Abstract #1037: Association of C-MYC, MYC target gene, and unfolded protein response (UPR) expression with clinical benefit from the oral aurora kinase A (AURKA) inhibitor, alisertib (A), in combination with paclitaxel (P) compared with P alone in patients (Pts) with HER2-negative metastatic breast cancer (MBC)

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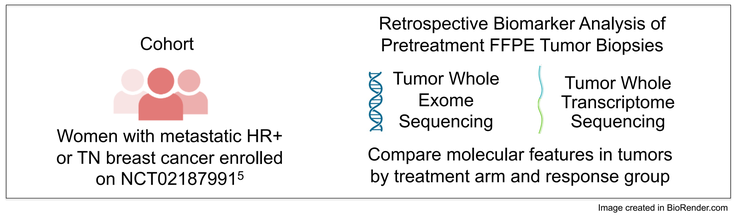
Background

AURKA is a key regulator of the mitotic spindle, G2/M checkpoint and epithelial-mesenchymal transition[1].

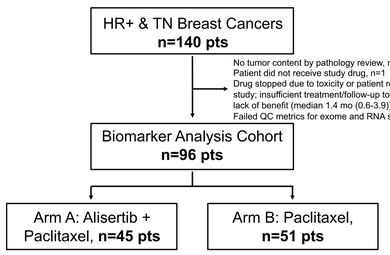
AURKA is amplified and/or overexpressed in breast cancer and is associated with therapy resistance and worse survival[2-4].

A randomized phase II trial in hormone receptor (HR)-positive, HER2-negative and triple negative (TN) metastatic breast cancer patients showed that addition of Alisertib to weekly Paclitaxel significantly improved progression-free survival (PFS) compared with Paclitaxel alone[5].

Here, pretreatment archival tissues from this clinical trial were analyzed for biomarkers associated with clinical benefit from Alisertib.



Methods



	Alisertib + Paclitaxel, n=45 pts	Paclitaxel, n=51 pts
Median Age, Years	63 (33-79)	62 (39-82)
Tissue Site	Primary: 28 (62%) Met: 17 (38%)	Primary: 28 (55%) Met: 23 (45%)
HR+ TN	35 (78%) 10 (22%)	42 (82%) 9 (18%)
Prior Chemotherapy	29 (64%)	38 (75%)
Prior CDK4/6i (HR+ only)	12 (34%)	10 (24%)

Figure 1. Biomarker Analysis Cohort. Full patient characteristics have been previously published[5]. Progression free survival (PFS) curves of this biomarker analysis cohort were concordant with the full study population. Median PFS A+P: 9.6 months; P: 6.0 months. HR+: Hormone receptor positive. TN: Triple negative. Pts: Patients. Met: Metastasis.

Sample Processing
Archival formalin-fixed, paraffin-embedded (FFPE) tumor tissue blocks (n=85) or slides (n=55) were available from primary or metastatic tumor sites, collected prior to treatment on the trial, for 140 participants in the NCT02187991 clinical trial[5]. Sample collection years ranged from 2006 to 2018. Blocks were sectioned and de-identified H&E stained slides were reviewed by an external pathologist for tumor content evaluation. Tumor content estimates were provided per sample, and areas of high tumor content marked for macrodissection to allow for enrichment of tumor. The median tumor content estimate was 90%.

Exome and RNA Sequencing
DNA and RNA were extracted from 10 FFPE tumor tissue sections using the Qiagen AllPrep kit. Exome libraries were prepared using the Twist Enzymatic Fragmentation 2.0 kit with exome target capture using Twist Exome 2.0 (Twist Biosciences). RNA libraries were prepared using whole-transcriptome random primed reverse transcription and ribodepletion with KAPA RNA HyperPrep with Riboerase kit (Roche). Sequencing was completed using the Illumina NovaSeq 6000 reagents and instruments. A minimum of 50X coverage for 50% of the exome target space was required for exome analysis and minimum of 50M reads required for RNA analysis. The average mean target coverage for the analysis cohort was 198X for tumor exomes, and average number of RNA reads was 315M. 88 samples passed QC measures for exome sequencing and 66 samples passed QC measures for RNA-sequencing analysis.

Variant Calling
Tumor-only variant calling was performed using TGen's Phoenix pipeline v1.2.0 (https://github.com/tgen/phoenix). Briefly, FASTQ files were aligned to the GRCh38 human reference genome using bwa. Single nucleotide variants and small insertions and deletions were called using a consensus approach, filtering to nonsynonymous coding variants called by three out of five variant callers (Lancet (v1.1), MuTect2, Octopus (v0.6.3), Strelka2 (v2.9.10), and varDict (v1.7.0)). Copy number analysis was performed using GATK CNV (v4.1.8.0) and a panel of unrelated normal samples. RNA FASTQs were aligned to GRCh38 using STAR and transcript quantification performed using Salmon (v1.2.1).

RNA Analysis
DESeq2 (v 1.32.0) was used to generate a normalized read count matrix for the analysis cohort. Log transformed normalized count expression values for specific genes of interest (*MYC*, *AURKA*, *TP53*, *FOXM1*, *MYBL2*, *E2F1*) were compared across treatment arms and treatment response groups. A tertile threshold was used to define tumors with high *MYC* expression (top tertile) and low *MYC* expression (bottom tertile). Gene set enrichment analysis was performed using GSEA v4.2.3 using the 50 Cancer Hallmark Gene Sets from the Molecular Signatures Database[6] and samples grouped by treatment arm and response.

Statistics
Progression-free survival of 6 months or greater was used to define response. Secondary analysis was performed focused on participants with exceptional response (PFS ≥12 mo) compared to those with PFS <6 mo PFS. Kaplan-Meier survival analysis was performed using Prism 9.5.0. A Fisher Exact test was used to compare the frequency of mutations in specific genes (categorical values, mutant or non-mutant) in treatment arm and response groups. An unpaired t-test was used to evaluate differences in expression values for single genes between two groups. Brown-Forsythe and Welch ANOVA tests were performed with Dunnett's T3 multiple comparisons test to compare expression of a single gene (log2 normalized counts) across multiple groups (treatment arms and treatment response). p-values <0.05 were considered significant.

Conclusions:

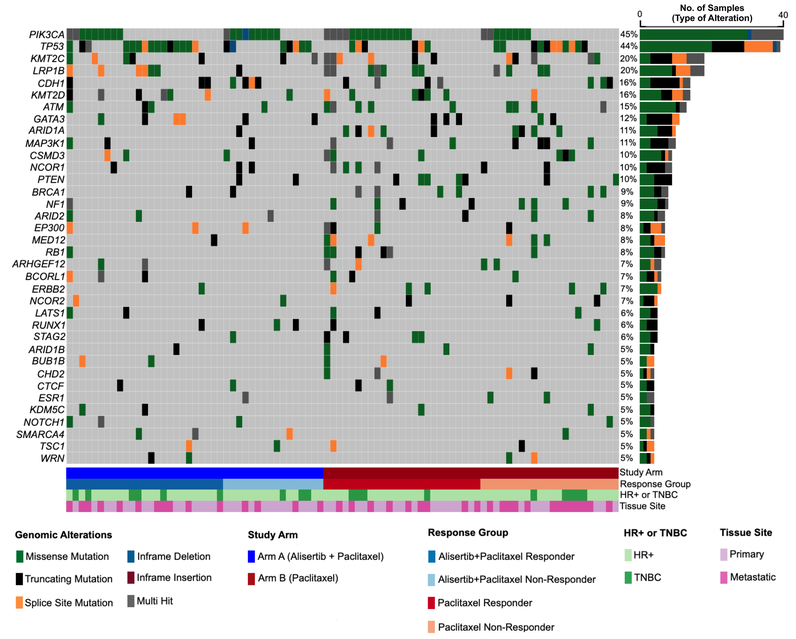
Patients whose breast cancers had increased *MYC* expression and high *MYC* activation derived greater clinical benefit from Alisertib + Paclitaxel than from Paclitaxel alone.

EMT signaling did not preclude prolonged response (≥12 months PFS) to Alisertib + Paclitaxel.

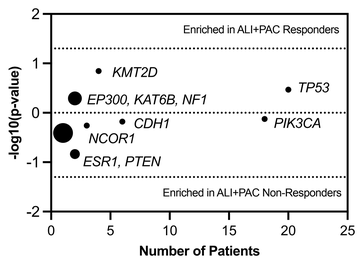
Results

While Genomic Alterations Were Not Significantly Associated with Response to Alisertib + Paclitaxel, the Presence of Common Oncogenic Mutations Did Not Preclude Response to Alisertib + Paclitaxel

A.



B.



C.

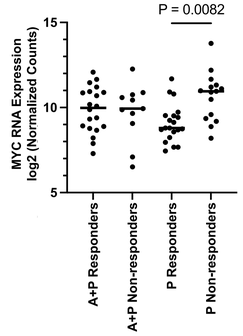
Gene	Alteration	Cancers (n)
AKT1	Hotspot missense mutation (E17K)	1
ARID1B	Truncating mutation	1
ATM	Truncating mutation	1
BRCA1	Truncating mutation	1
CCND1	Amplification	4
CDH1	Truncating mutations	3
EP300	Splice site mutations	2
ERBB2	Hotspot missense mutation (L755S)	1
GATA3	Truncating, Splice site mutations	3
KMT2C	Truncating, Splice site mutations	3
KMT2D	Truncating, Splice site mutations	4
MAP3K1	Truncating mutation	1
MDM2	Amplification	1
MYB	Amplification	1
NCOR1	Truncating mutation	1
NCOR2	Truncating mutation	1
NF1	Truncating mutation	1
NOTCH1	Truncating mutation	1
PIK3CA	Hotspot missense mutations (N345K, E542K, E545A/K, H1047R)	9
SMARCA4	Truncating mutation	1
TP53	Hotspot missense, truncating mutations	13
TSC1	Splice site mutation	1
WRN	Truncating mutation	1

Figure 2. Genomic alterations detected in archival breast cancers in the biomarker analysis cohort. (A) Oncoprint depicting genomic alterations detected in archival FFPE breast cancers collected prior to treatment on the NCT02187991 clinical trial. Response is defined as PFS ≥ 6 months. HR+: hormone receptor positive; TNBC: triple negative breast cancer. (B) Enrichment plot displaying associations between gene alterations and response to Alisertib + Paclitaxel. Enrichment is determined by Fisher's exact test with a p value <0.05. Size of the circle reflects the number of genes. Genes represented by the largest circle (n=11) are not listed. Dotted lines indicate the threshold for significance. (C) Table displaying oncogenic or likely-oncogenic variants detected in breast cancers from patients with a PFS ≥ 6 months in the Alisertib+Paclitaxel arm (n=25 tumors).

Results

MYC Expression and *MYC* Activation Were Associated with Response to Alisertib + Paclitaxel

A.



MYC tumor expression was elevated in cancers that did not respond to Paclitaxel alone. Most patients with high *MYC* tumor expression developed progressive disease within 6 months (9/12, 75%) with Paclitaxel alone.

The majority of patients with high tumor *MYC* expression on the Alisertib + Paclitaxel arm had PFS ≥ 6 months (7/10, 70%).

Three patients on the Alisertib + Paclitaxel arm had PFS ≥12 months in the presence of high *MYC* tumor expression. All patients on the Paclitaxel alone arm with high tumor *MYC* expression had disease progression within 12 months.

In Gene Set Enrichment Analysis, *MYC* targets and unfolded protein response gene sets were enriched in pretreatment cancers from Alisertib + Paclitaxel responders compared to Paclitaxel responders, and in Paclitaxel non-responders compared to Paclitaxel responders.

Wnt beta-catenin signaling was enriched in non-responders to both Paclitaxel alone and Alisertib + Paclitaxel.

B.

Enrichment Group	Enriched Hallmark Gene Sets	P-value
Enriched in **A+P Responders** (vs P Responders)	MYC Targets v2	0.024
	Unfolded Protein Response	0.028
	MYC Targets v1	0.092
Enriched in **A+P Non-Responders** (vs A+P Responders)	Wnt Beta-Catenin Signaling	0.086
Enriched in **P Non-Responders** (vs P Responders)	MYC Targets v2	0.008
	Unfolded Protein Response	0.006
	MYC Targets v1	0.037
	Wnt Beta-Catenin Signaling	0.030

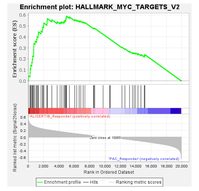


Figure 3. High *MYC* expression, *MYC* activation, and Unfolded Protein Response are associated with response to Alisertib + Paclitaxel (A+P) compared to Paclitaxel (P) alone. A. *MYC* RNA expression levels by trial arm and response group. 66 samples had RNA-sequencing data available (n=31 Arm A; n= 35 Arm B). Tumors in the Paclitaxel non-response group (<6 months PFS) showed elevated *MYC* expression. There was no significant difference in expression for other genes associated with AURKA activity (*AURKA*, *TP53*) or for regulators of mitotic transcription (*FOXM1*, *MYBL2*, *E2F1*) (not shown). High *MYC* expression was defined as the top tertile across the cohort (n=10 in Arm A, n=*12* in Arm B). Response to Alisertib + Paclitaxel or Paclitaxel alone was evaluated in the *MYC* high tumors. B. Gene Set Enrichment Analysis (GSEA) of the 50 Cancer Hallmark Gene Sets from the Human Molecular Signatures Database[6] by trial arm and response. Selected gene sets with p-values <0.1 are shown. An example enrichment plot for *MYC* targets is shown.

Cancers from Exceptional Responders to Alisertib + Paclitaxel Showed Enrichment for *MYC* and EMT Gene Sets

Enrichment Group	Enriched Hallmark Gene Sets	P-value
Enriched in **A+P Exceptional Responders (PFS ≥12mo)** (vs P Exceptional Responders)	MYC Targets v1	<0.001
	MYC Targets v2	<0.001
	Epithelial Mesenchymal Transition	<0.001
Enriched in **A+P Exceptional Responders** (vs A+P Non-Responders)	MYC Targets v1	<0.001
	Epithelial Mesenchymal Transition	<0.001
Enriched in **A+P Non-Responders** (vs A+P Exceptional Responders)	Wnt Beta-Catenin Signaling	0.022
Enriched in **P Non-Responders** (vs P Exceptional Responders)	MYC Targets v1	<0.001
	MYC Targets v2	<0.001
	Wnt Beta-Catenin Signaling	0.002
	Epithelial Mesenchymal Transition	<0.001
	E2F Targets	<0.001
	G2M Checkpoint	<0.001
	Mitotic Spindle	<0.001

Cancers from exceptional responders on Alisertib + Paclitaxel showed **elevated *MYC* and EMT activation** compared to cancers from patients whose disease progressed within 6 months of initiating Alisertib + Paclitaxel or those with exceptional response to Paclitaxel alone.

Figure 4. Tumors from patients with ≥12 month PFS with Alisertib+Paclitaxel show enrichment for MYC targets and EMT. Gene Set Enrichment Analysis of the Cancer Hallmark Gene Sets by trial arm and response was performed. Selected gene sets with p-values <0.1 are shown. Alisertib+Paclitaxel exceptional responders (PFS ≥12mo) n=12; Alisertib+Paclitaxel non-responders (PFS<6mo) n=11; Paclitaxel exceptional responders (PFS ≥12mo) n=4; Paclitaxel non-responders (PFS<6mo) n=15.

Acknowledgements

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