Serial monitoring of Circulating Tumor Cell Genomic Alterations in ER Positive/HER2 Negative Advanced Breast Cancer During Endocrine Therapy

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ABSTRACT

Background:

Nearly all estrogen receptor (ER) positive (POS) metastatic breast cancers become refractory to endocrine (ET) and other therapies leading to evolution of lethal disease presumably due to emerging genomic alterations. Timely monitoring of the molecular events associated with response/progression in serial tissue biopsies is logistically difficult. Use of liquid biopsies, including circulating tumor cells (CTC) might provide informative, yet easily obtainable evidence for better precision oncology care.

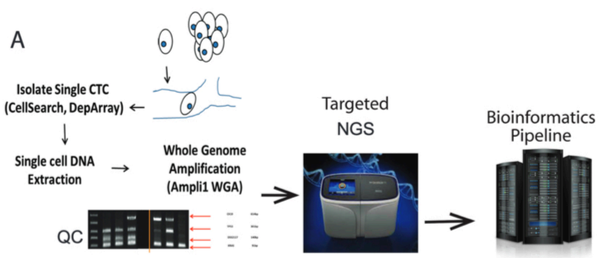
Methods:

Whole blood (WB) specimens were collected at serial time points from patients with advanced ER POS/HER2 negative (NEG) advanced breast cancer in a Phase I trial of AZD9496, an oral selective estrogen receptor degrader ET. Individual CTC were isolated from WB using tandem CellSearch®/DEPArray™ technologies and genomically profiled by targeted single-cell DNA next generation sequencing (scNGS).

Results:

High-quality CTC (n=123) from 12 patients profiled by scNGS showed 100% concordance with ctDNA detection of driver ESR1 mutations. We developed a novel CTC-based framework for precision medicine actionability reporting that incorporates clonal predominance and zygosity of targetable alterations, both unambiguously identifiable by CTC scNGS. Thus, we nominated opportunities for targeted therapies in 73% of patients, directed at alterations in PIK3CA, FGFR2, KIT, and BRAF. Intra-patient, inter-CTC genomic heterogeneity was observed, sometimes present between timepoints, especially in subclonal alterations.

Figure 1



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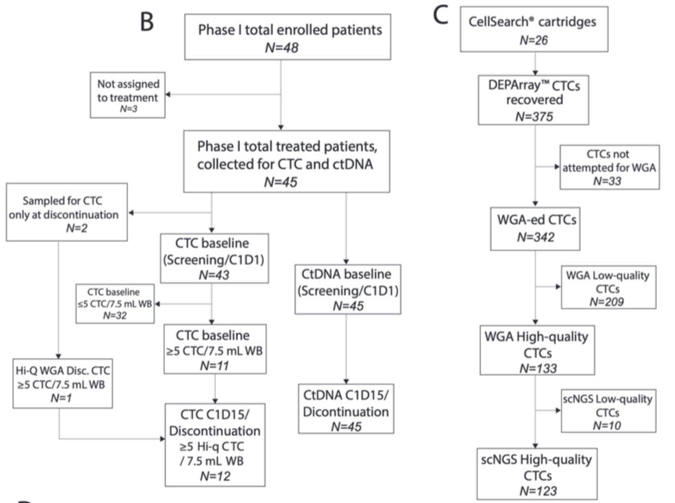


D

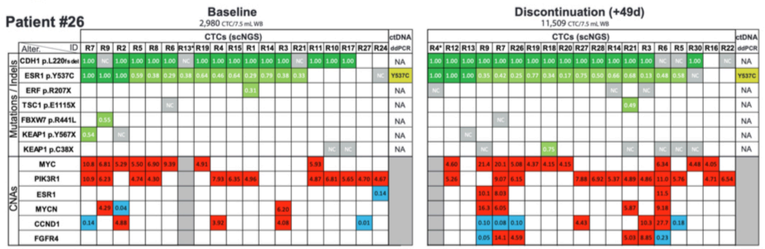
Patient ID:	***#06***	***#13***	***#17***	***#20***	***#26***	***#27***	***#30***	***#31***	***#34***	***#35***	***#36***	***#44***
# Cartridges[$]:	3	2	3	1	2	2	2	2	2	2	2	2
#CTC recovered[$]:	21	16	102*	17	61	20	16	13	41	30	10	19
#Hi-Q WGA CTC :	4	2	30	7	42	3	6	3	20	10	4	2
#Hi-Q scNGS CTC :	4	2	30	7	38	3	3	3	20	10	1	2

E

Sample	Mapped Reads	On-Target Reads	Mean Depth	Uniformity	Mean Read Length (bp)
Mean (Range)	2,932,356 (644,022 - 4,973,416)	90.5% (24.1 - 96.2%)	983x (47 - 1,711x)	40.1% (25.4 - 63.8%)	141 (99 - 152)

Figure 1. Consort diagram of AZD9496 phase I study patients and their CTC and ctDNA obtained at each time point.
A) Workflow of CTC enrichment (CellSearch®), isolation (DEPArray™), whole genome amplification (WGA), quality control (QC), tageted next generation sequencing (NGS) and analysis. B) Consort diagram of patients attempted for CTC and ctDNA collection showing cases with informative analytes for each time point. C) Flow chart of the total number of the cartridges and CTCs in each of the categories in panel 1A at each processing step. D) Details of CTC isolation for each patient at each processing step, showing numbers of: CellSearch® cartridges assayed, DEPArray™-recovered cells, high-quality whole genome amplified cells and high-quality scNGS cells. One cartridge (9 recovered cells) from panel 1B, not included here belongs to a discontinuation-only patient with no high-quality CTC who was excluded from further study. *33 of 102 CTCs for patient #17 were not WGA'd, in order to minimize unnecessary costs. E) ScNGS of 123 high-quality patient CTCs generated sequencing data. Table shows mean of totalreads mapped (to the refererence human genome), on-target reads (mapping to the panel target regions), mean depth of sequencing, uniformity (measuring distribution of reads throughout target regions and defined as percentage of reads having at least 0.2x of the mean coverage. Uniformity determination includes all panel amplicons, including those afected by WGA restriction enzyme treatment). Mean read length in base-pairs (bp) is shown.

Figure 2



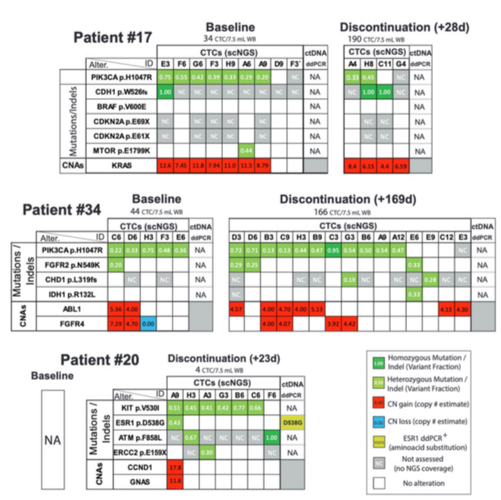


Figure 2. Integrative heatmap of putative driver genomic alterations detected by CTC scNGS and ctDNA ddPCR. Comprehensive genomic analysis of individual CTCs in four of the 12 patients. For each patient, total CTC count at each time point is shown. Columns represent individual CTCs. White boxes indicate adequate coverage and absence of the variant. For mutations/indels (top of each table) colored boxes indicate mutation presence, with dark and light green representing homo- and heterozygous mutations, respectively. Numbers inside colored boxes represent the variant read fraction. Grey "NC" boxes indicate no NGS coverage for that position. Mutations private to single cells are shown only for select cases. For CNAs (bottom of each table), estimated copy number is calculated back from the log2(Tumor/Normal Copy Ratio) value. High-confidence, high-level copy changes (<0.25 or >4.0 estimated copies) are shown. Only high-level CNAs present in >1 CTC are shown. *Cells with suboptimal CN data. Patient #17 baseline CTC F3' is from C1D1 whereas the rest form screening. DdPCRESR1 LBD mutation presence/absence and amino acid change are shown at the right end of each table. Later time points for ddPCR consist of C1D15 samples only. NA, not available (Patient #20 was only drawn for CTC at discontinuation). Empty gray box indicates low quality copy number data. Not all CTC harboring only single private alterations, or epithelial cells without alterations, are shown.

Table 1

	ESR1 LBD mutation	
Patient ID	ctDNA (ddPCR)	CTC (scNGS)
#26	+	+
#20	+	+
#35	+	+
#06	+	+
#31	+	+
#17		
#34		
#13		
#27		
#36		
#44		

Table 1. Concordance between CTC and ctDNA ESR1 LBD mutations. Concordance for the presence of identical ESR1 LBD mutation between CTC and ctDNA for each of the 11 evaluable patients is shown. Green (+) represents mutation presence in at least one time point.

Table 2

	Evidence of Actionability				
Patient ID	Alteration#	Tier	Description	Drug	Trial ID
34	PIK3CA p.H1047R	1-CLA	FDA-approved in aBC	Alpelisib	
17	PIK3CA p.H1047R	1-CLB	FDA-approved in aBC	Alpelisib	
20	KIT p.V530I	2-CLA	FDA-approved, off-label	Imatinib	
13	CCND1 amplification	3-CLA	Clinical Trial	Palbociclib	NCT02465060: Targeted Therapy Directed by Genetic Testing in Treating Patients With Advanced Refractory Solid Tumors, Lymphomas, or Multiple Myeloma (The MATCH Screening Trial)
35	MYC amplification	3-CLB	Clinical Trial	M6620 (VX-970)	NCT03718091: M6620 (VX-970) in Selected Solid Tumors
06	ESR1 p.Y537N	3-CLB*	Clinical Trial	Lasofoxifene	NCT03781063: Evaluation of Lasofoxifene Versus Fulvestrant in Advanced or Metastatic ER+/HER2- Breast Cancer With an ESR1 Mutation
26	MYC amplification	3-CLB	Clinical Trial	M6620 (VX-970)	NCT03718091: M6620 (VX-970) in Selected Solid Tumors
27	MYC amplification	3-CLB	Clinical Trial	M6620 (VX-970)	NCT03718091: M6620 (VX-970) in Selected Solid Tumors
31	None				
36	None				
44	None				

One representative alteration from the best evidence-level tier is shown if multiple actionable targets are present. If multiple from the best possible tier are present in a patient, examples different from those in other patients are chosen to illustrate a variety of alternatives. $At the time of reporting

Tier of Evidence Level	Description
1	FDA-approved in MBC
2	Recommended off-label use by practice guidelines for FDA-approved drugs in MBC or other cancer indications
3	Currently-recruiting[$] clinical trials investigating a therapy targeted to the alteration or immediate pathway
4	Strong biological plausibility and preclinical evidence

Tier: 1, 2, 3, 4, Null

Subtier	Clonality
CLA	>80%
CLB	50-80%
CLc	<50%

Zygosity
★ = Homozygous/ LOH/complete deletion

Table 1. Targetable alterations identified in CTC via a novel platform. One representative alteration from the best evidence-level tier and subtier is shown if multiple actionable targets are present#. If multiple alterations from the best possible tier are present in a patient, examples different from those in other patients are chosen to illustrate a variety of alternatives. $At the time of reporting.

CONCLUSIONS

- **Serial monitoring of the CTC genome is feasible and enables real-time tracking of tumor evolution**
- **Considerable intra-patient, inter-CTC genomic heterogeneity was observed, at times present between timepoints.**
- **We developed a novel CTC-based framework for precision medicine actionability reporting**
- **It incorporates clonal predominance and zygosity of targetable alterations**
- *CTC genomic profiling can complement ctDNA as a liquid biopsy platform suited to detection and monitoring of tumor clonal evolution, gene level CNA and alteration zygosity.*