Clinical Paper
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Application of epigenetic therapies in combination with cyclin dependent kinase inhibitors (CDKIs) in patients with metastatic breast cancer to reverse tumor resistance: two case reports

Abstract

CDKIs are widely used to treat breast cancer and recently suggested in treating manyother types of cancer, such as ovarian cancer, pancreatic cancer, and gliomas. Unfortunately however, resistance to such drugs develops in almost all cases after a few months to a year; thisgives the tumor selective advantage by allowing it access to other passenger promoter genes. Several mechanisms of resistance have been explored in the literature ranging from Rb1 mutations to epigenetic aberrancies as a result of using this class of drugs. Here we go over two cases of advanced stage four breast cancer treated with Palbociclib with initial response but eventual resistance. Patients were then started on epigenetic therapies to reverse resistance whichwas met with improved treatment response as evident by clinical and radiological measures. We conclude that the addition of multitargeted epigenetic therapies (MTET) to this class of drugs is clinically impactful by preventing/reversing tumor resistance and further providing possible synergistic effects of biological modifiers.

Keywords: cyclin dependent kinase inhibitors, palbociclib, epigenetic therapies, case report, breast cancer, ctDNA

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M Amin Nezami, Steven Hager DO, Aryan Chaychian BS
Sahel Oncology, Orange Coast Medical Center of Hope, USA

Correspondence: M Amin Nezami, Sahel Oncology, Orange Coast Medical Center of Hope, USA, Email amnezam@yahoo.com

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Introduction

The true mechanism of action of CDK-A is cell advancement of the cell cycle from G2→M.[1] CDK-A *is similar to cell hibernation* in that when inhibited, it causes cells to metabolically switch to utilizing fat for energy insteadof glucose, prolonging the stem cell's life span.[2] Thisphenomenon is also seen when hypoxia inducible factor one (HIF-1) is activated. Therefore, the effect of CDK on DNA repair system is one with a wide range of biological effects.[3–5] The cyclin-D-CDK complex phosphorylates and inactivates the tumor suppressor retinoblastoma protein (Rb) and its homologs p107 and p130.[4,6] This inactivation initiates a feedback loop, leading to an eventualinhibition of the E2F transcription factor, thus activating E2F-binding to DNA promoter regions, allowing the transcription of E2F-dependent genes such as cyclin A and cyclin E. These cyclins form a complex with CDK-2,activating its kinase activity. Cyclin(A,E)-CDK-2 complexes further phosphorylate Rb resulting in its complete inactivation.[6] The increase in cyclins and the activated cyclin(D,E)-CDKs(2/4/6) complexes are essential to drive cells from G1 to the S phase and commit the cell to proliferation. On the other hand, the absence of growth factorsreduces the activity of the RAS (MAPKs) and PI3K/Akt pathways, thus leading to the activation of the glycogensynthase kinase 3 beta (GSK3β), which halts the cell cycle by phosphorylation and subsequent degradation of cyclin-D. Degradation of cyclin D reduces cyclin(D)-CDK[4,6] complex formation followed by an increase in activated Rb, which ultimately strongly suppresses the formation of E2F transcriptionfactor and downstream genes. Cyclin D thus comprises a rate-limiting factor of cell cycle progression through G1.[7,8]

One main cause of resistance is inactivation of Rb1. Retinoblastoma 1(Rb1) is very rare but impactful in growth of breast tumors carrying such alterations. Its role is to arrest cell division from G1→S. Mutations in Rb1 also make breast tumor resistant to certain other methods of treatment, including standard chemotherapies, thus making it essential to explore other therapeutic approaches. Epigenetic drugs, specifically demethylating agents, have been studiedin this scenario. Additionally, CDK inhibition leads to stem cell quiescence and increased intracellular HDAC, ultimately making all CDKi drugs ineffective after a certain period of time. The addition of epigenetic drugs, mainly HDACi, may reduce excess HDAC produced by CDKi in cancer stem cells, inhibiting CSC quiescence, and leaving them vulnerable to apoptosis via negative regulatormolecules such as p53.[9] Multitargeted epigenetic therapy is a combination of HDACi and demethylating agents, and it thus has shown potential to accomplish this challenging, yet groundbreaking task.

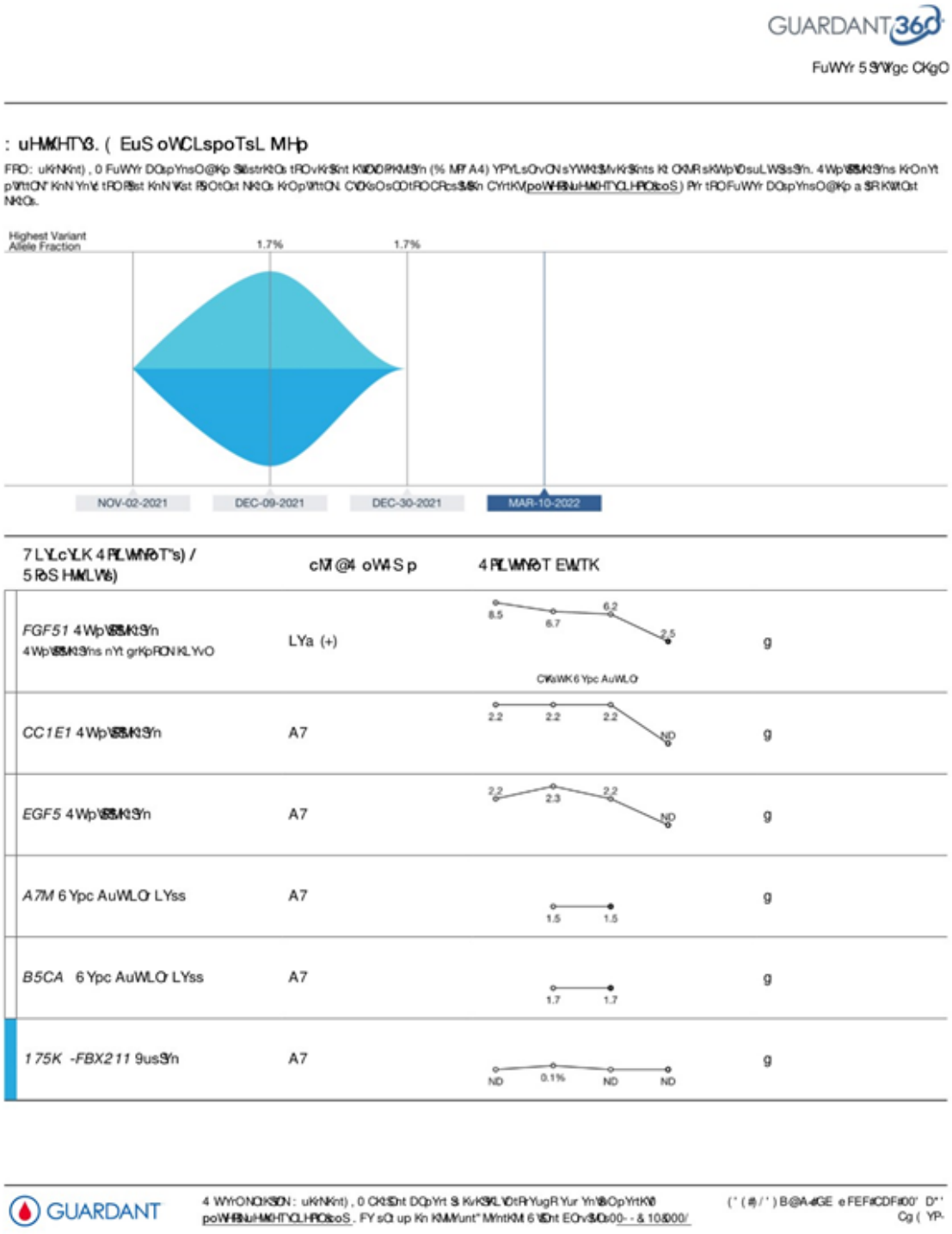
Methods and materials

Patients were informed and consented and subsequently enrolled in a phase II clinical trial or treated off the trial.Patients were treated on standards of good clinical practice and compassionate basis, after obtaining appropriate written consent forms in accordance with regional legislation and principals of declaration of Helsinki. Treatments were provided using patented intravenous administration of NP-Q (Nano particles of Quercetin) on predeterminedoses, at daily frequency and labs repeated a least 14 days after therapy initiation.

Case 1

40 years old female with history of invasive ductal carcinoma ER/PR ++ diagnosed in 2016 status post mastectomy, refused conventional therapies altogether, status post recurrence of her disease in stage four metastasized to the chest wall, ribs, and both lungs, currently seeking alternative therapies for her care. Her main concern is the sleep issue and pain in the sternum. Her initial findings confirmed agermline mutation at SMAR, SNF/SWI. She is reporting that the pain had started to subside in her chest wall, and the mass was less pressing to the sternum after the therapies. Her QOL has improved post treatments. Her chestdiscomfort was almost completely gone

post therapy. In exam it shrunk 50 percent. She was restaged with a PET scan which confirmed stable to improved findings. Her c DNA reported reduction of FGFR from 8.5 to 3.5 and other alterations (EGFR and CCNE1 became non detectable, after 15 days of the trial (measured on 11/29/2021). Further her FGFR1 dropped down to 2.5 on 3/11/22, as she continued the care with maintenance IV therapies at once a week schedule. Her CEA also dropped to 16. On March 10th, she was re-evaluated and her Guardant showed complete resolution of CCNE1 and EGFR and reduction of her FGFR1 down to 2.5(please see below). She was restaged on 5/13/22 with whole body PET scan which showed partial metabolic response in her large sternum mass(SUV down from 8 to 4.9), interval resolution of left pleural effusion, as well as partial response in her wide spread metastatic pulmonary disease; right axillary, internal mammary and hilar lymph adenopathies all responded to the interval therapies. For example left posterior medial lung lobe lesion decreased from 3 to 2 cm and activity from 6.2 to 3.4. She continues to improve with the therapies and significant response manifested in all hermarkers and scan (Figure 1).



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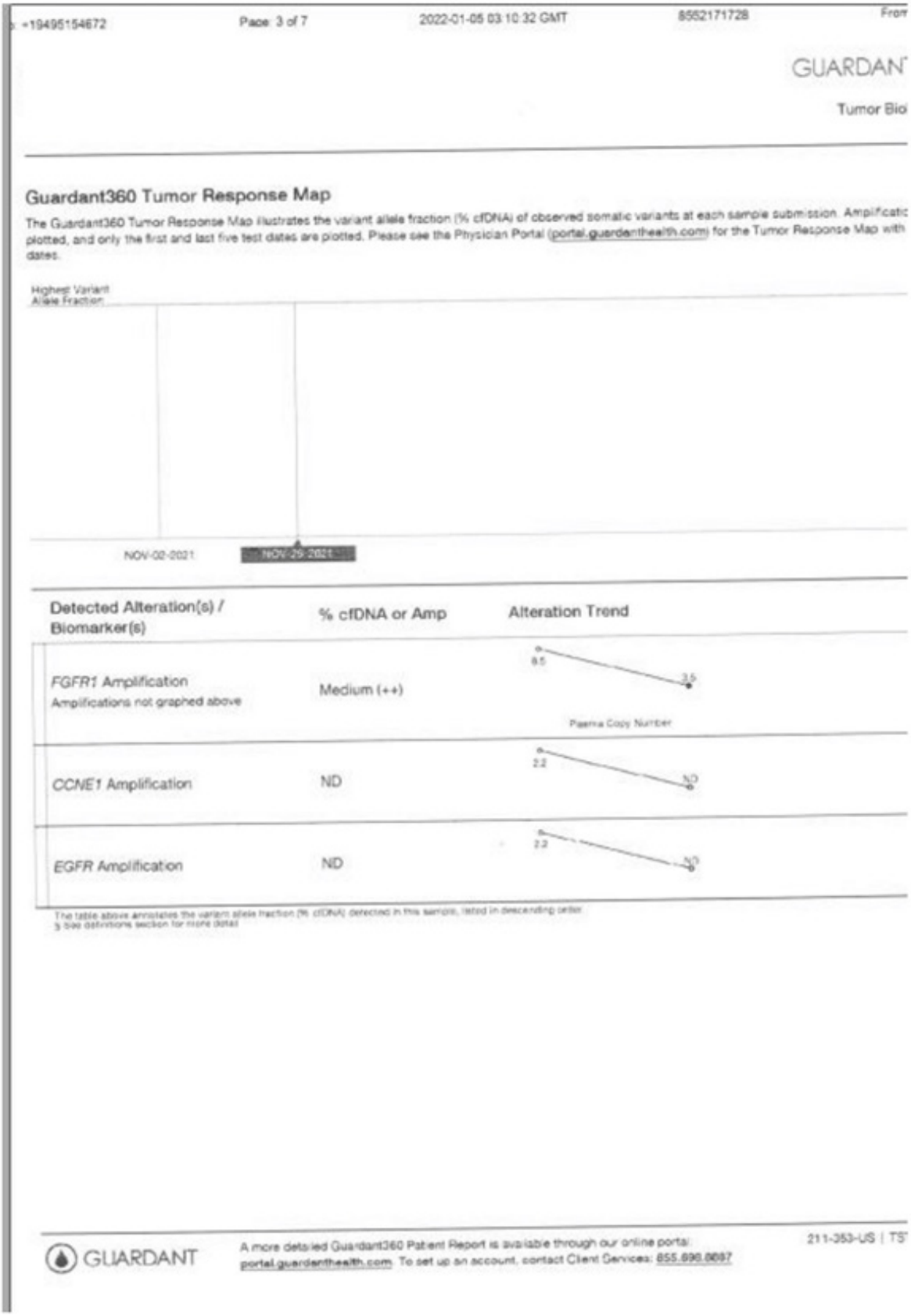

Figure 1 Guardant 360 tumor response map

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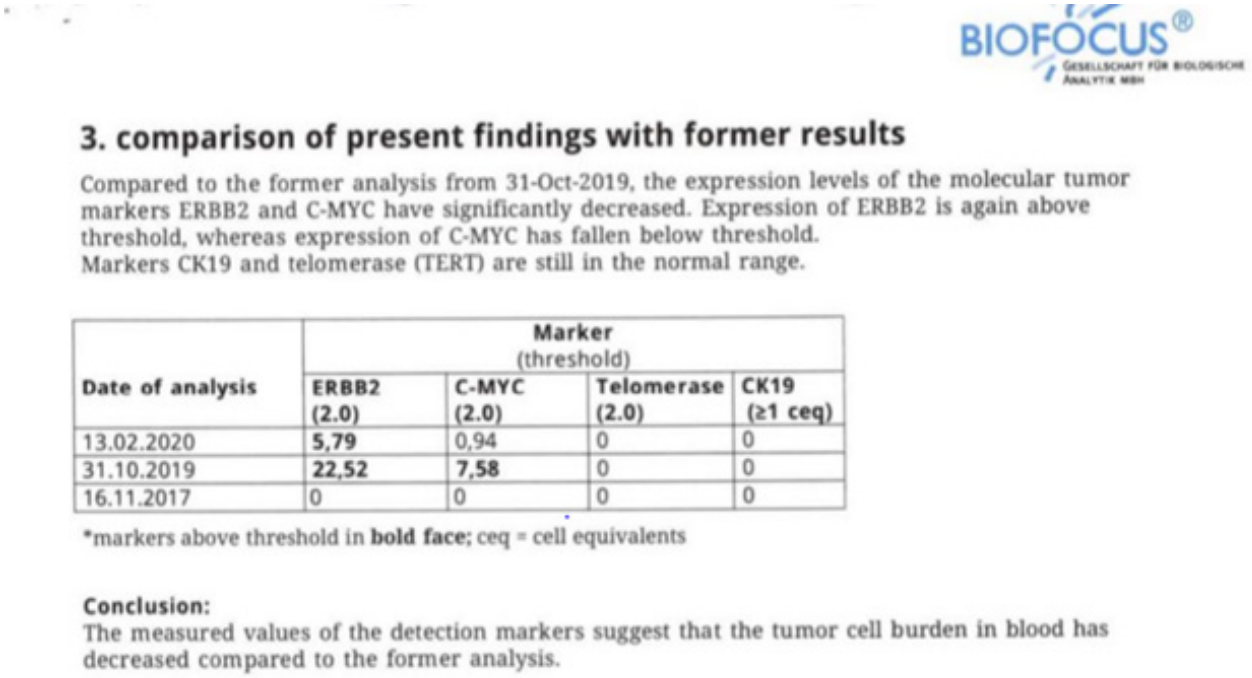
Case 2

61 years old female with history of bilateral breast ductal cancer initially at right side, diagnosed in 2008, status post two recurrences, initially in 2009, after she had a right lumpectomy, and completed chemo/CT regimen for four cycles as well as radiation in 2010, further with recurrence in left axillary node, biopsy proven ductal carcinoma with ER/ PR positive (strongly positive) and Her 2 negative disease. She had a PET scan in July 2017,which did not show any distant mets. She was referred by her oncologist to us for evaluation and treatments. She was seen at LAC+ USC for her conventional care which at that time had been projected to be chemotherapy and radiation again. Since she refused these options she was started on Ibrance which she received at the time she wasseen at our clinic. Although she was taking this drug her imaging was positive for tumor activity and her labs showed increased markers for tumor growth and dissemination. In 10/2019 before she saw us her scan had shown apositive breast mass with size of 20 mm abutting the left breast implant as well as left axillary node that was positive for metastatic disease. As such the decision was made to combine Ibrancewith epigenetic therapies in 2020, and further stop Ibrance and only receive Epigenetic therapies. Initial labs showed increased TGF- Beta 1,as well as VEGF at 9856 and 123 respectively, measured on 10/27/19. She was immediately started on IV epigenetic therapies, which she received on daily basis for four weeks, and her labs repeated. Her CTC was also positive beforestarting the therapies on 11/2019. After twenty treatments her VEGF dropped down to 43 and herTGF dropped down to 5587 (normal range), measured on 2/11/20. Her c DNA was non detectable. Her CTC responded to thetherapy after 20 days (Figure 2). She underwent several PET/CT imaging, in 9/29/2021 and 12/2022 and all confirmed completeresponse to the therapy and no sign of tumor recurrence or residual activity. Post surgical changes were observed. She has been off Palbociclib for over 2 years at the time of this article, withno sign of diseaserecurrence and continues care at monthly frequency at the clinic with no side effects and has a normal quality of life.



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3. comparison of present findings with former results

Compared to the former analysis from 31-Oct-2019, the expression levels of the molecular tumor markers ERBB2 and C-MYC have significantly decreased. Expression of ERBB2 is again above threshold, whereas expression of C-MYC has fallen below threshold.
Markers CK19 and telomerase (TERT) are still in the normal range.

Date of analysis	Marker (threshold)			
	ERBB2 (2.0)	C-MYC (2.0)	Telomerase (2.0)	CK19 (≥1 ceq)
13.02.2020	**5,79**	0,94	0	0
31.10.2019	**22,52**	**7,58**	0	0
16.11.2017	0	0	0	0

*markers above threshold in **bold face**; ceq = cell equivalents

Conclusion:
The measured values of the detection markers suggest that the tumor cell burden in blood has decreased compared to the former analysis.

Figure 2 Levels of molecular tumor markers ERBB2 and C-MYC

Discussion

Here we discuss advanced cases of metastatic breast cancer which had already progressed despite hormonal blockers, and targeted therapies (cyclin dependent kinases). We also discussed and correlated the main mechanismbehind resistance to such targeted therapies. The issue with using CDK inhibitors is that they cause cell quiescence, similar to HIF activation by hypoxic environment. Hypoxia induces EMT and causes CTC dissemination, which can trigger anaerobic glycolysis of tumor cells, induce angiogenesis, promote the proliferation, invasion, and migration of tumor cells, leading to multidrug resistance.[10] Although the data on the effect of CDKi on HIF is unclear, and some suggest there is anti HIF activity by this class of drugs, the combination of epigenetic modifiers that block both HSP (Heat Shock proteins) and HIF with CDKi has synergistic effect on inhibition of HIF.[11] This phenomenon is specifically important in Rb deficient breast cancercells, as Rb1 deficiency seems to be a main reason behind CDK failure.[12,13] In this study we specifically were interested in Palbociclib, compared to other two common CDKi drugs Ribociclib and Abemaciclib, as there are studies which suggest there could be activation of Wnt pathway by Abemaciclib (as well as CDK9 inhibition causing GI symptoms),[14] and that Ribociclib can induce chemo resistance. Additionally, Palbciclib dose modifications have been better clinically tolerated compared to both Ribociclib and Abemaciclib. We hypothesized that addition of HIF inhibitors (NP-Q) in MTET protocol will function both as HDAC and HIF inhibition. Further we establish a protocol based on proposed mechanisms of resistance, (including Rb1 mutation) using epigenetic therapies. The clinical outcome of these cases encourages further research on a larger scale on the long-term clinical outcome of combining epigenetic protocol with targeted CDKi drugs.

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Conclusion

Addition of multi targeted Epigenetic Therapy (MTET) to cyclin dependent kinases is feasible and clinically impactful to patients with advanced hormonal receptor positive breast cancer. Further longitudinal studies are suggested on a larger sample size to investigate the long- term outcome of implementation of combination therapy, perhaps in early-stage disease, to prevent drug resistance and slow down disease progression.

Acknowledgments

None.

Conflicts of interest

The authors declare that there is no conflict of interest.

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