# Research Week 2022

# The FLT3 F691L Gatekeeper Mutation Promotes Clinical Resistance to Gilteritinib + Venetoclax in AML

Setareh Sharzehi, MD, MS, Sunil Joshi, PhD, Janet Pittsenbarger, BA, Daniel Bottomly, Shannon McWeeney, PhD, Jeffrey W. Tyner, PhD, Elie Traer, MD, PhD.
sharzehs@ohsu.edu
Oregon Health and Science University, Portland, OR; Knight Cancer Institute, Oregon Health and Science University, Portland, OR

## Keywords

Acute Myeloid Leukemia (AML), FLT3, resistance, Microenvironment, Kinase inhibitors

## Abstract

**Back ground:** Acute Myeloid Leukemia (AML) is an aggressive cancer with poor overall survival due to frequent relapse. FMS-like tyrosine kinase (FLT3) is the most commonly mutated genes in AML, and leads to higher relapse rates. Several FLT3 inhibitors have been developed, including gilteritinib. Our lab developed a two-step model of early and late resistance to study the mechanism of resistance and relapse to FLT3 inhibitors. **Early resistant** AML cells are dependent upon the marrow microenvironment for survival. Over time, intrinsic resistance allows independent growth and results in **late resistance**, leading to disease relapse.

Current clinical trials are testing combination therapies in FLT3 mutated AML. Gilteritinib + venetoclax (GILT+VEN) is very active in these patients, however the mechanism of resistance to this combination remains unknown.

**Results:** Early resistance cell cultures to GILT+VEN were created by culturing MOLM14 cells in 25 nm of GILT+VEN in presence of the microenvironmental ligands, or in media alone (control). After 25 weeks, only the cultures with ligands resumed growth. Ligands were then removed from these early resistance cultures to induce late resistance. Immunoblot analysis of GILT+VEN early and late resistance cultures demonstrated restoration of FLT3 phosphorylation, as well as downstream AKT/MAPK signaling. Whole exome sequencing revealed that all resistance cultures had F691L gatekeeper mutations, which prevents gilteritinib binding to FLT3. As confirmation, we exposed resistance cell lines to FF10101, which binds FLT3 at a different site and is not blocked by F691L. FF10101 largely restored sensitivity our resistance cultures. In addition to F691L, PI3KR4 mutations were found in all resistance cell lines, suggesting mutational activation of the PI3K/AKT signaling pathway may also contribute to resistance.

As clinical validation, we obtained samples from two relapsed/refractory AML patients treated with GILT+VEN that eventually relapsed. One was found to have developed a FLT3

F691L mutation, and the other a FLT3 N701K mutation, which acts as a gatekeeper mutation.

**Conclusion:** Our robust cell line model of early and late resistance and early patient data predicts that F691L mutations are more important for GILT+VEN. This contrasts to gilteritinib monotherapy resistance, which is more commonly driven by NRAS mutations.