Exploring drug resistance mechanisms in different drug resistance models of an AML cell line using ResolveOME™ combined genomics and transcriptomics chemistry.

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Abstract

Examining single-cell heterogeneity is critical in understanding tumor heterogeneity, cancer clonal evolution, and drug resistance mechanisms. ResolveOME™ enables simultaneous assay of genomic and transcriptomic signatures of single cells. We applied ResolveOME to delineate the genomic and transcriptomic level changes leading to potentially varied drug resistance mechanisms in an AML model cell line called MOLM13. Using ResolveOME, we previously analyzed the genomic and transcriptomic information in a constant dose model of MOLM13 (2R). We treated non-resistant MOLM13 parental(P) cells with 2nM of quizartinib until the cells became resistant to the drug. In the current study, we attempt to apply ResolveOME to a dose escalation model, where we treated the MOLM13P cells with Quizartinib in 100pM dose step-up every week to where they become resistant to 2nM quizartinib. We aim to uncover the differences and similarities among drug-resistant mechanisms adapted by these two models using ResolveOME.

Initial CNV analysis of the WGS data showed that all 15 parental controls showed Chr.5, 6 & 13 trisomies and pentasomy of Chr.8, similar to the parental cell line control in the previous model.

Moreover, some single resistant cells in the previous 2R and the current step-up models did not show the Chr.5 aneuploidy. However, they acquired gain in 19q, indicating their potential involvement in drug resistance. Interestingly, 5/11 of step-up resistant cells showed quadruplication of a part of chr.1q and a small deletion in the Chr.2p; those we did not see in any resistant cells from the previous continuous dosage (2R) model. Markedly, Step-up resistant cells with 1q gain and 2p deletion specifically did not show Chr.5 aneuploidy or the gain in 19q, indicating the adaptation of a different mechanism of drug resistance in the step-up model from the previous 2R model. All the cells in the first 2R model cells have a key secondary mutation N841K in the Quizartinib target FLT3; however, this mutation is not present in some step-up resistant cells. We are further analyzing if the differential ploidy in the two models correlates with the presence and absence of key mutations in the two models.

In addition to these divergent CNV paradigms for these two drug-resistant models of the MOLM-13 cell line, using ResolveOME data, we are currently analyzing the SNV profiles and transcriptional adaptation of these cells for both types of drug treatment. We emphasize the AXL pathway bypass of FLT3 signaling inhibition via GAS6 upregulation in resistant cells. Finally, with this ResolveOME enabled multi-omic analysis, we strive to build a clear picture of the mechanisms of drug resistance adapted in the MOLM13 cell line.

Methods

ResolveOME™ unifies genomic and transcriptomic examination of single cells in one workflow.

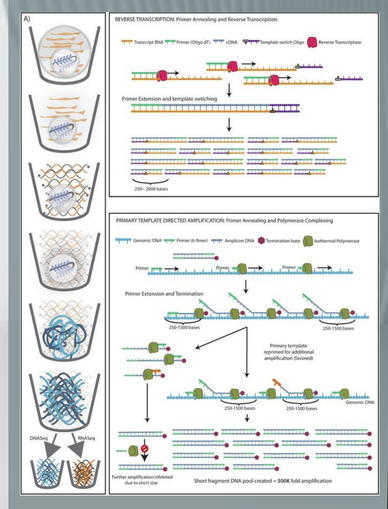


Figure 1. **ResolveOME™ combined whole genome and transcriptome schematic** (2) **: (A)** Single cells undergo 1st strand cDNA synthesis of cytosolic full-length mRNA molecules using a 5′ template-switching reverse transcription process. After the reverse transcription, whole–genome amplification from the nuclei is conducted (1&2). First-strand cDNA products are then enriched from the pool of amplified genomic DNA, prior to library preparation using the BioSkryb ResolveOME library preparation system. Analysis of the sequencing data is done with BaseJumper™ software.

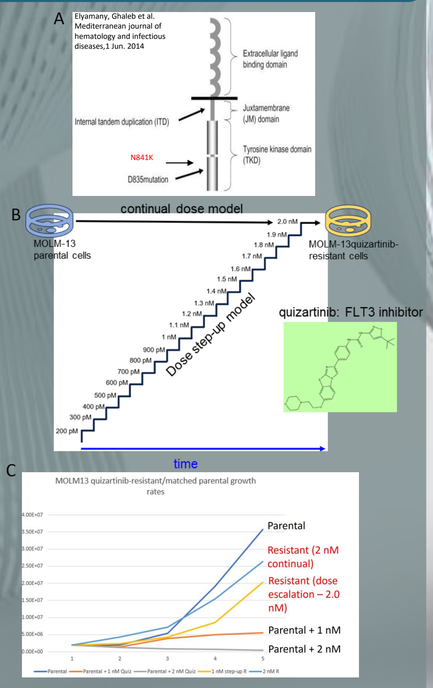


Figure 2: **Generation of acute myeloid leukemia resistance models:** (A) MOLM-13 cells harbor the internal tandem duplication (ITD) in-frame insertion mutation present in ~30% of AML patients and associated with poor prognosis (3&4). We verified the presence of this mutation in all parental and quizartinib-resistant cells and, identified a secondary *FLT3* mutation N841K in all resistant cells in the continuous dosage model. (B) MOLM-13 AML cells are sensitive to the FLT3 inhibitor quizartinib. We created two different quizartinib-resistant MOLM-13 lines. Firstly, a continual dosage model was created, with one month of constant treatment of 2 nM quizartinib.
(C). In the second model, MOLM13 drug-naïve cells were treated with 200 pM quizartinib, escalating by 100 pM ~ every 7-10 days to where they became resistant to 2 nM quizartinib (C).

Results

The WGA arm of ResolveOME™ uncovers heterogeneous copy number variations in two models of quizartinib resistance.

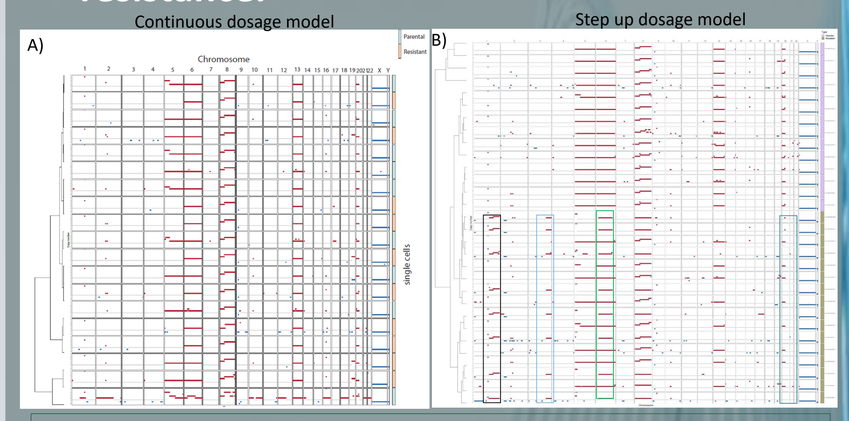


Figure 3: Copy number profiles of MOLM-13 drug resistance models: A) Copy number alterations of individual MOLM-13 parental and resistant cells from the continuous dosage model. Continuous dosage cells (rows) from parental (turquoise) and resistant (salmon) cells. Bin size is set at 500kb with Ginkgo.
B) Step up dosage cells(rows), parental (purple), and resistant (green) using a bin size of 500kb with Ginkgo. Key CNV changes in the SU model are highlighted with colored boxes.
MOLM-13 matched parental cell lines from both continuous dosage (CD) and Step-up (SU) models exhibited defined characteristics of the cell line such as trisomy of Chr.6 and Chr. 13 (A&B).
The dendrogram was generated based on the distance of each bin's average fold change from 2N.

- We found that none of the resistant cells from the CD model exhibited the additional 5p gain seen in the parental cohort, and 7/10 of CD-resistant cells did not have any amplification of Chr. 5 as a diploid 2n state, suggesting that this was selected to mediate drug resistance via gene expression changes in Chr.5-resident genes. In addition, we observed 19q gain uniquely in 4/10 resistance cells (Fig3 A).

- Interestingly, 8/13 resistant cells from the SU model showed gains in 1q & 3q that are not found in any of the CD-resistant cells. These 8 cells with 1q & 3q gain did not show trisomy of Chr. 6, pentasomy of 8, and 19q gain observed in the CD-resistant cells (Fig 3B). This CNV paradigm indicates that the drug resistance mechanism in the SU model could stem from a different set of copy number changes and associated gene expression alterations apart from the CD model of resistance.

A fraction of the MOLM-13 Quizartinib-resistant cells from the Step-up model show different SNV states and structural variations in the *FLT3* gene compared to the continuous dosage model.

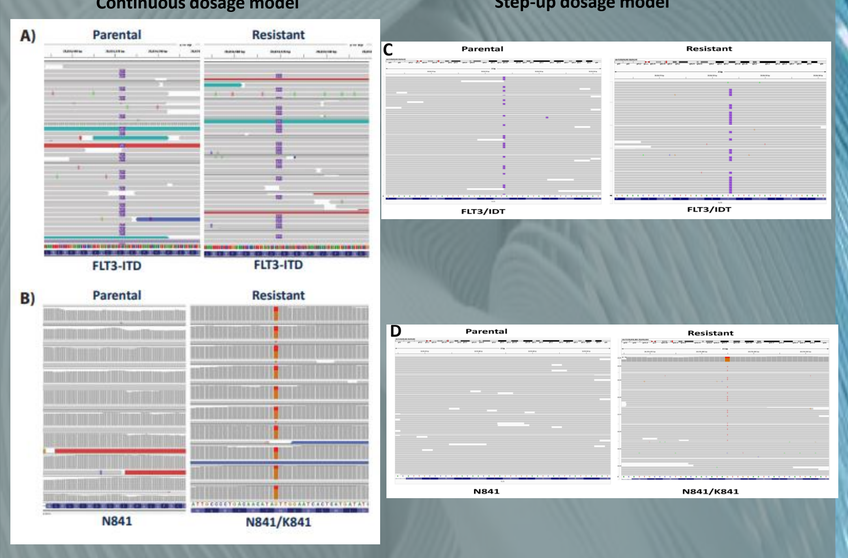
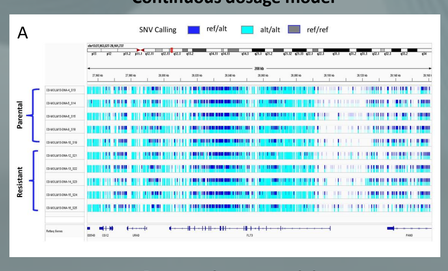


Figure 4. FLT3 gene in MOLM-13 cells: fms-like tyrosin kinase (FLT3) gene is on Chr 13q12 in humans. FLT3 ITD mutation is identified in parental and quizartinib-resistant single cells from both CD and SU models (A &C). (B&D) shows a novel FLT3 secondary mutation N841K in the tyrosine kinase domain exclusively in quizartinib-resistant cells from the CD model but absent in some of the resistant cells(2 cells at the end shown in figure D) from the SU model.
We hypothesize that the presence and lack of this key secondary mutation might be driving the mechanistic variation of drug resistance in these two models. Further experiments are needed to determine the exact contribution of this mutation to the FLT3 function and in the development of drug resistance.

Continuous dosage model



Step-up dosage model

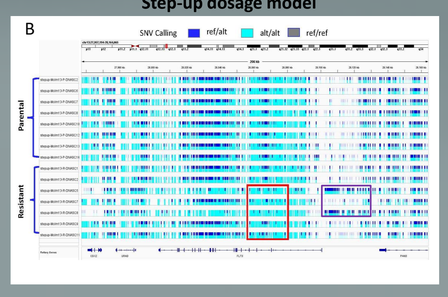
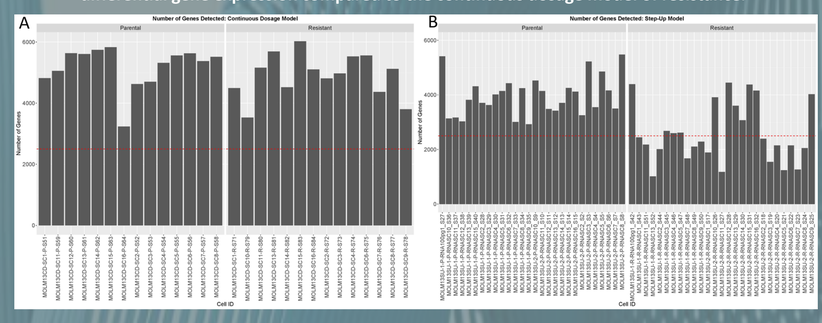


Figure 5: Structural variation in the *FLT3* Gene in Quizartinib-resistant MOLM-13 Cells:
We observed variations in allelic identities and exons between certain quizartinib-resistant and parental cells. This might point to structural variation discovered particularly in the Step-Up model (boxed in B) as a mechanism of AML or the treatment model that is not detected in the continuous dose model (A), and vice versa (possibly at exons 20 and 14 (N841/K841 and IDT)).

Results

Transcriptional signatures of the continuous and step-up dosage models of quizartinib resistance revealed by ResolveOME™.

The quizartinib dose step-up expressed a smaller number of genes and less defined differential gene expression compared to the continuous dosage model of resistance.



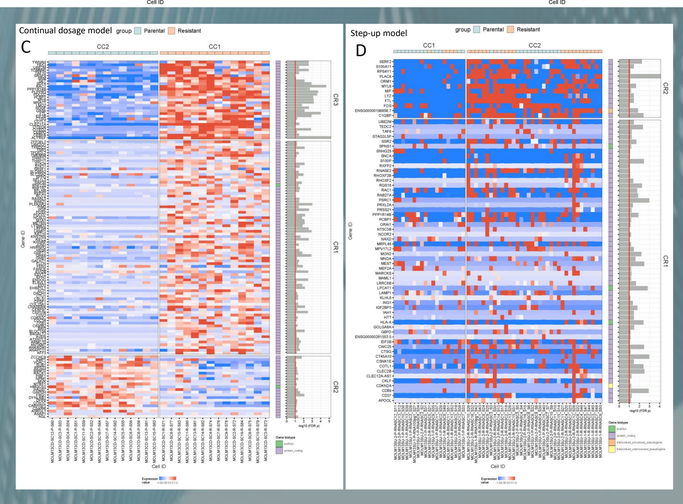
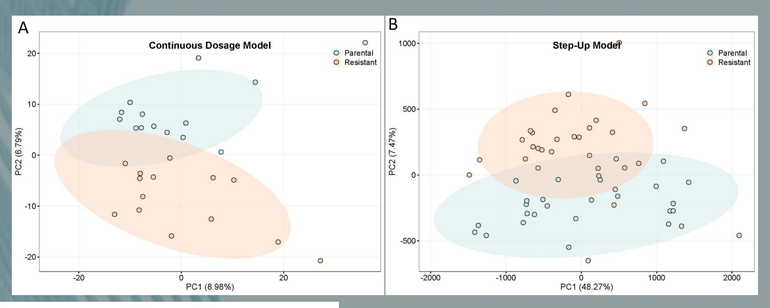


Figure 6: Transcriptional signatures of single cells revealed by the RNAseq arm of the ResolveOME™: (A) In the CD model of resistance parental and resistant cells did not show a significant difference in the number of genes expressed and both cell types expressed more than 2500 genes. (B) However, in the SU model, many resistant cells (16/26) expressed a low number of genes compared to the matched parental cells. (C&D) Resistant cells from the SU model showed fewer differentially expressed genes compared to the resistant cells from the CD model.



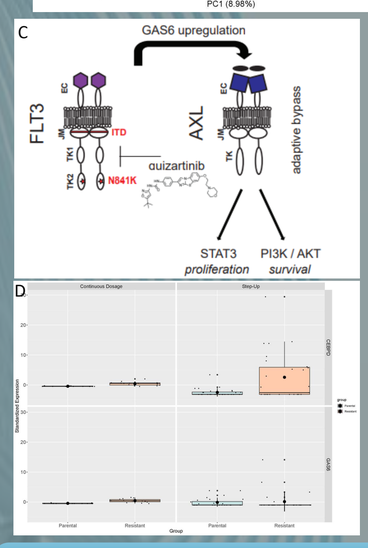


Figure 7: Principal component analysis for MOLM-13 Quizartinib resistance: Continuous Dosage and step-up models: (A&B) In both drug-resistance models parental and resistant cells are projected differentially although with different patterns depending on the mode of dosage.
(C) Schematic showing that upon *FLT3* inhibition by quizartinib, *GAS6*, the ligand for the receptor tyrosine kinase AXL, is upregulated in resistant MOLM-13 cells to drive growth and survival
(D) *GAS6 and CEBP* (a tumor suppressor & a transcription factor gene on Chr.19q13.1. Truncation mutations of *CEBP* gene found in some AML patients(5)) genes are slightly upregulated in the resistant cells from the CD model, supporting the transcriptional bypass by *GAS6* proposed in (C). However, upregulation of *GAS6 & CEBP* is not observed in many cells from the SU model indicating that a different mode of resistance is potentially adapted in the SU model.

Summary

- ResolveOME™ whole genome amplification arm enabled the unraveling of distinctive copy number variations between continuous dosage and step-up dosage models of quizartinib drug resistance in the MOLM-13 AML cell line.
- Further analysis of whole genome sequencing data using BaseJumper™ resolved differences in a key mutation and structural differences in AML-associated gene *FLT3* between the resistance cells of the two models.
- The RNA-seq arm of the ResolveOME™ enabled the delineation of variations in the transcriptional signatures in resistant cells from continuous dosage versus step-up dosage.
- Simultaneous examination of genomic and transcriptomic changes using ResolveOME™ in the MOLM-13 AML cell line indicates that distinctive mechanisms of resistance is employed by the cells based on the method of dosage with quizartinib.

References

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