Targeting the PIM kinases in combination with BTK inhibition is synergistic in preclinical models of B-cell malignancies

I. Abstract

BTK inhibitors (e.g., ibrutinib) have significantly impacted the treatment of B-cell malignancies in a positive way. Single agent response rates with ibrutinib are 65% or higher in B-cell lymphomas and chronic lymphocytic leukemia with the majority of patients enjoying a prolonged duration of response. Continued clinical development is needed, however, as most patients achieve only a partial response from their treatment and ultimately patients become refractory to ibrutinib leading to disease progression. Targeted combinations with ibrutinib could potentially increase the number of patients undergoing complete remission and combat emergent resistant mechanisms. The PIM family (1, 2, and 3) are serine/threonine kinases that have proven to be oncogenic in-part due to their ability to suppress c-Myc induced apoptosis. The PIM kinases have emerged as important regulators of drug resistance in multiple cancer types. Tolero Pharmaceuticals's second generation PIM Kinase inhibitor, TP-3654 has exhibited favorable activity in preclinical models of prostate cancer, AML, and lymphoma. Due to the signaling crosstalk between BTK and PIM through the STAT transcription factors, we hypothesized that synergies may arise through the simultaneous targeting of both kinases. Here, we report a significant increase in drug activity when a BTK inhibitor (ibrutinib) was combined with TP-3654 in various lymphoma cell lines. In Granta-519 cells, the IC50 of ibrutinib decreased 3.5-fold, from 0.7 mM to 0.2 mM, when cultured in combination with a subtoxic concentration of TP-3654 (100 nM). Similarly, the IC50 of TP-3654 decreased 6-fold, from 2.4 mM to 0.4 mM, when cells were cultured in combination with a subtoxic concentration of ibrutinib (100 nM). BTK is known to attenuate the activity of the transcription factor STAT3, a major regulator of PIM kinase levels in cells. Due to this, mechanistic studies focused on analyzing the STAT3 pathway are ongoing to determine the downstream effects of ibrutinib and TP-3654 in combination. Several lymphoma xenograft studies are also ongoing to further this combination in vivo. These results provide a strong rationale that inhibitors of PIM and BTK could be used in combination for the treatment of B-cell malignancies and other B-cell mediated diseases.

II. Background

Figure 1: BTK and PIM are modulated through STAT signaling

Figure 2: TP-3654 shows activity against multiple cell types and lines

III. Results

Figure 3: TP-3654 is active against all 3 isoforms of PIM, especially 1 and 3

Figure 4: TP-3654 is active against P38, as evidenced by decreased p-AKT levels

Figure 5: TP-3654 causes decreased expression in c-Myc as evidenced by western blot and RT-PCR data

IV. Conclusions

• We have developed a potent and specific PIM inhibitor, TP-3654
  • TP-3654 shows strongest potency against PI3K isoforms, PIM-1 and PIM-3
  • TP-3654 is also active against P38
  • TP-3654 treatment decreases expression of c-Myc
  • TP-3654 has single agent activity against multiple B-cell malignancies
  • Shifting IC50 curves suggests synergism between TP-3654 and ibrutinib
  • We observe a dose dependent increase in relative caspase activity with combination therapy
  • Our data suggest that STAT3 signaling, is inhibited by the TP-3654/ibrutinib combination treatment
  • PIM expression is also increased following combination treatment, consistent with the notion of PIM expression as a resistance mechanism to BTK inhibition
  • Levels of p-IRF4 decrease with dual BTK PIM inhibition, but increase with PIM inhibition, suggesting a potential reciprocal PIM resistance mechanism

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