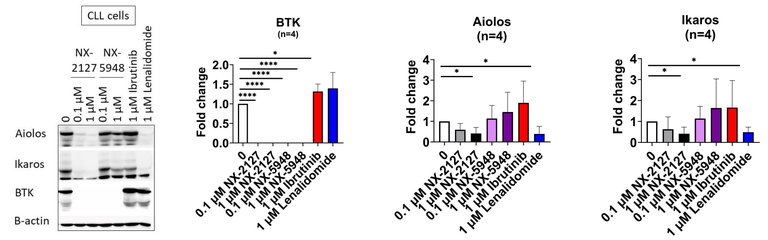
Results

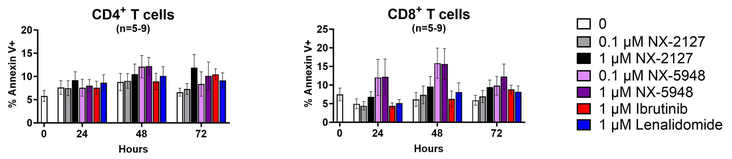
FIGURE 1. DEGRADATION OF BTK, AIOLOS, AND IKAROS, IN PRIMARY CLL CELLS



- NX-2127 and NX-5948 degrade BTK in primary CLL cells.
- NX-2127, but not NX-5948, induces dose-dependent degradation of Aiolos and Ikaros (known CRBN substrates).
- This confirms that only NX-2127 possesses CRBN immunomodulatory activity.

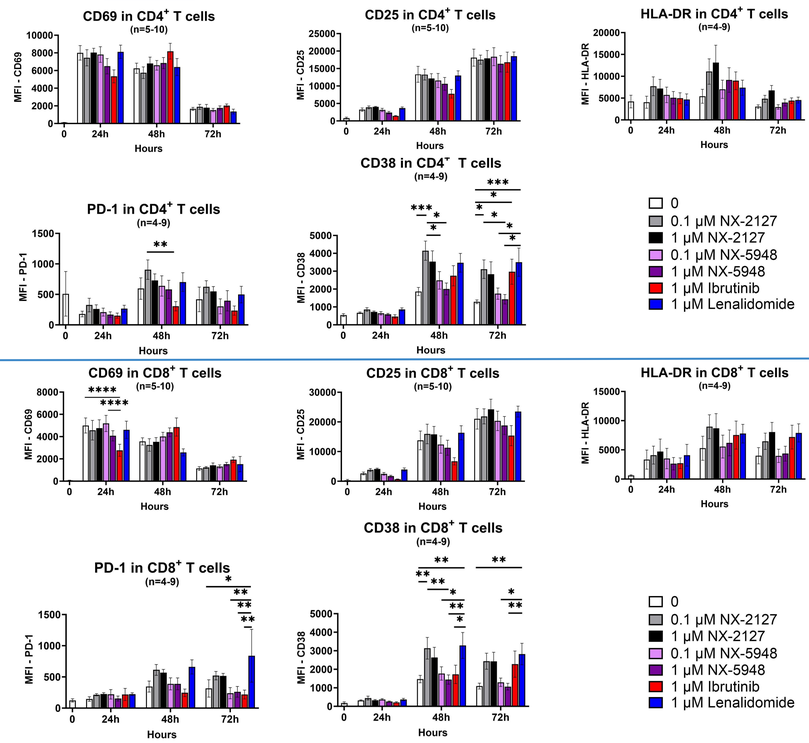
Primary CLL cells were treated with NX-2127 or NX-5948 for 24 hours. Bars represent mean ± SEM.

FIGURE 2. NX-2127 AND NX-5948 DO NOT INDUCE APOPTOSIS NOR REDUCE CELL PROLIFERATION IN HUMAN T CELLS



Surface expression by flow cytometry of Annexin V in $CD4^+$ (left) and $CD8^+$ (right). T cells isolated from primary CLL samples, stimulated with αCD3/CD28 mAbs, and treated with NX-2127 or NX-5948 for 72 hours. Bars represent mean ± SEM.

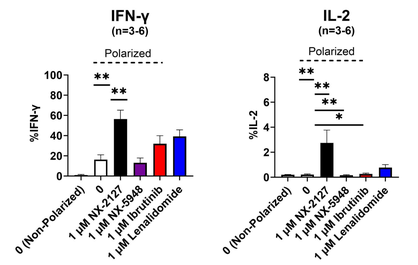
FIGURE 3. THE EFFECTS OF BTK DEGRADERS ON T CELL ACTIVATION



- NX-2127 and NX-5948 do not affect the early (CD69) or late (CD25, HLA-DR, and PD-1) activation markers in both $CD4^+$ and $CD8^+$ human T cells.
- NX-2127, but not NX-5948, increases the activation marker CD38 within $CD4^+$ and $CD8^+$ human T cells.

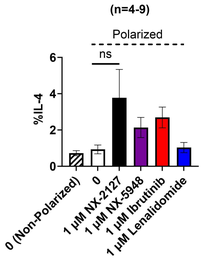
Surface expression by flow cytometry of various activation markers in $CD4^+$ (top) and $CD8^+$ (bottom). T cells isolated from primary CLL samples, stimulated with αCD3/CD28 mAbs, and treated with NX-2127 or NX-5948 for 72 hours. Bars represent mean ± SEM.

FIGURE 4. NX-2127, BUT NOT NX-5948, SIGNIFICANTLY UPREGULATES BOTH IFN-γ AND IL-2 UNDER T_H1-POLARIZING CONDITIONS



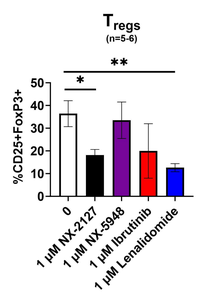
Intracellular expression by flow cytometry of IFN-γ and IL-2 in naïve $CD4^+$ T cells that were FACS-sorted from primary CLL samples. Naïve $CD4^+$ T cells were stimulated with αCD3/CD28 mAbs, treated with NX-2127 or NX-5948, and supplemented with human cytokines to drive T_H1 polarization over 7 days. Bars represent mean ± SEM.

FIGURE 5. NX-2127 AND NX-5948 DO NOT ALTER T CELL DIFFERENTIATION UNDER T_H2-POLARIZING CONDITIONS AS INDICATED BY UNCHANGED IL-4 EXPRESSION



Intracellular expression by flow cytometry of IL-4 in naïve $CD4^+$ T cells that were FACS-sorted from primary CLL samples. Naïve $CD4^+$ T cells were stimulated with αCD3/CD28 mAbs, treated with NX-2127 or NX-5948, and supplemented with human cytokines to drive T_H2 polarization over 14 days. Bars represent mean ± SEM.

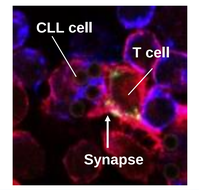
FIGURE 6. NX-2127, BUT NOT NX-5948, SIGNIFICANTLY REDUCES THE DIFFERENTIATION OF TREGS



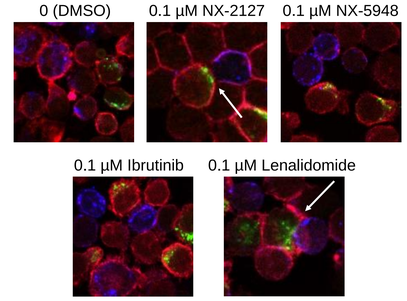
Intracellular expression by flow cytometry of $CD25^+FoxP3^+$ Tregs from naïve $CD4^+$ T-cells that were FACS-sorted from primary CLL samples. Naïve $CD4^+$ T cells were stimulated with αCD3/CD28 mAbs, treated with NX-2127 or NX-5948, and supplemented with human cytokines to drive Treg differentiation over 7 days. Bars represent mean ± SEM..

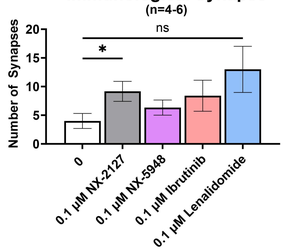
FIGURE 7. NX-2127, BUT NOT NX-5948, ENHANCED SYNAPSE FORMATION TO LEVELS COMPARABLE TO THOSE OF LENALIDOMIDE

Representative Synapse Photo



F-actin: T cells and CLL cells
Cell Tracker: CLL cells
Granzyme B: Synapse





Autologous T cells and CLL cells from primary CLL samples were treated with NX-2127 or NX-5948 for 24 hours. T cells were simultaneously stimulated with αCD3/CD28 mAbs, while CLL cells were activated with CD40L-conditioned media. After 24 hours, T cells and CLL cells were co-cultured at a ratio of 1:1 for 10 minutes and stained with the above antibodies.

Top: Representative images of immunological synapses formed between T cells and CLL cells obtained through immunofluorescent confocal microscopy. Synapses (depicted by granzyme B) are denoted by a white arrow.

Bottom: Quantification of the number of immunological synapses formed between T cells and CLL cells. Bars represent mean ± SEM.

Conclusion

CRBN-recruiting BTK degraders NX-2127 and NX-5948:

- Induce degradation of BTK in primary CLL cells
- Do not interfere with T-cell activation and survival *in vitro*

NX-2127-mediated immunomodulatory activity:

- Upregulates CD38 (an IFN response gene)
- Promotes T-cell differentiation towards a T_H1 phenotype
- Downregulates Treg differentiation
- Facilitates immunological synapse formation

Our findings offer strong rationale for continued investigation of these BTK degraders in CLL and lymphoid malignancies.

Questions? Please email Tiana Huynh at tihuynh@coh.org