Dual inhibition of JAK/STAT and MAPK Pathways Results in Synergistic Cell Killing of JAK-Mutated Pediatric Acute Lymphoblastic Leukemia

Santi Suryani, PhD,1 Keith C.S. Sia,1 Lauryn S Bracken,1 Herman Carol, PhD,1 Kathryn Evans,1 Raushan Kurmasheva, PhD,1 Peter J. Houghton, PhD,1 Malcolm A. Smith, MD, PhD1 and Richard B. Lock, PhD1

1Children’s Cancer Institute Australia for Medical Research, University of New South Wales, Sydney, Australia; 2Nationwide Children’s Hospital, Columbus, OH; 3NICI/CC/NC, National Institutes of Health, Bethesda, MD

ABSTRACT

Activating mutations in the pseudokinase/cruciform domain of Janus kinase (JAK) 1, 2 and 3 are present in ~10% of high-risk cases of relapsed/refractory pediatric acute lymphoblastic leukemia (ALL) patients and are associated with poor outcome. Moreover, JAK mutations correlate with high expression of cytokine receptor-like factor 2 (RFL2) and result in constitutive activation of downstream signaling pathways including JAK/STAT, Akt and MAPK.

ATP-competitive small molecule inhibitors of JAK activity, such as AZD1480 (JAK1/2 Inhibitor), offer an opportunity to improve the treatment of patients with JAK-mutated ALL. As part of the Pediatric Preclinical Testing Program (PPTP) we previously showed that AZD1480 alone was unable to induce objective responses in xenografts established in immune-deficient mice from direct patient explants of JAK-mutant ALL.

In this study we show that xenografts established from JAK-mutated ALL exhibit increased signaling via the JAK/STAT and MAPK pathways compared with JAK-wild-type ALL. In Ba/F3 cells, JAK inhibitor BPK-ALL was assessed by phosphoepitope analysis. Ex vivo exposure of xenograft cells to AZD1480 resulted in selective inhibition of the JAK/STAT signaling pathway, offering a potential explanation for the lack of single-agent AZD1480 cell killing effects both in vivo and ex vivo. However, this finding also provides rationale for dual targeting of the JAK/STAT and MAPK pathways. Our data show that the combination of AZD1480 and the MEK inhibitor, AZD6244, exerted strong synergistic cytotoxicity against JAK-mutant ALL xenograft cells in vivo, suggesting that this combination may have utility in improving the treatment options for patients with JAK-mutated ALL.

AIM

To develop synergistic drug combination regimens through rational analysis of signaling pathways to improve treatment options for high-risk pediatric ALL.

METHODS

Synergistic development and in vivo responses. Xenografts were established from direct patient explants of "typical" BCP-ALL or JAK-mutated ALL in NOD/SCID or NOD mice as previously described (Lien et al. Blood 103: 2366-2376, 2004). For in vivo AZD1480 efficacy testing, AZD1480 was administered (BID X 5 days 10mg/kg, SID X 2 days 15 mg/kg) via subcutaneous injection (SC) daily. Drug exposure was measured by time course exposure at indicated concentrations. Drug sensitivity to combination of drugs for 72 hours at the indicated concentrations. Drug sensitivity was assessed by mitochondrial activity assay (Alamar Blue).

Molecular analyses. Whole cell lysates were separated by SDS-PAGE and transferred to PVDF membrane and probed with the relevant monoclonal antibodies.

RESULTS

JAK-mutated ALL exhibits increased signaling via the JAK/STAT and MAPK pathways. The combination of AZD1480 and AZD6244 exerts strong synergistic cytotoxic effect against JAK-mutated ALL xenografts ex vivo. The combination of AZD1480 and AZD6244 caused a decrease in phosphorylation of JAK/STAT and MAPK signaling proteins.

CONCLUSIONS

AZD1480 is unlikely to exert significant single agent activity in the treatment of JAK-mutated pediatric ALL. JAK-mutated ALL xenografts exhibited constitutive activation of the JAK/STAT and MAPK signaling pathways. The combined treatment of JAK-mutated xenograft cells with AZD1480 and AZD6244 resulted in marked inhibition of the JAK/STAT and MAPK signaling pathways, and synergistic cytotoxicity.

Dual targeting of JAK/STAT and MAPK signaling pathways offers an opportunity to improve the treatment options for patients with JAK-mutated ALL.