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RESEARCH ARTICLE

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Epigenetic Aberrancies as the Exclusive Driver of Oncogenic Amplifications

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ABSTRACT

Three genetic mechanisms, including mutations, gene amplification, and chromosome rearrangements, have been recognized as drivers of oncogene activation in human neoplasms. These mechanisms result in either an alteration of protooncogene structure or an increase in protooncogene expression. While the role of epigenetic aberrancies in carcinogenesis has been previously described, the biological implications of epigenetic therapies for cancer prevention and the underlying mechanisms remain a mystery to clinicians. Moreover, there is a lack of biomarkers to track the steps of carcinogenesis before cancer develops, leading to a deficiency in proactive and preventive measures. All recommendations in preventive oncology are either incomplete, blindly made, or via screening methods which are only able to detect cancer after it has already initiated. In this article, we present a novel approach that bridges the gap between clinical findings and research, generating advanced hypotheses on the development of cancer.

We explore the relationship between the host and tumor cells, highlighting the role of specific cancer stem cell pathways and proposing the use of multi-targeted epigenetic therapies and off- label medications to inhibit tumor initiators. By considering this complex biological network, we believe that the treatment of cancer can be revolutionized. Additionally, we discuss the novel idea of epigenetic abnormalities as the main cause of tumor oncogenic amplifications, shedding light on the promise of epigenetic therapies to reverse and treat these aberrancies.

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Introduction

Centuries have passed since Abu Ali Sina (Ibne Sina/Avicenna) became the first physician to study a breast cancer patient and introduce the concept of metastasis in his book "Canon of Medicine" [1]. We now know that cancer metastasis is the cause of morbidity in at least 66.6% of cancer deaths, although that number has been historically been reported as up to 90% in literature [2]. In recent times, scientists worldwide have made a groundbreaking discovery— cancer stem cells, which are now recognized as a primary cause of relapse in almost all cancer types, often times develop resistance to the traditional chemotherapy drugs and lead to an eventual metastasis [3,4].

Traditionally, cancer has been defined as the growth of mutated cells driving tumor progression, and efforts have focused on targeting these mutated cells to achieve regression or slow down tumor growth. However, no therapies have been developed to specifically target cancer stem cells or address their close relationship with the body as most conventional chemotherapy medications fail to destroy them [5].

The concept of epigenetic therapies aligns with the idea of treating the cause rather than the effect. Epigenetics emphasizes that the cause of tumor growth is not solely genome mutations but rather gene transcription influenced by the microenvironment [6]. Gene methylation, for instance, can lead to gene mutation, yet traditional genetic testing fails to detect non-mutated genes that can be methylated and lead to similar genetic aberrancies. Not long ago, the impact of the host's microenvironment on tumor growth was acknowledged, and now we are realizing that epigenetic changes in the microenvironment actually initiate tumor production and carcinogenesis [7].

This article explores a possibly revolutionary theory that delves into two neglected aspects of current oncology practice: stem cells and the microenvironment. It suggests that tumor mutations may be secondary to specific growth factor activations, rather than the other way around. Furthermore, it posits that the majority of tumor gene amplifications are caused or reversed by pure epigenetic mechanisms. While a few scientists have previously proposed that gene amplifications occur before mutation events, providing preliminary evidence that general mutagenesis is a side effect of gene amplification, the biological dissection and its correlation with growth factors have not been described in the literature [8]. This novel perspective aligns with the definition

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of gene amplification as over-expressive transcription, which is precisely observed with epigenetic gene transcription.

In this article, we hypothesize that genetic amplifications and epigenetic abnormalities are early steps in carcinogenesis, and advanced cases of cancer can be driven solely by amplifications rather than mutated cells. We discuss several cases in which this concept was applied to treatment of patients with remarkable success, thus supporting this idea. Furthermore, gene amplifications are commonly observed after the use of chemotherapy agents due to tumor selective advantages and pressure, which we believe may be tampered via epigenetic therapies. This warrants an active area investigation of the subject by the scientific community.

Objectives

In recent years, clinicians have turned their attention to liquid biopsy, a technique that captures real-time tumor genomic changes and measures tumor burden [9]. These technologies have helped assess the heterogeneity of tumor genomics and its ability to adapt and switch driver genes under different selective pressures. For example, based on the mutated forms observed in liquid biopsy after first-line treatment, drugs targeting EGFR can be switched accordingly. Liquid biopsy also allows the identification of gene amplifications in tumors with deranged genome stability, which was a technique used as a primary screening tool in this study [10].

The aim of the treatment protocols used here was to inhibit the three following cancer stem cell driver genes: Hedgehog, Notch1, and Wnt. The involvement of growth factors, such as FGF and TGF, in cancer stem cell activation and the epithelial-to-mesenchymal transition (EMT) warranted the design of these protocols to inhibit the Wnt and Notch1 pathways [11].

Wnt was specifically targeted using Lithium, which inhibits GSK-Beta and halts the cell cycle by reducing RAS (MAPKs) and PI3K/Akt pathway activity. By inhibiting these targets, the treatment was aimed to reverse the cause of cancer through epigenetic mechanisms. Moreover, the heightened presence of Tumor Growth Factor Beta 1 (TGF-B1) in the blood specimen of patients with high number of mutations prompted an exploration of strategies to lower TGF-Beta1 levels. In relation to this, the use of blood thinners has been recommended in the literature to reduce tumor growth, and the mechanism behind this action is related to the urokinase plasminogen activator's similarity to TGF [12,13].

Similarly, the hedgehog (HH) pathway is essential for embryonic development, but has also been linked to various types of cancer when overactivated. This makes this pathway one of the main drivers of stem cell development, including cancer stem cells. Thereby, the inhibition of the hedgehog pathway was of a particular interest to us. Several compounds, including Veratrum and certain teratogens, have shown the ability to inhibit this pathway in the past [14]. Notably, the effects of Ibrutinib and nicotine were of interest as they have shown intriguing effects in inhibiting the invasion of cancer cells in ovarian cancer, despite some studies mentioning that nicotine can cause progression and recurrence in certain cancer types [15-17]. As one of the most important embryonic development features, HH is also a major driver in cancer stem cell proliferation. As such, the treatment protocols described in this study were also aimed to inhibit the hedgehog gene, along with Notch1, and Wnt.

Methods and Materials

Patients presented in this series were treated through protocol : ICMS-2017-001, " pilot study of quality of life and survival measures for patients with terminal cancer, by using nutritional intervention, Quercetin as a nutraceutical agent" and/ or treated on standards of good clinical practice and compassionate basis, were informed and consented for the therapy and the results were collected and analyzed by independent party. Each compound was manufactured by prescription of an MD under sterile techniques in FDA approved facilities. Patients were after obtaining appropriate written consent forms in accordance with regional legislation and principals of declaration of Helsinki. Patients received therapies through mediport with sterile techniques, and no side effects or toxicities were reported throughout the duration of treatment.

Results

Case 1

A 40-year-old female with a history of invasive ductal carcinoma, estrogen receptor (ER) and progesterone receptor (PR) positive, was diagnosed in 2016. She underwent mastectomy but declined conventional therapies. The disease recurred as stage four metastasis to the chest wall, ribs, and both lungs. Seeking alternative therapies, her main concern was the pain in the sternum, where a large tumor was located. Initial findings revealed a germline mutation at SMAR, SNF/SWI.

After undergoing alternative therapies, the patient reported a decrease in pain in the chest wall, with the tumor pressing less on the sternum. Her quality of life (QOL) improved post-treatments, and her chest discomfort was nearly eliminated. Upon examination, the tumor had shrunk by 50 percent. A PET scan confirmed stable to improved findings, leading to restaging.

The patient's cDNA analysis showed a reduction in FGFR from 8.5 to 3.5, along with non- detectable levels of EGFR and CCNE1, after 15 days of the trial (measured on 11/29/2021). Further, her FGFR1 dropped to 2.5 on 3/11/22, as she continued with maintenance IV therapies once a week. The cancer marker CEA also decreased to 16.

On March 10th, a reevaluation using the guardant test revealed complete resolution of CCNE1 and EGFR, and a reduction of FGFR1 to 2.5 (Figures 1 and 2). A restaging PET scan on 5/13/22 showed a partial metabolic response in the large sternum mass (SUV down from 8 to 4.9). Additionally, there was an interval resolution of left pleural effusion and a partial response in widespread metastatic pulmonary disease. Lesions in the left posterior medial lung lobe decreased from 3 to 2 cm, with activity decreasing from 6.2 to 3.4.

The patient continues to show improvement with the therapies, and significant responses are observed in all her markers and scans.

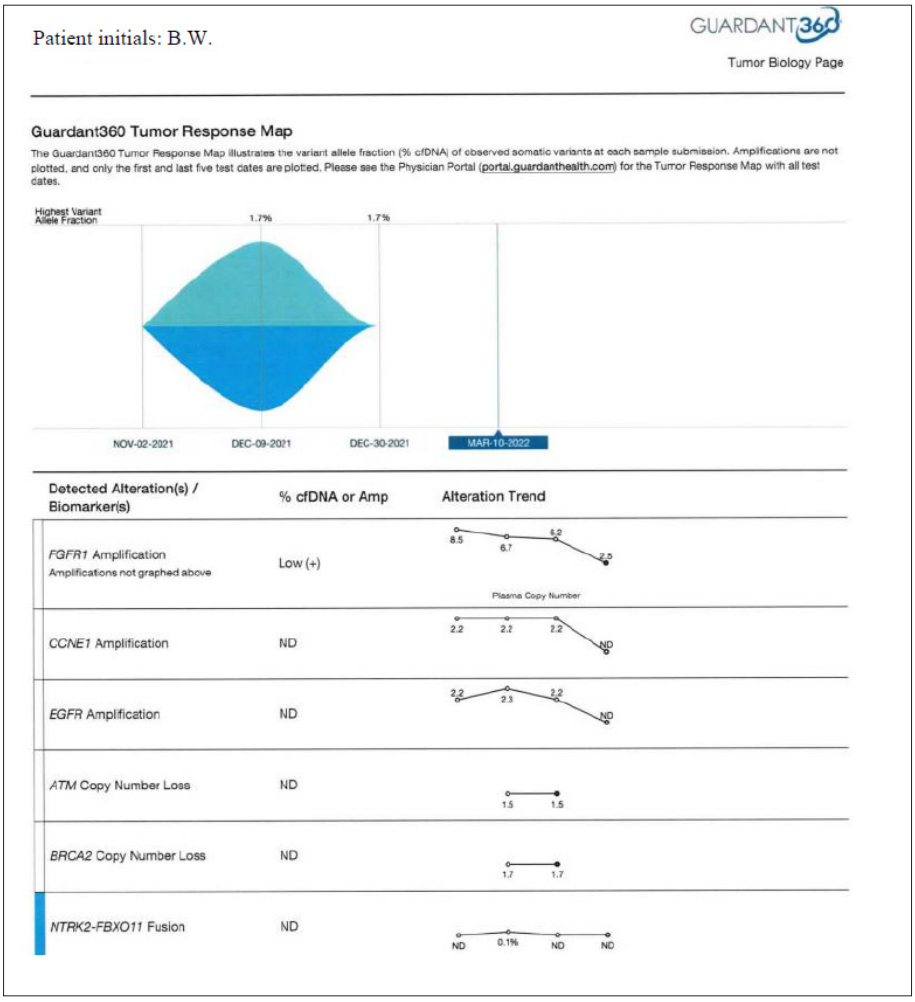

Figure 1: Results of Guardant360 blood test obtained for patient 1 regularly over months, showing the significant reduction in FGFR1 amplification from 8.5 down to 2.5, and CCNE1 and EGFR from 2.2 to nondetectable amounts.

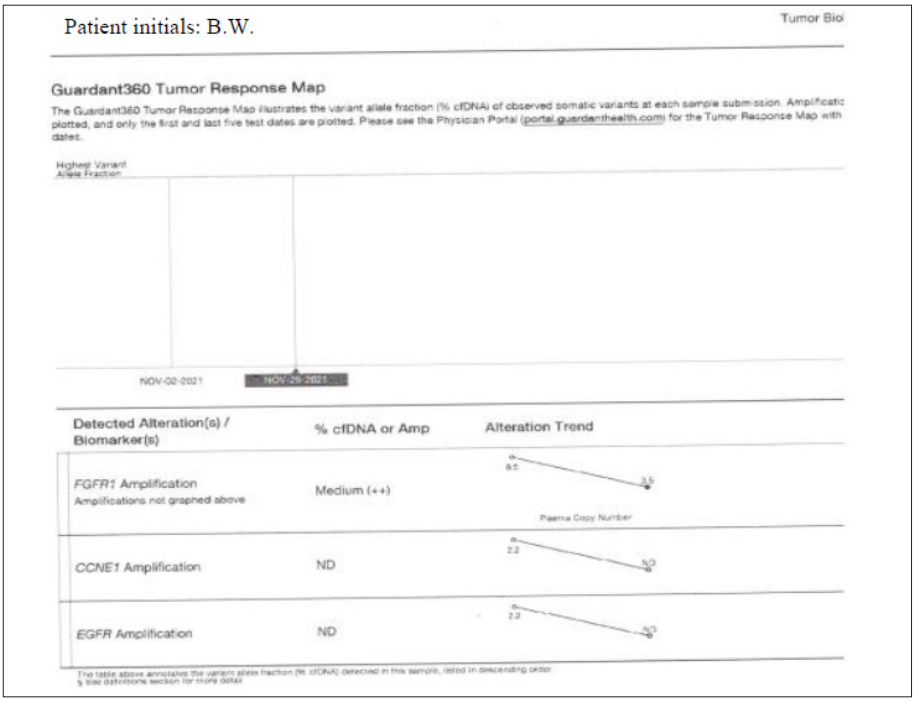

Figure 2: Results of Guardant360 blood test obtained on 11/29/2021 for patient 1 via measured ctDNA from blood samples, showing improvements in all previously detectable amplifications. CCNE1 and EGFR dropped from 2.2 to nondetectable amounts, and FGFR1 amplification dropped from 8.5 down to 3.5.

Case 2

A 50-year-old female with a history of invasive ductal carcinoma in her right breast, diagnosed in 2010. The tumor was estrogen receptor (ER) and progesterone receptor (PR) positive, HER2 negative, with a Ki-67 of 30 percent. She underwent a right mastectomy without hormonal blockade. However, the disease recurred in 2015. She initially responded to tamoxifen but later switched to aromatase inhibitors (AI). In 2020, treatment with Faslodex failed, and the patient began everolimus and exemestane. Unfortunately, liver lesions were detected in a scan conducted in August 2022. The patient had previously tried various alternative therapies, such as IV vitamin C, poly MVA, turmeric, ozone, and mistletoe, and sought a second opinion.

The patient underwent a scan on 11/22, which confirmed progressive liver disease. Laboratory results revealed elevated LDH at 319 and increased tumor markers, CA 27.29 and CEA, with

levels of 84. Further analysis of cDNA demonstrated amplifications of EGFR, FGFR, and BRAF, along with a mutated ESR1. The circulating tumor cells (CTC) test was positive, showing a high CK 19 at 114.

For treatment, patient was immediately started on daily IV epigenetic therapies. Remarkably, she experienced no side effects and maintained an active lifestyle, hiking about 3 miles every weekend. The treatments significantly improved her quality of life (QOL). After two weeks, labs were repeated on 12/13/22, showing stable to decreased tumor markers (CEA at 82) and LDH (299, further reducing to 263). CA 27.29 dropped from 1856 to 1782 (measured on 11/21 and 12/12/22). However, liver enzymes (ALK-P and AST/ALT) increased.

The cDNA analysis revealed a reduction in FGFR1 from 2.9 to 2.4. The mutated ESR1 had a lower mutation allele frequency (MAF), indicating a decrease in its presence. Notably, BRAF amplifications and EGFR amplifications completely resolved (Figure 3).

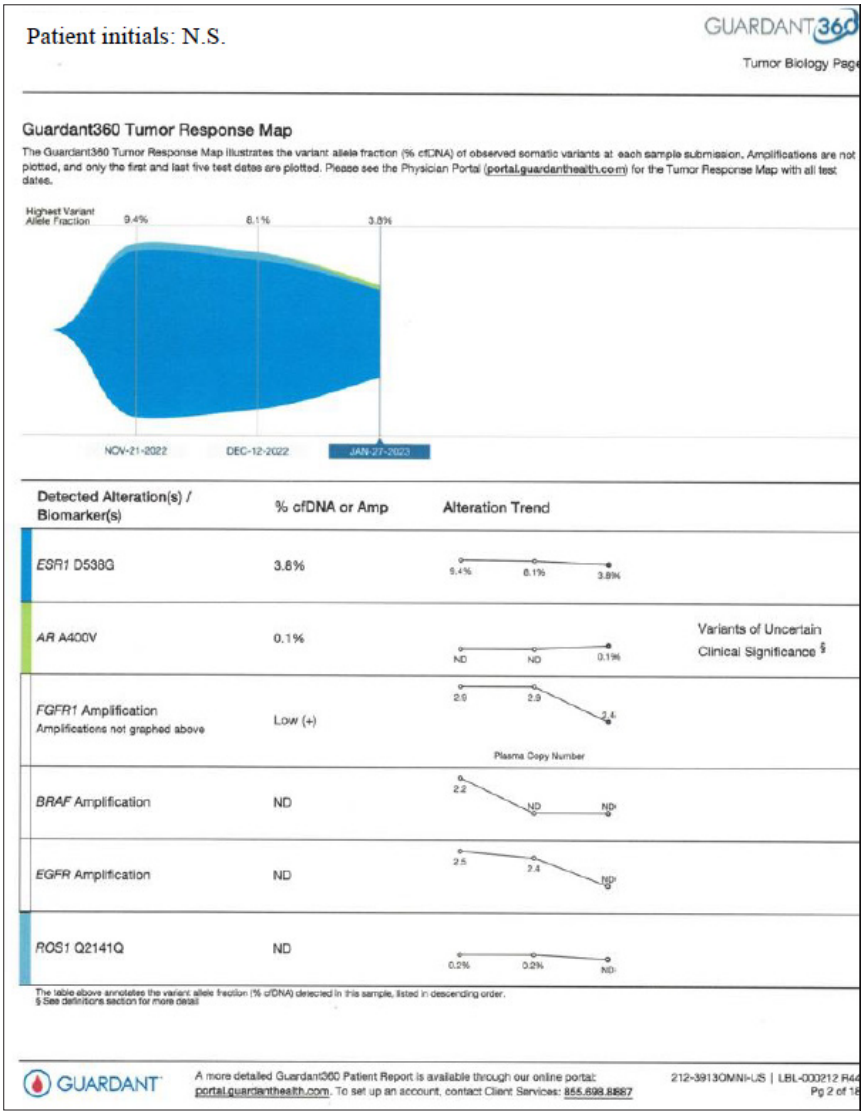

Figure 3: Results of Guardant360 blood test obtained over months for patient 2 via measured ctDNA from blood samples, showing improvements in all previously detectable amplifications. BRAF and EGFR dropped from 2.2 and 2.5, respectively, down to non-detectable levels. ESR1 and FGFR1 reduced from 9.4% and 2.9, to 3.8% and 2.4, respectively.

Discussion

Notably, the cases discussed in this article exhibited simultaneous mutated genes and multiple gene amplifications or were driven solely by gene amplifications. Interestingly, regardless of the gene type, the amplifications of genes responded significantly better to epigenetic therapies, as indicated in the figures above. Additionally, an intriguing observation was made regarding the correlation between transforming growth factor (TGF)-Beta1 levels in the blood and the presence of tumor amplifications versus mutations: TGF-Beta1 levels were consistently elevated in cases with genetic mutations (oncopromoter genes), while they remained within the normal range in patients carrying only gene amplifications. This raised questions about the relationship between tumor gene mutations and increased TGF-Beta1 levels and how they influence each other. This prompts further studies on the significance and effect of epigenetic therapies on TGF-Beta1 levels.

Epigenetic therapies administered to cases with genetic mutations in this case series led to a near-complete reduction or normalization of TGF levels, although the correlations were not direct. Further investigation into the activation mechanisms of TGF revealed its association with the hedgehog pathway, indicating that TGF induction and its receptor attachment are regulated by epigenetic mechanisms, particularly ubiquitination [18]. Compounds used in epigenetic therapies, such as Quercetin, have shown the ability to activate ubiquitination proteasome function on various oncogenes, including HER2. Other medications and compounds like Curcumin, Resveratrol, Metformin, and Avandia, have also been suggested in the literature to increase ubiquitination of TGF-Beta1 [19-22]. Epigenetic therapies containing Quercetin can inhibit DNMT and counteract carcinogenesis through this mechanism [23].

Conclusion

Based on our comprehensive literature review and subsequent hypothesis development, we have successfully formulated a protocol aimed at reducing TGF levels and inhibiting the hedgehog pathway. By examining the biological response and its correlation with clinical outcomes, our research indicates a direct causal relationship between the activation of TGF-Beta 1 and the occurrence of tumor mutations. Conversely, tumors lacking TGF production exhibit amplifications instead of mutations. Notably, our study demonstrates that epigenetic therapies can effectively impede tumor growth in these amplification-driven tumors, as evidenced by liquid biopsies. To the best of our knowledge, this investigation represents the first exploration of the impact of epigenetics on gene amplification in human subjects, as prior studies have primarily focused on primitive organisms such as drosophila flies rather than cancer cells in humans [24]. Nevertheless, the concurrence of our study's findings with previous research on humans with cancer can be rationalized by the evolutionary conservation of DNA replication machinery in both drosophila and humans.

With epigenetics becoming more recognized as a major cause for cancers, therapies should focus on reversing the epigenetic abnormalities via the same concept. While mechanisms triggering epigenetic abnormalities remain a subject of discussion, they certainly present promise as the epitome of future cancer therapies with a much higher likelihood of success than conventional non-epigenetic therapies. Factors such as hypoxia are currently being extensively studied due to its roles in angiogenesis, tumor resistance, acquisition of epithelial-to-mesenchymal transition phenotype, and changes in metabolism of cancer cells that lead to therapy resistance and cell quiescence [25,26]. This presents new avenues for investigating the impact of hypoxia on cancer pathogenesis and corresponding therapeutic interventions, thereby expanding our comprehension of the underlying mechanisms to cancer and facilitating the development of targeted treatment strategies.

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