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POSTER ABSTRACTS

604.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: MYELOID NEOPLASMS

Identifying Targeted Therapies for *CBFA2T3- GLIS2* Acute Myeloid Leukemia

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CBFA2T3- GLIS2 fusion positive pediatric acute myeloid leukemia (AML) remains one of the worst prognostic AML subgroups. Children with this subtype of AML frequently experience upfront chemoresistance with a high cumulative incidence of relapse (42-83%) and a dismal overall survival (~20%) even with intensive therapy. New treatment approaches are urgently needed.
By interrogating the Broad Institute's Cancer Dependency Map (DepMap), a data set composed of genome-scale CRISPR-Cas9 screens in over 1000 cancer cell lines, we discovered that the *CBFA2T3- GLIS2* AML model M-07e was highly dependent on the kinase *JAK2*. Indeed, M-07e was one of the most *JAK2* dependent models in the data set. Using the same genome-scale library, we next performed CRISPR-Cas9 screens in WSU-AML and CMS, two additional AML cell lines harboring this fusion, and revealed *JAK2* as a strong dependency shared by the 3 cell lines.
Using a doxycycline-inducible knockout (KO) system, we validated *JAK2* KO by western blot in three AML cell lines and cells from a patient-derived xenograft (PDX) harboring the fusion. Using two different sgRNAs targeting *JAK2* (sgJAK2) and a non-targeting (sgNT) control, we validated *JAK2* dependency in all 4 AML models in competitive growth assays. We also observed impaired proliferation and induced apoptosis upon *JAK2* KO.
To evaluate a role for *JAK2* dependency *in vivo*, we intravenously injected NSGS mice with WSU-AML either transduced with doxycycline-inducible sgJAK2 or sgNT. After confirmation of engraftment by bone marrow evaluation, doxycycline chow was initiated. After 2 weeks of doxycycline treatment, we observed a significantly decreased tumor burden in mice with *JAK2* KO AML compared to sgNT as measured by a decrement in human CD45% cells: peripheral blood (2.8% versus 12.9%), bone marrow (37.4% versus 56.3%,) and spleen (19% versus 79.5%).
We next assessed chemical inhibitors of JAK2. Both the type I JAK2 inhibitor ruxolitinib and the type II JAK2 inhibitor CHZ868 exhibited strong activity *in vitro* in *CBFA2T3- GLIS2* cell lines and PDX cells, many resistant to cytotoxic chemotherapy. Because ruxolitinib is FDA-approved for other indications, we evaluated this drug in a cell line and PDX model of *CBFA2T3- GLIS2* AML *in vivo*. As previously reported in myeloproliferative neoplasms, *JAK2* target inhibition with ruxolitinib treatment was incomplete, and accordingly, *in vivo* response was modest with leukemia ultimately progressing. Evaluation of CHZ868 *in vivo* is ongoing.
Because single agent targeted therapy is typically insufficient for durable response in the treatment of most cancers, we utilized CRISPR-Cas9 anchor screening to identify candidate resistance mechanisms and potential synergistic combinations with JAK2 inhibitors in CMS and WSU-AML cells. We compared the changes in abundance of sgRNAs in the cells either treated with ruxolitinib (IC_{50}) or vehicle for 14 days. The correlation of hits between the two cell lines was strong, and we found that sgRNAs targeting multiple negative regulators of the MAPK pathway were significantly enriched in the ruxolitinib-treated arm. In parallel, we generated resistant CMS sublines by chronic exposure to ruxolitinib. Ruxolitinib-resistant CMS cells were found to have a pathogenic *NRAS* missense mutation and an associated increase in levels of phosphorylated MEK. Accordingly, they were responsive to the MEK inhibitor trametinib. Both approaches converge on activation of the MAPK pathway as a resistance mechanism to JAK2 inhibitors in *CBFA2T3- GLIS2* AML.
We next hypothesized that targeting the MAPK pathway could overcome resistance to JAK2 inhibitors in this disease context. Indeed, treatment with ruxolitinib and the MEK inhibitor trametinib exhibited a synergistic effect when used in combination in cell lines and PDX cells harboring the fusion *in vitro* with *in vivo* testing as a next step.

In summary, we validated *JAK2* as a strong dependency in *CBFA2T3- GLIS2* fusion positive pediatric AML. We demonstrated that *JAK2* KO or small molecule inhibitors impaired cell viability and induced apoptosis *in vitro*, and *JAK2* KO was highly efficacious in decreasing leukemia burden *in vivo*. Activation of the MAPK pathway renders resistance to JAK2 inhibitors and the combination of a JAK2 with MEK inhibitor is highly synergistic.

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