ISSN 2475-529X

Case Reports

Journal of Nursing & Healthcare

Personalized Therapy based on Several Parameters of Comprehensive Tumour Profiling: a Challenge in NEBC

Dörthe Schaffrin-Nabe[1]*, Anke Josten-Nabe[1], Stefan Schuster[2], Andrea Tannapfel[3], Rudolf Voigtmann[1]

[1]*Praxis für Hämatologie und Onkologie, Bochum, Germany.*

[2]*Datar Cancer Genetics Europe GmbH, Eckersdorf, Germany.*

[3]*Institute of Pathology, Ruhr-University Bochum, Bochum, Germany.*

***Corresponding author**
Dörthe Schaffrin-Nabe,
Praxis für Hämatologie und Onkologie, Bochum, Germany.

Submitted: 09 Dec 2022; **Accepted**: 20 Dec 2022; **Published**: 30 Dec 2022

Citation: Dörthe Schaffrin-Nabe, Anke Josten-Nabe, Stefan Schuster, Andrea Tannapfel, Rudolf Voigtmann, (2022). Personalized Therapy based on Several Parameters of Comprehensive Tumour Profiling: a Challenge in NEBC, *J Nur Healthcare*, 2022, 7(4): 85-92.

Abstract

Primary neuroendocrine breast cancer (NEBC) is a rare entity accounting for <0.1% of all breast carcinomas and <1% of all neuroendocrine tumours. In most cases, treatment strategies in NEBC are empirical in absence of prospective trial data on NEBC cohorts. Herein, we present two case reports diagnosed with anaplastic large and small cell NEBC. After initial therapies analogue to those of breast cancer (BC) or neuroendocrine cancers (NEC) had failed, comprehensive tumour profiling was applied, leading to individualized treatment options for both patients. Due to targetable mutations and important co-alterations, the PIK3-mTOR pathway was highlighted from different aspects in both patients. The epicrisis of the two patients exemplifies how to manage the challenge of rare and difficult to treat cancers and how new diagnostic tools contribute to medical management.

Introduction

Primary neuroendocrine breast cancer (NEBC) represent a kind of tumour with low incidence (0.1%) among all breast cancer subtypes [1]. Diagnosis of NEBC is often challenging, as other neuroendocrine tumours, but also lung, gastrointestinal and pancreatic cancers as primary extramammary tumours need to be excluded [2-5, 8]. Generally, morphology remains the base of pathologic classification, but NEBC do not exhibit a definite and unequivocally recognizable morphology. Diagnosis often relies on IHC (Immunohistochemistry) for CGA (Chromogranin A) and SYN (Synaptophysin) which are sometimes expressed in other non-NE tumours [9]. Adjunctive staining of e.g. Mammaglobin, GATA3 (GATA binding protein 3) and GCDFP15 (Gross cystic disease fluid protein 15) allows to confirm mammary origin [3]. As above mentioned, IHC is not conducted routinely, making it difficult to evaluate the real incidence of NEBC.

Observation shows a neuroendocrine differentiation in up to 20% of all breast cancers [5], but inconsistent to this fact, NEBC are diagnosed in less than 0,1% of all breast carcinomas [1], based on the latest WHO classification in 2019, 5th edition [9].

Due to low prevalence of these tumours and successive changes in their diagnostic criteria over the years, no therapeutic guidelines have been established to date. In most cases treatment strategies in NEBC are empirical in absence of prospective trial data on NEBC cohorts. NEBCs are mostly associated with poor long-term survival and with rapid therapy resistance development [1, 48, 52, 54].

Currently the surgical intervention is the mainstay of therapeutic approach [5, 6].

Treatment strategies are chosen dependent on TNM status, aggressiveness, age, general condition and comorbidities of the patient [7]. If (neo-)adjuvant chemotherapy is necessary, NEBC is being treated either analogue to adenocarcinomas of the breast or to SCLC [8, 9]. Previously, Ki67 was used as a decision tool in NEBC: Ki67 < 15% led to a breast cancer analogue therapy, for Ki67 > 15% the therapy was orientated to SCLC/neuroendocrine treatment [7]. Promising results were seen when combining sur-

gery, radiotherapy and chemotherapy [6].

The development of molecular tumour profiling in recent years increasingly provides the opportunity for the use of targeted therapies, taking into account the involved activation and inhibition of the signal transduction pathways [12–14]. This tool is particularly useful for rare tumours without existing therapy guidelines and for tumours that are refractory to therapy.

We want to illustrate the diagnostic and therapeutic challenges with presenting the epicrisis of two patients diagnosed with NEBC in these above-mentioned situations.

Patient 1

The first patient was a 67-year-old female patient (Figure 1), diagnosed with a primary NEBC of the small cell subtype confirmed by histopathology. Undergoing surgery and considering definitive tumour stage (pT2, pN1a, L1, V0, G3, Ki67 60%) she received six cycles of Carboplatin and Etoposide, tailored to high proliferation index, followed by radiotherapy of the breast.

17 months later, pronounced bilateral pleural metastases without effusion were detected and one brain metastasis on the left occipital side progressed, which was surgically removed. Considering micrometastases of the brain, the patient received Topotecan due to its ability to cross the blood-brain barrier.

Further brain metastases, progressive lung metastases with effusion, metastatic spread to bone and thyroid gland were discovered by MRI two months later during ongoing chemotherapy.

Consequently, tumour profiling was performed with the Exacta® test using peripheral blood to detect genetic alterations by NGS gene expression profiles, targetable markers by immunocytochemistry staining, pharmacogenetics of tumour specific medication and chemotherapy sensitivity testing using circulating tumour associated cells [15–17].

Patient 2

The second 51-year-old female patient suffered from a NEBC anaplastic large cell subtype (Figure 2). After breast conserving therapy and sentinel lymphonodectomy, the definitive tumour stage was pT2, pN0, G3, Ki 67 40%, L0, V0, Pn0, ER 30%, PR neg, Her2/neu neg. In the adjuvant setting Carboplatin and Etoposide were applied with extremely poor clinical tolerability. Shortly after completion of adjuvant radiotherapy, hepatic filiae appeared in the right liver lobe. The planned atypical liver resection was rejected, due to intraoperatively detected diffuse spread into the left lobe.

Histopathologic confirmation revealed highly proliferating liver metastasis with a Ki67 of 80%, poorly differentiated, associated with the known NEBC.

Tumour profiling was performed using Exacta® analysis based on liver biopsy and blood. Waiting for results, a diffuse bone metastasis with infiltration of the spinal canal with corresponding clinical signs was observed and consequently radiotherapy was applied.

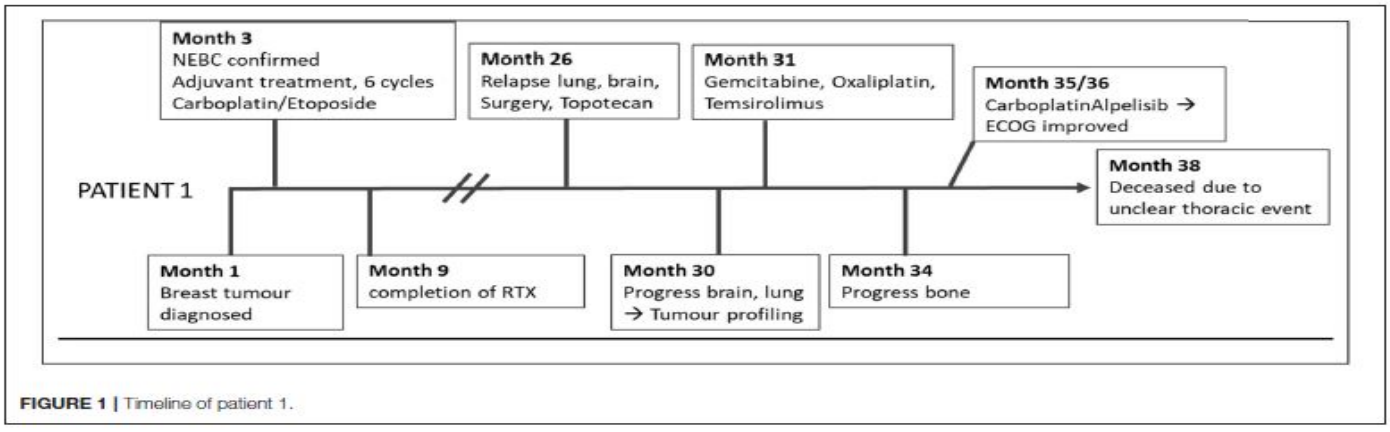

FIGURE 1 | Timeline of patient 1.

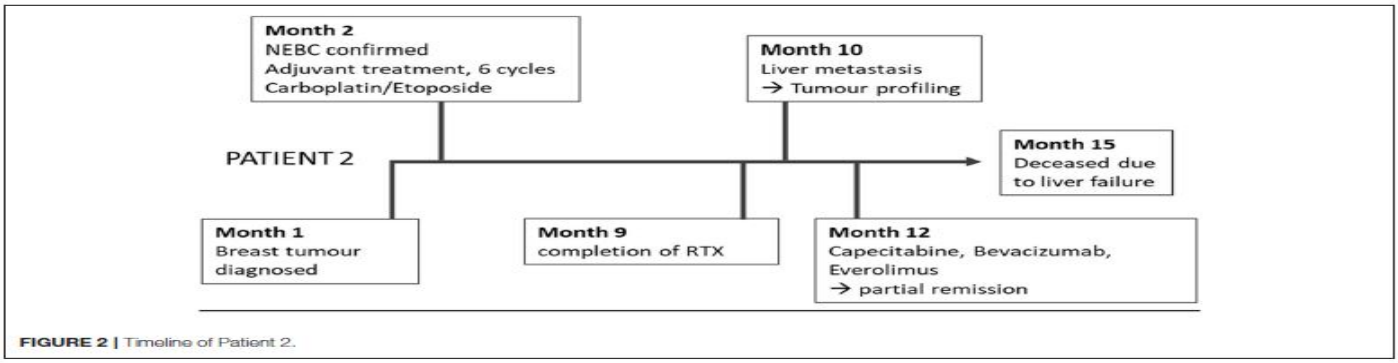

FIGURE 2 | Timeline of Patient 2.

Discussion

The reported aggressive scenario in both patients is consistent with the high grading and high proliferation index (Ki67 >40%). After failing of initial chemotherapies, question raised up for novel therapy strategies. Dotatate-based PET-CT as an experimental diagnostic and therapeutic alternative was rejected by the patient [18]. In this situation tumour profiling was performed, considering that treatment recommendations for genomic alterations exist for certain tumour types, but not for NEBC. At no stage guidelines or clinical trials were available, only the individual approach remained as an option.

In case of the first patient, Exacta® revealed an activating mutation of PIK3CA (p,E545K), which is one of the most mutated genes and has been found to play a crucial role in several cancer types, but information about the incidence in NEBC is inconsistent in the literature [19–21]. The PI3K/AKT/mTOR pathway is highly important for proliferation, migration and cell survival and alterations are quite frequent in other NETs [22]. This mutation therefore suggested a therapeutic benefit from mTOR or PIK3CA inhibition. Due to extended metastasis (pleura, neurocranium, bone) and high Ki67, Gemcitabine and Oxaliplatin were added, based on the chemosensitivity result to the mTOR inhibitor Temsirolimus [16, 23]. Even though the therapy was tailored to individual tumour characteristics, the patient progressed, developing new pulmonary metastasis and lymphangiosis as well as pronounced pleural effusion. Despite molecular genetic evidence together with an upregulation at mRNA level of AKT, an important activator of mTOR, suggesting potential benefit from mTOR inhibitors, no response was seen. Resistance mechanisms to mTOR inhibitors, for example, caused by disruption of the negative feedback loop between IRS1 and PI3K signalling followed by AKT activation, could explain treatment failure [26–28]. Furthermore, RHEB (RAS homolog enriched in brain) as an mTOR activator was downregulated, together with mTOR downstream activating pathway components, like eIF4B (eukaryotic initiation factor 4B) and S6 (ribosomal subunit S6 = RPS6) mainly effected by Temsirolimus [29, 30].

It seemed that the proliferation promoting influence was not triggered via mTOR. Only by focussing on molecular base this was not predictable. Subsequently, the patient underwent pleurodesis on both sides. TROP2 overexpression relating to Sacituzumab-Govitecan [24, 25] or biomarkers for immune checkpoint inhibitors were not observed.

Since NGS revealed PIK3CA as the only targetable alteration and AKT together with transcriptions factors, such as BCL2 were upregulated, it appeared that the proliferation promoting influence was obviously not triggered via the mTOR pathway. The therapy was focused on the PIK3CA mutation, again (Figure 3). We evaluated possible intrinsic resistance factors for PIK3CA inhibitors and no PTEN loss neither amplification of FGFR1 was detected [27, 31].

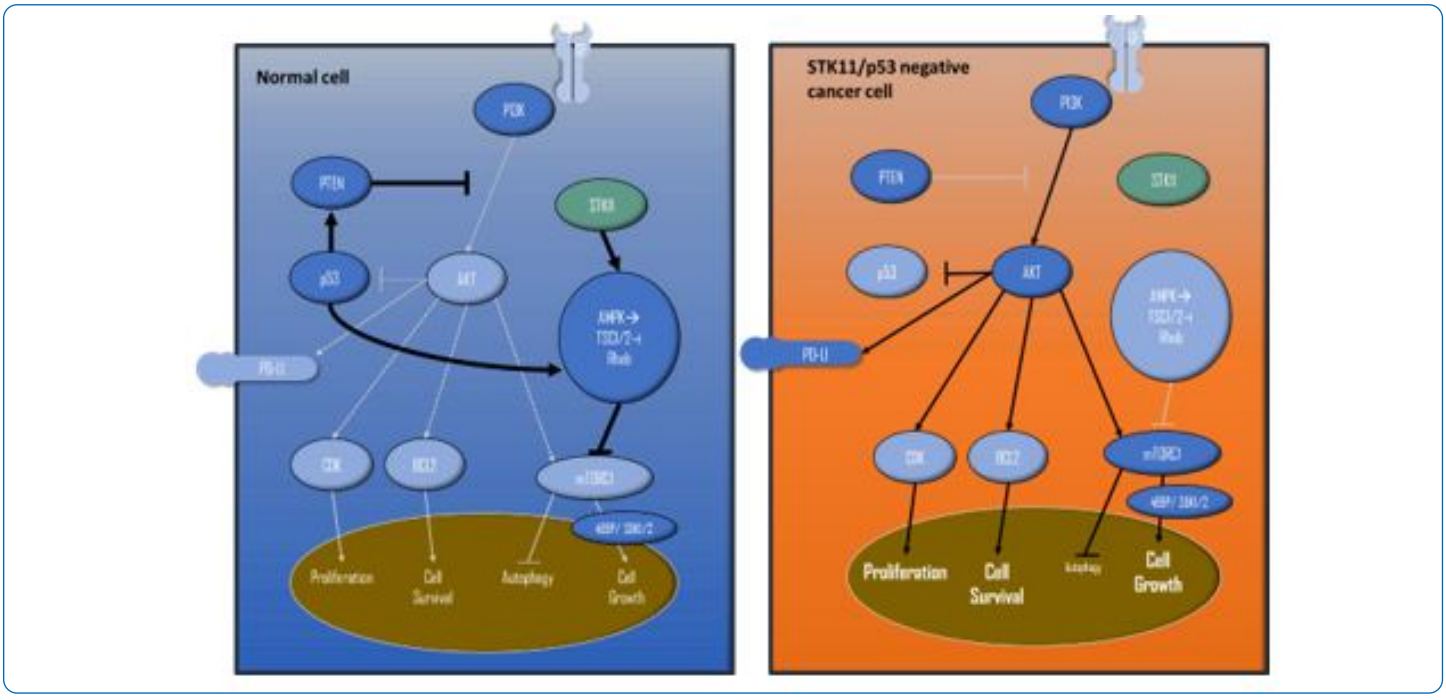

Figure 3: Simplified PIK3/AKT/mTOR pathway and interactions with STK11 and p53. The left shows a wildtype cell, the right cell displays how STK11 and p53 loss of function lead to extensive proliferation/cell survival and cell growth, because of the missing negative feedbacks and activations

Case 1	
Genetic mutations/amplifications	PIK3CA p.E545K activating
Chemosensitivity cell death rate [%]	Gemcitabine 72%, Oxaliplatin 59%
	Carboplatin 55%
	Vinblastine 58%
	Etoposid, no response
	Topotecan, no response
IHC Staining (PD-L1, EGFR, VEGFA, mTOR)	EGFR
MMR/MSI	Negative
Tumour Mutational Burden	0,59 mutations/Mb blood based
Pharmacogenetics (altered metabolism)	ERCC1; rs11615 GG, NT5C2; rs11598702 TT

Case 2	Liver tissue	Blood
CNV/ fusions/ SNV / Indel	STK11 p.Y131*(l.o.f) Tier II levelC TP53 p.R290fs (l.o.f) Tier I level B FGFR1 amplification 22 cop Tier I level B FGFR2 amplification 12 cop Tier II level C VEGFA amplification 5,61 cop	---------- TP53 p.R290fs (l.o.f) FGFR1 amplification 7cop FGFR2 amplification 5cop ----------
Pathway modelling (mRNA based)	Increased signaling of TUBB2B, PGF, VEGFA, HIF1	
Chemosensitivity Cell Death Rate [%]	Gemcitabine +Carboplatin 85% Etoposid 79% Gemcitabine 60% 5-Fluoruracil 56% Carboplatin 55%	
ICH staining	PDL negative	VEGFA
MMR/MSI	MSI stable	
Tumour mutational burden	2,21 mutations/Mb tissue based	
Pharmacogenetics (altered metabolism)	ERCC1 -> rs11615A6 + MTHFR; rs180113366 and DPYD*1/*5	

Table 1: Main results of the tumour profiling of patient 1 and 2

The analysis of the PI3K pathway, including the peripheral effector components involved at molecular and mRNA level, indicated that the application of the PI3K inhibitor Alpelisib would not suffice to inhibit the complete PI3K/AKT pathway. This assumption is supported by the fact that important components of tumour metabolism like PEPCK (phosphoenol-pyruvate-carboxykinase), of cell cycle progression like CDK20, MYC and factors of cell survival like Mcl1, BIM were not upregulated. In addition, IRS family member 4, which constitutively can hyperactivate the PI3K/AKT pathway, was downregulated on mRNA level. Components of the cross-linked oncogenic MAPK pathway [22, 42], such as RAS, were not upregulated, therefore inhibition of this pathway did not appear promising [32]. To address this issue and to take into account the high proliferation rate, a cytostatic agent was administered in analogy to the study NCT04215003 together with Alpelisib.

For the first time a remarkable therapeutic effect was observed - the patient´s condition changed from ECOG II towards ECOG 0 within three weeks, also because oxygen saturation had improved from 57 to 70mm/Hg. Sonographically, the effusion was not traceable anymore. Seven weeks later the patient suffered from an etiologically unclear thoracic pain event and died unexpectedly.

The second patient presented herself with a hepatic progress shortly after completing adjuvant therapies. Tumour profiling was performed based on liver biopsy and peripheral blood. A STK11p.Y131 mutation with clinical relevance was found. STK11/LKB1 mutations are reported in neuroendocrine tumours such as large cell subtypes [33–35], but rare in breast cancers with an incidence of 0,2-1,0% [35].

STK11 alterations are associated with a lack of PDL1 expression, and as in addition, the patient had low TMB and MSI stability, he could not benefit from checkpoint inhibitors at all [24].

The detected STK11mutation is considered to be a loss-of-function mutation resulting in possible activation of mTOR, as it is additionally induced by the detected p53 alteration (Figure 3). Functional loss of p53 activity can contribute to higher activity of the PI3K/AKT/mTOR pathways [36, 37].

To evaluate further the mTOR effect, we investigated additional peripheral effectors at mRNA level. Due to STK11 loss, the mTOR activation was most likely triggered via S6K1/2 (ribosomal S6 protein kinase 1/2) which was partially upregulated, stimulating proliferation by eIF4B and S6. Consequently, we applied the mTOR inhibitor Everolimus in this situation [35, 38–40].

Everolimus itself is approved by the FDA for hormone receptor positive and Her2 negative breast cancer. It is also standard of care for NETs in NCCN guidelines [41].

But mTOR inhibition as monotherapy based on allosteric inhibitors of mTORC1, like Everolimus, may lead to decreased therapeutic efficacy due to several resistance mechanisms: this could be incomplete inhibition of mTORC1 by effecting mainly mTORC1 substrates such as S6 rather than stronger factors like eIF4B [56], suppression of negative feedback loops, for example via increased IRS 1, which activates PI3K/AKT or interconnected oncogenic pathways like MAPK may be stimulated, just to mention a few of possible resistance factors [22, 27, 42]. There is evidence of potential synergism with angiogenetic inhibitors. Taking into account the presence of upregulation of VEGFA and HIF-alpha-pathway, Bevacizumab was added to Everolimus [26, 50, 53, 55, 57].

Due to the highly proliferating disease and extent metastasis, Capecitabine was administered in accordance with the test results [46]. This is not suprising as Capecitabine is standard of care to treat breast cancer and it is also mentioned in German guidelines for treating colorectal NETs or NETs with pancreatic origin. The therapy combination of Everolimus, Bevacizumab and Capecitabine was well tolerated. Imaging showed partial remission for three months. Then the tumour progressed dramatically and the patient died soon due to liver insufficiency.

Conclusion

The above explained complex content illustrates the advantage of evaluation of tumour specific targetable characteristics. This comprehensive tool was used in two different patients suffering from a highly aggressive cancer type after failing of empirical therapies in analogy to recommendations for BC and NEC.

To date, molecular profiling is used especially in breast, lung, colorectal, prostate and gastric cancer [47]. Here we demonstrate the scenario of two patients with a rare tumour entity as a role model to illustrate the benefit to which extent a broader molecular tumour profiling can offer a significant contribution - not only for diagnosis but also to the therapeutic regime.

In case of these two examples, therapy-relevant mutations were uncovered by analysing numerous tumour-relevant genes (>400) and pharmacogenomics. Specifically the intelligent combination of immunocytochemistry/-histochemistry, chemosensitivity testing on tumour cells, DNA alterations and expression profiles, could be detected and delivered valuable insights in tailored therapy.

Hence the rate of ineffective and cost-intensive therapies could be diminished and would improve the so far available personalized targeted therapies. Currently, application of solitary genetic testing deliver advantages only to a minority of patients [49–51]. The first basket trials (especially the SHIVA trial) mainly failed because molecular filters were applied [49]. Newer trials like the RESILIENT trial, had beneficial outcomes even in late stage patients with several previous therapies, applying enhanced molecular analysis comprising also cytological features and other cancer characteristics [15].

Promising new options are especially required for rare tumour entities, exemplified by NEBC, which remains a major diagnostic and therapeutic challenge today. The rarity of this tumour type makes it imperative to apply sensitive diagnostic tools for effective treatment options.

It would also be important for patients in whom empirical therapy has not shown any effect and possible therapies are being explored on the basis of tumour-specific profiles, taking into account possible resistance mechanisms. Viewed in isolation, not only the targets might be considered for the choice of therapy, but if possible, the context of the whole pathway network together with other biomarkers, too.

Questions that have to be asked are, whether the therapeutic effect justifies the application of comprehensive tools in these cases. In both intensively pretreated patients, actionable targets were discovered together with findings from ICC, chemosensitivity and pathway modelling leading to a treatment which was well tolerated and with an improvement of the overall situation. But both patients suffered from a highly aggressive cancer subtype leading early into metastatic situation where curative treatment is virtually not possible and the effects of the treatment did not last longer than a few months. Especially in rare cancers, where the prognosis is unfavorable from the beginning, we should think about using tailored therapies based on comprehensive tumour characteristics at an earlier stage, as only then we can find out whether this approach might provide a benefit. Trials to combine several rare cancer types and extensive profiling could hold the key for a successful treatment.

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