Robust *In vivo* Synergy Between the Anti-CD33 ADC IMGN779 and the FLT3 Inhibitor Quizartinib in Human FLT3-ITD AML Models

Abstract
PF190

Krystal Watkins, Lee Flaherty, Rebecca McCarthy, Katherine Francisco, Sharlene Adams, Callum Sloss, Angela Romanelli
ImmunoGen, Inc., Waltham, MA, USA

INTRODUCTION

- Acute myeloid leukemia (AML) patients harboring a FLT3 Internal Tandem Duplication (FLT3-ITD) have a poor prognosis and high rates of relapse following treatment with standard agents.
- Midostaurin has been recently approved for the treatment of newly-diagnosed FLT3-mutant AML patients. Due to efficacy and toxicity concerns with midostaurin, additional FLT3 inhibitors, such as the more potent and specific quizartinib, are currently being evaluated in clinical trials for frontline and relapsed AML patients.
- In pre-clinical models, FLT3 inhibitors decrease anti-apoptotic signaling and re-sensitize FLT3-ITD-positive leukemia to cytotoxic drugs[1].
- IMGN779 is a next-generation anti-CD33 antibody-drug conjugate (ADC) with a novel DNA-alkylating IGN payload and a cleavable s-SPDB linker, currently in Phase 1 development for AML (NCT02674763).
- IMGN779 has been shown pre-clinically to be highly active in FLT3-ITD AML cells *in vitro*[2].
- Our goal was to investigate the anti-leukemic efficacy of the combination of IMGN779 and quizartinib in FLT3-ITD mutant AML models.

Methods

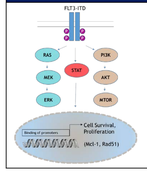
In vitro Studies:

FLT3-ITD AML cell lines (MV4-11 and Molm-13) were cultured in supplier recommended media. For combination studies, MV4-11 cells were treated with treatment vehicle, or IMGN779 (77 pM), or quizartinib (4.5 nM), or the combination of IMGN779 and quizartinib. Molm-13 cells were treated with treatment vehicle, or IMGN779 (32 pM), or quizartinib (3 nM), or the combination of IMGN779 and quizartinib. Cell viability (LIVE/DEAD cell viability dye, Thermo), apoptosis (cCaspase3 and cPARP), and Tyrosine Kinase Signaling (Mcl-1, p-Stat5, Rad51, p-Erk1/2) were measured by flow cytometry and/or immunoblotting. All antibodies were purchased from Cell Signaling, BD Biosciences, or Abcam.

In vivo Studies:

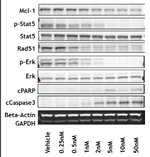
Two disseminated FLT3-ITD AML cell lines (MV4-11 and Molm-13) were engrafted into immunodeficient mice. Mice were treated with: vehicle control; quizartinib daily for 14 days; IMGN779 weekly for 3 weeks; or the combination of both drugs. Mice were terminated for weight loss >20% or clinical signs. Treatment began 7 days after AML cell engraftment in the Molm-13 model and 21 days in the MV4-11 model.

FLT3-ITD



- Constitutive autophosphorylation of FLT3 induces activation of STAT5 and the downstream MAPK, PI3K signaling pathways, leading to suppression of apoptosis and increased cell proliferation.
- The anti-apoptotic protein Mcl-1 has been shown to be upregulated in FLT3-ITD cells[3].
- Patients with FLT3-ITD mutations have been shown to have increased Rad51 (a key homologous recombination effector) expression[4].
- Does FLT3 inhibition sensitize AML cells to DNA damage (IMGN779)?

Quizartinib Decreases Tyrosine Kinase Signaling in a Dose-Dependent Manner



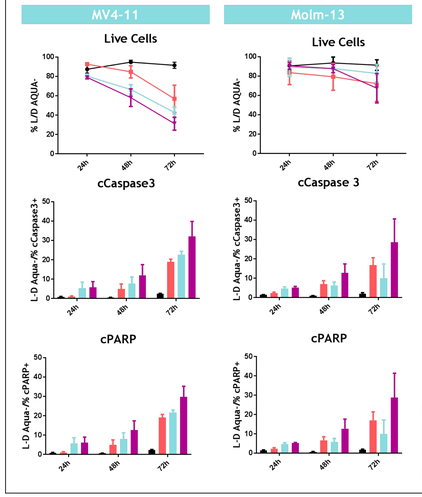
Treatment with quizartinib led to dose- dependent decreases in the following proteins:

- Mcl-1 (anti-apoptotic)
- p-Stat5 (pro-survival, pro-proliferative)
- p-Erk (pro-proliferative)
- Rad51 (DNA repair protein)

In addition quizartinib induced a dose dependent increase in apoptosis as shown by an increase in expression of the following proteins:

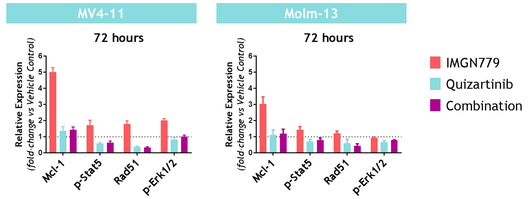
- Cleaved Caspase3 (induces apoptotic signaling)
- Cleaved PARP (Inactivates DNA repair, induces apoptotic signaling)

The Combination of IMGN779 + Quizartinib Decreased AML Cell Viability and Increased Apoptotic Signaling



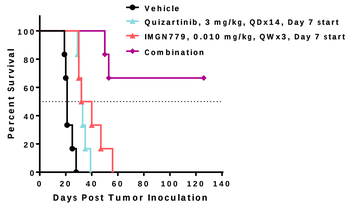
- Treatment with combination of IMGN779 and quizartinib led to fewer viable cells than either single agent treatment at 72 hours
- Treatment with combination of IMGN779 and quizartinib induced more apoptotic signaling than either single agent treatment at 72 hours

Quizartinib Reverses IMGN779 Induced Increases in the Anti-Apoptotic Protein, MCL-1



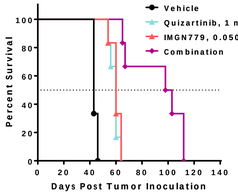
- Treatment with IMGN779 increased Mcl-1 levels
- Treatment with the combination of quizartinib and IMGN779 decreased Mcl-1, p-Stat5, p-ERK1/2, and Rad 51 in a manner similar to that of quizartinib alone
- Crucially, in spite of treatment with IMGN779, the combination-treated cells do not show the upregulation of the anti-apoptotic protein Mcl-1.
- Reduced levels of Mcl-1, decreases in a cell's DNA damage response (Rad51) and pro-survival signaling (p-STAT5), may be responsible for the increased cell killing of IMGN779 when combined with quizartinib

The Combination of IMGN779 + Quizartinib Prolongs Survival in Molm-13 Disseminated Xenograft Model



Treatment	Median Survival Time (Days)	Tumor Growth Delay (Days)	% Increased Life Span	Result
Vehicle	21	-	-	-
IMGN779	36	15	71.4	Highly Active
Quizartinib	32.5	11.5	54.8	Highly Active
Combination	>126	>105	>500	Highly Active

The Combination of IMGN779 + Quizartinib Prolongs Survival in MV4-11 Disseminated Xenograft Model



Treatment	Median Survival Time (Days)	Tumor Growth Delay (Days)	% Increased Life Span	Result
Vehicle	43	-	-	-
IMGN779	60	17	39.5	Minimally Active
Quizartinib	60	17	39.5	Minimally Active
Combination	110.5	57.5	133.7	Highly Active

CONCLUSIONS

- The combination of IMGN779 and quizartinib results in a robust anti-leukemic effect *in vitro*.
- Mcl-1 is upregulated with IMGN779 treatment; the addition of quizartinib precludes this increase.
- The combination effect *in vitro* correlates with quizartinib-driven decreases in anti-apoptotic signaling, and decreases in the DNA repair effector Rad51.
- *In vivo* studies confirm a combination benefit in two disseminated AML xenograft models, suggesting cooperativity of the two drugs' distinct anti-leukemic mechanisms of action.
- These findings support testing the combination of IMGN779 and FLT3 inhibition in a clinical trial in FLT3-ITD mutant AML patients.

References:
1. Kasper, et al. Targeting MCL-1 sensitizes FLT3-ITD-positive leukemias to cytotoxic therapies. Blood Cancer Journal, 2012.
2. Whiteman, et al. The Antibody-Drug Conjugate (ADC) IMGN779 Is Highly Active In Vitro and In Vivo Against Acute Myeloid Leukemia (AML) With FLT3-ITD Mutations. Abstract 2321, 56th Annual Meeting of the American Society of Hematology, Dec 6-9, 2014.
3. Yoshimoto, et al. FLT3-ITD up-regulates MCL-1 to promote survival of stem cells in acute myeloid leukemia via FLT3-ITD-specific STAT5 activation. Blood, 2009
4. Seedhouse, et al. DNA repair contributes to the drug-resistant phenotype of primary acute myeloid leukaemia cells with FLT3 internal tandem duplications and is reversed by the FLT3 inhibitor PKC412. Leukemia, 2006.