CLINICAL VIGNETTE

Atypical responses to neoadjuvant chemotherapy combined with accelerated partial breast tumor-directed radiotherapy: two cases and considerations for future clinical trials

Nhu Hanh To[1–4], Myriam Kossai[5, 6], Nabila Ouidir[7], Noemie Grellier[1], Elias Assaf[8], Isabelle Gabelle-Flandin[9], Yazid Belkacemi[1–4, 10]*, Nina Radosevic-Robin[5, 6, 10]*

[1]Department of Radiation Oncology and The Henri Mondor Breast Center, The Henri Mondor University Hospital, Creteil, France
[2]University of Paris-Est Créteil (UPEC), Creteil, France
[3]INSERM Unit 955, Immunoregulation and Biotherapy (I-Biot) Team, The Mondor Institute for Biomedical Research (IMRB), Creteil, France
[4]Transatlantic Radiation Oncology Network (TRONE), Créteil, France
[5]Department of Pathology, Centre Jean Perrin, Clermont-Ferrand, France
[6]University Clermont Auvergne, INSERM Unit 1240 (IMoST), Radiopharmaceuticals & Biomarkers (RoBust) Team, Clermont-Ferrand, France
[7]Department of Pathology, The Henri Mondor University Hospital, AP-HP, Creteil, France
[8]Department of Medical Oncology, The Henri Mondor University Hospital, Creteil, France
[9]University Clinic of Cancerology-Radiotherapy, The Grenoble Alpes University Hospital Centre, La Tronche, France
[10]Association of Radiation Oncologists in the Mediterranean region (AROME), Creteil, France
*These authors contributed equally to this work.

Neoadjuvant radiotherapy (RT) has been increasingly tested in clinical trials due to its well-documented capacity to induce and/or boost the anticancer immune response [1]. The introduction of irradiation in breast cancer (BC) neoadjuvant treatment has gotten particularly facilitated by recent technical advances in RT, which allow more precise radiation delivery and fewer postoperative complications [2].

Neoadjuvant association of accelerated partial breast tumor-directed irradiation (APBTI) and chemotherapy in BC is expected to fully exploit the synergy of radiation and cytotoxic drugs, with acceptable side effects, especially on long-term cosmetic outcomes. This approach is currently being evaluated in a French multicentric randomized phase 2 trial, NeoAPBI-01 (NCT02806258). The trial compares patients with triple-negative (TN) or luminal B/HER2- locally advanced BC receiving a standard anthracycline-taxane-based regimen and patients receiving the same regimen sequentially combined with a short-course APBTI (5 consecutive days, 2.5 Gy bi-daily).

Here, we present two patients from the Neo-APBI-01 trial, one with an exceptionally good and the other with an exceptionally poor response to the regimen with APBTI. We elaborate on tumor tissue characteristics, and blood cell counts, which could have predicted such unusual responses to therapy, and provide suggestions for improvement of patient management in future trials of neoadjuvant APBTI in BC.

Address for correspondence: Pr. Yazid Belkacemi, MD, Ph.D. Department of Radiation Oncology and The Henri Mondor Breast Center, The Henri Mondor University Hospital, AP-HP, 1 rue Gustave Eiffel, 94010 Creteil, France, tel: (+33) 149814522, fax: (+33) 149812589; e-mail: yazid.belkacemi@aphp.fr

Patient 1: Exceptional responder (ExR)

Clinical features (Supplementary File — Tab. S1): a 56-year-old African black woman without a family history of cancer or comorbidities was diagnosed with TNBC stage T3 N2 M0. She first received four cycles of 5-fluorouracil-epirubicin-cyclophosphamide (FEC) and then APBTI, followed by two cycles of docetaxel. The treatment was stopped due to several toxicities and, four weeks later, breast-conserving surgery with complete axillary LN dissection (ALND) was performed. Six weeks after breast surgery, adjuvant RT at a total dose of 50 Gy in 25 fractions of 2 Gy was delivered to the whole breast and the internal mammary and medial supraclavicular (IM-MS) LN regions without boosting the lumpectomy bed. The patient is alive and disease-free five years after enrollment into NeoAPBI-01.

Blood counts: at baseline, slight anemia (Hb: 10.6 g/dL) and leukopenia (3.5×10^9/L), normal platelet count (293×10^9/L). The neutrophil-to-lymphocyte ratio (NLR) and the platelet-to-lymphocyte ratio (PLR) were 1.1 and 205, respectively. The NLR remained relatively low throughout the NAT (Fig. 1A). Compared to the pre-APBTI value, the PLR doubled after the irradiation but returned to the pre-APBTI level before surgery (Fig. 1B).

Histopathology (detailed in Supplementary File — Tab. S2): at diagnosis, an invasive BC of non-specific type (IBC-NS), intermediate grade, without lymphovascular invasion (LVI). Immuno-histochemistry (IHC): a TN, basal-like BC (50% tumor cells expressing cytokeratin 5/6), diffusely and strongly positive for p53 (corresponding to
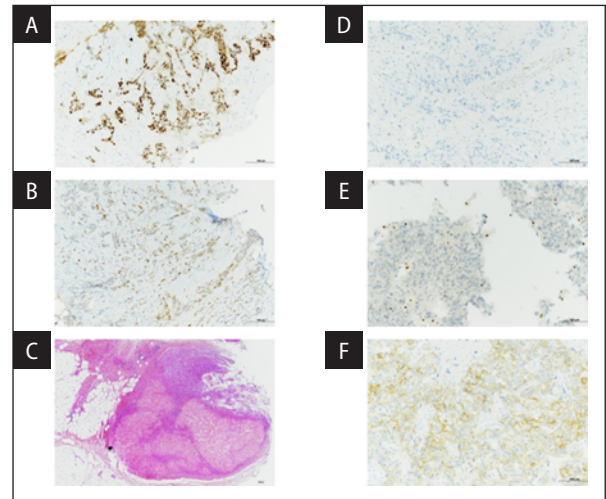
Figure 2. Histological and immunohistochemical characteristics of patients' pre-treatment biopsies and post-treatment surgical specimens. **A.** ExR patient, pre-treatment, immunohistochemistry (IHC) for p53, × 200; **B.** ExR patient, pre-treatment, IHC for pRb, × 200; **C.** ExR patient, post-treatment, axillary lymph node, hyaline change, H&E, × 40; **D.** ExNR patient, pre-treatment, IHC for p53, × 200; **E.** ExNR patient, pre-treatment, IHC for pRb, × 200; **F.** ExNR patient, post-treatment, an area of HER2 score 2, IHC for HER2, × 200

the missense type *TP53* mutation [10], Fig. 2A), with the retinoblastoma protein (pRb) present in all cells (Fig. 2B). After NAT, the breast contained less than 100 viable tumor cells, single or in small groups. Most of the tumor bed was replaced by scar tissue, with a high number of elastic fibers and foamy macrophages. Three out of 13 excised LNs were replaced by acellular tissue, corresponding to the hyaline change (Fig. 2C). In six other LNs, 30-80% of the lymphoid tissue was destroyed and replaced by fibrin deposits or hemorrhage.
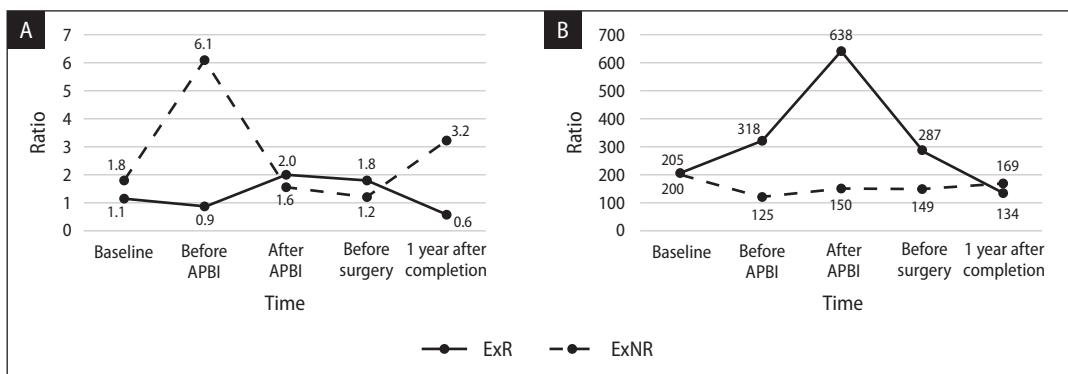
Figure 1. Dynamics of the neutrophil/lymphocyte ratio (**A**) and the platelet-lymphocyte ratio (**B**) and thoughout the clinical follow-up. APBI — accelerated partial breast irradiation; ExR — the exceptional responder patient; ExNR — the exceptional non-responder patient

Patient 2: Exceptional non-responder (ExNR)

Clinical features (Supplementary File — Tab. S1): a 31-year-old Caucasian woman without a family history of cancer or comorbidities was diagnosed with TNBC stage T2 N0 M0. After the first four cycles of FEC, a clinically suspected progression was confirmed by magnetic resonance imaging showing a bigger primary tumor and three centimetric satellite nodules. The NAT was continued with interceding APBTI between two of the four cycles of docetaxel, to be finalized by mastectomy and complete ALND. The surgical specimen contained a large residual tumor without involved LNs. Eight weeks post-surgery, adjuvant treatment consisted of RT only, at a total dose of 50 Gy normofractionally delivered to the chest wall and IM-MS regions. No further adjuvant systemic treatment was indicated according to national and institutional breast cancer management guidelines at that time. The patient developed a solitary metastasis in the right lower lung lobe three months after adjuvant RT. The lesion was not accessible for a biopsy to exclude lung cancer, so the treatment was continued with three cycles of a carboplatin-paclitaxel-bevacizumab regimen, to be completed with a lobectomy and mediastinal lymphadenectomy. Histopathological analysis confirmed BC metastasis without therapeutic effect. Three months later, new metastases in the lungs, the pleura, and the mediastinal LNs were observed by computerized tomography. The patient died 15 months after the lung surgery and three years after the BC diagnosis.

Blood counts: at baseline, Hb, leucocyte, and platelet counts were within the normal range; NLR and PLR were 1.8 and 200, respectively. The NLR and PLR dynamics were strikingly opposite to the one of the ExR patient: a very high NLR (6.1) right before APBTI dropped to 1.6 post-irradiation (Fig. 1A) while the PLR remained relatively stable throughout the therapy (Fig. 1B).

Histopathology (detailed in Table 2): at diagnosis, an IBC-NS of high grade, without LVI. IHC: a TNBC without basal-like characteristics (< 1% tumor cells CK5/6+). Both p53 and pRb were absent (Fig. 2DE), indicating the presence of *TP53* mutation of the "null" type [10] and *RB1* loss or a "null" mutation. The post-NAT residual tumor was high-

ly histologically heterogeneous, with >30% represented by loose epithelial cells and sarcomatous tissue. IHC: absence of hormone receptor expression, with many zones of HER2 score 1 or 2 (Fig. 2F) but without HER2 gene amplification (details in Supplementary File — Tab. S2). The tumor cells expressed CK5/6, CK8/18, KIT(CD117), CD56, SOX10, and ZEB1 in multiple large foci, SOX2 in small foci, while being negative for EGFR, BCL-2, androgen receptor, chromogranin, synaptophysin and PD-L1 (Tab. S2). This IHC profile corresponded to a basal-like TNBC in epithelial-mesenchymal transition.

According to our best knowledge, this is the first report of an exceptionally good or poor BC response to a NAT containing RT, which is not a salvage treatment.

No evaluated baseline clinico-pathological feature could indicate that the ExR patient will respond by the total elimination of tumor cells in the non-irradiated LNs. The hyaline change observed in some LNs after NAT indicates tissue destruction that happened well before the surgery and suggests an early response to the treatment, which could have been induced by chemotherapy, the abscopal effect of the APBTI, or both. The increased tumor cell sensitivity to DNA damaging agents, well documented in the basal-like TNBC subtype [3], could have resulted in sufficient activating of the cancer-immunity cycle [4] and almost total elimination of malignant cells over time. This underlines the need for an extensive assessment of DNA damage repair (DDR) proficiency before any DNA damage-inducing therapy, as the DDR pathway deficiencies are likely among the strongest predictors of good response to this type of anticancer treatment.

The only pre-treatment feature indicative of potential high resistance to treatment of the ExNR patient's tumor was the p53-/pRb- status, unique among the first 25 patients enrolled in the trial (data not shown). The simultaneous inactivation of the p53 and the pRb pathway has been shown to predict breast cancer resistance to DNA damage in vivo [5]. In addition, *RB1* deficiency is implicated in promoting stemness and metastatic progression [6] and is associated with poor clinical outcomes in several cancer types [7]. Interestingly, prostate cancers with p53/pRb loss, resistant to many therapeutics [8], were radiosensitized by PARP1 inhibitors

(PARP1i) [9], making a combination of PARP1i and APBTI worth clinical testing in p53-/pRb- breast cancers.

Our patients markedly differed in blood parameters right before APBTI. The ExNR patient had more than 3-fold higher NLR than the ExR patient, mainly due to a much higher neutrophil count. Neutrophilia, alone or combined with lymphopenia, is well demonstrated to be strongly unfavorable for response to treatment and prognosis in breast and other cancers [10]. While it remains to be validated, we believe that a high neutrophil count in a patient under an experimental therapy and with suspected progression should be discussed by a multidisciplinary team (MDT) as a potential stop signal to prevent harmful effects of the upcoming treatment.

Without available on-treatment tumor biopsies, we cannot conclude whether APBTI stimulated the metastatic progression of the ExNR patient's tumor. Resistance to chemotherapy was suspected well before the APBTI started, so re-biopsying the breast tumor was already indicated at that time. That could have revealed the HER2$_{low}$ tumor status, observed only after NAT, and initiated a discussion about the exclusion of the patient from the trial and her eventual enrollment into a trial of HER2 antibody-drug conjugates, shown to be efficacious in HER2$_{low}$ BCs [11].

Molecular tumor profiling before treatment (for example, PAM50 gene panel for determination of molecular subclass and the BRCAness assays) would have been helpful in better elucidating why these unusual responses occurred. In addition, if the patient agrees, analyses of the germline mutational status should be undertaken in all situations of unexpected/unusual/exceptional response to a novel treatment. Certain germline anomalies, like mutations in *BRCA1/2* or/and other genes involved in DDR, can be responsible for particularly good responses to chemo- or/and radiotherapy [12].

In conclusion, the p53-/pRb- tumor status and blood cell counts are biomarkers worth testing in future trials of neoadjuvant chemoradiation for BC. By this report, we encourage MDTs to demand additional tumor tissue and/or blood samples in any situation of atypical response to neoadjuvant anticancer treatment and to connect with the consortia dedicated to a deep exploration of such cases.

Conflicts of interest

Funding

References

1. Ho AY, Wright JL, Blitzblau RC, et al. Optimizing Radiation Therapy to Boost Systemic Immune Responses in Breast Cancer: A Critical Review for Breast Radiation Oncologists. Int J Radiat Oncol Biol Phys. 2020; 108(1): 227–241, doi: 10.1016/j.ijrobp.2020.05.011, indexed in Pubmed: 32417409.

2. Corradini S, Krug D, Meattini I, et al. Preoperative radiotherapy: A paradigm shift in the treatment of breast cancer? A review of literature. Crit Rev Oncol Hematol. 2019; 141: 102–111, doi: 10.1016/j.critrevonc.2019.06.003, indexed in Pubmed: 31272045.

3. Lehmann BD, Bauer JA, Chen Xi, et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest. 2011; 121(7): 2750–2767, doi: 10.1172/JCI45014, indexed in Pubmed: 21633166.

4. Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. Immunity. 2013; 39(1): 1–10, doi: 10.1016/j.immuni.2013.07.012, indexed in Pubmed: 23890059.

5. Knappskog S, Berge EO, Chrisanthar R, et al. Concomitant inactivation of the p53- and pRB- functional pathways predicts resistance to DNA damaging drugs in breast cancer in vivo. Mol Oncol. 2015; 9(8): 1553–1564, doi: 10.1016/j.molonc.2015.04.008, indexed in Pubmed: 26004085.

6. Zacksenhaus E, Shrestha M, Liu JC, et al. Mitochondrial OXPHOS Induced by RB1 Deficiency in Breast Cancer: Implications for Anabolic Metabolism, Stemness, and Metastasis. Trends Cancer. 2017; 3(11): 768–779, doi: 10.1016/j.trecan.2017.09.002, indexed in Pubmed: 29120753.

7. Chen WS, Alshalalfa M, Zhao SG, et al. Novel RB1-Loss Transcriptomic Signature Is Associated with Poor Clinical Outcomes across Cancer Types. Clin Cancer Res. 2019; 25(14): 4290–4299, doi: 10.1158/1078-0432.CCR-19-0404, indexed in Pubmed: 31010837.

8. Nyquist MD, Corella A, Coleman I, et al. Combined TP53 and RB1 Loss Promotes Prostate Cancer Resistance to a Spectrum of Therapeutics and Confers Vulnerability to Replication Stress. Cell Rep. 2020; 31(8): 107669, doi: 10.1016/j.celrep.2020.107669, indexed in Pubmed: 32460015.

9. Fan Y, Fan H, Quan Z, et al. Ionizing Radiation Combined with PARP1 Inhibitor Reduces Radioresistance in Prostate Cancer with RB1/TP53 Loss. Cancer Invest. 2021; 39(5): 423–434, doi: 10.1080/07357907.2021.1899200, indexed in Pubmed: 33683975.

10. Qian Yi, Tao J, Li X, et al. Peripheral inflammation/immune indicators of chemosensitivity and prognosis in breast

cancer patients treated with neoadjuvant chemotherapy. Onco Targets Ther. 2018; 11: 1423–1432, doi: 10.2147/OTT. S148496, indexed in Pubmed: 29588597.

11. Modi S, Park H, Murthy RK, et al. Antitumor Activity and Safety of Trastuzumab Deruxtecan in Patients With HER2-Low-Expressing Advanced Breast Cancer: Results From a Phase Ib Study. J Clin Oncol. 2020; 38(17): 1887–1896, doi: 10.1200/JCO.19.02318, indexed in Pubmed: 32058843.

12. Bianchini G, Balko JM, Mayer IA, et al. Triple-negative breast cancer: challenges and opportunities of a heterogeneous disease. Nat Rev Clin Oncol. 2016; 13(11): 674–690, doi: 10.1038/nrclinonc.2016.66, indexed in Pubmed: 27184417.

https://journals.viamedica.pl/rpor