TK216, a novel, small molecule inhibitor of the ETS-family of transcription factors, displays anti-tumor activity in AML and DLBCL

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ABSTRACT

The ETS family of transcription factors is critical for development, differentiation, proliferation, and plays an important role in apoptosis and tissue remodeling. Transcriptional activity of ETS family proteins deregulation by overexpression, gene fusion, and modulation by miRNA and RNA binding proteins is associated in normal cell functions, and leads to increased proliferation, sustained angiogenesis, invasion, and metastasis. Overexpression of ETS family fusion proteins have been reported in acute myeloid leukemia (AML) and diffuse large B cell lymphoma (DLBCL). In AML, the TK216 target has been identified as an oncogenic partner in the transformation of disease, leading to the deregulation of ETS family members, C11 and FLI1. Additionally, in AML, the overexpression and translocations of ETS, an ETS family member, has been associated with poor prognosis in complex or normal karyotypes.

TK216 is a first in class, small molecule that directly binds EWS-FLI1 inhibiting the biological activity of ETS family transcription factor oncogenes and is currently under clinical investigation in patients with Ewing sarcoma (NCT01703090). The EWS-FLI1 family of transcription factors has been shown to be the driver of Ewing sarcoma (ES). In preclinical potency models, TK216 blocked the binding between EWS-FLI1 and FLI1 responsive promoter. In AML, TK216 showed a significant transcriptional decrease in COX7 cells transfected with a EWS-FLI1 responsive promoter (EC50 = 100 µM), and inhibited the proliferation of AML cell lines (TK216 expressed as IC50 in nanomolar concentration) at nanomolar concentrations (EC50 = 200 µM). Here we show that TK216 has potent proliferative effects on a panel of AML and DLBCL cell lines with deregulated ETS family members. We report an upregulation of FLI1 and/or ERG ETS family members in 5 of 5 myeloid cell lines evaluated (HL-60, Kasumi-1, ML-2, MOLM-13, MOLM-16, THP-1). Treatment with TK216 showed a decreased in cellular viability and induced dose-dependent apoptosis of cells at 48 hours. Similarly in a panel of DLBCL cell lines (TMD-8, HBL-1, L-292, DOHH2, DOHH2-DCLCL1, OCI-LY1, OCI-LY-3), TK216 treatment resulted in a decrease in cellular proliferation and an increase in apoptosis. In vivo efficacy studies in xenograft models of DLBCL are underway; the preliminary anti-tumor activity that is being seen encouraging and consistent with our in vitro findings.

We believe that our findings provide compelling evidence of the utility and potential efficacy of TK216 in the treatment of AML and DLBCL by targeting the aberrant expression and transcriptional activity of the ETS-family of transcription factors, which contribute to the pathogenesis of the disease.

RESULTS

4.560

Altered Gene Expression:

3

software was used to determine

1.0

Combination of TK216 with BCL2 and/or BTK inhibitors in DLBCL cell lines leads to synergistic activity and allows for more effective inhibition of cell proliferation.

Table 1. TK216 IC50 in AML Cell Lines.

Figure 2. TK216 Treatment Inhibits EWS-FLI1 Protein Interactions Leading to a Decrease in Transcription and Proliferation.

Figure 3. TK216 Displays Anti-Proliferative Activity in AML Cell Lines.

Figure 4. TK216 Displays Anti-Proliferative Activity in DLBCL Cell Lines.

Figure 5. Induction of Apoptosis by TK216 in DLBCL Cell Lines.

Figure 6. TK216 Reduces Transcriptional Activity In COS7 Cells Co-Transfected with EWS-FLI1 and FLI1 Responsive Promoter.

Figure 7. TK216 Displays Anti-Tumor Activity in a DLBCL Xenograft Model.

Figure 8. TK216 Inhibits Proliferation and Induces Apoptosis in the TMD-8 xenograft model.

CONCLUSION

• TK216 is a first in class, small molecule that directly binds EWS-FLI1 inhibiting the biological activity of ETS-family transcription factor.

• In AML and DLBCL cell lines with deregulated ETS-family members, treatment with TK216 results in potent inhibition of proliferation and the induction of apoptosis.

• Combination of TK216 with BCL2 and/or BTK inhibitors in DLBCL cell lines leads to synergistic activity and allows for more effective inhibition of cell proliferation.

• Daily, oral administration of TK216 potently inhibits tumor growth in xenograft models of DLBCL with deregulated expression of ETS-family members, and is well-tolerated.

• The inhibition of the EWS-FLI1 oncogene offers a promising approach for the treatment of Ewing Sarcoma and is currently in Phase I clinical trials in patients with relapsed/refractory Ewing Sarcoma (KT-0267005).

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