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Integrating Whole Exome Sequencing Data with RNASeq And Quantitative Proteomics to Better Inform Clinical Treatment Decisions in Patients with Metastatic Triple Negative Breast Cancer

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Background

- The integration of next-generation sequencing (NGS) into clinical practice has significantly advanced personalized medicine for patients with breast cancer
- NGS enables the precise identification of clinically relevant genomic alterations, allowing physicians to select appropriate targeted therapies for their patients
- NGS has also highlighted the heterogeneity of breast cancer, and there remains the challenge of understanding whether and how tumor heterogeneity confounds molecular analysis and treatment decisions
- The Intensive Trial of OMics in Cancer study (ITOMIC; NCT01957514) enrolls patients with metastatic triple negative breast cancer (mTNBC) who are platinum-naïve and scheduled to receive cisplatin
  - Primary objective: to establish the safety and feasibility of collecting, analyzing, and storing panomic and other data from serially monitored patients with TNBC
- We investigated intrapatient and temporal tumor heterogeneity among 12 patients enrolled in the ITOMIC study to determine the potential benefit of panomic analysis to inform treatment decisions

Patients and Methods

Study Design

- Eligibilty: mTNBC, platinum-naïve, scheduled to receive cisplatin, ECOG PS of 0–1, no known brain metastases, no bleeding disorders, and life expectancy of at least 6 months
- Multiple biopsies of up to 7 metastatic sites are performed before administration of cisplatin and repeated upon completion of cisplatin and following subsequent therapies
- A subset of tumor specimens is chosen for DNA sequencing, RNA sequencing, and quantitative proteomics

Genomic Analysis

- Between 10 and 103 tissue samples/biopsy specimens were obtained from each patient from 1–21 different time points.
- Blood samples were collected for matched tumor-normal genomic analysis
- DNA sequencing data were processed using Contraster[1] and MuTect.

Proteomic Analysis

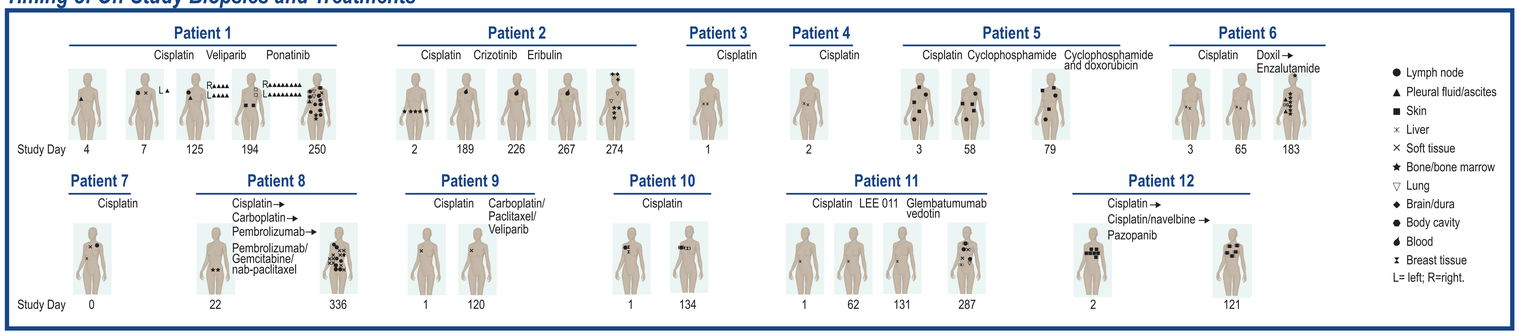
- Proteomics analysis was done using a quantitative, multiplexed, selected reaction monitoring-mass spectrometry assay comprising a panel of 54 proteins
- A subset of FFPE tumor samples (for which sufficient material was available) were laser microdissected, solubilized, and enzymatically digested
- Absolute quantitation of proteins was accomplished through the simultaneous detection of endogenous targets and identical, synthetic, labeled heavy peptides; protein levels were normalized to total protein extracted from each sample

References

1. Sanborn JZ, Salama SR, Grifford M, et al. *Cancer Res.* 2013;73:6036-6045.
2. Vaske CJ, Benz SC, Sanborn JZ, et al. *Bioinformatics.* 2010;26:i237-245.
3. Sedgewick AJ, Benz SC, Rabizadeh S, Soon-Shiong P, Vaske CJ. *Bioinformatics.* 2013;29:i62-70.

Results

Timing of On-Study Biopsies and Treatments

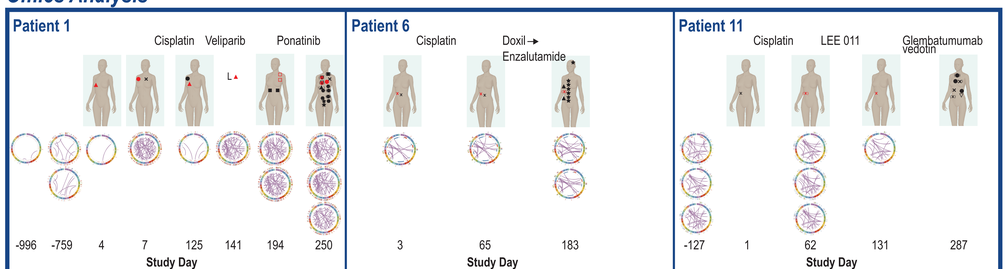


Patient Baseline Characteristics

Characteristic	All Patients (N=12)
Age, median (range) years	57 (37–77)
Prior therapy, n (%)	
Surgery	9 (75)
Radiotherapy	8 (67)
Systemic therapy	11 (92)
Number of prior systemic therapies, n (%)	
0	1 (8)
1	4 (33)
2	0
≥ 3	7 (58)
Lesions, n (%)	
Lymph node	12 (100)
Bone	6 (50)
Liver	5 (42)
Lung	1 (8)
Soft tissue/other organ	1 (8)

- There were no adverse events grade > 2 attributable to biopsies (23 procedures performed on 12 patients)
- 8 of 12 patients (67%) received treatments (post-cisplatin) based on the molecular profile of their tumors
  - Three patients achieved partial responses (PR): one with two *FGFR2* activating mutations treated with ponatinib; one with a germline *BRCA2* mutation treated with carboplatin/paclitaxel/veliparib; one with highly expressed GPNMB treated with glembatumumab vedotin
  - Four patients showed progressive disease when treated with crizotinib, enzalutamide, cyclophosphamide, or pembrolizumab

Omics Analysis



Patient 1

45-year-old woman diagnosed with TNBC early 2011; poor response to neoadjuvant chemotherapy after left mastectomy. Subsequent involvement of right breast, radical mastectomy late 2012, then metastases involving bone and lymph nodes 2013. She received multiple rounds of prior chemotherapy.

DNA Sequencing

2 separate activating mutations of *FGFR2* (S252W and Y376C) after neoadjuvant chemotherapy; mutation in *PIK3C2G* (D1298N); 7-fold amplification of *KRAS*.

Quantitative Proteomics

Autopsy sample: FGFR2 not detected; EGFR 687 amol/µg; KRAS not reportable.

Treatments and Response

Treated with PARP inhibitor veliparib (based on somatic loss of BRCA1) with no response, then FGFR inhibitor ponatinib with a PR. Autopsy samples demonstrated persistence of FGFR2 (S252W; Y376C) despite PR to FGFR2 inhibitor.

Patient 6

67-year-old woman diagnosed with ER+/PR+ stage 1 breast cancer in 2006. Developed mTNBC in 2013, despite hormonal therapy, with involvement of the liver, bones, and lymph nodes. Before enrollment, progressed despite treatment with radiation, nab-paclitaxel, and capecitabine.

DNA Sequencing

PIK3CA mutation (H1047R) at all time points; 24-fold amplification of *KRAS*; 20-fold amplification of *PI3KC2G*

Quantitative Proteomics

Autopsy sample: HER2 688 amol/µg; AR not detected, although AR+ at day 65; KRAS not reportable.

Treatments and Response

Treated with enzalutamide because an early biopsy was AR+; became resistant to enzalutamide; liver metastasis at time of death was AR- and HER2+.

Patient 11

41-year-old woman diagnosed with mTNBC (left breast mass) in 2014. Received 6 cycles of TAC followed by a left mastectomy (showed significant residual disease) and local radiation. Late 2014 she developed metastases involving the bone and liver and was enrolled into the study.

DNA Sequencing

6.7-fold amplification of *IL6*; *KDR* G335V mutation emerged in a subclone and increased from 4% at day 62 to 37% at day 131; *CDK6* amplification at day -127; LOH of most of chromosome 13, including the *Rb* locus.

Quantitative Proteomics

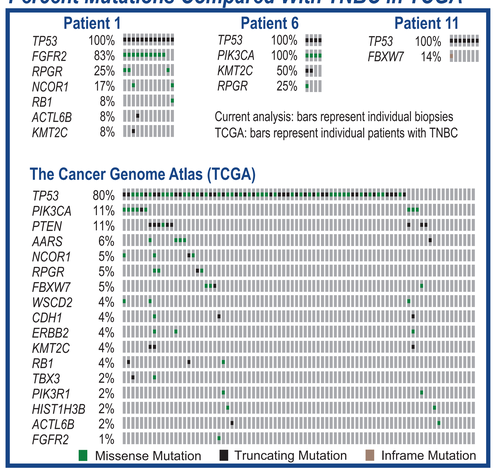
Three samples from mastectomy at day -127 post neoadjuvant TAC: expression of MRP1, ERCC1, P16, GPNMB.

Treatments and Response

LEE011 administered based on amplification of *CCND1* with no response; glembatumumab vedotin administered based on high expression of GPNMB, resulting in a PR.

Red symbols denote sites that underwent whole exome sequencing + whole genome sequencing.

Percent Mutations Compared With TNBC in TCGA



Conclusions

- In this feasibility clinical study, comprehensive omic analysis of patients with multiple biopsies shows high heterogeneity within and among primary and metastatic sites and illustrates the evolution of mutagenesis over time.
- Patient 1 responded to ponatinib as predicted by the presence of activating genomic mutations in *FGFR* but subsequently progressed, potentially due to loss of FGFR protein expression.
- Patient 6 responded to enzalutamide but subsequently progressed, potentially due to a reduction in AR expression over time. Although she was diagnosed with TNBC, borderline HER2 overexpression was detected at time of death, suggesting that she could have received an anti-HER2 therapy.
- Patient 11 responded to glembatumumab vedotin as predicted by high expression of GPNMB. Of note, she did not respond to LEE011 despite amplification in *CDK6*, potentially due to loss of *Rb* (predicted by overexpression of P16) or monoallelic amplification of *CDKN2A*, which encodes for P16.
- Our experience to date indicates that intensive longitudinal monitoring in patients with metastatic cancer appears to be safe and feasible.
- Whole genome and quantitative proteomic analyses of multiple biopsies reveal extensive heterogeneity over space and time and reveal molecular signatures that may lead to a lack of response/development of resistance to identified targeted therapies.

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