Biological Modulation of Parp Inhibition in Triple Negative Breast Cancer, a Combinational Approach Implementing Multitargeted Epigenetic Therapy (Mtet) with Parp Inhibition, in Advanced Breast Cancer: A Case Study

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Abstract

The introduction of PARP inhibitors as active agents to inhibit the DNA repair was a revolution in the cancer therapeutics, however, such approach only has shown promising results for a short time in majority of cases due to secondary mutations and promoter gene methylation, and most of patients with triple negative breast cancer when treated with such agents only benefit for a short time, until the tumor shows resistance and further the therapy fails [1]. Considering this category of drugs and their mechanism of action in DNA repair [2] [3], several recent studies have focused on combination of PARP inhibitors with chemotherapy, immune therapy and interestingly relevant to this article, epigenetic therapies [4]. That said, to our knowledge the human data in this regard is missing. Here we discuss a case report of a patient with stage four refractory and resistant BRCA1 mutated triple negative breast cancer who responded in matter of two weeks to a combinational therapy, consisting of PARP inhibitor and epigenetic therapies. As the patient already had exhausted the PARP inhibitor by excessive presence of BRCA positive altered circulatory DNA, the response merely reflects the epigenetic therapy as back bone of treatment. The liquid biopsy repeated after two weeks of combination therapy showed complete disappearance (resolution of positive BRCA gene/cDNA), reflecting a synergism by proposed modulation of resistance as mechanism of action. (The initial cDNA showed 93 percent mutation allele fraction of BRCA gene.) To our knowledge, this is the first study on combinational therapy in human. The finding in this case could potentially change the standard of care in treating BRCA positive tumors, by providing a superior treatment to current standards.
Keywords
Triple Negative Breast Cancer, PARP Inhibition, Epigenetic Therapies

1. Background

Triple negative breast cancers compromise about 15% of breast cancers overall, about 70% of breast cancers in individuals harboring a germline BRCA1 mutation, and 20% in BRCA2 mutation carriers, vice versa, the presence of germ line and/or somatic mutations in BRCA has been widely recognized in triple negative breast cancer. As of January 2016, there were more than 6 PARP inhibitors under development for BRCA positive tumors (See Table 1).

That said, a common failure of phase III trials has been improved progression free or more importantly the overall survival in the group of patients with triple negative breast cancer treated with single PARP inhibitors. A good example of this is phase III trial using Inaparib in patients with triple negative breast cancer who also received cytotoxic chemotherapy and showed no improvement in survival (phase II trial however showed promising results). A common rationale on the failure of the drug is the epigenetic aberrancies involving the BRCA gene and acquired resistance explained by secondary mutations.

In Oct., 2016, the preclinical work of Dr. Baylin at John Hopkins showed that PARP inhibitors work according to how intensely and durably the PARP

<table>
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<th>Table 1. List of all PARP inhibitors.</th>
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<tr>
<td>Olaparib (AZD2281)</td>
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<td>Veliparib (ABT-888)</td>
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<tr>
<td>Niraparib (formerly MK-4827)</td>
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<td>Talazoparib (RMN-673)</td>
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<td>Rucaparib (formerly AG-14699)</td>
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<td>CEP-9722</td>
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enzyme is trapped at certain DNA damage sites. Therefore, by ramping up the
duration and intensity of this trapping, you could potentially increase the effica-
cy of the drug. Such understanding provides the rationale on combination of
epigenetic therapies with PARP inhibitors [5] [6]. That said, to our knowledge,
the combination of epigenetic therapies consisting of the protocol we used with
a known PARP inhibitor has never been clinically explored in an advanced case
of triple negative breast cancer and clinical relevance been discussed.

2. Methods

Epigenetic therapies, consisting of polyphenol, Quercetin as mainstay of therapy,
and phenyl butyrate, in a protocol called “multitargeted epigenetic therapies”
administered intravenously on daily basis.

3. Case Report

37 yr female w/metastatic breast ca to bone, lymph nodes, and brain, she was
Caucasian, had positive family history for maternal breast cancer, and was non
smoker.

Patient was initially diagnosed in July 2013 w/stage III right breast ca and 4
axilla lymph nodes. BRCA 1w/Met ER+. Pt received 4 cycles of ATC, then un-
derwent double mastectomy, followed by 36 rounds of radiation therapy, and
then prophylactic oophorectomy.

In June 2015, CA27/29 marker noted to be elevated and scans indicated mets
to left lung and left clavicle lymph nodes. Pt participated in UCLA trial w/PARP
inhibitor from July to October 2015. Then switched to Xeloda from Nov. 2015 to
Jan. 2016. From Feb. to March 2016 patient was on Aromatase inhibitor and
Ibrance. From April to May 2016 patient received Carbo/Gemzar but during this
time period patient developed pain in sacrum. In June 2016, she received radia-
tion to sacrum with relief of pain and also received radiation to two brain lesions
per cyberknife. In July to Aug. 2016 participated in Stanford trial BMO1103 and
received more radiation to another brain lesion.

She also received 10 rounds of radiation to lesions noted on neck. In Oct.
2016, patient started immunotherapy, PARP, and Ameridex. Her restaging PET
scan showed very large mass in the left lower lung, as well as severe lymphad-
epathies in her neck, thorax and pelvis, retroperitoneum with mass effect on
IVC, causing hydronephrosis and partial colon obstruction, due to peritoneal
carcinomatosis, along with sacrum large lesion with significant uptake, com-
pared to her PET scan in 5/15 (see Image 1).

She was referred by her oncologist to us for evaluation and treatments. On her
initial evaluation, she was in significant pain. Labs indicated 93 percent MAF of
c DNA with several alterations, including BRCA. CTC was positive for three out
of four markers, very extensively high.

Immediately after starting the multi targeted epigenetic therapies (MTET), she
was feeling better and breathing better and had more sense of well being after
the first two treatments. Pain was better. Less nauseous. More appetite. She did not experience any toxicity from the treatment.

Lab showed significant reduction in her tumor markers (CA 27.29 and CA 15.3) and LDH, measured on 11/22/16, compared to 11/7/16.

CA 15.3 at 179 from 249. LDH down from 1603 to 1348 and further down to 1298 (measured on 12/6/16) and 890 two weeks after. After two weeks of therapy all her CTC markers dropped including the CK19, telomerase, ERBB2 and c Myc.
Patient: 
Tumor: Breast CA

For the analysis, we performed the following work steps

1. Isolation of circulating tumor cells / micrometastases
In order to obtain circulating tumor cells from the patient's peripheral blood, large cells and cell-clusters as well as epithelial cells were isolated. A preparation of mononuclear cells (MNC) served as a control cell fraction. From all fractions mRNA was isolated. Afterwards, the expression of tumor-relevant genes was measured by quantitative real-time RT-PCR.

2. Molecular detection of circulating tumor cells
The following molecular markers were used to detect tumor cells:

<table>
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<td>Telomerase</td>
<td>The expression of the telomerase-gene can be increased in most tumor types, but not in normal tissue. An increased expression of the telomerase gene may be indicative for the presence of tumor cells in the circulation. pos: Overexpression of telomerase was detected in the isolated cells.</td>
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<tr>
<td>C-MYC</td>
<td>Overexpression of C-MYC indicates an increased proliferation-rate of the isolated cells. An increased proliferation-rate is a typical feature of tumor cells. pos: The expression level of C-MYC was elevated.</td>
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<tr>
<td>ERBB2</td>
<td>Overexpression of ERBB2 (HER2/NEU) is a trait of different types of cancers and may be observed also in breast cancer. Thus, the detection of ERBB2 overexpression may be indicative for the presence of circulating tumor cells. pos: Expression of ERBB2 was elevated.</td>
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<tr>
<td>CK19</td>
<td>The detection of an expression of the cytokeratin 19 (CK19) gene indicates the presence of epithelial cells and may thus be indicative for circulating tumor cells. pos: There was strong expression of CK19 detected.</td>
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Interpretation
In the isolated tumor cell fraction, expression of ERBB2, C-MYC and telomerase was above threshold (>2.0) and expression of CK19 was above threshold (>0) in the very high range (> 10000). This finding may indicate the presence of circulating tumor cells in the analysed blood sample.
This happened without using any chemotherapy (please see Figure 1).

Her circulatory DNA through Guardant dropped from 93 percent on BRCA to non detectable (See Figure 2). This substantial response confirms the synergistic effect of the applied combinational therapy, and noticed in only 14 days after the initiation of the therapy.

Further the PARP inhibitor was stopped and the polyphenol therapy was continued. The cDNA was repeated on 12/20/16 and it showed increased BRCA back up again at 86 percent (please see Figure 3).

Patient accomplished unexpected response to the combinational therapy of PARP inhibitors and epigenetic protocol, with improved progression free survival and quality of life. She was under our care for three months and did not encounter any toxicity form the therapy.

4. Conclusion

Application of dual inhibition of epigenetic modifier consisting of natural compounds implemented in multitargeted epigenetic therapy (MTET) and traditional PARP inhibition is feasible and clinically relevant. Such therapy in our study showed significantly effective in treating BRCA positive colonies in tumor in triple negative breast cancer. The correlation of liquid biopsy findings and clinical response to such combinational therapy is also important as it can be used as a companion diagnostic for the therapy. We conclude that such therapy could replace the current standard of care in advanced triple negative breast cancer harboring BRCA deficient somatic or germ line mutations.
Guardant360 Tumor Response Map

The Guardant360 Tumor Response Map illustrates the mutant allele percentage (% cDNA) of observed somatic variants at each sample submission time point. The "Somatic Alteration Burden" value below refers to the maximum % cDNA detected at each time point. Amplifications are not plotted, and only the first and last four test dates are plotted. Please see the Physician Portal (https://portal.guardanthealth.com) for the Tumor Response Map with all test dates.

Summary of Somatic Alterations & Associated Treatment Options

The percentage of altered cell-free DNA (% cDNA) circulating in blood is related to the unique tumor biology of each patient. Factors that may affect the % cDNA of detected somatic alterations include tumor growth, turn-over, size, heterogeneity, vascularization, disease progression, and treatment.

For a more detailed Guardant360 Patient Report, log onto: https://portal.guardanthealth.com or to set up an account, contact Client Services: 855.698.8887
Figure 2. Circulating DNA (11/22/2016).
Guardant360 Tumor Response Map

The Guardant360 Tumor Response Map illustrates the mutant allele percentage (% ctDNA) of observed somatic variants at each sample submission time point. The "Somatic Alteration Burden" value below refers to the maximum % ctDNA detected at each time point. Amplifications are not plotted, and only the first and last four test dates are plotted. Please see the Physician Portal (https://portal.guardanthealth.com) for the Tumor Response Map with all test dates.

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**Figure 3.** Circulating DNA (12/20/2016).
Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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