Institution: University of Cambridge		
Unit of Assessment: UOA1		
Title of case study: A blood test to detect lung cancer mutations and monitor treatment response: from concept to clinical application.		
Period when the underpinning research was undertaken: October 2009-July 2020		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	**Role(s) (e.g. job title):**	**Period(s) employed by submitting HEI:**
Nitzan Rosenfeld	Director of Research	Sept 2009 - July 2020
Davina Gale	Senior Research Associate	Jan 2013 - present
James D. Brenton	Professor of Ovarian Cancer Medicine	May 2001-Aug 2006 Jan 2013- present
Period when the claimed impact occurred: Sept 2014 to July 2020		
Is this case study continued from a case study submitted in 2014? N		

1. Summary of the impact (indicative maximum 100 words)

Lung cancer is a leading cause of cancer-related death worldwide. Detecting DNA mutations in tumours can help assign therapy, particularly if the mutations can be targeted directly with drugs. However, limited access to tumour material, both before and during treatment, constrains this approach. Cambridge researchers invented a blood test (liquid-biopsy) that detects mutant tumour DNA circulating in the blood of patients with non-small cell lung cancer (NSCLC). This test was further developed into the InVisionFirst™-Lung Liquid Biopsy (IF-LLB) platform through Inivata Ltd., a spinout company launched by the researchers that has raised USD129,400,000 (~GBP96,800,000) and employs >80 staff within the UK and US. IF-LLB detects 37 NSCLC-mutant genes, 10 of which are actionable, and six linked to FDA approved therapies. It is now a leading precision cancer medicine test used in routine clinical practice; is Medicare reimbursement-approved in the US; and has been used in the management of [text removed for publication].

2. Underpinning research (indicative maximum 500 words)

Precision cancer medicine includes the detection, monitoring and targeting of DNA mutations in each individual patient's tumour. These mutations can be detected in tumours using next generation sequencing (NGS). However, this is not possible for patients whose tumours are surgically inaccessible. Furthermore, since repeat biopsies of primary tumours is neither practical nor acceptable, then serial analysis of mutations in tumours cannot be used to measure treatment response. These challenges are exemplified by NSCLC–the commonest form of lung cancer–that is diagnosed in 230,000 UK and US patients each year (Cancer Research UK; American Society of Clinical Oncologists). Although up to 69% of NSCLC contain mutations that can be targeted with drugs (Zhang *et al*., Journal of Hematology & Oncology, 2019), the limitations of primary tumour NGS place most of these beyond detection.

Development and application of novel non-invasive cancer liquid-biopsies: Tumours shed mutant DNA (ctDNA) that can be detected in peripheral blood (Sorenson, GD *et al.* 1994). Initial approaches to detect mutant ctDNA required prior knowledge of the mutations present in the primary tumour, presenting a clinical 'Catch 22'. To address this, Cambridge researchers invented Tagged-Amplicon Deep Sequencing (TAm-Seq), which detects tumour-derived mutations in ctDNA without requiring knowledge of the primary tumour DNA sequence [1]. They then used TAm-Seq to create the InVision™ liquid biopsy platform that simultaneously detects multiple tumour-derived mutations in ctDNA. Their study of 30 women with metastatic breast cancer confirmed that measuring ctDNA using InVision™ quantifies tumour burden with greater precision, and over a larger dynamic range, than existing tumour biomarkers [2]. This research enabled the Cambridge team to found Inivata Ltd., a spinout company launched in 2014. They then showed that the InVision™ liquid biopsy platform can detect mutations in ctDNA to identify drug targets for, and track primary tumour treatment response, of NSCLC [3].

***Clinical implementation of liquid biopsy technology**:* Translation of InVision™ into routine clinical practice required real-world verification of its sensitivity and specificity. Through work in two independent laboratories, the Cambridge team showed that in optimal samples (input DNA average of 53 ng of DNA), InVision™ detects 94% of tumour mutations at an allele fraction of only 0.25%-0.33%, and ~90% of mutations are detected in samples with very low amounts of DNA (~6.6 ng), both with high levels of specificity [4]. In a subsequent prospective clinical study that analysed both ctDNA and tumour DNA simultaneously among >250 patients with NSCLC, the Cambridge team identified 97.8% of primary tumour DNA mutations in ctDNA using InVisionFirst™ (70.6% sensitivity and 99.2% specificity) [5]. To complete translation of InVisionFirst™ to a NSCLC clinical test, the researchers developed InVisionFirst™-Lung Liquid Biopsy (IF-LLB) that detects mutations in 37 NSCLC-relevant genes, 10 of which are actionable and six linked to FDA-approved therapies. In their clinical study of IF-LLB, the team detected mutations in the ctDNA of 77% (165/214) of patients with NSCLC (81% sensitivity and 97% specificity) [6]. Critically, clinically actionable mutations were detected by IF-LLB in 17% of 103 patients for whom no primary tumour was available. These patients went on to receive personalised treatment. Further, IF-LLB measurement of ctDNA mutations correlated with response rate and progression-free survival [6].

3. References to the research (indicative maximum of six references)

Evidence of research quality: *Research published in peer-review journals. Research was supported by competitively won grants.

* **[1]** Forshew T, …, Brenton JD, **Rosenfeld N.** Noninvasive identification and monitoring of cancer mutations by targeted deep sequencing of plasma DNA. **Science Transl Med**. 2012; 30;4(136):136ra68. doi: 10.1126/scitranslmed.3003726. PMID: 22649089.

* **[2]** Dawson SJ, …, **Gale D**, …, **Rosenfeld N.** Analysis of circulating tumor DNA to monitor metastatic breast cancer. **N Engl J Med**. 2013; 368(13):1199-209. doi: 10.1056/NEJMoa1213261. Epub 2013 Mar 13. PMID: 23484797.

* **[3]** Remon J, …, **Gale D**, …, Besse B. Osimertinib benefit in EGFR-mutant NSCLC patients with T790M-mutation detected by circulating tumour DNA. **Ann Oncol**. 2017; 28(4):784-790. doi: 10.1093/annonc/mdx017. PMID: 28104619.

* **[4] Gale D**, …, **Rosenfeld N.** Development of a highly sensitive liquid biopsy platform to detect clinically-relevant cancer mutations at low allele fractions in cell-free DNA. **PLoS One**. 2018;13(3):e0194630. doi: 10.1371/journal.pone.0194630. eCollection 2018. PMID: 29547634.

* **[5]** Pritchett M, …, **Rosenfeld N**, Morris CD, Govindan R. Prospective Clinical Validation of the InVisionFirst-Lung Circulating Tumor DNA Assay for Molecular Profiling of Patients With Advanced Nonsquamous Non–Small-Cell Lung Cancer. **JCO Precision Oncology**; published online April 25, 2019: doi: 10.1200/PO.18.00299. PMID: 32914040.

* **[6]** Remon J, …, **Rosenfeld N**, …, Besse B. Real-World Utility of an Amplicon-Based Next-Generation Sequencing Liquid Biopsy for Broad Molecular Profiling in Patients With Advanced Non–Small-Cell Lung Cancer. **JCO Precision Oncology**; published online March 6, 2019: doi: 10.1200/PO.18.00211. PMID: 32914037

Funding

ERC Starter Award: Cancer Exomes in Plasma, 2014-2020. Awarded **GBP1,474,484**. Principal Investigator: **Nitzan Rosenfeld.**

CRUK Early Detection Programme Award A26886, 2018-2023. Awarded **GBP821,941.** Co-Leads: **Nitzan Rosenfeld** and Douglas Easton.

CRUK Early Detection Programme Award A27548, 2019-2024. Awarded **GBP1,500,000** of total requested **GBP2**,500,000 Co-Leads: **Nitzan Rosenfeld** and Robert Rintoul.

AstraZeneca, 2013-2019: **GBP183,617.** Co-Principal Investigators: **Nitzan Rosenfeld** and Simon Pacey.

Patents

- Rosenfeld N, Forshew T, *et al*. A method for detecting a genetic variant – Tam-Seq: International publication number: WO 2016/009224A1, 21 January 2016.
- Fisher E …. Rosenfeld N, *et al*. Improvements in variant detection: International publication number: WO 2019170773A1, 12 September 2019.

- Plagnol V, Forshew T, et al. Method the Analysis of Minimal Residual Disease: Publication number: 20200157604, 21 May 2020. US Patent: US20200157604A1

Rosenfeld N, Forshew T, *et al*. Method for Detecting Tumor DNA in a cfDNA Sample Collected from a Patient that has Previously Undergone Cancer Therapy: Publication number: 20200232021, 23 July 2020.

4. Details of the impact (indicative maximum 750 words)

Lung cancer accounts for 1,760,000 cancer deaths world-wide each year (World Health Organisation Statistics, 2018). Cambridge University-led research has established ctDNA liquid biopsy as a cornerstone of precision lung cancer medicine, with the aim of reducing this figure.

Impact on practitioners and the delivery of professional services

A clinically approved blood test for managing patients with NSCLC:

In 2018, the International Association for the Study of Lung Cancer (includes 9,000 lung cancer specialists in over 100 countries) convened a multidisciplinary panel of experts in the field of thoracic oncology to produce a set of recommendations for the use of liquid biopsy in the clinical management of advanced NSCLC patients. The panel reviewed a number of studies that had applied liquid biopsy technology to detect cancer-causing mutations (including [2]), and recommended that liquid biopsy be considered at the time of initial diagnosis in all patients with advanced NSCLC who need tumour molecular profiling, particularly when tumour tissue is scarce, unavailable, or for patients in whom invasive procedures may be risky or contraindicated. They also recommended that liquid biopsy be conducted at the time of initial diagnosis if the turnaround time for tissue biopsy is anticipated to be longer than 2 weeks [A].

Subsequently in 2019, IF-LLB received Medicare reimbursement approval in the US, providing the US population with routine access to a comprehensive genomic profiling test for NSCLC (one of only five such approved tests in the US) [B]. In February 2020, the US National Comprehensive Cancer Network guidelines, recommended use of liquid biopsies in advanced or metastatic NSCLC patients when tissue biopsy is not available: "*If there is insufficient tissue to allow testing for all of EGFR, ALK, ROS1, and BRAF, repeat biopsy and/or plasma testing should be done*." [C]. Liquid biopsies are now regarded as routine clinical practice as part of cancer care in the US. Furthermore, a 2020 systematic review which featured Pritchett *et al.*, 2019 [5] noted that: *"ctDNA molecular profiling is an accurate and reliable tool for the detection of clinically relevant molecular alterations in advanced NSCLC patients. Clinical outcomes with targeted therapies endorse the use of liquid biopsy by amplicon-based NGS ctDNA analysis in first line and relapse testing for advanced NSCLC patients."* [D].

[Text removed for publication] [B]. Two of these studies, including a large prospective EU clinical trial, investigated the potential of IF-LLB to reduce the time patients waited for treatment and to select treatments [E]. This work has underpinned the increased use of liquid biopsies in the routine management of patients with NSCLC lung cancer.

Leading international lung cancer oncologists attest to the positive impact of Inivata's assays on treatment selection and patient care; an Associate Professor at the Stanford Cancer Institute says: *"Data presented by Inivata has helped demonstrate the validity of their technology. This adds to the supportive data which is important in increasing the awareness and adoption of liquid biopsies for patient benefit."* [F]. An Associate Professor of Medicine at Harvard Medical School commented, *"The impact of Inivata is clear. The company, based off your* [Cambridge] *research, has proven the validity and reliability of amplicon-based plasma NGS…had led the development of methods to monitor cancer noinvasively and use this monitoring to improve patient care.."* [F].

The benefit to patients is further illustrated in an interview with the first patient to receive treatment based on his IF-LLB results in 2015, who said: *"This test in fact triggered a change in therapy, which gave me almost 18 months of progression free survival until my cancer was mutated again. The liquid biopsies give me visibility, we're not guessing anymore and can make an informed decision."* [F].

Supporting research of novel patient care pathways and therapies: In addition to use in routine clinical care, IF-LLB is also being used in ongoing studies to improve the NSCLC patient care pathway and develop new therapies. Inivata Ltd has established collaborations with Genomics England to assess ctDNA samples from the 100,000 Genomes Project to explore the potential of liquid biopsies to improve cancer management and patient outcomes in the UK [G]. IF-LLB will also be deployed in a GBP10,000,000 early cancer detection study of 15,000 participants (iDx-LUNG project). This national collaboration between academia and several major diagnostics and informatics companies will test ways to detect cancer at a stage when it can be cured. IF-LLB will be used to analyse blood samples from patients with inconclusive CT scan results participating in NHS England Targeted Lung Health Checks[G].

Impact on commerce and the economy

In 2014, Dr Rosenfeld and colleagues in Cambridge founded the spinout company Inivata Ltd, as a global clinical cancer genomics company developing its proprietary, industry-leading liquid biopsy platform to transform patient care [E]. The company has raised USD129,400,000 (~GBP96,800,000) and employs >80 highly-skilled individuals at its headquarters and R&D centre in Cambridgeshire and its certified clinical laboratory in North Carolina [H]. In March 2015, the further development of Inivata Ltd. technology was enabled through a collaboration with MedStar Health, a US not-for-profit health care provider [I]. To commercialise IF-LLB in the US, in May 2020 Inivata Ltd., then formed a strategic partnership with Neogenomics that made a USD25,000,000 equity investment in UK-based Inivata Ltd., Cambridge [I]. In July 2019, Inivata Ltd., signed a distribution agreement with IPS Genomix, part of the IPS Group, to provide cancer patients and partners in the Middle East and Africa with access to its IF-LLB test and other liquid biopsy tests [I].

5. Sources to corroborate the impact (indicative maximum of 10 references)

[A] Impact on international cancer recommendations:
Rolfo C, *et al*. J Thorac. Oncol. 2018. Sep;13(9):1248-1268. doi: 10.1016/j.jtho.2018.05.030. Epub 2018 Jun 6. (p1249), cites [2], ref 138.

[B] Impact on cancer services in the USA:
(i) Evidence of Medicare reimbursement-approval for routine use of IF-LLB in the management of advance NSCLC patients in the USA. Press release, One Nucleus, 05 March 2019. (ii) Medicare Local Coverage Determination documents for InVisionFirst, Liquid Biopsy for Patients with Lung Cancer. (iii) A complete list of Medicare documents regarding Inivata Ltd. technologies. (iv) Evidence the Inivata platform is now one of the leading ctDNA tests used in treatment recommendation by doctors in the US.

[C] Impact on US clinical guidelines:
National Comprehensive Cancer Network: NCCN Clinical Practice Guidelines in Oncology. NSCLC. Version 3.2020 (p33, 36, 112 and 136).

[D] Evidence of impact on clinical application of liquid biopsy technology:
Remon J, *et al*. PLoS ONE 15(6): e0234302. 11 June 2020. doi.org/10.1371/journal. pone.0234302. (p8)

[E] Clinical trials involving Inivata technology:
(i) Press releases from EORTC and Genomeweb confirming the use of the InVisionFirst®-Lung test in a Pfizer-funded Phase II trial to analyze resistance in patients treated with lorlatinib, 29 October 2019. (ii) Evidence of the range of clinical trials assessing Inivata liquid biopsy technology. Inivata Press releases, Oct 2016 to November 2019.

[F] Impact on patient health and wellbeing:
(i) Testimonial letter from CRK Faculty Scholar & Associate Professor, Stanford Cancer Institute, Stanford University School of Medicine (ii) Testimonial letter from Associate Professor of Medicine, Harvard Medical School.. (iii) Transcript of a YouTube video with the first patient to receive treatment based on the results of his IF-LLB screen in 2015: https://www.youtube.com/watch?v=IzM87uxWPvQ

[G] Impact on national cancer services:

(i) Evidence of established collaborative efforts between Inivata and Genomics England to improve patient outcomes in the UK. Genomics England press release, 16 April 2019 and Genomeweb press release, 13 October 2017. (ii) Inivata's Liquid Biopsy Technology Included in Unique GBP10m Research Collaboration for Early Detection of Lung Cancer, Inivata press release, 18 December 2020

[H] Evidence of commercial impact of Inivata Ltd. liquid biopsy technology:

Inivata Ltd. Funding and staffing information from Crunchbase, and Craft.info

[I] Evidence of the global reach of Inivata technology in clinical cancer management:

(i) A strategic collaboration with Neogenomics for the commercialisation of Inivata Ltd.'s InVisionFirst®-Lung test in the US. Press release, 360Dx, May 2020. (ii) Signing of a distribution agreement with IPS Genomix, to provide cancer patients and partners in the Middle East and Africa with access to its InVisionFirst®-Lung and InVisionSeq™ liquid biopsy tests. Press release, Consilium Strategic Communications, July 2019. (iii) Establishment of a collaboration with MedStar Health to assess the potential of liquid biopsy and ctDNA analysis in management of lung cancer patients in Washington D.C., USA. Press release, Medstar Health, 15 March 2018.