Investigation of Cytarabine Resistance in AML

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INTRODUCTION

•Acute Myeloid Leukemia is a cancer derived from myeloid progenitor cells.

•Standard treatment for AML consists of a "3+7" regimen: 3 days of daunorubicin and 7 days of cytarabine. However, relapses are common and often accompanied by chemoresistance.[1-2]

•Investigation of various chemotherapy combinations and dosing schedules over the past 30 years has failed to significantly improve survival in patients >60 years old where majority of the disease burden remains.[2-4]

•Elderly patient have the worst prognosis and often fail to tolerate induction therapy. [2]

•Our approach: identify targets that may sensitize cells to Ara-C using RNA-interference.

•Our Goal: Use an siRNA library of the human kinome (572 different kinases) in leukemia cell lines in order to identify targets that increase Ara-C sensitivity.

METHODS

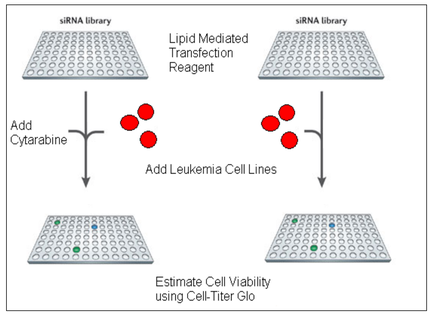

Figure 1: siRNA library screening procedures. A kinase library consisting of 2x si-RNA for 572 kinases was printed onto 384 well plates. A lipid mediated transfection reagent was used to deliver the siRNA's to the cells. Leukemia cell lines (THP-1 and TF-1) were added to the plates and allowed to incubate with the siRNA's for 48 hours. After 48 hours cytarabine was added to achieve an approximate IC_{30} for the cell lines. After 48 hours of incubation with cytarabine, cell viability was indirectly measured using Cell Titer Glo. Controls consisted of using the transfection reagent alone, siRNA's without cytarabine, Non-Silencing siRNA, and UBB siRNA

Verification of kinase screen and Verification of "hits.": 4x siRNA kinase screens were performed in the same cell lines of sensitizing kinases. Verification of target knockdown was done by RT-PCR and Western Blot.

Drug Dose Response Curves: Identified sensitizers were used in siRNA drug dose response (siDDR) experiments utilizing 4x siRNA. The siRNA for the identified hit was transfected as previously described. An 11-point range of cytarabine concentrations were added to the cells at the 48 hour time point. After 48 hours of drug incubation Cell Titer Glo was used to measure cell viability. From this data an IC_{50} was calculated.

RESULTS

Kinome Screen:

	Number of Sensitizing Kinases			
	2/4 siRNA	3/4siRNA	4/4siRNA	Total (% of Screen)
TF1	5	4	1	10 (1.7)
THP1	7	1	0	8 (1.4)

Table 1: Number of Sensitizing Kinases Identified In Kinome Screen. Log(siRNA/siRNA+AraC) ratios were calculated for the 2xsiRNA from 2 different screens giving a total of 4 data points. The median of the plate and screen was calculated and any kinase with >2/4 points that were >2SD from the median were considered hits. Above shows the total number of kinases that had 2/4, 3/4, or 4/4 kinases identified as "hits" as well as the total identified sensitizing kinases for each cell line.

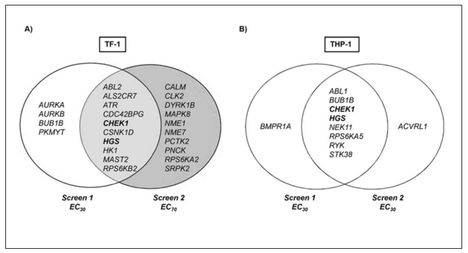

Figure 2: siRNA Screen Hits. Venn-diagram of hits from primary siRNA-kinome sensitizer screens with Ara-C. Genes/hits depicted in bold are common Ara-C sensitizers identified in both TF-1 and THP-1. (Figure taken from Tibes et al).[5]

Verification of Sensitizing Kinases:

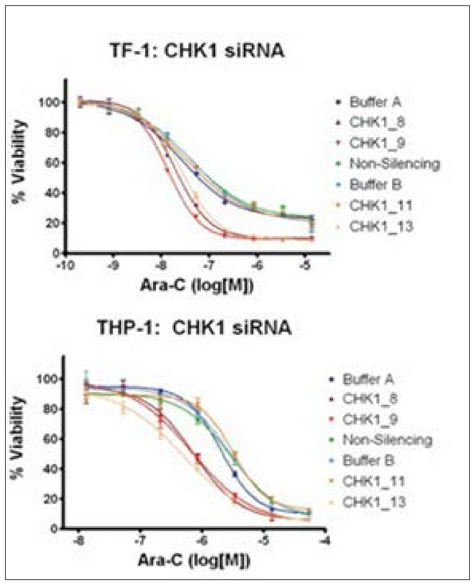

Figure 3: Drug Dose Response Curves of CHK1 siRNA + Ara-C. 4x siRNA of CHK1 was screened in THP-1 and TF-1 cells at varying concentrations of Ara-C. The IC_{50} was calculated for each of the siRNA's and compared to the negative control (Non-Silencing RNA) and buffer alone.

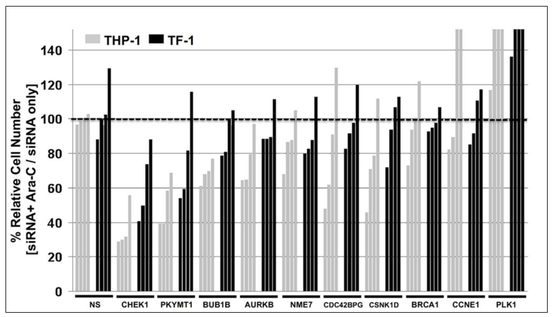

Figure 4: Validation of Hits by Secondary si RNA Screens. Validation of selected top hits from primary siRNA screens with 4x siRNA against each gene. Reduction in relative cell number expressed as Ara-C with siRNA versus Ara-C alone. NS: Non silencing control. Darkly-shaded bars correspond to TF-1 and lightly-shaded bars to THP-1. (Figure taken from Tibes et al).[5]

DISCUSSION

Kinase Screen:

▪Overall, we investigated Cytarabine resistance using high-throughput RNAi and identified multiple kinases that decrease cell viability when treated concurrently with cytarabine.

▪These kinases may be targets for new drug development and the data gathered from the screen gives us a platform to identify effective combination therapies

CHK-1 Validation Experiments:

▪Verification of drug sensitization was performed with additional siRNA sequences to ensure target specificity in drug dose response experiments utilizing an 11-point range of cytarabine concentrations.

▪CHK-1 shows a significant sensitization in THP-1 and TF-1 cells.

▪Currently CHK-1 inhibition is being tested in combination with Ara-C in clinical trials.

▪In addition, further investigation identified WEE1 kinase (not originally included in the kinome screen) as another potent sensitizer to Ara-C treatments. WEE1 similarly works at the G2/M DNA repair checkpoint and is being tested in various solid tumors in combination with cytotoxic chmotherapies.

▪Development of a clinical trial with WEE-1 inhibitors in combination with Ara-C in AML is currently underway.

Future Directions:

▪Further investigation is warranted to look at different combinations of small molecule inhibitors in AML. An siRNA platform can be used to identify rational drug and small molecule combinations in AML.

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