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Synergistic inhibition of FLT3-ITD Acute Myeloid Leukaemia cell growth *in vitro* using combination of FLT3 inhibitor quizartinib and standard chemotherapeutic agent cytarabine (also known as AraC)

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INTRODUCTION

- Acute myeloid leukaemia (AML) with the internal tandem duplication (ITD) mutation of the Fms-like receptor tyrosine kinase 3 (FLT3) (FLT3-ITD) is the most frequent mutation (~25%) in normal karyotype AML.
- In recent clinical studies, few patients display prolonged remissions with receptor tyrosine kinase (RTK) inhibitors, such as FLT3 inhibitors therapy, highlighting a substantial unmet need for novel effective treatment[1].
- FLT3-ITD mutation leads to the constitutive activation of FLT3 kinase and its downstream pathways, strongly associated with cell survival, proliferation and differentiation[2].
- Combination of FLT3 inhibitor with standard chemotherapeutic agents may synergise in AML cells and present a potential therapeutic strategy for FLT3-ITD AML.
- In this preliminary study, we aim to study the efficacy of the FLT3 inhibitor quizartinib in combination with chemotherapeutic agent AraC (also known as cytarabine) against FLT3-ITD AML cells *in vitro* using human cell lines.

METHOD

Cell culture and drugs Human THP1, MOLM-13 and MV4-11 cell lines were cultured in RPMI 1640 supplemented with 10% FBS and 1% L-Glutamine. Drugs were purchased from Selleckchem.

Cell viability To measure live cells, fluorometric/colourimetric resazurin assay was performed.

Flow cytometry Apoptosis and cell cycle state was assessed with annexin V/DAPI and propidium iodide/RNase staining, detected by flow cytometric analysis.

Synergy Combination indices (CI) were calculated using the Bliss Independence method using the following equation: $CI = (E_A + E_B - E_A \times E_B)/E_{AB}$ where E_A is the effect of drug A, E_B the effect of drug B and E_{AB} the combined effect of drug A and B. CI <1, =1, and >1 indicates synergism, additive effect and antagonism, respectively.

CONCLUSIONS

- Quizartinib reduced the viability of the FLT3-ITD AML cell lines MOLM-13 and MV4-11 cells, but had minimal impact on the growth of FLT3wt AML cell line THP1, suggesting that quizartinib exerts potent antileukaemic activity selectively on FLT3-ITD AML.
- Combination of quizartinib and AraC synergistically inhibited cell viability of FLT3-ITD AML cells. As cytarabine inhibits synthesis of DNA when the cell is in the S phase, its mode of action could be impeded by quizartinib-induced cytostatic effects. Sequential administration of drugs may further enhance synergistic effects.

REFERENCES

[1]Daver N et al. Targeting FLT3 mutations in AML: review of current knowledge and evidence. *Leukemia. 2019 Feb;33(2):299-312*

[2]Staudt D et al. Targeting Oncogenic Signaling in Mutant FLT3 Acute Myeloid Leukemia: The Path to Least Resistance. *Int J Mol Sci*. 2018 Oct 16;19(10):3198.

Quizartinib exerts potent antileukaemic activity selectively on FLT3-ITD AML cell *in vitro* and induces moderate G1 cell cycle arrest and apoptosis.

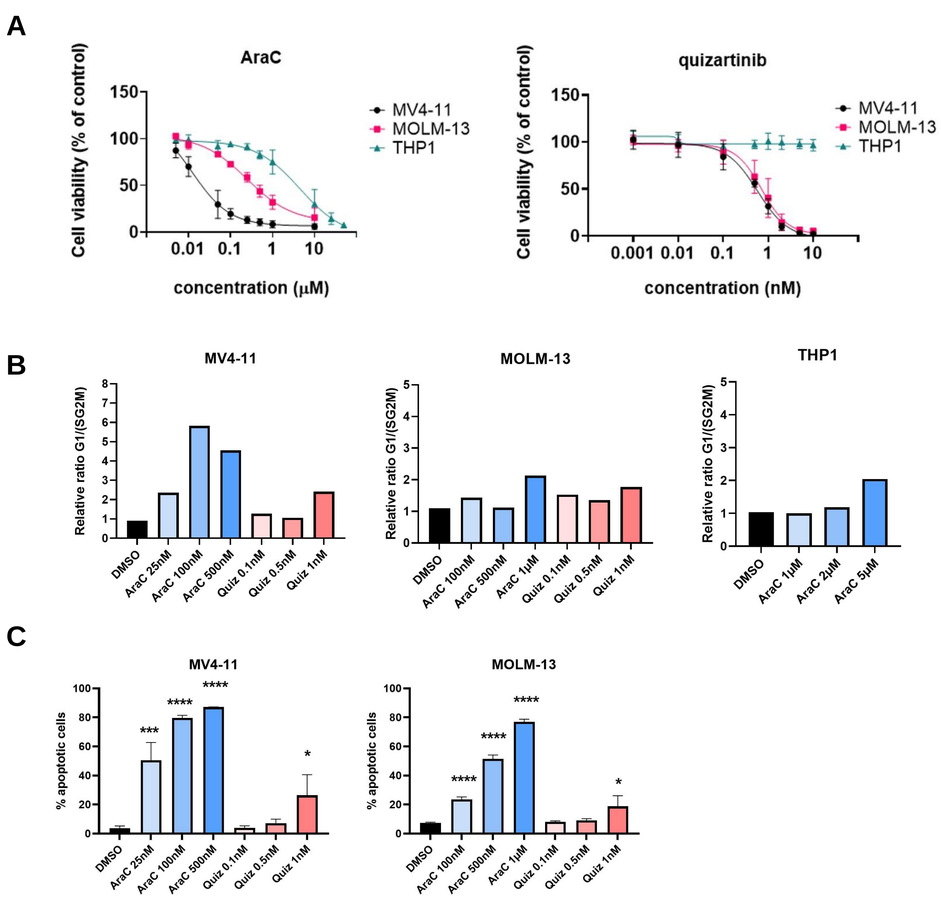
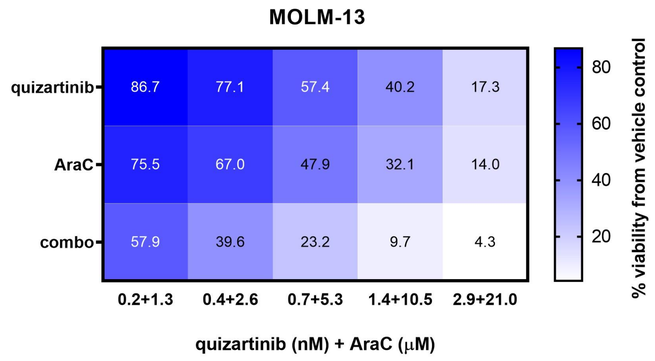


Figure 1. The effect of AraC and quizartinib on FLT3-ITD AML versus FLT3 wildtype (wt) cells.
A) Concentration-cell viability curves for AraC and quizartinib as monotherapy in MOLM-13, MV4-11 (FLT3-ITD) and THP1 (FLT3wt) cell lines at 48h. **B)** Effect on cell cycle and **C)** apoptosis of increasing concentrations of AraC and quizartinib around the respective IC50 (determined by resazurin assay) at 48h. Statistics determined by ordinary one-way ANOVA. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$. $p < 0.05$ is considered significant. n=3 +/- SD.

Quizartinib in combination with AraC synergistically inhibits cell viability in FLT3-ITD AML cells.

A



B

0.82	0.80	0.94	0.96	1.04	Bliss Independence

C

Fa	Dose Quizartinib (nM)	Dose AraC (μM)	DRI Quizartinib	DRI AraC
0.5	0.90	4.53	3.75	2.58

Figure 2. The effect of quizartinib in combination with AraC on FLT3-ITD AML cells.
A) Heatmap of concentration-cell viability for quizartinib as monotherapy or in combination with AraC in MOLM-13 cells at 48h (n=2). Drugs were added simultaneously at t=0h. **B)** Combination index (CI) was generated using the Bliss Independence method where CI<1, =1, and >1 indicates synergism, additive effect and antagonism, respectively. **C)** Dose reduction index (DRI) values for quizartinib and AraC combinations calculated using CompuSyn software. The dose reduction index assessment for quizartinib indicates that dose reduction of 3.75 fold can be obtained for a 50% growth inhibitory effect in combination setting as compared to monotherapy. Fa: Fraction affected.

CONTACT INFORMATION

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