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605.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: LYMPHOID NEOPLASMS

Ex Vivo Drug Sensitivity Evaluation of a ZMYM2::FGFR1 Fusion-Positive 8p11 Myeloproliferative Syndrome (EMS) Leukemia

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Introduction : Fibroblast growth factor receptors (FGFRs) are conserved single transmembrane receptor tyrosine kinases (RTKs) with an extracellular ligand-binding domain and a cytoplasmic tyrosine kinase domain. They play vital roles in cellular proliferation, survival, development, fetal organogenesis, metabolism, and tissue repair. 8p11 myeloproliferative syndrome (EMS) is a hematologic malignancy caused by a translocation of the fibroblast growth factor receptor 1 (FGFR1) gene on chromosome 8p11-12. The most common fusion partner of FGFR1 is the zinc-finger domain ZNF198 (also known as ZMYM2) on chromosome 13q12. Pemigatinib is approved for FGFR fusion positive myeloid/lymphoid neoplasms (MLNs), and other FGFR inhibitors are being tested in clinical trials. However, EMS patients still face a poor prognosis and drug resistance, necessitating further research to overcome these challenges. This study evaluated *ex vivo* drug screening assay data generated from ZMYM2::FGFR1 positive primary leukemia samples throughout the course of an EMS patient's clinical response and validated these findings on a ZMYM2::FGFR1 positive cell line.

Methods : *Ex vivo* and drug screening experiments were performed on freshly isolated primary patient samples and a ZMYM2::FGFR1-expressing cell line. The fusion was confirmed using FISH, and additional clinical specimens were collected with informed patient consent. *Ex vivo* drug sensitivity assays were conducted on patient mononuclear cells isolated from blood and bone marrow. IL-3 dependent BaF3 cells were infected with retrovirus containing a ZMYM2::FGFR1-V5 construct. ZMYM2::FGFR1 transformed BaF3 cells were evaluated with single agent and drug combination panels. Sanger sequencing of the FGFR portion of the fusion was performed on patient samples to confirm the breakpoint and identify potential mutations affecting sensitivity to Ponatinib throughout the course of treatment.

Results : Sample were obtained with informed consent from a 36-year-old man diagnosed with 8p11 myeloproliferative syndrome and a ZMYM2::FGFR1 fusion along with a RUNX1 ^{R201Q} mutation. *Ex vivo* drug screens performed on isolated mononuclear cells demonstrated sensitivity to Ponatinib and Pemigatinib. He underwent treatment with HyperCVAD plus Ponatinib and subsequently HyperCVAD with Ponatinib and Velcade (Bortezomib) to CR1, FISH for FGFR1 rearrangement was undetected. He was scheduled to proceed with hematopoietic stem cell transplant (HSCT), however his pre-transplant bone marrow biopsy showed return of his FGFR1 translocation by FISH. *Ex vivo* drug screening assays confirmed resistance to Ponatinib, but continued sensitivity to Pemigatinib. He was transitioned to treatment with single agent Pemigatinib and was able to re-clear his FGFR1 fusion by FISH, and has since undergone hematopoietic stem cell transplantation. He remains in remission on post-transplant Pemigatinib maintenance therapy. Sanger sequencing performed at the time of progression on Ponatinib identified a nucleotide alteration resulting in an amino acid substitution (FGFR1 ^{F686L}; ZMYM2::FGFR1 ^{F1171L}) within the kinase domain that could contribute to decreased Ponatinib sensitivity. Bortezomib and Axitinib exhibited high efficacy on the patient's sample. *Ex vivo* drug assays performed on ZMYM2::FGFR1 transformed BaF3 cells confirmed the efficacy of Pemigatinib and Ponatinib. Other FGFR inhibitors, including Olverematinib, AZD4547, Axitinib, Cediranib, Dovitinib, and Lenvatinib, also demonstrated exquisite sensitivity. Despite extensive screening, no other single agents or drug combinations exhibited increased effectiveness in the ZMYM2::FGFR1 transformed BaF3 cells except for Trametinib, a MEK inhibitor, and the combination of Belvarafenib (RAF inhibitor) and Gilteritinib (FLT3 inhibitor).

Conclusions : *Ex vivo* drug sensitivity assays demonstrated the highly selective efficacy of FGFR inhibitors in ZMYM2::FGFR1 fusion-positive leukemia cells and a fusion-expressing BaF3 cell line. Mutations in the FGFR1 kinase domain (ZMYM2::FGFR1 ^{F1171L}) could contribute to Ponatinib insensitivity. These *ex vivo* drug screening results provide further support for ongoing clinical trials which are investigating the use of single agent Pemigatinib and other FGFR1 inhibitors for the treatment of patients with FGFR1-fusion positive leukemias.

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