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Combining Histone Deacetylase Inhibitors (HDACi) in a Specific Protocol Called Multi Targeted Epigenetic Therapy (MTET), Bruton's Tyrosine Kinase Inhibitors (BTKi), and Carbonic Anhydrase Inhibitors (CAi) in Cancer Treatment: Clinical Case Studies Highlighting Promising Results and Therapeutic Potential

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Abstract

Carbonic anhydrase inhibitors (CAi), histone deacetylase inhibitors (HDACi), and Bruton's tyrosine kinase inhibitors (BTKi) have demonstrated anticancer properties and the ability to target cancer stem cells. To the best of our knowledge, there is currently no existing in vivo or clinical literature investigating the exclusive combination of these three agents as a therapeutic approach for solid tumors. In this case series, we administered a combination of Acetazolamide, Tegretol, and Multi targeted epigenetic therapy (MTET protocol) consisting of quercetin, sodium phenyl butyrate, and Ibrutinib to end-stage patients with cancers of ovarian, colon, glioma, and sarcoma. Following treatment, a substantial reduction in tumor burden was consistently observed in all cases, as evidenced by labs –consisting of tumor markers and liquid biopsies (circulating tumor cells, and circulating DNA)– and imaging (whole body PET/CT). These findings collectively indicate that the combined use of HDACi, CAi, and BTKi as targeted epigenetic modifiers holds significant promise and clinical potential for improving the prognosis of patients with advanced-stage solid tumors across various cancer types.

Keywords: ***Multi targeted epigenetic therapy (MTET); Carbonic anhydrase inhibitors; Histone deacetylase inhibitors; Bruton's tyrosine kinase inhibitors; Epigenetic modifiers; Solid tumors; Cancer stem cells.***

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1. Introduction

Tumor resistance is a recurring challenge encountered in the treatment of cancer when relying on monotherapy approaches. An effective and cost-efficient strategy is to employ combination therapy involving epigenetic modifiers, some of which may have originally been developed for the treatment of non-cancerous conditions. Recent evidence suggests that carbonic anhydrase inhibitors, such as acetazolamide, can enhance the in vitro anti-tumor efficacy of histone deacetylase inhibitors (HDACi) in neuroblastoma cell lines [3]. Additionally, a preprint study demonstrated the therapeutic potential of combining HDAC inhibitors (panobinostat, vorinostat, entinostat, and pyroxamide) with a CA IX inhibitor (SLC-0111) against intrinsic pontine glioma (DIPG) cell lines [4]. Ruzzoini et al. conducted a study revealing that the combination of HDACi SAHA and CA IX inhibitor SLC-0111 exerted a more substantial blockade on cell viability and colony formation in breast, colorectal, and melanoma cancer cells compared to either treatment alone [5]. In an in vitro study conducted by Amengual et al., resistance of a genetically-modified cell line to Ricolinostat, an HDAC6 inhibitor, was reversed when combined with Ibrutinib, leading to a synergistic inhibition of cell proliferation [6]. Although these studies were promising, they were all conducted invitro and could not propose a clinical protocol to be implemented with established guidelines.

Moreover, anti-epileptic medications such as Tegretol and Acetazolamide, which have shown potent carbonic anhydrase inhibition abilities [7], have also shown anti-cancer abilities when combined with other epigenetic modifiers via enhancing chemosensitivity in a range of cancer cells both in Hep-2 cell cultures [8], and in-vitro in colorectal HT-29 and MC-38 carcinoma cells [9]. This calls into action the vast potential of other CAi drugs such as Tegretol and Acetazolamide to be used in combination with other epigenetic therapies. Ultimately, the utilization of these three agents in combination offers the prospect of enhanced therapeutic benefits compared to individual agents alone, as it may mitigate the development of treatment resistance, potentiate synergistic effects, necessitate lower drug dosages, and result in reduced adverse effects [10]. Consequently, superior clinical outcomes and improved disease prognosis can be anticipated for patients with solid tumors.

We conjecture that CAi agents indirectly cause a pH drop around solid tumors via blocking $CO_2 \rightarrow CO_3H^-$ conversion, which is normally catalyzed by carbonic anhydrases in the presence of excess CO_2. The sequential inhibition of carbonic anhydrases allows the activation of other epigenetic modifiers, such as HDACi and BTKi, and enhances their effectiveness at preventing tumor growth. Particularly, carbonic anhydrase IX has demonstrated induced overactivity in response to tumor-associated hypoxia [11], suggesting further investigation on the molecular mechanism of action pertinent to solid tumor growth when carbonic anhydrase IX is inhibited.

In order to gain deeper insights into the potential clinical impact of a combination therapy comprising carbonic anhydrase inhibitors (CAi), histone deacetylase inhibitors (HDACi), and Bruton's tyrosine kinase inhibitors (BTKi), we treated four patients in a pilot study with combination of Acetazolamide, Tegretol, Quercetin, Phenyl butyrate, with and without Ibrutinib. The decision to combine HDACi with BTKi was based on its potential synergistic effects by targeting multiple pathways, including regulating the expression of the transcription factor c-Myc and the protein cyclin D1, both of which play pivotal roles in aberrant cell cycle progression observed in various cancer types [12].

2. Materials and Methods

Prior to the initiation of the study, each patient was provided with detailed information regarding the treatment, and informed consent was obtained. Subsequently, the patients were enrolled in a phase II clinical trial or received treatment off trial. The administration of each treatment adhered to the standards of good clinical practice and was conducted on a compassionate basis. Written consent forms were obtained from the patients in accordance with the relevant regional legislation and the principles outlined in the Declaration of Helsinki.

All treatments utilized in the study were patented and administered orally or intravenously at predetermined doses on a daily basis. The treatment regimen consisted of patented histone deacetylase inhibitors, namely NP-Q (Nano particles of Quercetin) and phenyl butyrate, delivered intravenously. In addition, oral Acetazolamide, Tegretol and Ibrutinib were administered as carbonic anhydrase inhibitors (CAi) and Bruton's tyrosine kinase inhibitors (BTKi), respectively. The dosages and frequency of administration were predetermined and maintained consistently throughout the study.

Laboratory examinations were conducted within a month following the initiation of therapy. These examinations were repeated regularly as specified, and the results are reported in the corresponding sections of this article. Each case's medical history, physical examinations, and subsequent follow-ups were thoroughly documented and discussed in detail, ensuring comprehensive evaluation and analysis of the treatment outcomes.

3. Results

3.1 Case (1)

A 53-year-old female with a medical history of poorly differentiated left breast adenocarcinoma confirmed by biopsy in February 2011, presented to our clinic for assessment and treatment. The patient had not received any conventional therapies prior to seeking our care. Upon examination, she exhibited an asymptomatic state, except for a palpable oval mass measuring 4-5 cm at 12 o'clock in the left breast. Fibrocystic changes were also observed in other areas of the breast, as well as in the right breast. The patient declined all conventional treatment options and immediately commenced intravenous targeted epigenetic and antioxidant therapies on a daily basis. Initial evaluation revealed normal tumor markers, elevated copper levels, and low Vitamin D3 levels. The patient's Natural Killer (NK) cell activity was measured at 135, which fell within the normal range. Previous imaging had indicated the presence of two masses in the left breast, both of which were biopsied and diagnosed as infiltrating poorly differentiated (Nottingham grade 3)

ductal carcinoma with ductal carcinoma in situ (DCIS). Additionally, the patient tested positive for BRCA1 polymorphism.

After completing a series of ten treatments, the patient underwent reassessment. Her quality of life showed improvement, as measured by the Eastern Cooperative Oncology Group (ECOG) functional status and memory function. Upon examination, no palpable mass was detected in the left breast after six treatments. Tumor marker levels remained within the normal range. A follow-up scan conducted on November 15, 2011, after 25 days, revealed no evidence of tumor at the site of the previous biopsy in the left breast. Only one of the two previously diagnosed malignancies was visible, with a size of 1.9 x 1.2 x 1.7 cm on MRI and 1.3 cm on high-resolution CT scan, representing a reduction from the initial measurement of 4 x 2 cm on October 20, 2011, just prior to treatment. The PET scan indicated an SUV (standardized uptake value) of 5.3, without prior scans available for comparison. No local or distant invasive sites were identified.

Follow-up laboratory tests conducted on February 1, 2012, confirmed stable tumor markers (CA 15-3 at 15.6 compared to 17.6), normalized lactate dehydrogenase (LDH) levels at 123 from 179, and copper levels returned to normal at 108 from 187. Physical examination revealed no detectable tumor. On April 26, 2012, the patient's CA 15-3 marker dropped further to 9.4.

Subsequently, the patient left California and did not receive any further therapies from our clinic for a year. Upon her return, in July 2013, she underwent restaging PET imaging and laboratory tests, which confirmed disease progression despite hormonal therapies. At that point, she commenced treatments at our clinic, receiving therapy twice a week for 12 weeks. A restaging PET scan conducted on September 26, 2013, showed stable disease activity with no significant change (23.4 versus 23.2). The tumor size increased slightly from 3.9 cm to 4.4 cm, but the rate of growth had decreased. No new lesions were identified, and the staging remained stable. The patient was scheduled for left modified radical mastectomy with lymph node dissection, and further maintenance treatments once a week, with subsequent scans every three months.

The patient consented to the left modified radical mastectomy, including lymph node dissection, which was performed in October 2013. She declined radiation and chemotherapy. Pathological examination conducted in November 2013 revealed invasive ductal carcinoma measuring 3.8 cm, grade III, with one positive lymph node out of 12 examined. An Oncotype DX test performed on the tumor indicated a recurrence score of 50%, suggesting the requirement for chemotherapy, which the patient refused. The patient continued therapy at our clinic on a weekly basis. A restaging PET scan conducted on March 12, 2014, showed no residual disease, with normal uptake, after six months of remission. The patient continued receiving maintenance-level therapies for one year before temporarily discontinuing.

In January 2016, the patient's tumor marker CA 125 showed an increase, leading to an ultrasound and PET scan that suspected bilateral masses in the ovaries, which were subsequently confirmed to be malignant ovarian adenocarcinoma. The patient also tested positive for somatic BRCA1 mutations, as well as ERBB4, NF-1, PDGFR, and MSH6 mutations. Circulating DNA measurements identified actionable targets, including Myc, TERT, Met, and NTRK1.

These alterations were found to be epigenetically driven and were detected in liquid biopsy, guiding the clinician's decision-making process. The patient received IV epigenetic therapies following the MTET protocol, without any additional therapies such as chemotherapy. The liquid biopsy indicated near-complete resolution of circulating DNA, and this resolution was further confirmed through testing conducted by Guardant Laboratories in September 2017.
The patient continued to receive IV therapies at a frequency of one to three times a month between 2019 and 2022, maintaining stable disease. However, she temporarily stopped therapies between February 2022 and December 2022 due to financial constraints. Upon restarting the therapies, scans and laboratory tests revealed disease progression, with a CA 125 level of 339 on December 20, 2022. Subsequently, the patient resumed IV epigenetic therapies, along with acetazolamide, Ibrutinib, and Tegretol, on a weekly basis in December 2022. Notably, her CA 125 level significantly dropped, measuring 231 on March 28, 2023, representing a 30% decrease in three months. By 9/25/17, all alterations once detectable in patient's ctDNA were nondetectable, as shown in Fig. 1 below.

As of the present, the patient continues to receive therapies at our clinic, yielding superior results.

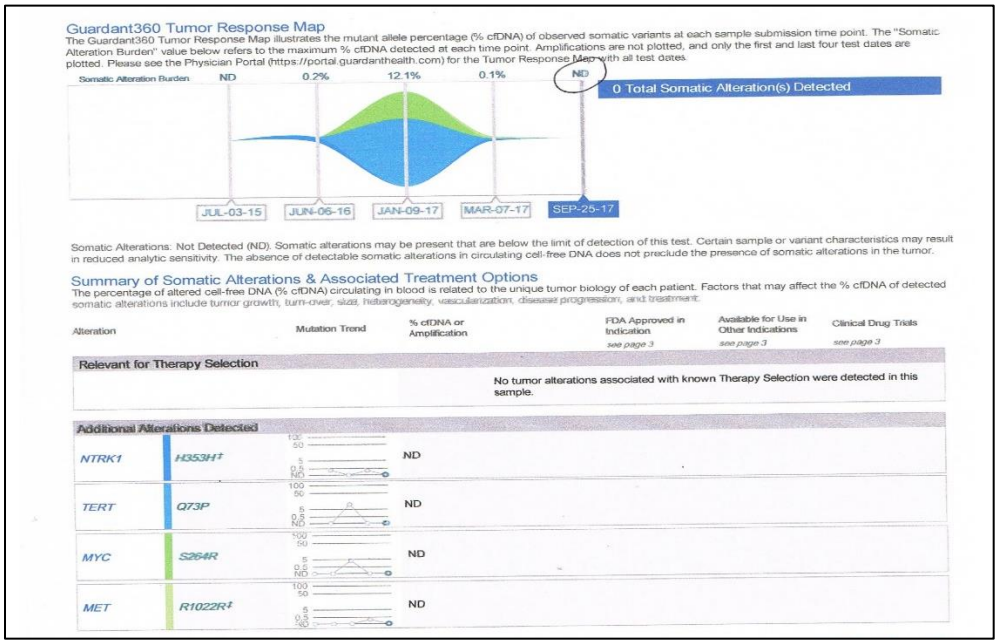


Fig. 1. Guardant360 Test results for patient 1, demonstrating a significant drop in alterations of NTRK1, TERT, MYC, and MET genes detected in cfDNA of patient collected via blood samples.

3.2 Case 2

31-year-old female with a medical history of colorectal cancer was diagnosed in September 2022 after experiencing months of ignored rectal bleeding. At the time of diagnosis, the patient was classified as stage four with metastatic disease in the lungs, chest lymph nodes, and abdomen. Given the advanced stage of the disease, the patient was advised to initiate chemotherapy for palliative purposes.

The patient had been experiencing intermittent rectal bleeding for several months. Laboratory tests were conducted immediately, revealing elevated frequencies of mutations in APC, TP53, FANCA, BRAF, PI3KCA, and EGFR genes. The patient was promptly started on intravenous epigenetic therapies administered on a daily basis. Additionally, she

received an oral combination of acetazolamide, Ibrutinib, and Tegretol, along with Phenyl butyrate twice a week at a dosage of 5 grams per day. After approximately one month of therapy, her lab tests were repeated on January 24, 2023, demonstrating a significant reduction in the mutant allele frequency (MAF) of the altered genes. Please refer to Fig. 2 below.

A follow-up scan was performed on March 23, 2023, revealing a reduction in the tumor burden associated with metastatic disease. The transforming growth factor level decreased from 17,905 (measured on January 24, 2023) to 4,305 (measured on February 23, 2023). Further she underwent a whole body restaging PET/ CT on 8/8/23, which showed a partial metabolic response on all metastatic disease.(for example reduced SUV activity from 7.6 to 5.2 in inferior upper lobe lung mass, left lower lobe from 6.4 to 5.7, original tumor in rectosigmoid from 23 to 16.9, left retroperitoneal lymph nodes from 3.1 to 2.4, left external iliac SUV down from 3.8 to 2.9.(although the sizes of the lesions had increased but since the metabolic activity had decreased, it was interpreted as tumor necrosis). At this time patient had almost completely stopped bleeding from rectal mass, and her transfusion requirement had dropped significantly. The patient continues to receive care at our clinic without experiencing any negative side effects.

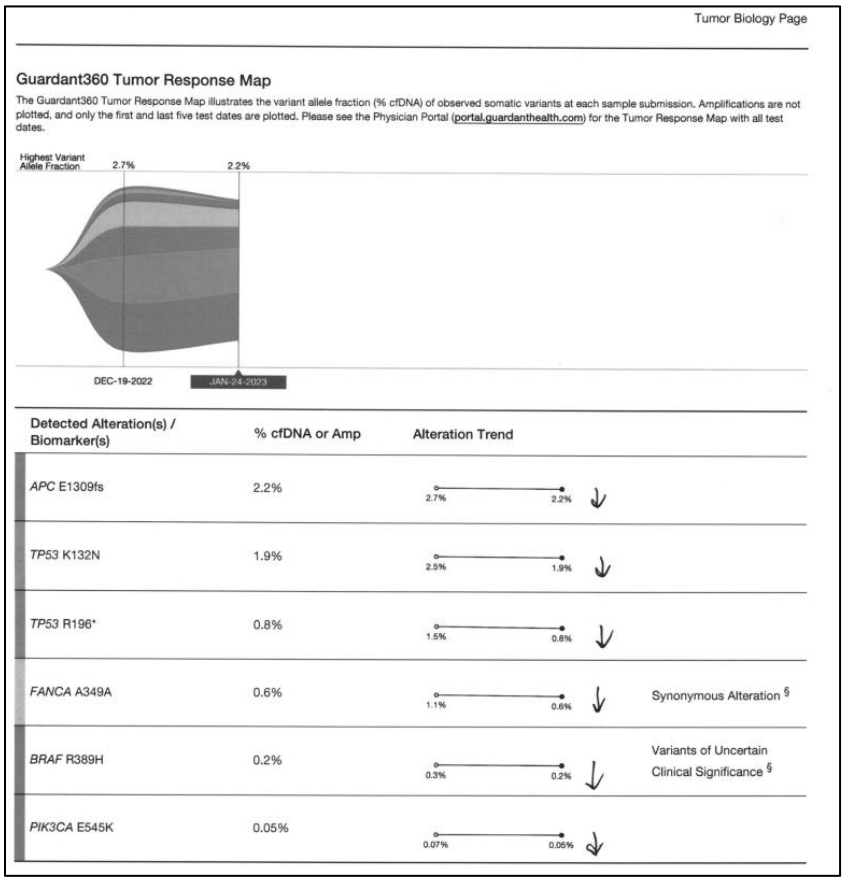


Fig. 2. Guardant360 Test results for patient 2, demonstrating a significant drop in alterations of APC, TP53, FANCA, BRAF, and PIK3CA genes detected in cfDNA of patient collected via blood samples.

3.3 Case 3

A 68-year-old male with a medical history of glioma was diagnosed in June 2022 after undergoing an incomplete resection of a large parietal lobe mass. The patient declined standard therapies such as Temodar and radiation and opted for alternative treatments, including glutamine, carnitine, dendritic vaccine, and injections of activated natural killer (NK) cells. Despite these alternative therapies, the tumor continued to grow, prompting the patient to seek treatment from Dr. Rubio in Mexico, where further alternative therapies were attempted. Further analysis of his tumor genomics revealed deletions of IDH/1q/19P and H3K27me3 mutations. The tumor had somewhat responded positively, as confirmed by the MRI, which revealed a stable mass measuring 1.8 cm in diameter. Concurrently, the patient received metformin, imatinib, and dexamethasone (1.75 mg) once a week.

He was referred to our clinic while still experiencing left leg numbness, the initial symptom leading to his diagnosis. Initial laboratory tests indicated the presence of circulating tumor cells (CTCs) exhibiting positive epigenetic marks and the presence of ERBB2. His tumor epigenetic alterations of H3K27me3 mutations activated telomerase, contributing to a better prognosis based on low P53 mutation rates (less than 3 percent), the absence of co-polysomy with deletions, and positive IDH status. However, the patient's overall survival was estimated to be approximately 16 months from the time of diagnosis.

Upon evaluation, the patient was immediately initiated on daily intravenous epigenetic therapies combined with CAi. After six weeks, his laboratory tests were repeated, revealing a complete response of the CTCs to the treatments (please refer to the results below). The patient experienced a significant improvement in his quality of life, with enhanced mental clarity, improved concentration, and resolution of leg weakness.

He was restaged by Brain MRI and PET scan on 3/27/23. The results showed a complete response with no sign of residual disease activity compared to prior MRI (refer to Figs. 3 and 4 below). The patient continues to receive care at our clinic, demonstrating superior results in his ongoing treatment.

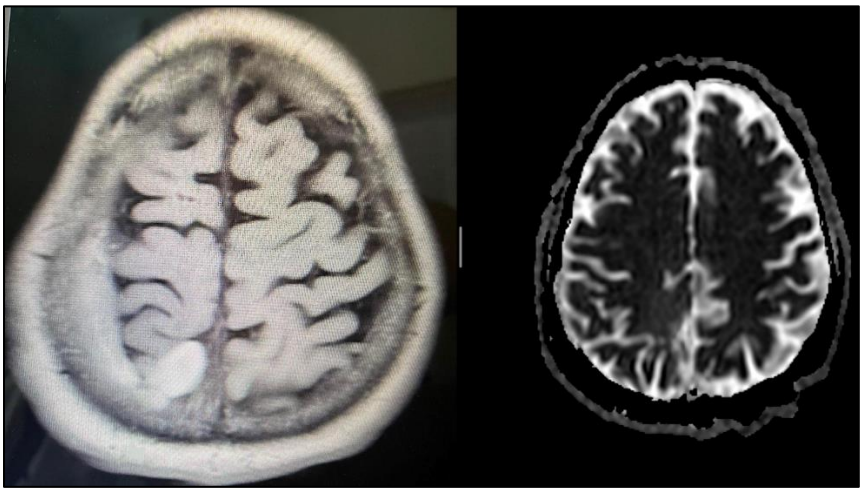


Fig. 3. Comparative MRI imaging of patient 3, pre-treatment (left) and post-treatment restaging (right). In contrast to the left image, the right image reveals a complete disappearance of the cephalohematoma and proximal glioma

located in the bottom right quadrant of the brain (note that the imaging is flipped). This observation suggests the absence of any remaining disease activity following the treatment.

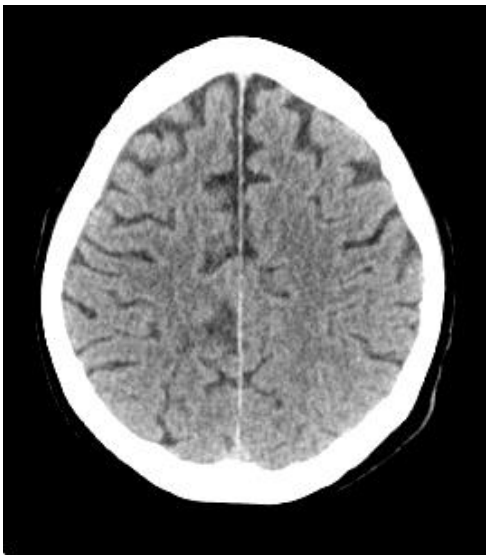


Fig. 4. PET scan post-treatment and surgical resection of tumor, confirming the full resolution of tumor and cephalohematoma.

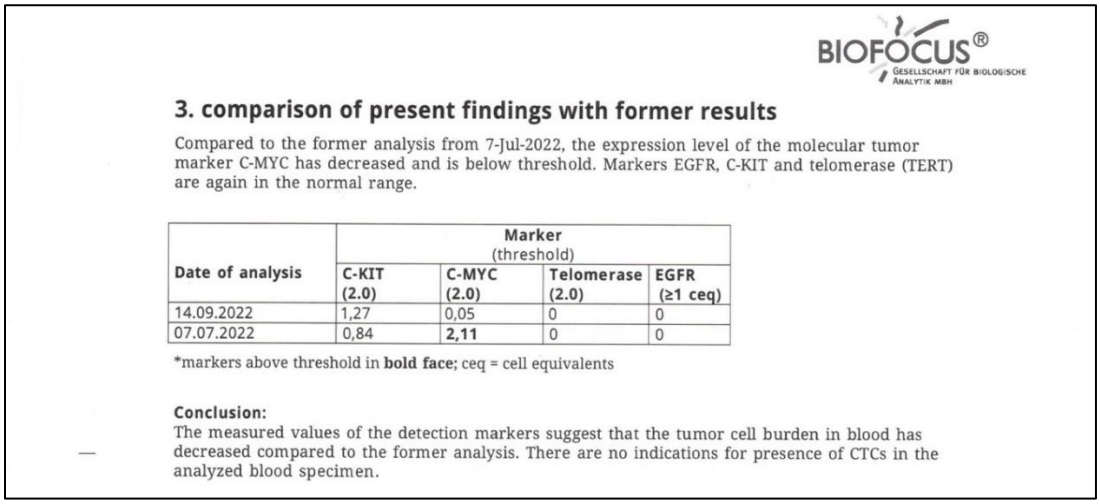
3.4 Case 4

In June 2022, the patient was referred to our clinic with a right ankle mass measuring approximately 3-4 cm. The mass had been present for several months, initially ignored but now growing rapidly. The patient's laboratory tests revealed positive IDH1/2 circulating DNA (cDNA) and positive circulating tumor cells (CTCs). The ankle lesion was painful and caused discomfort upon examination, appearing as a firm 4 cm round mass located laterally.

Confirmatory laboratory analysis confirmed the presence of CTCs in the patient's blood (Fig. 5), along with circulating DNA exhibiting the IDH2 altered gene (Fig. 6). Considering the patient's family history of cancer, with her mother and sister having lymphoma and melanoma, respectively, germline testing was performed and identified SUFU 423A>G mutation. Blood results also indicated an elevated level of TGf-beta 1, measuring 18,000, which was planned to be addressed through epigenetic therapies.

Chondrosarcomas commonly exhibit the IDH mutation in approximately 50 percent of cases, and the risk of recurrence tends to be higher than usual. However, these tumors typically have a slow growth rate. Additionally, there is an association with the CIMP (CpG island methylator phenotype) phenotype, suggesting the potential benefit of utilizing epigenetic therapies. Furthermore, activation of HIF (hypoxia-inducible factor) in these tumors suggests potential benefits from multitargeted epigenetic therapies. Consequently, the patient was promptly initiated on IV epigenetic therapies in combination with CAi. Over the course of 20 IV treatments, the patient's laboratory tests were repeated, revealing a significant reduction in TGf-beta 1 levels to 5,304 on 8/26/22. The CTCs completely resolved with the therapy (please refer to Fig. 5 below).

Post-therapies, the patient's circulating DNA completely disappeared (Fig. 6). Subsequently, the patient underwent surgical resection on November 15, 2022, after completing 35 treatments. The size of the mass decreased to 1.6 x 1.4 cm, and the pathology results showed no malignancy. Only a small number of cells exhibited SMA+ (smooth muscle actin), leading to the diagnosis of treated sarcoma/hemangiosarcoma (ERG+). Furthermore, patient's IDH1 alteration was no longer detectable by 9/20/2022 (refer to Fig. 7 below). Currently, the patient remains in complete remission, indicating a positive treatment outcome.



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3. comparison of present findings with former results

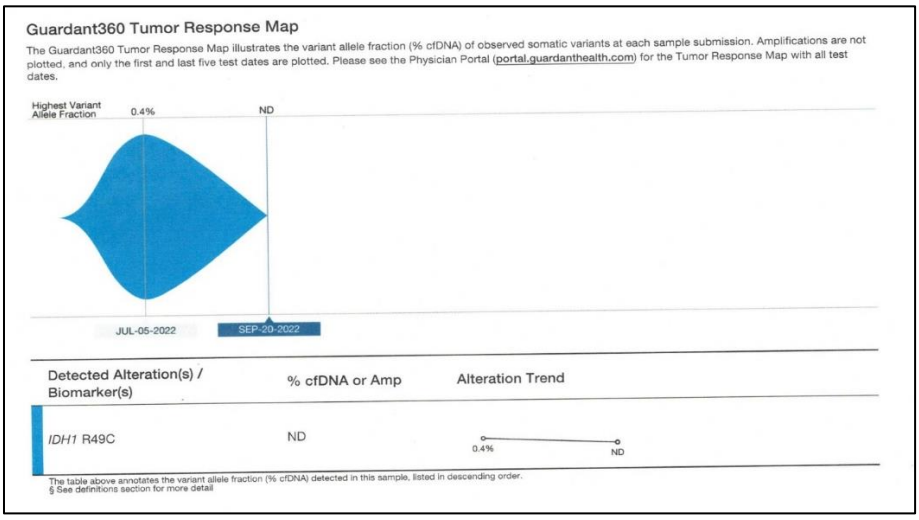
Compared to the former analysis from 7-Jul-2022, the expression level of the molecular tumor marker C-MYC has decreased and is below threshold. Markers EGFR, C-KIT and telomerase (TERT) are again in the normal range.

Date of analysis	Marker (threshold)			
	C-KIT (2.0)	C-MYC (2.0)	Telomerase (2.0)	EGFR (≥1 ceq)
14.09.2022	1,27	0,05	0	0
07.07.2022	0,84	**2,11**	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. There are no indications for presence of CTCs in the analyzed blood specimen.

Fig. 5. BioFocus test result for patient 4, demonstrating a significant drop in the molecular tumor marker C-MYC and lack of presence of any circulating tumor cells in the collected blood sample from the patient.



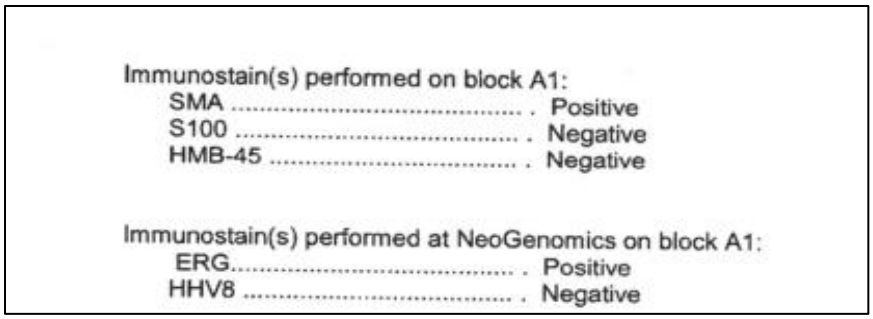
Guardant360 Tumor Response Map

The Guardant360 Tumor Response Map illustrates the variant allele fraction (% cfDNA) of observed somatic variants at each sample submission. Amplifications are not plotted, and only the first and last five test dates are plotted. Please see the Physician Portal (portal.guardanthealth.com) for the Tumor Response Map with all test dates.

Detected Alteration(s) / Biomarker(s)	% cfDNA or Amp	Alteration Trend
IDH1 R49C	ND	0.4% → ND

The table above annotates the variant allele fraction (% cfDNA) detected in this sample, listed in descending order.
§ See definitions section for more detail

Fig. 6. Guardant360 test result for patient 4, demonstrating a significant drop in alteration of IDH gene detected in cfDNA of patient collected via blood samples.


Immunostain(s) performed on block A1:
SMA ..... Positive
S100 ..... Negative
HMB-45 ..... Negative

Immunostain(s) performed at NeoGenomics on block A1:
ERG..... Positive
HHV8 ..... Negative

Fig. 7. Pathology report for patient 4, demonstrating positive response to treatment with no malignancies.

3.5 Case 5

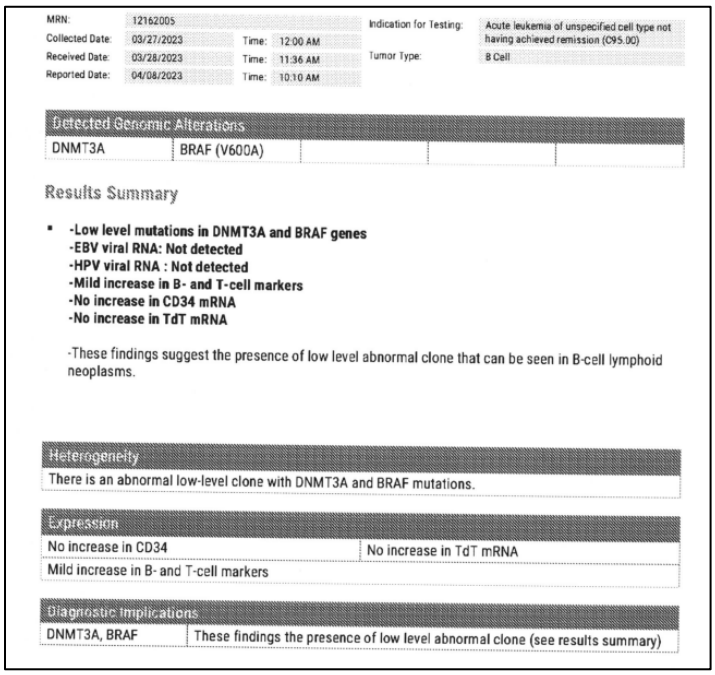
A 17 year old male with history of stage four high risk Acute lymphoblastic leukemia (ALL) initially diagnosed in 2019. Cytogenics remarkable for CRLF2 fusion (optic nerve leukemic infiltration) treated with standard chemo therapies, with initial response, in remission since 1/2022 when he completed his regimen; until March 2023, for last three weeks with left eye vision loss, due to infiltrated optic nerve (CNS involvement) seen his oncologist who recommended intrathecal chemotherapy as well as CAR T therapy, parents were hesitant to do this and had been referred by their PMD here for second opinion. At the time of arrival in march 2023, he was blind in left eye, and was on Decadron 24 mg a day. Appeared with Cushing syndrome, hypertension and diabetes (secondary to high dose steroids) and elevated liver enzymes (NASH). He also had mouth thrush secondary to fungal infection caused by high steroid immune suppression.

His bone marrow biopsy had shown a 1.6 percent leukemic cells, and he was technically relapsing. His Beta 2 micrglobulin, IL-2 soluble receptor, were normal, but his LDH was elevated and his liquid biopsies by Genomics lab detected abnormal colonies with mutated BRAF and DNMT3A.

We started him on IV epigenetic therapies to demethylate the DNAMT3A, along with hydralazine (both to reduce his blood pressure as well as demethylating DNA), included off label use of RU-486 and were able to taper down his steroids to prednisone at only 20 mg a day. (RU 486 synergizes with steroids on destruction of leukemic cells). Other off label drugs used were Vismodegib, (to inhibit the HedgeHog pathway), Quercetin (to inhibit Notch 1 pathway) and Ibrutinib, Acetazolamide, and Tegretol (to inhibit Cyclin D1) and phenyl butyrate to enhance the cytotoxic efficacy of Quercetin. The combination of HDACi and CAi (with or without Ibrutinib) has been published in our studies.

His labs were repeated after three weeks of therapies. His leukocytosis resolved. His liver enzymes are normalized, his hypoalbuminemia resolved and his LDH dropped from 457 (5/4/23) to 228(5/17/23) to 170 (6/12/23). His vision improved but he still had compromised vision in the left eye. His Cushing syndrome was resolved. His Genomic Lab was repeated on 6/14/23 (Fig. 9) which showed no detectable

abnormalities. All mutated DNA were resolved. (Compared to Fig. 8). Patient maintained a positive response to treatment throughout, and is continuing to receive care at our clinic, without the need for cytotoxic therapies.



MRN: 12162005
Collected Date: 03/27/2023 Time: 12:00 AM
Received Date: 03/28/2023 Time: 11:36 AM
Reported Date: 04/08/2023 Time: 10:10 AM
Indication for Testing: Acute leukemia of unspecified cell type not having achieved remission (C95.00)
Tumor Type: B Cell

Detected Genomic Alterations

DNMT3A	BRAF (V600A)			

Results Summary

- **-Low level mutations in DNMT3A and BRAF genes**
  -EBV viral RNA: Not detected
  -HPV viral RNA : Not detected
  -Mild increase in B- and T-cell markers
  -No increase in CD34 mRNA
  -No increase in TdT mRNA

-These findings suggest the presence of low level abnormal clone that can be seen in B-cell lymphoid neoplasms.

Heterogeneity

There is an abnormal low-level clone with DNMT3A and BRAF mutations.

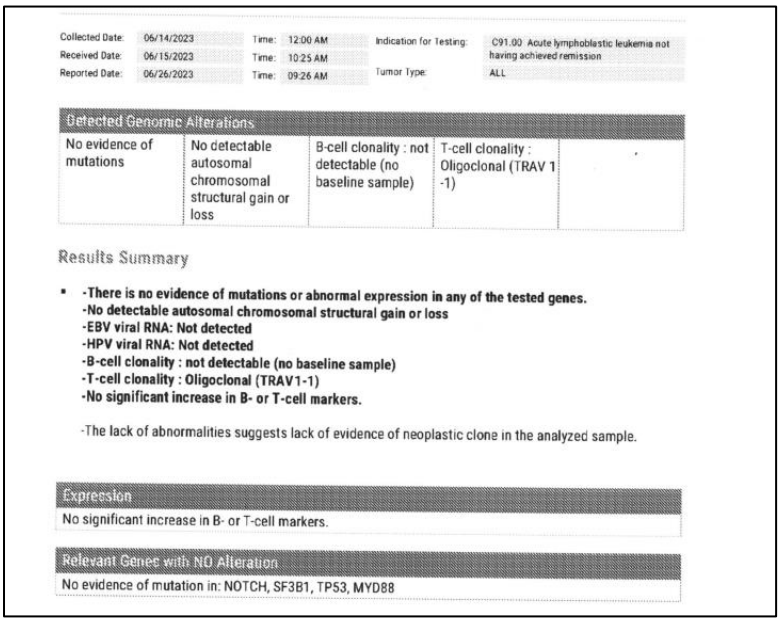
Expression

No increase in CD34	No increase in TdT mRNA
Mild increase in B- and T-cell markers	

Diagnostic Implications

DNMT3A, BRAF	These findings the presence of low level abnormal clone (see results summary)

Fig. 8. Liquid biopsy obtained via blood specimen before treatment initiation for patient 5. The results showed genomic alterations in DNMT3A and BRAF genes, as well as increases in B- and T-cell markers. This was indicative of the presence of abnormal B-cell lymphoid neoplasm clones.



Collected Date: 06/14/2023 Time: 12:00 AM
Received Date: 06/15/2023 Time: 10:25 AM
Reported Date: 06/26/2023 Time: 09:26 AM
Indication for Testing: C91.00 Acute lymphoblastic leukemia not having achieved remission
Tumor Type: ALL

Detected Genomic Alterations

No evidence of mutations	No detectable autosomal chromosomal structural gain or loss	B-cell clonality : not detectable (no baseline sample)	T-cell clonality : Oligoclonal (TRAV 1 -1)	

Results Summary

- **-There is no evidence of mutations or abnormal expression in any of the tested genes.**
  -No detectable autosomal chromosomal structural gain or loss
  -EBV viral RNA: Not detected
  -HPV viral RNA: Not detected
  -B-cell clonality : not detectable (no baseline sample)
  -T-cell clonality : Oligoclonal (TRAV1-1)
  -No significant increase in B- or T-cell markers.

-The lack of abnormalities suggests lack of evidence of neoplastic clone in the analyzed sample.

Expression

No significant increase in B- or T-cell markers.

Relevant Genes with NO Alteration

No evidence of mutation in: NOTCH, SF3B1, TP53, MYD88

Fig. 9. Liquid biopsy obtained via blood specimen after 10 rounds of treatment for patient 5. The results showed no genomic alterations or presence of increased B- and T- cell markers. This was indicative of the resolution of the previously detected neoplastic clones in the patient's blood.

4. Discussion

Clinical and laboratory examination results presented in this study reveal the synergistic effects of CA inhibitors in combination with BTKi (Bruton's tyrosine kinase inhibitors) and HDACi (histone deacetylase inhibitors) epigenetic modifiers. The study utilized liquid biopsy techniques via Guardant360 and Biofocus laboratories, which demonstrated significant reductions in ctDNA (circulating tumor DNA) and CTC (circulating tumor cells). These biomarkers (collectively called liquid biopsies) have shown significant promise for cancer screening in all stages of cancer [13], particularly when used for cancers of breast [13], [14], colorectal [15], [16], and bone [17]. Patients 1,2, and 4 were therefore appropriate candidates for this type of screening, which was selected to both monitor the efficacy and guide the treatment approach for all patients. Notably, treatment with the combination of mentioned medications resulted in a substantial decrease in the CMyc mRNA expression of CTC in one patient, while another patient experienced a greater than 30% reduction in CA125 levels from 339 to 231. The observed significant reduction in c-Myc as measured by BioFocus in case 4 aligns with the hypothesized synergistic effects of CA inhibitors, HDACi, and BTKi on c-Myc, highlighting the need for further investigation into their long-term impact on both c-Myc and cyclin D [18].

To the best of our knowledge, this is the first clinical study that examined the combined use of these three agents and their direct effects on c-Myc levels. The significant reduction seen in CA125 also calls for further molecular investigation of the combined use of the aforementioned agents on the expression levels of MUC16 gene. While preliminary studies have been done in the individual impact of Bruton’s tyrosine kinase inhibitors [19], and of course carbonic anhydrase inhibitors [20] on the MUC16 gene, no studies have been performed on the combined effects of CAi, BTKi, and HDACi, or individually on HDACi.

In all four cases, all tumor markers exhibited significant reductions two weeks after initiating the therapy, as determined by Guardant360 testing. This response can be attributed to the ability of the combined therapy to target multiple pathways, thereby reducing the likelihood of tumor resistance compared to individual therapeutic agents. Moreover, this combination therapy may induce hyperacetylation, leading to enhanced expression of two critical onco-suppressor genes, p53 and histone H4. Both of these genes play pivotal roles in DNA damage repair and maintaining cellular homeostasis by preventing uncontrolled proliferation [5], [21].

The collective findings from the four case studies highlight the promising effects of combining HDAC inhibitors (HDACi), BTK inhibitors (BTKi), and CA inhibitors (CAi) in cancer treatment. These case studies demonstrate the potential of this targeted and multifaceted approach in improving patient outcomes and quality of life.

In Case Study 1, the combination of patented HDAC inhibitors, NP-Q, phenyl butyrate, and oral CAi and BTKi resulted in significant improvements for a patient with breast adenocarcinoma. The delivery of these therapies on predetermined doses and daily frequency, along with regular monitoring of labs, led to tumor size reduction and enhanced quality of life.

Case Study 2 highlighted the benefits of incorporating HDACi and CAi along with targeted agents in a patient with colorectal cancer. The use of these therapies resulted in a reduction in mutated allele gene frequencies in liquid biopsy and a positive response in tumor burden, underscoring the potential of this combination approach.

In Case Study 3, the administration of IV epigenetic therapies, including HDACi, to a patient with glioma demonstrated positive imaging outcomes. The patient experienced improved quality of life, a complete response in circulating tumor cells, and complete response documented in his brain PET and MRI, highlighting the potential efficacy of this comprehensive treatment approach.

Case Study 4 showcased the success of combining HDACi, CAi, and BTKi in the treatment of a patient with spindle cell sarcoma. Through this tailored approach, which targeted specific genetic alterations, complete pathological response was achieved.

Lastly, Case study 5 was a case of leukemic relapse that responded very positively to epigenetic treatments with complete eradication of neoplastic clones and resolution of DNMT3A and BRAF gene mutations, which were likely the previous drivers of the patients' relapse symptoms.

5. Conclusion

The study's findings collectively suggest that the combination of HDAC inhibitors in MTET protocol, BTK inhibitors, and CA inhibitors holds promise in cancer treatment. By specifically targeting epigenetic alterations and relevant signaling pathways, this approach may offer enhanced efficacy and improved patient outcomes. While these case studies provide encouraging results, further research and larger-scale clinical trials are needed to validate these findings and establish optimal protocols for combining HDACi, BTKi, and CAi. Nevertheless, these studies contribute to the growing body of evidence supporting the potential benefits of this targeted therapeutic approach in cancer treatment.

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7. Conflict of Interest

The authors declare no conflict of interest in preparing this article.

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