Safety, Feasibility, and Merits of Longitudinal Molecular Testing of Multiple Metastatic Sites to Inform mTNBC Patient Treatment in the Intensive Trial of Omics in Cancer

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PURPOSE Patients with metastatic triple-negative breast cancer (mTNBC) have poor outcomes. The Intensive Trial of Omics in Cancer (ITOMIC) sought to determine the feasibility and potential efficacy of informing treatment decisions through multiple biopsies of mTNBC deposits longitudinally over time, accompanied by analysis using a distributed network of experts.

METHODS Thirty-one subjects were enrolled and 432 postenrollment biopsies performed (clinical and study-directed) of which 332 were study-directed. Molecular profiling included whole-genome sequencing or whole-exome sequencing, cancer-associated gene panel sequencing, RNA-sequencing, and immunohistochemistry. To afford time for analysis, subjects were initially treated with cisplatin (19 subjects), or another treatment they had not received previously. The results were discussed at a multi-institutional ITOMIC Tumor Board, and a report transmitted to the subject’s oncologist who arrived at the final treatment decision in conjunction with the subject. Assistance was provided to access treatments that were predicted to be effective.

RESULTS Multiple biopsies in single settings and over time were safe, and comprehensive analysis was feasible. Two subjects were found to have lung cancer, one had carcinoma of unknown primary site, tumor samples from three subjects were estrogen receptor–positive and from two others, human epidermal growth factor receptor 2–positive. Two subjects withdrew. Thirty-four of 112 recommended treatments were accessed using approved drugs, clinical trials, and single-patient investigational new drugs. After excluding the three subjects with nonbreast cancers and the two subjects who withdrew, 22 of 26 subjects (84.6%) received at least one ITOMIC Tumor Board–recommended treatment.

CONCLUSION Further exploration of this approach in patients with mTNBC is merited.

INTRODUCTION Breast cancer (BC) is the most common malignancy in women worldwide excluding skin cancer.1 Triple-negative BC (TNBC), defined by features that it lacks—overexpression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2)—comprises about 10% of BCs in non-Hispanic White women and 21% of BCs in non-Hispanic Black women.2 TNBC is more aggressive and is associated with a poorer survival at 5 years than other BC subtypes.3,5 Patients with metastatic TNBC (mTNBC) have especially poor outcomes, with median overall survivals ranging from 8.9 months6 to 13.3 months.7,8 Research advances using next-generation sequencing, computational biology, and other technologies have significantly advanced our understanding of mTNBC; however, insights from these efforts are rarely deployed in a manner that has the potential to immediately benefit patients.10,11 A growing number of institutions have established molecular tumor boards to recommend treatments on the basis of the results of molecular profiling,12-15 and clinical trials have assessed the benefits of this approach.16-21 Multidimensional molecular analysis is typically confined to single tumor samples analyzed at single points in time; however, heterogeneity is inherent to almost all
cancers and the molecular features of cancers evolve with disease progression\textsuperscript{22}; therefore, patients may also benefit from longitudinal profiling. Additionally, the results considered by molecular tumor boards are typically derived from tests performed in Clinical Laboratory Improvement Amendments (CLIA)–approved facilities, limiting the scope of potentially useful information.

We launched the Intensive Trial of Omics in Cancer (ITOMIC; ClinicalTrials.gov identifier: NCT01957514) in October 2013 to capture differences between different tumor samples taken from the same patient at the same time and longitudinally at different times, to access both clinically-validated and research-based tests, to enable analysis by a distributed network of experts, and to provide results to oncologists and their patients. Outside of ITOMIC, the University of Washington Center for Cancer Innovation assisted patients and their oncologists to access treatments that were predicted to be effective. The experience of one subject in this trial has been described previously.\textsuperscript{23} Here, we describe the experiences of 31 patients enrolled in the trial.

\textbf{METHODS}

\textbf{Study Design, Subjects, and Tissue Collection}

The design of ITOMIC is depicted schematically in Figure 1. Thirty-one (31) patients with a prestudy diagnosis of mTNBC seen at Northwest Medical Specialties or the Seattle Cancer Care Alliance were enrolled. A diagnosis of mTNBC was established on the basis of the most recent pathology report(s) from clinical specimens.

Upon enrollment, biopsies were taken from multiple metastatic sites, if possible. Archival tissues were analyzed when study biopsies were not feasible (or not successful as occurred in subject 8 whose disease was confined to bone). Archival tissues were either from primary or metastatic sites, and in a few instances, from both. Samples chosen for analysis were based on representativeness and tumor content, and analysis of the most recent biopsy sample was prioritized. Select specimens of sufficient size (typically > 5 mm in length) and tumor content (typically > 50\%) were comprehensively analyzed. To afford time for analysis, subjects were initially treated with cisplatin (19 subjects),\textsuperscript{24,25} or another treatment that they had not received previously at the discretion of their physician.

The results of analyses across platforms and laboratories were reviewed at a virtual meeting of a multi-institutional ITOMIC Tumor Board (ITB), and a report describing findings was returned to the subject’s oncologist who in turn provided the results to the subject for discussion. An example of a report is provided in the Data Supplement. Assistance in accessing a recommended treatment was provided upon request by the University of Washington Center for Cancer Innovation. If the subject declined or was unable to avail themselves of the recommended treatment, the physician provided standard-of-care (SoC) treatment at their discretion or, in some instances and at their discretion, the physician would combine the ITB recommended treatment with SoC therapy at doses and schedules previously demonstrated to be safe.

If disease progressed on the first ITB (or alternative physician-recommended) treatment, the subject underwent additional biopsies for analysis, ITB review, and recommendation. This process was repeated as feasible.

\textbf{Biopsy-Related Adverse Events}

Adverse events (AEs) were graded by the investigator according to the Common Terminology Criteria for Adverse Events (version 4.03) 1 day and 7 days after the study-directed biopsy. A data safety monitoring board reviewed AEs.

\textbf{CONTEXT}

\textbf{Key Objective}

To enhance treatment options for patients with metastatic triple-negative breast cancer, we performed molecular analyses that included research as well as approved assays on biopsies of existing and emergent metastases collected over time. Findings were provided to the Intensive Trial of Omics in Cancer Tumor Board, which then made recommendations to the oncologist and patient for their consideration on the basis of identified targets.

\textbf{Knowledge Generated}

Longitudinal molecular testing of biopsies from multiple metastatic sites of patients with metastatic triple-negative breast cancer was found to be safe and feasible, and changes in tumor/metastases molecular profiles over time provided, in some cases, new therapeutic targets.

\textbf{Relevance}

Assessment of changes in tumor/metastasis molecular character during the course of disease is, along with use of research assays and experimental treatments, an approach to precision medicine that has the potential to leverage our increasing knowledge of tumor biology.
Analyses
Selected samples were analyzed using whole-genome sequencing (WGS) or whole-exome sequencing (WES), RNA sequencing (RNA-seq), deep sequencing of panels of cancer-associated genes, immunohistochemistry (IHC) and, in some instances, other studies as described in the Data Supplement. Germline sequencing was performed in all patients and somatic mutations identified by comparing results from germline and tumor sequencing.

RESULTS
Patient Characteristics
Demographics of all 31 subjects who enrolled in ITOMIC and prestudy treatment histories are shown in Table 1. Additional information on subject screening is found in the Data Supplement. The median age at enrollment was 57 years (range: 35-77 years) and 84% of subjects were White. The median number of prior treatments was 2 (range 0-7). All but five participants had received at least one prior therapy.

Study-Directed Biopsies
Figure 2 depicts the timing and anatomic sites for postenrollment biopsies for all 31 participants. Up to five adequate tumor samples were obtained from a single metastatic site. If an adequate tissue sample could not be obtained, the most recent prestudy clinical specimen was analyzed. Details on biopsy collection numbers and assessments are provided in the Data Supplement.

Adverse Events
AEs related or possibly related to the 332 study-directed biopsies performed on 77 occasions were evaluated one day and 7 days post-biopsy. There were six grade II AEs for pain and one grade III AE for pain associated with extensive cutaneous inflammatory BC; the patient’s symptoms had previously been alleviated by bathing, which was temporarily interrupted after she underwent several skin punch biopsies, necessitating a 5-day hospitalization for pain control.

Changes in Diagnosis
Subjects were eligible for enrollment in ITOMIC if the most recent pathologic evaluation of a metastatic site was reported as mTNBC. Subjects 21 and 27 were subsequently determined to have metastatic lung cancer on the basis of analysis of postenrollment biopsies, and subject 30 was determined to have a cancer of unknown primary; all three were removed from the study. Subjects 3 and 7 withdrew following the first set of biopsies. Of the remaining 26 subjects, four (Nos. 2, 5, 6, and 18) had prior histories of...
| Subject | Age (years) | Race | BC Diagnosis (year) | Receptor Status at Diagnosis | Therapy Before Enrollment | mTNBC Diagnosis Date | Enrollment Date | Baseline CTC (in 7.5 mL) |
|---------|-------------|------|--------------------|----------------------------|--------------------------|----------------------|-----------------|----------------------|
| 1       | 45          | White | 2011               | TNBC                       | Paclitaxel, sunitinib, followed by ddAC | Capecitabine         | Vinorelbine     | May 28, 2013         | October 24, 2013     | 20-40               |
| 2       | 56          | White | 2007               | ER+/PR+                    | Docetaxel, doxorubicin, cyclophosphamide | Anastrozole           | Letrozole, fulvestrant | Capecitabine, paclitaxel | June 25, 2013         | October 28, 2013     | 11,840              |
| 3       | 52          | White | 2012               | TNBC                       | Paclitaxel, bevacizumab   | Cyclophosphamide, fluorouracil, doxorubicin, insulin | April 26, 2012 | February 19, 2014 | 2                    |
| 4       | 54          | White | 2010               | TNBC                       | Docetaxel, cyclophosphamide | Capecitabine           | Abraxane         | April 25, 2012 | February 25, 2014 | 8                    |
| 5       | 77          | White | 1996               | ER+/PR+                    | Tamoxifen                 | Anastrozole           | Fulvestrant | Paclitaxel, docetaxel, cyclophosphamide | Epirubicin | February 28, 2014 | April 7, 2014 | 7                   |
| 6       | 67          | White | 2006               | ER+/PR+                    | Anastrozole               | Exemestane           | Abraxane          | Capecitabine | April 29, 2013 | April 21, 2014 | 2                    |
| 7       | 40          | Native Hawaiian or Other Pacific Islander | 2006 | ER+/PR+ | Paclitaxel | May 9, 2014 | May 20, 2014 | 18 |
| 8       | 62          | White | 2013               | TNBC                       | Docetaxel, doxorubicin, cyclophosphamide | June 11, 2013 | September 23, 2014 | 0 | (study day 22) |
| 9       | 37          | Native Hawaiian or Other Pacific Islander | 2011 | TNBC | Docetaxel, doxorubicin, cyclophosphamide | September 23, 2014 | October 13, 2014 | 0 | |
| 10      | 71          | White | 2014               | TNBC                       | None                      | December 23, 2014 | December 29, 2014 | 0 | |
| 11      | 42          | White | 2014               | TNBC                       | Docetaxel, doxorubicin, cyclophosphamide | December 17, 2014 | January 14, 2015 | 4 | |
| 12      | 46          | White | 2014               | TNBC                       | Docetaxel, doxorubicin, cyclophosphamide | March 16, 2015 | March 24, 2015 | 2 | |
| 13      | 57          | White | 2014               | TNBC                       | Paclitaxel, doxorubicin, cyclophosphamide | January 14, 2015 | February 3, 2016 | 5 | |
| 14      | 54          | White | 1999               | TNBC                       | Doxorubicin, cyclophosphamide | Paclitaxel plus ipatasertib/placebo | Eribulin | September 21, 2015 | March 7, 2016 | 0 |
| 15      | 66          | White | 2003               | TNBC                       | Docetaxel, doxorubicin, cyclophosphamide | Paclitaxel plus ipatasertib/placebo | Abraxane | July 23, 2015 | March 15, 2016 | 5 | |
| 16      | 56          | White | 2016               | TNBC                       | None                      | June 27, 2016 | July 11, 2016 | 4 | |
| 17      | 35          | African American, Asian or Pacific Islander | 2016 | TNBC | Doxorubicin, cyclophosphamide | May 25, 2016 | July 15, 2016 | 5 | |

(Continued on following page)
| Subject | Age (years) | Race        | Year | BC Diagnosis (year) | Receptor Status at Diagnosis | Therapy Before Enrollment | mTNBC Diagnosis Date | Enrollment Date  | Baseline CTC (in 7.5 mL) |
|---------|-------------|-------------|------|--------------------|-----------------------------|---------------------------|----------------------|------------------|-------------------|
| 18      | 62          | White       | 2013 | TNBC               | focal ER+                    | Cyclophosphamide, methotrexate, fluorouracil, femara | September 20, 2016  | October 10, 2016 | 18                |
| 19      | 52          | White       | 2010 | TNBC               | Doxorubicin, cyclophosphamide, tamoxifen | Paclitaxel plus ipatasertib/ placebo | December 14, 2015   | October 19, 2016 | 10                |
| 20      | 67          | White       | 2016 | TNBC               | None                         | None                       | December 14, 2016   | December 29, 2016 | 0                 |
| 21      | 50          | White       | 2016 | TNBC               | None                         | Vinorelbine                | December 5, 2016    | January 9, 2017  | 10                |
| 22      | 64          | White       | 2008 | TNBC               | Doxorubicin, cyclophosphamide | Paclitaxel plus ipatasertib/ placebo | November 20, 2015   | February 21, 2017 | 36                |
| 23      | 56          | White       | 2015 | TNBC               | Docetaxel, doxorubicin, cyclophosphamide | Cisplatin, herceptin           | February 9, 2017    | March 8, 2017    | 10                |
| 24      | 58          | African American | 2016 | TNBC               | Paclitaxel, doxorubicin, cyclophosphamide | Vinorelbine, methotrexate, capecitabine | May 23, 2016       | March 20, 2017   | 6                 |
| 25      | 60          | White       | 2007 | TNBC               | Paclitaxel, doxorubicin, cyclophosphamide, herceptin | Vinorelbine, methotrexate, capecitabine | March 20, 2017      | March 29, 2017   | 42                |
| 26      | 64          | White       | 2011 | HER2+              | Carboplatin, docetaxel, herceptin | Capecitabine, lapatinib, Herceptin, paclitaxel, perjeta | July 13, 2017       | July 18, 2017    | Assay failed     |
| 27      | 73          | White       | 2017 | TNBC               | None                         | None                       | September 1, 2017   | September 12, 2017 | 2                 |
| 28      | 36          | Asian or Pacific Islander | 2014 | TNBC               | Doxorubicin, cyclophosphamide | Cisplatin, paclitaxel     | Paclitaxel, paclitaxel, capecitabine | January 15, 2016   | October 26, 2017 | 0                 |
| 29      | 75          | White       | 2017 | TNBC               | Paclitaxel                   | None                       | December 22, 2016   | December 6, 2017 | 15                |
| 30      | 64          | White       | 2000 | HER2+              | Perjeta, herceptin, carboplatin, docetaxel | Herceptin                | December 4, 2017    | December 27, 2017 | 0                 |
| 31      | 61          | White       | 2015 | TNBC               | Docetaxel, doxorubicin, cyclophosphamide | None                       | December 21, 2017   | January 18, 2018  | 0                 |

NOTE. Patients were enrolled in the ITOMIC trial between October 2013 and January 2018. The median age of enrolled subjects was 57 years and race is as listed. Four subjects were ER+/PR+ and two were HER2+ at the time of their original BC diagnosis, but all had a TNBC diagnosis at the time of enrollment. Subjects 10, 16, 20, 21, and 27 presented with mTNBC and were immediately enrolled in the trial.

Abbreviations: BC, breast cancer; CTC, circulating tumor cells; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; mTNBC, metastatic triple-negative breast cancer; PR, progesterone receptor; TNBC, triple-negative breast cancer.
ER-positive BC. ER-positivity was again detected in postenrollment biopsies from subject 6, whereas postenrollment biopsies from subjects 2, 5, and 18 were consistently ER-negative. ER-positivity was also detected in postenrollment biopsies from subjects 16 and 17, and weak ER staining affecting 1% of cells was detected in one of six postenrollment biopsies from subject 15 who was categorized as ER-negative. Subject 26 had a prior history of HER2-positive cancer, and persistence of HER2-positivity was confirmed in postenrollment biopsies. Subject 25 had a history of TNBC; however, postenrollment biopsies demonstrated HER2-positivity. Although subject 14 had a history of TNBC, a left BC was documented to be ER/PR- and HER2-positive, whereas a synchronous right BC and metastatic right cervical lymph node were both TNBC. In aggregate, eight of 31 study participants (No.s 6, 16, 17, 21, 25, 26, 27, and 30; 26%) enrolled with a diagnosis of mTNBC were found during postenrollment evaluation to have a different diagnosis.

**ITB Recommendations**

ITB meetings (Data Supplement) began with a presentation of the patient’s relevant medical history, followed by results of IHC, cancer gene panel sequencing, WES, WGS, and RNA-seq from multiple biopsy specimens obtained at the same time from different metastatic sites. The results from research, non-CLIA-approved assays, along with standard assays, were considered by the ITB, as consented to by the patient and permitted in the institutional review board–approved framework. Correlating the variant allele frequency of a somatic mutation with the estimated tumor cell content across samples was taken into consideration in assessing whether a variant was likely to be present in most or all tumor cells, thereby presenting a reasonable therapeutic target. Samples with high tumor content were the most useful in evaluating RNA-seq signatures, and confidence in assessments of the relative expression level of an mRNA transcript increased if the results were similar across different samples. The sequencing depth associated with cancer gene panels provided results when tumor cell frequencies were too low to permit evaluation by WES or WGS. Germline sequencing was used to assess whether variants of undetermined significance identified in cancer gene panels were somatic or germline in origin. Germline sequencing also allowed for predictions of enhanced toxicity in the setting of specific chemotherapeutic agents.26

**FIG 2.** Anatomic sites and timing of postenrollment biopsies. (A) The anatomic locations of postenrollment biopsies (red dots) for all 31 enrolled subjects are shown. Black squares depict instances in which only prestudy biopsies were analyzed. (B) The timing of tissue collections is shown. Black squares depict prestudy tissue collections. Orange squares depict subjects who received cisplatin as the first postenrollment therapy.
FIG 3. ITB treatment recommendations. Numbers of ITB meetings, molecular lesions targeted, treatments administered, and duration of therapy for patients enrolled in Intensive Trial of Omics in Cancer (ITOMIC) with confirmed triple-negative breast cancer who received at least one treatment. Numbers in superscript denote method of drug access: 1Investigational drug accessed via single patient investigational new drug (three instances; light blue); 2Investigational drug accessed via an existing clinical trial (five instances; beige); 3On-label indication for an approved drug (three instances; pink); 4Off-label indication for an approved drug (17 instances; gray). Light green shading indicates a treatment duration of 20-40 weeks, and dark green shading indicates a treatment duration > 40 weeks. Patients came off therapy if there was disease progression, toxicity, or death. Yellow shading denotes subjects who were still alive as of June 1, 2021. Subjects found to show receptor-positive postenrollment, subjects 3 and 7 who withdrew, and subject 4 who died before the first postenrollment treatment are not shown. Subjects 12, 20, and 31 died before receiving the first ITB-Rx. ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; ITB, ITOMIC Tumor Board; ITB Rx, ITOMIC Tumor Board recommended treatment; mTNBC, metastatic triple-negative breast cancer; NA, not applicable; PD-L1, programmed death ligand-1; Rx, recommended treatment.

availability of ongoing clinical trials appropriate for an identified target—uniformly targeted molecular features specific to somatic tissues. When the ITB recommended multiaiagenregimens, the dose and schedules of component drugs were adopted from published or active clinical trials. For example, subject 23 received a recommendation for combined neratinib and temsirolimus therapy on the basis of a reported clinical trial.29

Figure 3 shows recommendations for 19 patients confirmed to have mTNBC, excluding two patients who withdrew (subjects 3 and 7) and one who died before the first postenrollment treatment (subject 4). Significant findings were conveyed to the oncologist ahead of the ITB meeting if indicated by clinical urgency. Details of the numbers of biopsies that underwent assessment can be found in the Data Supplement.
Initially, there was focus on identifying clinical trials across the United States for which a patient might qualify; however, patients were almost uniformly unwilling to travel. For example, subject 12’s tumor was found to have two activating mutations affecting the Notch pathway—a NOTCH2 mutation resulting in a predicted R2400X truncation,28 and homozygous deletion of exons 3-27 within NOTCH1—predicted to confer susceptibility to gamma secretase inhibition. A clinical trial testing a gamma secretase inhibitor at the University of Chicago (ClinicalTrials.gov identifier: NCT02299635) was recommended; however subject 12 did not wish to travel. Subsequently, focus was placed on local clinical trials.

As ITOMIC’s processes and areas of emphasis evolved, assessments of RNA-seq results grew in importance, with an emphasis on assessing relative expression levels of transcripts encoding proteins targeted in locally available clinical trials. Assessment of expression level is complicated by variation in the cellular composition of a tumor specimen, tissue processing, and batch effects.30 Analysis of multiple samples collected over time for comparison and the use of XENA proved especially useful in triaging subjects to locally available trials targeting GPNMB (ClinicalTrials.gov identifier: NCT01997333), LIV-1 (ClinicalTrials.gov identifier: NCT01969643), and ROR-1 (ClinicalTrials.gov identifier: NCT02706392).

Responses to ITB-Recommended Treatments

As a feasibility study, ITOMIC was not designed to demonstrate efficacy, and therefore, assessments of responses using RECIST criteria were not performed. mTNBC has an aggressive clinical course requiring continuous treatment, and the duration of a given therapy provides a useful surrogate for assessing the duration of response. The durations of ITB-recommended treatments are presented in Figure 3. The durations of some ITB-recommended treatments administered to subjects 13, 14, 15, and 24 lasted between 20 and 50 weeks, whereas the durations of some ITB-recommended treatments for subjects 9, 10, 14, 23, 25, and 29 surpassed 50 weeks. Subjects remained on treatment until disease progression or toxicity. Treatment was not changed solely on the basis of a new recommendation from the ITB. We note that although treatment responses to single agents were observed in some of the first subjects enrolled in ITOMIC, they were short-lived. For example, subject 1 was found to have two somatic activating mutations affecting FGF2, with predicted amino acid substitutions at S252W and Y375C.34 Off-label treatment with ponatinib produced a significant but short-lived reduction in cutaneous tumor infiltrates, lasting only 7 weeks. These early findings lead to the adoption of multiagent regimens.

Treatments recommended by the ITB sometimes failed. For example, a ROS1 point mutation identified in subject 2 (encoding Y2092C) failed to confer responsiveness to crizotinib despite the best efforts of domain experts,23 and an FGR2/EIF3A fusion expressed at high levels failed to confer responsiveness to an investigational FGFR2 inhibitor available through the NCI-MATCH trial.35 Although it is not intended to be a presentation of formal assessment of survival, Figure 4 shows the duration of disease pre-enrollment and survival postenrollment up to the end of the 2-year study period and beyond.

Integrating ITB-Recommended Treatments With Clinical Care

Adjustments to ITB treatment recommendations were frequently required because of treatment toxicity and a lack of or loss of treatment responsiveness, as exemplified by subject 10’s clinical course, depicted in Figure 5. Subject 10 was age 71 years at the time of mTNBC diagnosis in December 2014. She immediately enrolled in ITOMIC and Fig 4. Time from TNBC diagnosis to study enrollment and survival postenrollment. The duration of prestudy disease is shown in blue and poststudy survival in red for the 21 of the 31 enrolled subjects with confirmed metastatic TNBC who received at least one Intensive Trial of Omics in Cancer Tumor Board–recommended treatment. The end of the 2-year Intensive Trial of Omics in Cancer study participation is demarcated in white. Subjects 14, 15, and 23 (orange arrows) were still alive as of June 1, 2021. TNBC, triple-negative breast cancer.
received cisplatin followed by bilateral mastectomies and a right axillary lymphadenectomy. Adjuvant cisplatin was subsequently discontinued because of neuropathy and tinnitus. New metastases were detected in June 2015 and were treated with radiation therapy. Because of disease progression (December 2015), she was treated with ITB-recommended nivolumab and nab-paclitaxel beginning in January 2016. Nivolumab was recommended on the basis of ImmunoSEQ profiling, which identified a dominant clonal population of infiltrating T cells, and research results from metastatic melanoma suggesting that this pattern may be associated with an increased likelihood of responding to programmed cell death protein-1 blockade.36 A complete response was noted and nivolumab continued while nab-paclitaxel was discontinued because of toxicity. In 2017, several brain metastases were treated with gamma knife radiation/surgery and recurrence in July 2018 prompted the addition of capecitabine and nivolumab. Disease progression in November 2018 prompted discontinuation of capecitabine and initiation of the second ITB recommended treatment, olaparib (because of a signature 3-associated mutation profile37) plus eribulin, combined with ongoing nivolumab therapy. Eribulin was discontinued because of infusion-associated dyspnea, substituted by nab-paclitaxel with continued olaparib and nivolumab in September 2019. Continued disease progression prompted a switch back to lower-dose eribulin to January 2020, followed briefly by gemcitabine and doxorubicin treatment before her death in May 2020.

Utility of Serial Biopsies

Although many of molecular features of biopsies remained stable throughout a patient’s disease course, there were two ways in which serial biopsies proved useful. The first is related to molecular features only detected in later biopsies. For example, subject 14’s first postenrollment biopsies revealed focal ER-positivity, resulting in the inclusion of antiestrogen therapy in her regimen and a second study-related biopsy revealed focal HER2-positivity, resulting in the addition of trastuzumab. Additionally, CCND2 amplification was first detected in subject 15 in her third post-enrollment biopsy, leading to treatment with palbociclib. Finally, serial biopsies revealed an increase in tumor mutation burden (TMB) over time in subjects 19, 20, 24, 26, 28, and 29. For subjects 24 and 28, the increase in TMB resulted in the incorporation of immune checkpoint inhibitors to their treatment regimens. In subject 24, TMB rose from 4.32 mt/MB and 4.74 mt/MB on initial study biopsies to 12 mt/MB on a later study biopsy, and in subject 28 the TMB increased from 2.6 mt/MB on an initial study biopsy to 16.1 mt/MB in a later biopsy.

A second way in which serial monitoring proved useful was that it allowed for the application of analytic methods not available at the time of previous evaluations. Over the course of ITOMIC, we incorporated methods for estimating levels of mRNA transcripts encoding proteins for which targeted therapies could be accessed via clinical trials. In subject 9, LIV-1A transcript levels were in the 55th percentile of compared with 122 other mTNBC samples, and she was enrolled in ClinicalTrials.gov identifer: NCT01969643. Subject 9 was also found to have high ROR-1 transcript levels (in the 93rd percentile). ROR-1 protein expression was confirmed by IHC, and she was accepted for participation in a CAR-T trial targeting ROR1; however, she elected to receive hospice care. In another example, subject 10 was found to have a mutational signature suggestive of loss of BRCA1 or BRCA2 on tissue obtained from her third set of biopsies, and a poly (ADP-ribose) polymerase inhibitor was added to her regimen.

![FIG 5. Schematic depiction of the >5-year clinical course of subject 10. Cancer treatments and CA 15-3 levels (a surrogate marker of tumor burden) are shown. Clinician-directed modifications are described in the Results section. Bilat Mast. R Ax. LND, bilateral mastectomies and right axillary lymph node dissection; CA, cancer antigen; ITB Rx, Intensive Trial of Omics in Cancer Tumor Board–recommended treatment.]
DISCUSSION

ITOMIC was a feasibility study and, as such, lacked rigorous, predefined end points. Its aim was to establish and test a framework for delivering a best effort to understand the innerworkings of a patient’s cancer that transcended technology platforms, scientific disciplines, and institutions. The addition of research-based tests to clinically validated tests significantly improved the ITB’s ability to guide oncologists and their patients to potentially effective therapies, as exemplified by the estimation of relative levels of specific mRNA transcripts for experimental agents targeting the encoded proteins. The results of surveys describing the attitudes of ITOMIC participants, which reflect their overall support for the innovative aspects of the study, have been reported previously.38

Surprisingly, ITOMIC analyses revealed that 26% of subjects thought to have mTNBC were subsequently found to have other cancers (three subjects) or other BC subtypes (five subjects). In addition, in some, increases in TMB with time were detected. These observations are clinically significant and point to the merits analyzing multiple biopsy specimens in single settings and over time in patients with mTNBC. These findings underscore the frequent heterogeneity of ER, PR, and HER2 expression in BCs, both spatially and temporally,29 the frequent difficulty of distinguishing mTNBC from other metastatic cancers, and support the merits of performing multiple biopsies.

Perhaps the greatest success of the work described here was the high frequency with which subjects enrolled in ITOMIC were able to access ITB-recommended therapies. However, despite these successes, many instances remained in which treatments predicted to be effective could not be accessed, as exemplified by subject 2 for whom we were unable to acquire venetoclax.12

ITOMIC highlights critical limitations associated with a clinical trial system that is inaccessible to most patients. Urgently needed are mechanisms that afford greater flexibility, allowing patients to access investigational drugs at the point of care, combined with a framework that enables learning by capturing their experiences for the benefit of future patients.
subject matter of this manuscript. For more information about ASCO’s conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/po/author-center.

Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians (Open Payments).

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Speakers’ Bureau: Seattle Genetics, Hologics, Genentech/Roche, Puma Biotechnology
Research Funding: Genentech/Roche (Inst), SignalOne Bio, Agenda
Travel, Accommodations, Expenses: Seattle Genetics, Genentech/Roche, Puma Biotechnology
Open Payments Link: https://openpaymentsdata.cms.gov/physician/2511

Roy Ronen
Other Relationship: Data4Cure

Jackie Stilwell
Employment: Umoja Biopharma, Seattle Genetics
Stock and Other Ownership Interests: Seattle Genetics

Eric Kaldjian
Employment: RareCyte Inc
Stock and Other Ownership Interests: RareCyte Inc

Janusz Dutkowski
Patents, Royalties, Other Intellectual Property: Patents pending on methods and systems for data analysis including biomedical data analysis (Inst)
Other Relationship: Data4Cure Inc

Stephen Charles Benz
Employment: NantWorks, ImmunityBio, NantHealth
Stock and Other Ownership Interests: Novartis, Celgene, NantWorks, NantHealth, Pfizer, Regeneron, Bluebird Bio
Patents, Royalties, Other Intellectual Property: Patents issued and pending

Shahroz Rabizadeh
Employment: ImmunityBio, Sagittarius Bio
Leadership: NantBioScience Inc, NantWorks, ImmunityBio, Sagittarius Bio
Stock and Other Ownership Interests: NantHealth, ImmunityBio, Sagittarius Bio
Patents, Royalties, Other Intellectual Property: Made inventions that resulted in IP for NantBioScience and NantOmics; made inventions that resulted in IP for ImmunityBio; made inventions that resulted in IP for Sagittarius Bio

Patrick Soon-Shiong
Employment: ImmunityBio, NantWorks, NantHealth
Leadership: ImmunityBio, NantWorks, NantHealth
Stock and Other Ownership Interests: ImmunityBio, NantWorks, NantHealth
Patents, Royalties, Other Intellectual Property: NantWorks and Affiliates, NantKwest, ImmunityBio

C. Anthony Blau
Employment: All4Cure
Leadership: All4Cure
Stock and Other Ownership Interests: All4Cure
Consulting or Advisory Role: GlaxoSmithKline (Inst)
Travel, Accommodations, Expenses: Novartis (I)

No other potential conflicts of interest were reported.

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