A simplified, potentially point-of-care (POC), electrode method detects changes in the amount of cfDNA/ctDNA and evaluates the response of advanced cancer patients to therapy

Taub, F.[2] , Russo, M[1] , Tunney, S [2], Wright, S.[2] , Henry, C. [1] [1]Colorado State University [2]aiGENE, Inc.

Abstract

Rapid assessment of therapy for advanced cancer, especially immunotherapy (IO), is critical. For advanced cancers the majority of therapies, even targeted/personalized ones fail. Even worse, IO has been reported to cause more than doubling in the cancer growth rate (defined as hyper-progressive disease or HPD) in 5-40% (typically ~20%) of patients. **Early identification of HPD, *or any inactive therapy,* and switching to an effective therapy can be lifesaving.** Though a consensus has not emerged, there are a number of reports that describe sensitization to chemotherapy following failed immunotherapy, this suggests that, at least in some cases, switching results in dramatic benefit. Due to inflammatory infiltrates causing "pseudoprogression" (false readings of cancer progression) early during treatment with IO, ineffective IO is often continued for 4-6 months before imaging can definitively say the IO is failing. During this time progressive, potential fatal cancer growth, as well as medical and financial side effects can occur. The cost of this ineffective or counterproductive IO is typically $10,000 to over $20,000 a month.

There are numerous reports of highly sensitive mutation based, or methylation sequence based, cfDNA signatures and methods. Unfortunately, they are inherently difficult tests which typically cost thousands of dollars and do not report the results to clinicians for 1 to 2 weeks. They have not been adopted for routine use to monitor on an every visit basis and allow near real-time adjustment of therapy. **Rapid detection of failure can lead to lifesaving therapy changes; detection of benefit can enhance compliance and improve clinical outcomes.**

Previously a research lab style solid gold electrode, requiring artful polishing and reuse, has been reported to be selectively sensitive to cancer DNA due to ctDNA's altered pattern of methylation. Electrochemical detection of the binding of cfDNA, following a 10-minute incubation, has been published. Here the results of a dramatically revised and optimized binding methodology to increase cnDNA binding is shown. Cancer samples are better separated from normal with the new method. **The new method is unique in using inexpensive, clinically and POC suitable, disposable electrodes and standard silica extraction methods;** it is highly sensitive to clinical samples. 5 µL of a 10 pg/ul sample provides reproducible readings. cfDNA from normal persons generates readings significantly different than from those with cancer ($p < .01$).

Patient time course data illustrates the correlation of the electrode response with cancer burden and suggests this is a monitoring tool that should be compared to, and may have benefits over, previously approved monitoring tests for advanced cancer such as carcinoembryonic antigen (CEA).

Introduction

Therapy (Rx) for advanced cancer fails more often than it succeeds. Over half of even "personalized" treatments fail to result in meaningful responses.[1,2,3] Expensive, ineffective, toxic Rx may be given for months before imaging can determine it is not helping the patient, leading to both increased cost and decreased quality of life with no medical benefit. **A rapid, inexpensive, preferably POC, method to quickly detect ineffective Rx is critical.**

Tracking cancer progression is currently done using imaging that tracks tumor size, but requires large, expensive instrumentation, operated, and interpreted by highly trained individuals. Imaging is classically a "lagging" indicator. Critically, it fails to distinguish cancer cells from reactive stroma. In immunotherapy, clinicians delay diagnosing drug failure due to immune infiltration generated pseudo-progression being hard to distinguish from real progression.

Multiple clinical studies have found that circulating tumor DNA (ctDNA) correlates with tumor progression. Several technologies have been developed to and precedes imaging progression[4,5,6,7] monitor ctDNA, including specific mutations,[5] copy number,[8] specific viral sequences,[9,4] and specific methylation patterns.[10,11] These methods require large, expensive instruments, specialized labs, a week (or more), and a highly trained staff, typically resulting in lab costs

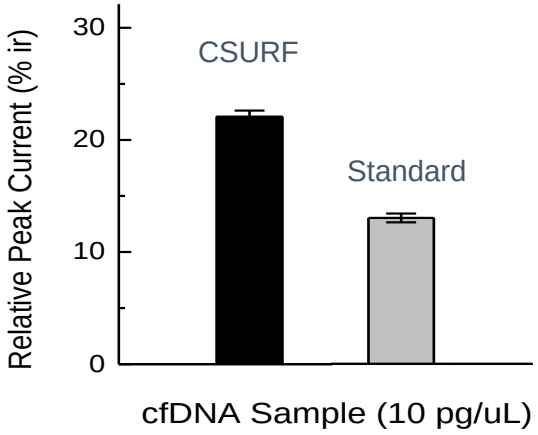
of >$1000. Published work indicates the altered methylation pattern causes cfDNA from cancer patients to have altered binding to solid gold electrodes.[12,13] aiGENE technology uses inexpensive, disposable, gold electrodes to assay differentiate normal cfDNA (cnDNA) and cancer DNA (ctDNA).

Prior work relied on problematic sample preparation, including organic extraction of DNA and solid gold electrodes that required complex and artful cleaning between measurements.[12] This is the first presentation of a gold electrode method for distinguishing ctDNA from cnDNA that does not require either of these.

Methods and Samples

The normal and patient (Pt) samples used were obtained at the University of Colorado Cancer Center Pathology Shared Resource Group (UCCPSR), except as noted. The longitudinal patient samples were kindly provided by Dr. Odd Terje Brustugun. Patients had advanced colorectal cancer (CRC) or non small cell lung cancer (NSCLC); they were age and sex matched with healthy donors. Blood was collected into EDTA tubes, centrifuged, and plasma was frozen at-80 degrees C until extraction. cfDNA was isolated using standard phenol chloroform extraction (PCI), except as noted. When pools used they were made by combining an equal volume of all samples of the same type (6 normals, 5 CRC, 5 NSLC). Additional normal plasmapheresis samples were obtained by Vitalant. Additional cancer Pt samples used for the QIAmp extraction were from NSCLC Pts before therapy; Pt 1 had squamous carcinoma and Pt 2 had adenocarcinoma care of Dr. Odd Terje Brustugun. Both the normal and cancer samples were extracted using Qiagen kit QIAamp ccfDNA MinElute in the lab of Dr. Srinivas Ramachandran at the Colorado University Anschutz Medical Campus. Extracted DNA was kept at-20 degrees C until use. All institutions collected blood following IRB approval and proper subject consent. Electrochemistry was performed as in Sina, et al 2018 (12), in some cases with modified buffer. CSURF is filing patents on this buffer, it is anticipated it will be fully described in our anticipated AACR annual meeting poster. Screen Printed Electrodes (SPE) were purchased from Metrohm, Thin Film Electrodes were purchased from Micrux. The latter were acid cleaned before use.

Increased Binding of cfDNA to the Electrode



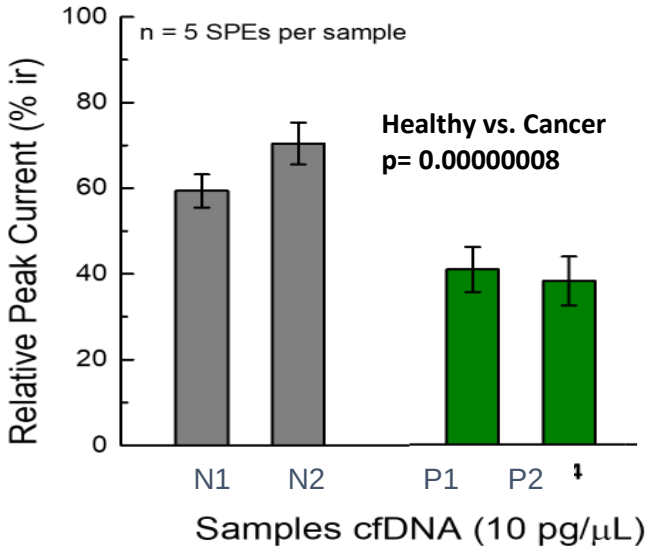
The described pool of cnDNA was bound to **Screen Printed Electrodes – SPEs** (n=3) for 10 min in standard (lighter grey) or CSURF buffer (darker grey). **Compared to previous methods an over 2-fold increase in cfDNA binding is shown with the CSURF buffer.**

Increased Separation of Cancer from Normal cfDNA

	CSURF	Standard
cnDNA (normal)	22.05	13.02
ctDNA (NSCLC)	7.73	6.77
% difference normal v. cancer	**185**	92

The described pools of PCI extracted cnDNA and cfDNA were bound to a SPE (n=3) electrodes for 10 min. **The novel CSURF buffer had a greater separation between cnDNA and NSCLC ctDNA.**

Cancer vs Normal in Qiagen Extracted cfDNA

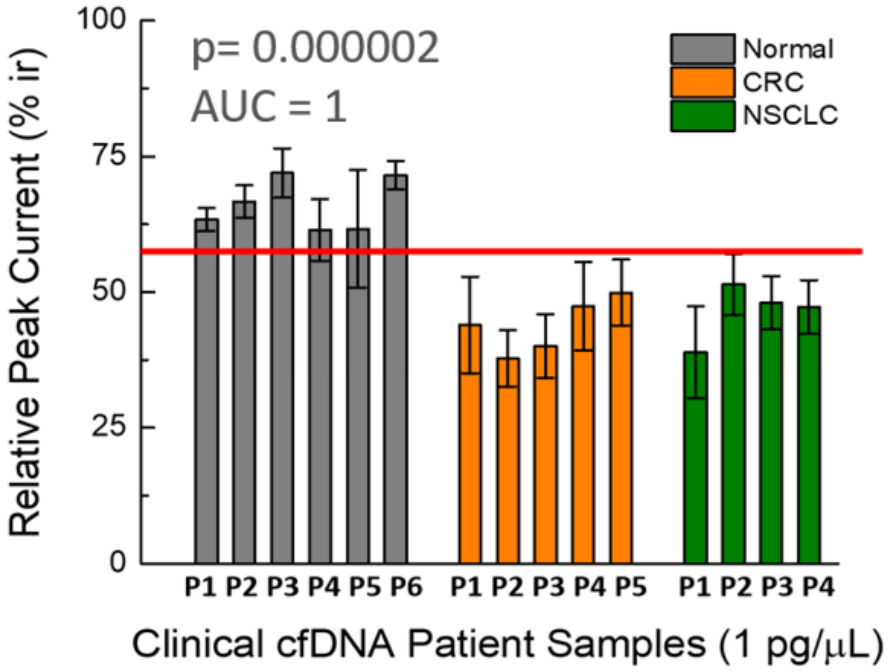


QiaAmp ccfDNA extracted cnDNA and cfDNA kindly provided by Ramachandran lab were bound to SPEs (n=5) electrodes for 10 min in CSURF buffer. Normal samples were from Vitalant.

A 65% difference between cnDNA and ctDNA was observed.

While published method required PCI extraction (Sina, personal communication), this method works with methods consistent with an automated clinical lab.

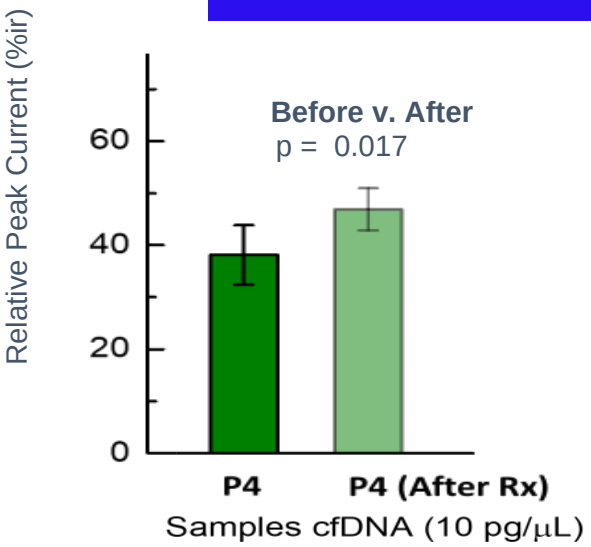
Cancer vs Normal TFE Electrodes



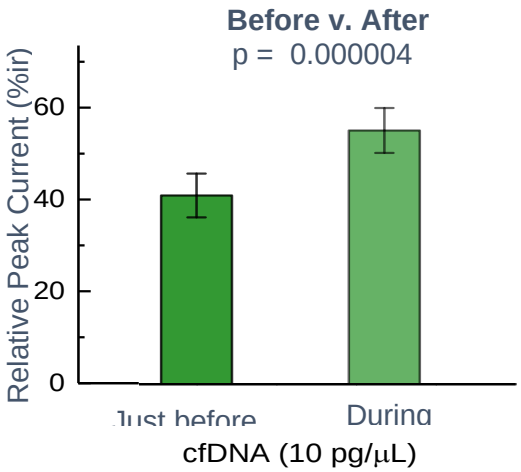
PCI extracted subject cfDNA (each person is one bar) are shown for the UCCSPRC samples. Thin Film Electrode, TFE, (n=4, the SD is shown by bars) response is shown for each sample following 10 min incubation. (This figure was updated based on January, 2023 data.) A difference in cancer and normal was observed.

p= 0.000002. AUC = 1

Do Electrode Measurements Correlate with Clinical Course?



A patient with adeno-NSCLC was tested before Rx and at day 34 during initial Rx. Imaging at 6 months determined the Pt was responding to Rx. The increase in signal/return toward normal values on the electrode (n= 5 each bar, p = < 0.02) on day 34 presaged the clinical benefit detected by imaging 5 months later.



The first time point for this lymphoma Pt. was 2.5 weeks before her first Dx; it shows a lower reading consistent with cancer. The second sample, after therapy, like the NSCLC Pt., shows a higher, more normal-like reading. The increase in signal/return toward normal values on the electrode (n= 10 each bar, p = 0.000004) is consistent with the clinical remission.

These patients suggest successful therapy returns electrode readings toward normal. These results are inconsistent with the theoretical possibility that the cancer treatment causes a release of cfDNA that mimics increasing cancer.

CONCLUSIONS & DISCUSSION

Modified methods improve detection of cfDNA.

Novel methods improve separation of cnDNA from ctDNA

Anecdotally, changes in electrode reading correlate with, and precede changes by imaging.

Clinicians now monitor blood counts and chemistry the morning of therapy to both prevent fatal side effects and in leukemia to assess cancer burden. We have made progress toward a POC ctDNA monitoring method that will allow similar close to real time evaluation of therapy effectiveness in lymphoma and solid cancers.

LIMITATIONS ~ FUTURE DIRECTIONS ~ PARTNERSHIPS

Only preliminary results and anecdotes of correlation with therapy effectiveness are currently available. We seek specimen banks, clinical sites with ongoing trials, and corporate partners for the next stages. Contact floydtaub@a-i-gene.com

REFERENCES

1. Peskov K, Azarov I, Chu L, et al. Front Immunol. 2019 Apr 30;10:924. doi: 10.3389/fimmu.2019.00924.
2. Mehnert JM, Monjazeb AM, Beerthuijzen JMT, Collyar D, Rubinstein L, Harris LN. Clin Cancer Res. 2017 Sep 1;23(17):4970-4979. doi: 10.1158/1078-0432.CCR-16-3063.
3. Buhl IK, Jensen PB, Buhl ASK, Knudsen S. Pharmacogenomics 2019 20:5, 307-309.
4. Yalçın B, Kutluk T, Ağbaba SK, Demir Ç, Talim B. Turk J Pediatr. 2020;62(4):541-550. doi: 10.24953/turkjped.2020.04.003. PMID: 32779406.
5. Herbreteau G, Vallée A, Charpentier S, Normanno N, Hofman P, Denis MG. J Thorac Dis. 2019;11(Suppl 1):S113-S126. doi:10.21037/jtd.2018.12.18
6. Gressel GM, Maggi EC, Harmon BE, et al. J Transl Med. 2020 Aug 27;18(1):323. doi: 10.1186/s12967-020-02493-8. PMID: 32854748; PMCID: PMC7457235.
7. Goldberg SB, Narayan A, Kole AJ, et al. Clin Cancer Res. 2018;24(8):1872-1880. doi:10.1158/1078-0432.CCR-17-1341.
8. Jensen TJ, Goodman AM, Kato S, et al. Mol Cancer Ther. 2019;18(2):448-458. doi:10.1158/1535-7163.MCT-18-0535.
9. Chera BS, Kumar S, Shen C, et al. J Clin Oncol. 2020 Apr 1;38(10):1050-1058. doi: 10.1200/JCO.19.02444. Epub 2020 Feb 4. Erratum in: J Clin Oncol. 2020 Oct 20;38(30):3579. PMID: 32017652; PMCID: PMC7106982.
10. Xu RH, Wei W, Krawczyk M, et al. Nature Mater. 2017; 16(11):1155–1161.
11. Hlady RA, Zhao X, Pan X, Yang JD, Ahmed F, Antwi SO, Giama NH, Patel T, Roberts LR, Liu C, Robertson KD. Theranostics. 2019 Sep 25;9(24):7239-7250. doi: 10.7150/thno.35573. PMID: 31695765; PMCID: PMC6831291.
12. Sina AAI, Carrascosa LG, Liang Z, Grewal YS, Wardiana A, Shiddiky MJA, Gardiner RA, Samaratunga H, Gandhi MK, Scott R, Korbie D, Trau M. Nat Commun 9, 4915 (2018).
13. Sina AA, Lin TY, Vaidyanathan, R.; Wang, Z.; Dey, S.; Wang, J.; Behren, A.; Wuethrich, A.; Carrascosa, L. G.; Trau, M. Nanoscale Horiz 2020, 5 (9), 1317-1323.