I. Abstract

Background: With 31,000 new cases expected in 2018 (US), and a 50% five-year overall survival rate, there is a significant unmet need in the treatment of patients with multiple myeloma (MM). The proteasomal inhibitor bortezomib is approved for the treatment of patients with multiple myeloma. Bortezomib inhibits the degradation of many proteins, including the pro-apoptotic protein NOXA. However, low basal levels of NOXA and/or high levels of the anti-apoptotic protein MCL-1 have been implicated in bortezomib resistance and negative patient outcomes. NOXA functions to sequester MCL-1 and prevent its interaction with the apoptosis-inducing proteins, BAK or BAX. The BCL-2 inhibitor, venetoclax, has also been investigated in clinical trials for the treatment of multiple myeloma. Increased MCL-1 expression has been shown to be key in the resistance to venetoclax (ABT-199). Considering the central role of MCL-1 to survival and treatment efficacy in myeloma, we investigated the ability of an MCL-1-factoring agent, namely the IDEC-Kinetics' TP-1287, to suppress tumor growth in non-clinical models of multiple myeloma.

Methods: Cell lines and human cell lines were treated with the complexed proteasome inhibitor, bortezomib, to evaluate the in vitro anti-tumor activity of TP-1287, bortezomib, and venetoclax. We utilized real-time PCR to measure gene expression changes in treated cells. We also measured protein expression changes following treatment, using standard gel electrophoresis and immunoblotting techniques. In order to assess the anti-tumor activity of these compounds in vivo, we initiated xenograft studies in the RPMI-8226 model for multiple myeloma.

Results: In cell viability assays, we observed IC50s ranging from 0.1 µM to 3000 nM with alvocidib or venetoclax treatments. The addition of up to 100 nM venetoclax resulted in a 2.8-fold reduction in the IC50 of alvocidib in the cultured OPM-2 cell line. Venetoclax potency was potentiated with the addition of alvocidib, resulting in a more than 500-fold decrease in the IC50 in the Bortezomib resistant OPA-2 cells. The cleared form of TP-1287, or alvocidib, was able to reduce MCL-1 protein and mRNA expression in multiple myeloma cell lines, to a time-dependent fashion. In this RPMI-8226 xenograft model for multiple myeloma, TP-1287 treatment frequency and dose level were evaluated, with administration of doses up to 7 µM. As a single agent, TP-1287 achieved tumor growth inhibition (TGI) of 50.1%, 76.6%, and 93.9% at doses of 0.5, 1.7, and 5.5 mg/kg, respectively. Additional in vivo antitumor efficacy was very limited, and TGI of 0.4% was observed at 0.5 mg/kg. To investigate the efficacy of alvocidib and venetoclax in the context of bortezomib resistance, where low NOXA might contribute to enhanced cell survival via MCL-1, we observed.

Conclusions: Taken together, our data suggest that the combination of alvocidib with venetoclax may constitute a novel therapeutic regimen in the treatment of MM. Further, it suggests that CDK9-mediated targeting of MCL-1 may offer a route to addressing intrinsic resistance in multiple myeloma patients.

II. Background

Figure 1: Proposed synergy of alvocidib and ABT-199 in multiple myeloma

- The proteasome inhibitor, bortezomib, induces cell death in part by increasing NOXA expression
- NOXA inhibits MCL-1, suppresses expression of MCL-1
- ABT-199 blocks MCL-1-mediated cell survival
- Alvocidib and ABT-199 may combine well in multiple myeloma, especially in tumors previously treated with bortezomib

III. Results

Figure 2: Alvocidib suppresses MCL-1 protein expression in multiple myeloma cells

A: OPM-2 cells treated with alvocidib alone, showing reduced expression of MCL-1.B: OPM-2 cells treated with alvocidib and venetoclax showing increased expression of NOXA and reduced expression of MCL-1.

Figure 3: MCL-1 suppression drives alvocidib activity in multiple myeloma cells

A: OPM-2 cells treated with alvocidib alone, showing reduced expression of MCL-1.B: OPM-2 cells treated with alvocidib and venetoclax showing increased expression of NOXA and reduced expression of MCL-1.

Figure 4: Bortezomib induces cell death in multiple myeloma via NOXA accumulation

A: OPM-2 cells treated with bortezomib alone, showing increased expression of NOXA.B: OPM-2 cells treated with bortezomib and alvocidib, showing increased expression of NOXA and reduced expression of MCL-1.

Figure 5: Increased BCL-2 expression in bortezomib resistant myeloma cells

A: OPM-2 cells treated with bortezomib alone, showing increased expression of BCL-2.B: OPM-2 cells treated with bortezomib and alvocidib, showing increased expression of NOXA and reduced expression of MCL-1.

Figure 6: Induction of apoptosis with combination treatment

A: OPM-2 cells treated with alvocidib and ABT-199 synergize to induce apoptosis in multiple myeloma cells.B: OPM-2 cells treated with bortezomib and alvocidib synergize to induce apoptosis in multiple myeloma cells.

Figure 7: Induction of apoptosis with combination treatment

A: OPM-2 cells treated with alvocidib and ABT-199 synergize to induce apoptosis in multiple myeloma cells.B: OPM-2 cells treated with bortezomib and alvocidib synergize to induce apoptosis in multiple myeloma cells.

IV. Conclusions

- TP-1287 is a potent, orally administered CDK9 inhibitor
- Alvocidib and TP-1287 suppress the mTRA and protein expression of a key anti-apoptotic protein, MCL-1, in multiple myeloma
- Suppression of MCL-1 coincides with induction of apoptosis in myeloma cells
- Bortezomb resistance in multiple myeloma can be achieved via several routes, namely NOXA suppression and BCL-2 induction
- Alvocidib and ABT-199 synergize to reduce cell viability in bortezomib-resistant OPA-2 cells, reduce apoptosis in MM-12 or HS929 cells, and modulate anti-apoptotic protein expression in OPA-2 cells
- TP-1287 is active in the RPMI-8226 model for multiple myeloma
- Alvocidib and ABT199 synergize to reduce tumor growth in the RPMI-2 model for multiple myeloma

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