The CDK9 inhibitor, alvocidib, potentiates the non-clinical activity of azacytidine or decitabine in an MCL-1-dependent fashion, supporting clinical exploration of a decitabine and alvocidib combination

I. Abstract

The hypomethylating agents (HMAs) azacytidine and decitabine exert biological activity via two distinct mechanisms, namely DNA demethylation and inhibition of DNA methyltransferase. Azacytidine and decitabine are indicated in the treatment of patients with myelodysplastic syndromes (MDS). As a result of DNA methyltransferase inhibition, it is hypothesized that HMAs may function by inducing expression of key pro-apoptotic proteins such as NOXA, which sequences with the anti-apoptotic protein MCL-1, potentiating apoptotic induction with the mitochondrial pore-forming proteins Bak/ BamI. Activity of the potent CDK9 inhibitor, alvocidib, is largely driven by targeting of CDK9-dependent MCL-1 expression. Alvocidib is under active clinical investigations, but has also demonstrated high complete response rates in newly diagnosed AML patients, particularly when administered as part of a cytarabine and mitoxantrone containing regimen (ACM regimen). Given the dual NOXA/MCL-1 targeting ability of combining alvocidib and azacytidine or decitabine, the combination may synergize therapeutically in the treatment of non-clinical models of AML, or MDS by means of transcriptional upregulation of NOXA and expression of MCL-1 expression.

Cell viability and induction of apoptosis was assessed following treatment with alvocidib, azacytidine, and decitabine in cells using the CellTiter-Glo and CytoTox-Glo assays. Genotoxic changes following treatment were assessed using quantification of FACS. Protein expression changes with treatment were also measured using standard immunostaining technique. To assess the in vivo anti-tumor activity of these compounds, xenograft studies in the MOLM13 and additional models of MDS, exploring sequencing and scheduling of azacytidine administration with HMAs, were performed. Treatment of AML cell lines with alvocidib inhibited both mRNA and protein expression of MCL-1 in a time and concentration-dependent fashion. Pre-treatment of cells with alvocidib, to repress MCL-1 expression prior to azacytidine treatment, reduced the azacytidine cell viability 4-5 fold, from 1.4 μM to 0.25 μM in MOLM-11 cells. The alvocidib/azacytidine combination also resulted in synergistic increases in caspase activity relative to either single agent within the combination, at multiple dose levels. The combination of azacytidine or decitabine with alvocidib was active in the MOLM13 xenograft model, yielding to 65.7% and 61.5% tumor growth inhibition (TGI) in the azacytidine or decitabine combination, respectively. Taken together, in vitro and in vivo studies indicated that decitabine was more effective at re-expressing NOXA and potentiating alvocidib activity compared to azacytidine.

These non-clinical data suggest that an alvocidib/HMA combination may constitute a viable therapeutic regimen whose rationale focuses on targeting of NOXA/MCL-1. Based on these non-clinical results, a Phase 1/2b clinical study of alvocidib administered in sequence following treatment in patients with intermediate to high risk MDS is being conducted (Zella 102). Patients will be enrolled in cohorts of 1-6 patients with decitabine administration as a 1-hour i.v. infusion daily on day 1 to a dose of 30 mg/m2/day followed by a single alvocidib treatment on day 8 as a loading dose over 45 minutes followed by a 4-hour infusion. Treatment will be repeated every 28 days until disease progression or unacceptable toxicity. Enrollment will include MDS patients (Phase 1b) with previously untreated MDS and patients who received fewer than 3 lines of prior therapies, as well as (Phase 2) untreated patients with de novo or secondary MDS. The primary objective is to determine the maximum tolerated dose and recommended Phase 2 dose of alvocidib administered in sequence with decitabine. Key Phase 2 endpoints will include complete response rate and improvement in transfusion dependency.

II. Background

Figure 1: Proposed synergy between alvocidib and HMAs

- Hypomethylating agents (HMAs) azacytidine and decitabine exert biological activity via two distinct mechanisms, namely DNA demethylation and inhibition of DNA methyltransferase.
- Azacytidine and decitabine are indicated in the treatment of patients with myelodysplastic syndromes (MDS). As a result of DNA methyltransferase inhibition, it is hypothesized that HMAs may function by inducing expression of key pro-apoptotic proteins such as NOXA, which sequences with the anti-apoptotic protein MCL-1, potentiating apoptotic induction with the mitochondrial pore-forming proteins Bak/BamI.
- Activity of the potent CDK9 inhibitor, alvocidib, is largely driven by targeting of CDK9-dependent MCL-1 expression. Alvocidib is under active clinical investigations, but has also demonstrated high complete response rates in newly diagnosed AML patients, particularly when administered as part of a cytarabine and mitoxantrone containing regimen (ACM regimen).
- Given the dual NOXA/MCL-1 targeting ability of combining alvocidib and azacytidine or decitabine, the combination may synergize therapeutically in the treatment of non-clinical models of AML, or MDS by means of transcriptional upregulation of NOXA and expression of MCL-1 expression.

III. Results

Figure 2: Alvocidib suppresses MCL-1 mRNA and protein expression

- Alvocidib inhibits the transcription of key anti-apoptotic genes, including MCL-1. Following alvocidib treatment at various doses, both mRNA (A) and protein (B) levels decreased in a dose-dependent fashion in the MCL-1 (MOLM-13) line cell.

Figure 3: Alvocidib shows clinical activity in secondary AML

- To determine the clinical activity of alvocidib in secondary AML, Alvocidib was administered to patients with secondary AML treated with HMAs. Alvocidib dosing was associated with significant improvement in OR rates in ACM treated patients. (A) Alvocidib dosing was performed in 1 hour treatment, every 28 days. (B) Improvement in OR rate in ACM treated patients when Alvocidib was dosed with HMAs. (C) Alvocidib dosing with HMAs in patients with secondary AML and AML with secondary AML.

- Alvocidib dosing with HMAs in patients with secondary AML and AML with secondary AML.

- Alvocidib dosing with HMAs in patients with secondary AML and AML with secondary AML.

IV. Conclusions

- Alvocidib suppresses MCL-1 expression in a dose-dependent fashion in vitro
- Alvocidib clinical activity correlates with NOXA BH3 profiling
- HMAs induce NOXA re-expression in cultured cells
- HMAs induce NOXA re-expression in xenograft models
- Combination of HMAs and alvocidib increased apoptosis in cultured cells
- Sequential dosing, and subsequent NOXA re-expression, are required for increased apoptosis
- HMAs synergize in xenograft models for MDS/AML