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# Effect of Triple IV therapy (Artesunate, Ascorbate, and Doxycycline) on Circulating Tumor Cells and DNA: Two Consecutive Cases

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## Background

Artesunate (ART) is synthesized from artemisinin, an extract from the sweet wormwood plant, Artemisia annua [1]. Historically it has been used in Chinese herbal medicine as an antipyretic and more recently as anti-malarial, but it also has been shown to have broad anti-neoplastic properties [2]. ART has been reported to induce apoptosis, differentiation and autophagy in colorectal cancer cells by impairing angiogenesis [3], inhibiting cell invasion and migration [4], inducing cell cycle arrest [5], upregulating ROS levels, regulating signal transduction [for example, activating the AMPK-mTOR-Unc-51-like autophagy activating kinase (ULK1) pathway in human bladder cancer cells [6] and blocking immune escape [7]. In addition, ART has been shown to restore the sensitivity of a number of cancer types to chemotherapeutic drugs by modulating various signaling pathways. For example, ART can improve the apoptosis of HCC by inhibiting the PI3K/AKT/mTOR pathway [8] and can increase liver cancer cell sensitivity to sorafenib via suppression of the MEK/ERK pathway [9]. For ovarian cancer specifically, ART has been shown to have clinical activity in treatment [10]. Although the amount of clinical data regarding the use of ART as an anticancer drug remains limited, preliminary results have been encouraging in terms of efficacy and tolerance [11-14].

Doxycycline is an FDA-approved drug, which first became available in 1967. It shows minimal side effects and is currently used world-wide as a broad-spectrum antibiotic. In recent years, pre-clinical and clinical data suggest that this tetracycline antibiotic could be repurposed to target, inhibit and eradicate cancer stem cells (CSCs) in multiple cancer types [15-16], particularly when used in combination with high-dose intravenous vitamin C (HDIVC) [15]. Preclinical data exists that doxycycline not only has an inhibitory effect on ovarian cancer, but also can increase sensitivity to cisplatin [17].

High dose intravenous vitamin C (HDIVC) has been evaluated as a potential treatment for cancer as an independent agent and in combination with standard chemotherapies. It was proposed to have anticancer effects as early as the 1950s, but earliest efforts to use high-dose vitamin C as a cancer treatment did not occur until the 1970s [18]. Two most studied mechanisms by which pharmacologic ascorbate concentrations have cytotoxic effects on tumor cells include increased pro-oxidant damage that is irreparable by tumor cells, and oxidation of ascorbate into dehydroascorbic acid (DHA), which is an unstable metabolite and can be cytotoxic [19]. In recent decades, data have been published that HDIVC up to 1.5g /kg/day appears to be well-tolerated [20], may improve the quality of life of terminal cancer patients [21], and reduce chemotherapy-associated toxicity in patients with ovarian cancer [23]. This led to a renewed interest in studying high-dose IV vitamin C as an anticancer treatment [20,22,24,25].

Comprehensive genomic profiling (CGP) is a next-generation sequencing (NGS) approach that uses a single assay to assess hundreds of genes including relevant cancer biomarkers, as established in guidelines and clinical trials, for therapy guidance [26]. While solid tumor DNA sequencing has been employed in conventional oncology for over two decades, liquid biopsies only gained traction in 2016, when FDA approved the first "liquid biopsy" test [27]. Evaluation of circulating tumor DNA (ctDNA) dynamics in advanced cancer patients is a real-time, precise, non-invasive method to assess treatment response and disease progression [28]. It is now possible to assess the efficacy of a new cancer treatment in as early as 4 weeks. This tool has revamped integrative oncology clinical research. Treatment response can now be assessed more rapidly and more frequently than the few and far between interval scans. Some tumor markers – CA19-9, CA27.29, CA125, PSA, CEA among others – have a long half-life and

may not accurately reflect tumor growth or tumor burden [29].

## Preliminary Data

### Case 1

A 58-year-old, stage IC ovarian cancer at the time of diagnosis, presented 4.5 years later with small rise in CA 125. Patient was initially diagnosed in July 2017 with high-grade, serous ovarian cancer, pathologic stage pT1c pN0. At the time of diagnosis her CA 125 was 77.5 U/mL. After a robotic hysterectomy with bilateral salpingo-oophorectomy, she went on to receive 6 cycles of adjuvant carboplatin/paclitaxel, tolerated considerably well and completed November 2017.

For the next 4 years her CA 125 was never above 15.1 U/mL and regular surveillance imaging correlated with her NED status. In October 2019, roughly two years after completing adjuvant chemotherapy, she began high dose intravenous vitamin C (HDIVC), Trametes versicolor oral immunotherapy, prompted by consistently low natural killer cell function (< 20 LU10). For the next two years she was receiving these weekly at first, eventually reducing the frequency down to monthly. During this period, CA-125 levels were monitored at routine visits as illustrated in Fig. 1. At one of the routine visits on 9/29/21 it was noted that her CA-125 was now 25 U/mL, which subsequently elicited further investigation with ctDNA. Baseline Signatera was done on 9/29/21, the result was positive but very low at 0.03 MTM/mL (Figure 1).

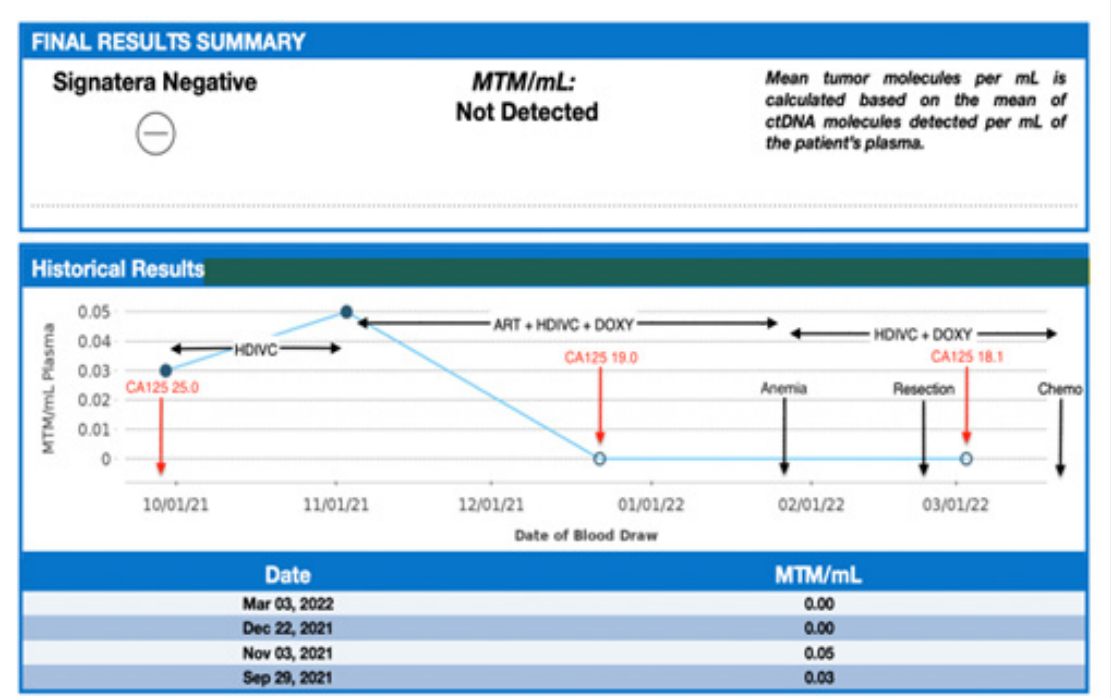


**Figure 1:** Signatera Recurrence and Treatment Monitoring for Ovarian Cancer.

Biweekly HDIVC was re-initiated. Six-week-interval Signatera on 11/09/21 indicated a very slight increase to 0.05 MTM/mL (Figure 1). Subsequently, a therapeutic trial of parenteral HDIVC + Artesunate + Doxycycline Biweekly initiated for another 6 wk. Six-week-interval Signatera testing on 01/04/2021 was now negative; 0.00 MTM/mL detected (Figure 1). Her CA125 on 12/22/21 had also decreased from 25 U/mL to 19 U/mL (Figure 2). CT/CAP and CT/PET scans performed in December 2021, revealed an enlarging hyper-metabolic right pelvic soft tissue nodule adjacent to the appendix, described as either peritoneal or a malignant pelvic lymph. On 2/23/22 she underwent laparoscopic resection of the right pelvic soft tissue mass, revealing an abnormal–appearing appendix, with firm nodular mass replacing the proximal third of the appendix, near the appendiceal/cecal junction. Pathology was consistent with high-grade serous carcinoma of gynecologic origin. Pelvic floor wash was negative. 3/3/22 CA-125 was 18.1 U/mL (Figure 1), Signatera, again, negative; 0.00 MTM/mL detected (Figure 1). Patient resumed HDIVC post-operatively and started adjuvant doxorubicin and carboplatin every 28 days, followed by PARPi. Of note, the patient did become anemic after 6 weeks of Artesunate. CBC from 1/26/22 significant for low RBC 3.47x10E6/uL, low Hgb 10.7 g/dL, low Hct 32.3%, all else within normal. Artesunate discontinued immediately and by 2/23/22 CBC was WNL.


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## Case 2

A 56-year-old postmenopausal cis-female with history of state IIA IDC of right breast estrogen receptor 95%, progesterone receptor 2%, HER-2 0%, Ki-67 9%, originally diagnosed September 2016. She is status post neoadjuvant chemotherapy with Dose Dense AC (DD-AC, Doxorubicin + Cyclophosphamide) and Dose Dense T (DD-T, Paclitaxel), bilateral mastectomy and right sentinel lymph node biopsy (1/3 SLN's positive for macro-metastasis). She declined adjuvant aromatase inhibitor (AI) plus ovarian function suppression (OFS) and was started on tamoxifen March 2017 but stopped after 6 months to 1 year. Unfortunately, right breast MRI in 4/2021 revealed a suspicious right breast mass along with bilateral axillary lymphadenopathy. June 2021 liquid biopsy via Guardant 360 released no reportable tumor-related somatic alterations (Figure 2). US-guided right axillary biopsy August 2021 was consistent with breast primary metastatic carcinoma. She declined additional axillary surgery and radiation. While considering her treatment options, she started anastrozole April 2021 and continued it through April 2022. March 2022 liquid biopsy via Guardant 360 detected 0.26% ctDNA with mutations for SMAD4 tumor-related somatic alterations (Figure 2). She was started on triple IV therapy (Artesunate, ascorbate, and doxycycline) and completed 14 IV infusions twice per week over months of May 2022 to June 2022. Interval Guardant 360 on 6/16/22 had no reportable tumor-related somatic alterations (Figure 2). Guardant 360 was repeated again on 9/1/22, again, showing no reportable tumor-related somatic alterations (Figure 2).

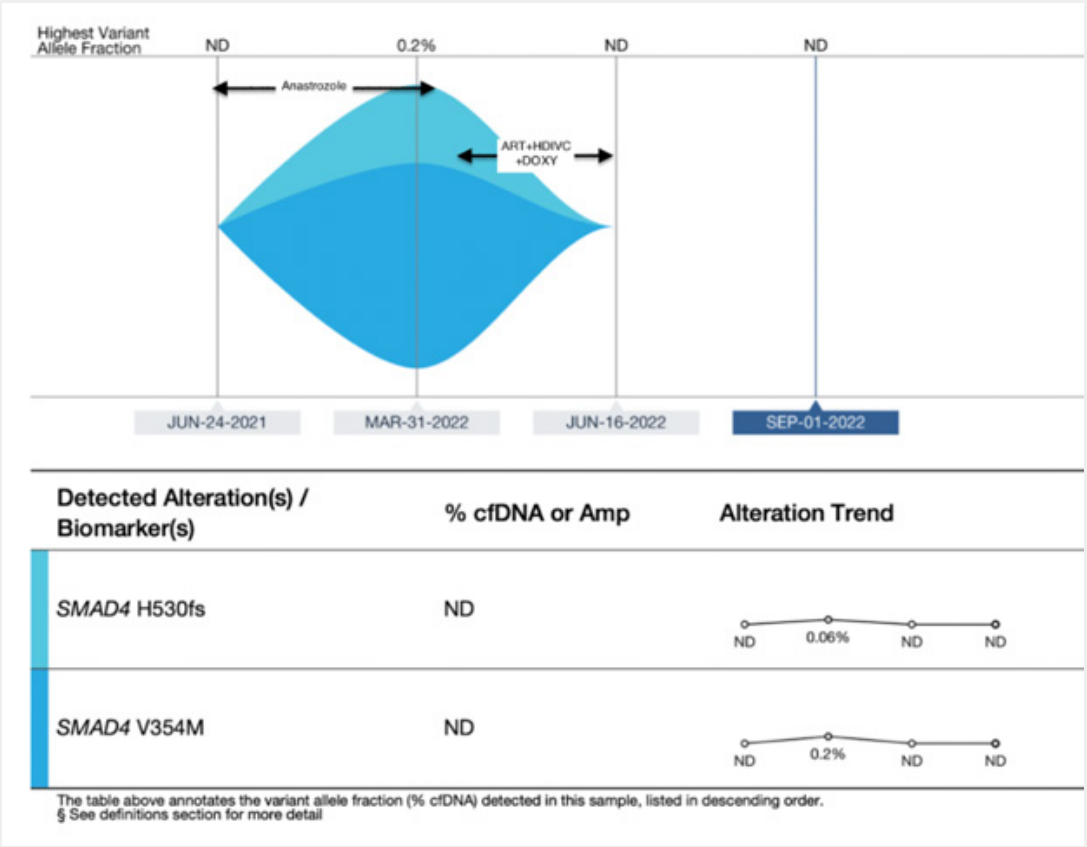


**Figure 2:** Guardant 360 Recurrence and Treatment Monitoring for Breast Cancer.

## Conclusion

Four-6 weeks of triple IV therapy with Artenusate + ascorbate + doxycycline can eradicate evidence of circulating tumor cells and circulating tumor DNA. We propose that this triple therapy may be useful in early recurrence of disease in early stage ovarian and breast cancer.

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