Case Reports in Oncology

Case Rep Oncol 2016;9:112–118

DOI: 10.1159/000443371
Published online: February 18, 2016

1662–6575/16/0091–0112$39.50/0
www.karger.com/cro

A Combination of Targeted Therapy with Chemotherapy Backbone Induces Response in a Treatment-Resistant Triple-Negative MCL1-Amplified Metastatic Breast Cancer Patient

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Key Words
MCL1 · Triple-negative breast cancer · BAP1 · Everolimus · Sorafenib

Abstract
After failure of anthracycline- and platinum-based therapy, no effective therapies exist for management of metastatic triple-negative breast cancer (TNBC). We report a case of metastatic TNBC harboring MCL1 amplification, as identified by comprehensive genomic profiling in the course of clinical care. *MCL1* is an antiapoptotic gene in the BCL2 family, and *MCL1* amplification is common in TNBC (at least 20%). A personalized dose-reduced regimen centered on a combination of sorafenib and vorinostat was implemented, based on preclinical evidence demonstrating treatment synergy in the setting of *MCL1* amplification. Although hospice care was being considered before treatment initiation, the personalized regimen yielded 6 additional months of life for this patient. Further rigorous studies are needed to confirm that this regimen or derivatives thereof can benefit the *MCL1*-amplified subset of TNBC patients.

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Case Presentation

A 51-year-old female was diagnosed in November 2008 with stage IIA, N0, M0, ER-/PR-, HER2-negative breast cancer with two lumpectomies. She received six rounds of cyclophosphamide and docetaxel from December 2008 to March 2009, followed by irradiation to the left breast in April/May 2009. She remained disease free for 2 years and then had a recurrence in the left breast in September 2011. The patient received two rounds of doxorubicin and paclitaxel, but she did not respond. In November 2011, she received carboplatin/nab-paclitaxel without response. In December 2011, a left total mastectomy was performed demonstrating a poorly differentiated breast adenocarcinoma measuring 7.1 cm in the greatest dimension located centrally in the specimen and with clear margins. The tumor was Nottingham grade 3, with lymphovascular invasion but no evidence of nipple and skin involvement. The left central node was identified and assessed as free of tumor, demarcating this as T3, N0, (clinical) M0 disease and as stage IIB, node-negative and triple-negative breast cancer (TNBC).

Over the next 2 months, the patient received two rounds of gemcitabine and carboplatin with additional irradiation to the skin on the left side. Six months later, PET scan imaging revealed suspicious lesions in the right (contralateral) breast with suspicious foci also in the operative site on the left. A right mastectomy was performed in March 2012, and the patient received four cycles of eribulin. Two months later, biopsy of the left neck was performed revealing metastatic carcinoma. Subsequent PET scan then revealed lesions in the axial skeleton consistent with metastatic disease.

Having exhausted standard-of-care treatment, the patient was entered into three clinical trials serially utilizing NOTCH inhibitors, MEK inhibitors, and a trial utilizing a CDK4/6 dual inhibitor. After rapid failure of each investigational agent in the course of 1 year, a CT performed on April 17, 2013, showed greater than 50% liver involvement of tumor; other sites also involved were the lymph nodes, lung, and skeletal systems. On May 9, 2013, the patient came to the Arlington Cancer Center for a fourth clinical opinion. At that time, tumor involvement in the liver had increased to greater than 85% per CT scan, and because of rapid progression in the liver, life expectancy was estimated to be 1 month. Earlier that year a breast tissue specimen was submitted for comprehensive genomic profiling which was used by us to identify options for treatment as all standard-of-care and clinical trials had been exhausted (see Methods). On May 14, 2013, personalized treatment based on the genomic profile of the tumor was initiated.

Methods

With patient permission, the left primary mastectomy specimen was submitted for comprehensive genomic profiling (FoundationOne), performed in a CLIA-certified, CAP-accredited, NYS-regulated laboratory (Foundation Medicine). DNA extracted from formalin-fixed paraffin-embedded tumor was analyzed by hybridization capture of 3,320 exons from 186 cancer-related genes and 37 introns of 14 genes commonly rearranged in cancer. At least 50 ng of DNA was isolated and sequenced to high, uniform coverage, as previously described [1]. All classes of genomic alterations (GA) consisting of base substitutions, short insertions, and deletions, focal gene amplifications, homozygous deletions, and select rearrangements were determined and reported for this case. Clinically relevant GA (CRGA) were defined as those which suggest benefit from targeted therapies, or mechanism-based clinical trials. Tumor response was measured by PET/CT and CT scans throughout the treatment.

Results

Hybrid capture-based CGP demonstrated that the tumor harbored *MCL1* amplification and base substitutions in *BAP1* C649fs*6 (a truncating alteration) and a *TP53* (a splice site mutation). On the basis of the genomic profiling results, the patient was treated with an intensive personalized multidrug regimen with targeted therapy including sorafenib (Nexavar) and vorinostat (Zolinza) to target *MCL1* [2–6], everolimus (Afinitor), cetuximab (Erbitux) to specifically target the mTOR and *EGFR* pathways [7, 8], as well as the chemotherapeutic agent nab-paclitaxel (Abraxane), which was selected by biomarker testing (SPARC poly- and monoclonal antibodies [9]), and denosumab (Xjeva), a RANKL binder, was added for bone metastases [10, 11] (table 1). Eight courses (C) were administered with variable dosing of vorinostat, sorafenib, and nab-paclitaxel. Dosing for each agent was altered on the basis of toxicity during treatment as observed by the treating physician.

C_1 showed a major response in the liver, lung, and lymph nodes, but not in the skeletal system (fig. 1). Toxicity was noted, mainly weakness, GI toxicity consisting of nausea, vomiting, and diarrhea, and bone marrow toxicity consisting of anemia, thrombocytopenia and neutropenia (grade 3). Platelets were kept artificially above 70,000/m^3 to avoid severe nose bleeding by sorafenib, and hemoglobin was kept above 8 g/100 ml to avoid fatigue due to anemia. Still there was significant total overall weakness, and the patient needed treatment respite of 4 weeks. After 4 weeks of rest, significant progression of disease was noted in the skeletal system, so denosumab was added to the previous regimen and then maintained throughout future courses. C_2 was started and in order to decrease toxicity, we shortened the length of treatment from 6 weeks to 3 weeks. Response was shown in C_2, but not as profound as after C_1. The doses of vorinostat and sorafenib were increased in $C_{3,4}$. A significant overall response was observed. However, side effects significantly increased including weakness, diarrhea, nausea, vomiting and bone marrow toxicities. In C_5 vorinostat was held, and the other drugs including sorafenib were decreased by 25%. Disease progression was observed in this cycle. In C_6 vorinostat was restarted at lower dose, and sorafenib and the other drugs were continued at the same level as C_5, resulting in stabilization of disease, including osseous metastases. In C_7 vorinostat and sorafenib were further increased, which resulted in overall decrease of disease. However, toxicity was significant and the treatment was interrupted for 1 month and the patient's disease further progressed. C_8 was started with lower doses of sorafenib, vorinostat, and nab-paclitaxel. Due to weakness and GI toxicity, treatment was discontinued at patient's request, and the patient expired thereafter.

Discussion

TNBC is an aggressive disease defined as breast carcinoma with negative slide-based assays for estrogen, progesterone receptor and HER2. Consistent with being a diagnosis of exclusion, TNBC is widely understood to encompass significant genomic heterogeneity [12]. Alterations in *BRCA1/2* that create defects in homologous recombination define an important subset of TNBC with sensitivity to first-line platinum treatment and PARP inhibitors, but for the remaining majority of TNBC not harboring *BRCA* alterations, no particular benefit can be ascribed to such therapies [13].

The metastatic TNBC case reported here harbored amplification of *MCL1*, and a truncating mutation in *BAP1*, as well as a mutated *TP53. MCL1*, a member of the BCL2 antiapoptotic gene family is amplified in 20–54% of TNBC cases [14]. Amplification of *MCL1* is thought to interfere with the physiologic induction of apoptosis, which is broadly consistent with a role

in oncogenesis [15]. *MCL1* amplification is one of three alterations observed in this tumor, potentially empirically consistent with this alteration being an oncogenic driver. Given the assumption of *MCL1* amplification serving as an oncogenic driver, no molecularly targeted therapies specific to this alteration are known to exist at present, highlighting a large unmet medical need in this subset of *MCL1*-amplified TNBC patients with a poor prognosis.

Sorafenib is a promiscuous multikinase inhibitor that has defied ready characterization of its antineoplastic in vivo effect. Preclinical studies have demonstrated that sorafenib can induce cell death/apoptosis mediated by the downregulation of *MCL1,* but it remains to be definitively demonstrated whether this is the relevant effect of sorafenib [2, 3]. Interestingly, a synergy of co-administration of sorafenib and vorinostat, a histone deacetylase inhibitor has been observed, and this proapoptotic effect is also thought to be routed through MCL1 [4–6].

For this patient, the clinical benefit of the regimen was quite remarkable, and the 'drop-out' of individual therapies does hint at the relevant importance of each component. However, it is impossible to define the most important components, and relevance of each therapy vis-à-vis the GA. Sorafenib has previously been investigated as a combination therapy in breast cancer, typically with a chemotherapy backbone, but differing conclusions as to clinical benefit have been reached, and definitive studies are ongoing [15, 16]. In such studies, even if biomarker stratification was performed, no genomic stratification was used as criteria for entry, so responders to sorafenib cannot be segregated on the basis of *MCL1* amplifications or other alterations [17].

No anecdotal clinical reports or completed clinical trials are currently available to guide treatment for *MCL1*-amplified breast cancers. The personalized treatment regimen utilized here resulted in the patient experiencing 6 months of extended survival. The administration of sorafenib and vorinostat in the presence of the *MCL1* amplification is essential to the success of this program. Given the high frequency of *MCL1* amplification in TNBC, the current case study potentially suggests that other such patients might benefit from a similar treatment regimen.

The importance of vorinostat and sorafenib is supported by results of C_5 when a decreased dose of sorafenib and deletion of vorinostat led to progression of disease, furthermore by results of C_6 which stabilized disease when vorinostat was reintroduced. In C_7 there was additional disease response when raising the dosage of the 2 drugs. In contrast to other cycles in which dropout of other agents, e.g., everolimus, were still effective in inducing disease response.

In conclusion, the efficacy of sorafenib and vorinostat in combination with a backbone of other nontargeted therapies in this *MCL1*-amplified TNBC is a provocative observation that warrants additional investigation, as at present, MCL1-amplified TNBC patients are unable to benefit from molecular targeted therapy in current practice paradigms.

Statement of Ethics

The patient and her husband gave informed consent to Dr. Karel Dicke at the time of treatment protocol, after full disclosure of risks, side effects and potential benefit.

Disclosure Statement

S.M.A., C.M., J.H.C., and J.S.R. are employees of and have equity interest in Foundation Medicine, Inc. K.W. is a former employee of, current consultant to, and has equity interest in Foundation Medicine, Inc. J.W. and K.D. have nothing to declare.

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Table 1. Treatment program

Target		Drug	Dose range
Enhance apoptosis by targeting MCL-1			
[4–6]	Histone deacetylase inhibitor/MCL-1	Zolinza (vorinostat)	200–600 mg/day
[2, 3]	Multiple receptor tyrosine kinases/MCL-1	Nexavar (sorafenib)	200–800 mg/day
Inhibition of mitosis (i.e. killing)			
[9]	Antimicrotubule agent/SPARC	Abraxane (nab-paclitaxel)	50–75 mg/m^2 weekly
Silencing the proliferation pathway			
[16]	EGFR inhibitor	Erbitux (cetuximab)	250 mg/kg weekly
[17]	mTOR inhibitor/rapamycin analogue	Affinitor (everolimus)	5 mg/day
Osteoclast activity mediation			
[10, 11]	RANKL binder	Xjeva (denosumab)	120 mg every 4 weeks

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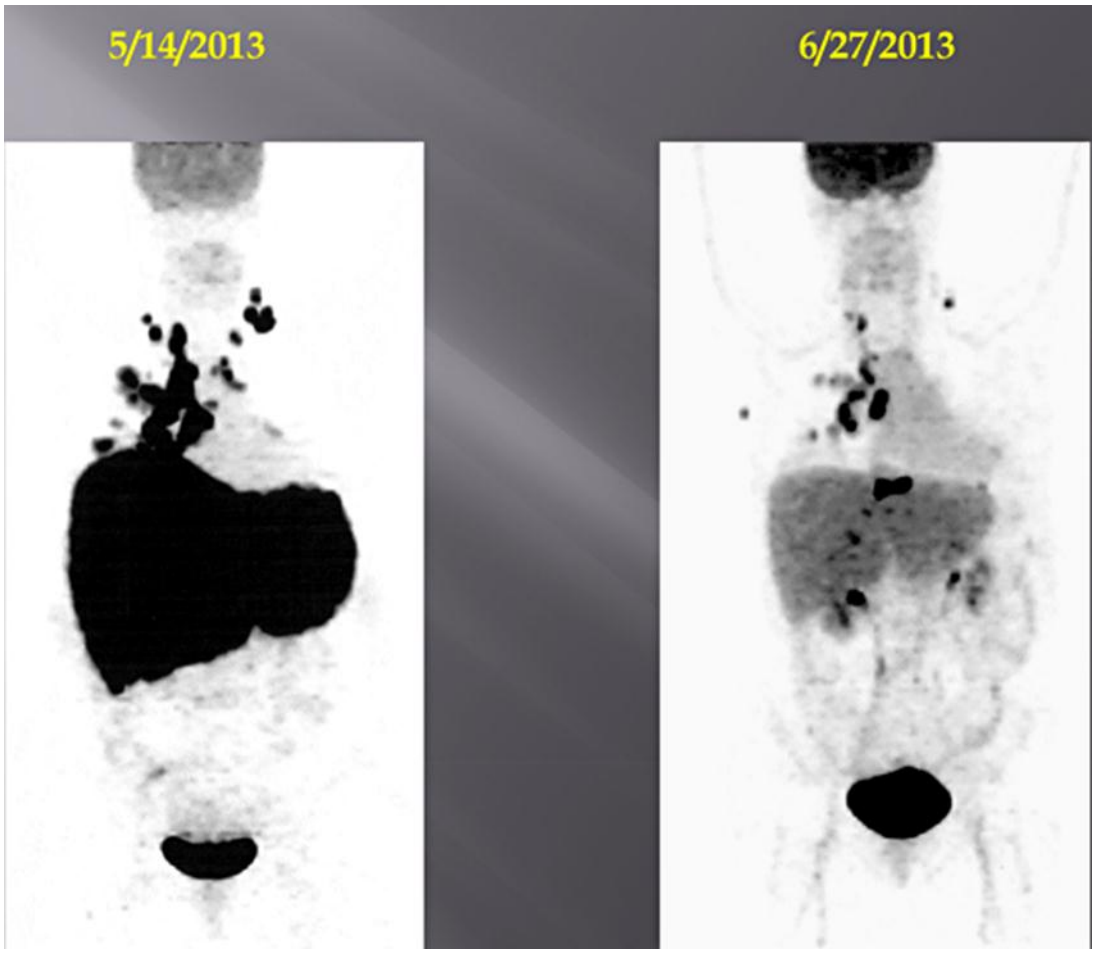

Fig. 1. Left: PET scan 5/14/2013 before treatment shows hepatomegaly with intense hypermetabolic FDG accumulation (SUV up to 20) throughout the entire liver with very low areas of normal hepatic parenchyma. Right: PET scan 6/27/13 after C_1 shows subsided hepatomegaly and decrease of liver metastases with a decrease in FDG activity (SUV between 4 and 6).