IRAK4 INHIBITOR CA-4948 POTENTIATES ANTITUMOR EFFECTS OF AZACITIDINE AND VENETOCLAX IN HUMAN ACUTE MYELOID LEUKEMIA

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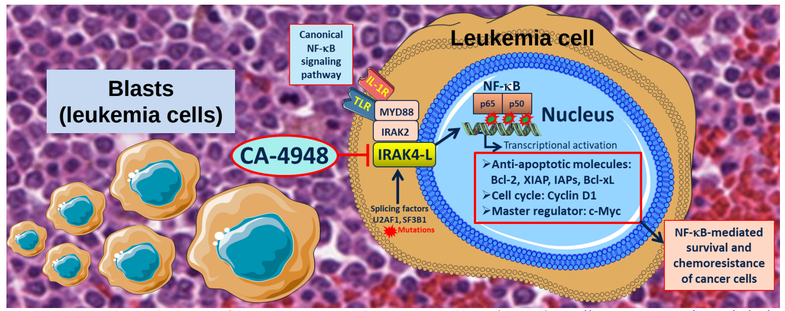
INTRODUCTION

Acute myeloid leukemia (AML) is known to be associated with high lethality despite intensive treatment. Previously, we have shown that CA-4948, a potent inhibitor of Interleukin-1 Receptor Associated Kinase 4 (IRAK4) and FMS-like Tyrosine Kinase 3 (FLT3), exhibited *in vitro* and *in vivo* antitumor activity against AML cell lines harboring FLT3-ITD and FLT3-ITD/D835Y mutations (1). Recent studies demonstrated that expression of IRAK4-L is mediated by mutant splicing factors U2AF1 and SF3B1, and is associated with oncogenic signaling in human AML and myelodysplastic syndromes (MDS) (2). Preliminary data from our ongoing Phase 1 dose escalation study demonstrated that treatment with IRAK4 inhibitor CA-4948 has shown early signs of clinical activity in patients with relapsed/refractory AML and high-risk MDS. Because IRAK4 plays a positive role in activation of NF-κB signaling pathway, inhibition of IRAK4 might help to overcome NF-κB-mediated chemoresistance of cancer cells. In present study, we evaluated whether IRAK4 inhibitor CA-4948 could potentiate antitumor effects of standard-of-care (SOC) AML drugs in AML cell lines which are not FLT3 mutated.



OBJECTIVE

Our goal was to identify an effective combination of CA-4948 with SOC AML drugs for the treatment of FLT3-WT AML.

METHODS

We used CellTiter Glo cell viability assay (Promega, Madison, WI) to evaluate the antitumor effects of CA-4948 in combination with AML SOC drugs daunorubicin, Ara-C, decitabine, azacitidine and venetoclax in AML cell lines THP-1, F-36P, OCI-AML2 and GDM-1 (which are not FLT3 mutated). Relative cell viability was measured by CellTiter Glo assay at 0 and 96 hrs. GI_{50} (the concentration for 50% of maximal inhibition of cell proliferation) was determined for each drug in AML cell lines. Either GI_{50} (below peak plasma concentration) or peak plasma concentration (if GI_{50} was higher than peak plasma concentration) of each drug was used as clinically relevant concentration for combination experiments. Cell viability assay data were analyzed with one-way ANOVA. P values less than 0.05 are considered significant. Statistical analysis was performed using GraphPad Prism 8.0 software.

RESULTS

Using CellTiter Glo cell viability assay, we determined sensitivity of AML cell lines to treatment with CA-4948 and SOC drugs. AML cell lines were treated with venetoclax, decitabine and azacitidine, as well as Ara-C and daunorubicin, either alone or in combination with CA-4948 (10 μM). Peak plasma concentration of venetoclax (1 μM), decitabine (1 μM) and azacitidine (4 μM) were defined as clinically relevant concentrations. We identified THP-1 and F-36P cell lines as resistant to clinically relevant concentrations of venetoclax, whereas OCI-AML2 cell line was resistant to the treatment with azacitidine and decitabine.

Synergistic effect of combining CA-4948 and Ara-C was observed in 3 of 4 AML cell lines (Table 1). We found that CA-4948 potentiated antitumor effects of hypomethylating agents in four AML cell lines (azacitidine) and in OCI-AML2 cell line (decitabine) (Table 1). The combination of venetoclax and CA-4948 inhibited the cell growth in 2 of 4 AML cell lines more effectively than either of the two agents alone (Table 1). Moreover, we found that CA-4948 significantly potentiated antitumor effects of azacitidine+venetoclax in all AML cell lines (Table 1).

AML cell line	Decitabine + CA-4948	Azacitidine + CA-4948	Venetoclax + CA-4948	Venetoclax + Decitabine + CA-4948	Venetoclax + Azacitidine + CA-4948
THP-1	NS	++	+	NS	+++
F-36P	NS	++	NS	NS	++
OCI-AML2	++	+	++	NS	+++
GDM-1	NS	++	NS	NS	++

AML cell line	Ara-C + CA-4948	Venetoclax + Ara-C + CA-4948	Daunorubicin + CA-4948	Daunorubicin + Ara-C + CA-4948
THP-1	+	++	NS	++
F-36P	++	NS	NS	NS
OCI-AML2	+	NS	NS	NS
GDM-1	NS	NS	NS	NS

Table 1. Antitumor effects of combination treatment with CA-4948 and SOC drugs in AML cell lines. *AML cells were treated continuously with GI_{50} (below peak plasma concentration) or clinically relevant drug concentration (if GI_{50} was higher than peak plasma concentration) for 96 hrs. Relative cell viability was measured by CellTiter Glo assay at 0 and at 96 hrs. All values are presented as mean ± SE. Cell viability assay data were analyzed with one-way ANOVA. P values less than 0.05 are considered significant. Statistical analysis was performed using GraphPad Prism 8.0 software. +, less than 50% growth inhibition, p<0.05; ++, 50%-100% growth inhibition, p<0.05; +++, 100% growth inhibition and induction of cell death, p<0.05; NS, not significant.*

RESULTS

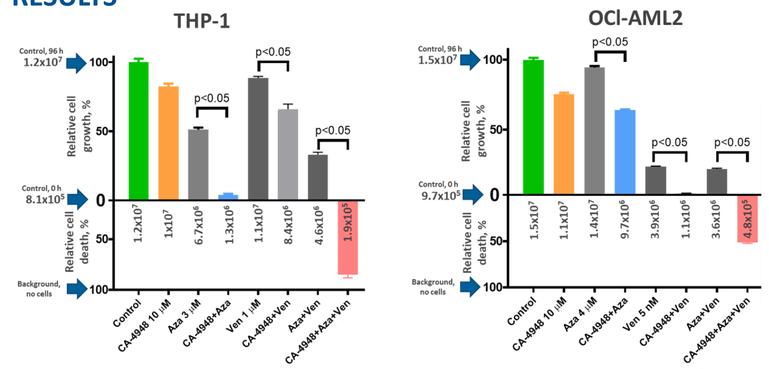


Figure 1. CA-4948 potentiates antitumor effects of azacitidine and venetoclax in AML cell lines. *AML cell lines were treated for 96 hrs. Relative cell viability was measured by CellTiter Glo assay at 0 and at 96 hrs. All values are presented as mean ± SE. Cell viability assay data were analyzed with one-way ANOVA. P values less than 0.05 are considered significant. Statistical analysis was performed using GraphPad Prism 8.0 software.*

CONCLUSIONS

Our results demonstrate that combination CA-4948+azacitidine and triple combination CA-4948+azacitidine+venetoclax exhibit synergistic activity in leukemia cells providing a rationale for clinical testing of this combinations in patients with AML.

REFERENCES

1. Booher R, Samson M, Borek M, Modafferi H, Martell R. CA-4948, an IRAK4/FLT3 inhibitor, shows antileukemic activity in mouse models of FLT3 WT and FLT3 mutated acute myeloid leukemia. *Abstract PS991, 24th EHA Congress, June 13-16, 2019.*
2. Smith MA, Choudhary GS, Pellagatti A, Choi K, Bolanos LC, Bhagat TD, et al.. U2AF1 mutations induce oncogenic IRAK4 isoforms and activate innate immune pathways in myeloid malignancies. *Nat Cell Biol. 2019;21(5):640-650.*

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