WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 8, Issue 11, 299-314. Review Article ISSN 2277– 7105

A REVIEW ON APPLICATION OF METABOLOMICS, LIQUID BIOPSY AND FOUNDATION ONE CDx IN CANCER

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Article Received on
26 July 2019,

Revised on 16 August 2019,
Accepted on 04 Sept. 2019,

DOI: 10.20959/wjpr201911-15835

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ABSTRACT

Cancer is a devastating disease that alters the metabolism of cells in the body. The metabolites can be detected using different techniques in which the novel one is metabolomics. These focus on metabolite profiling intra-cellularly or from circulating fluids of the body. Unlike the genomics and proteomics, the metabolomics is novel diagnostic tools that led to the development of precision medicine. On the other hand, to deliver precision medicine, the novel non-invasive techniques emerged in the field of oncology like liquid biopsies. The body fluids like saliva, urine, blood and stools can be utilized as diagnostic techniques for early detection of stages of cancer. The genetic mutations are most common in cancerous condition. To overcome this, foundation medicine has developed foundation one CDx (companion diagnostic) tool which is approved by Food and Drug Administration (FDA) that can detect 5 types of genetic alterations, deletions, insertions, substitutions (melanoma, non-small cell lung cancer, breast, ovarian and colorectal cancers) in a tumor cell. In this review, the emerging applications of metabolomics, various types of liquid biopsies and foundation one CDx are discussed.

KEYWORDS: Cancer, metabolomics, liquid biopsy, foundation one CDx, circulating tumor cells, applications.

INTRODUCTION

Cancer is a deadly disease which alters the metabolism of a cell and the surrounding tissue.[1] The invention of newer technologies in molecular science revealed about the signaling pathways and regulation of genes in cancer. New methods for classification of cancer and its subtypes, diagnosis, prognosis, response and resistance to the therapy have been developed.

The recent findings include detection of circulating tumor cells and circulating DNA in the blood stream and other body fluids. This led to the evolution of biopsies. These cellular and molecular technologies will provide us accurate and precise decision-making. The first discovery of changes in metabolism occurred a century ago. Metabolism is a set of catabolism and anabolism which involves the cellular building blocks (amino acids, lipids, nucleotides, etc.) through which the energy production is performed. The biochemical intermediates produced during the cellular processes are known as metabolites. These metabolites in turn effects the gene regulation, post transcription, pathway interactions. Metabolomics are the diagnostic tools which focus on profiling of metabolites in the sample either from intracellular or from circulating body fluids.[2] The metabolomics are significant analytical tools unlike genomics and proteomics that aims to measure molecules which have diverse physical properties.[3] In oncology the old saying 'one size fits all' has been overcome by pharmacogenomics research which led to the development of precision medicine.[4] As this needs the accurate sampling and diagnostic methods, non-invasive biopsies have been emerged in the field of oncology. Liquid biopsy is the novel and highly specific diagnostic tool that offers collection of robust and reproducible data in a simple and non-invasive manner. The liquid biopsies are based on detection and analysis of circulating tumor cells and tumor-derived extracellular vesicles. There are many types of applications of liquid biopsy like, saliva, blood, urine, stools, breath, cerebrospinal fluid biopsies.[5] Cancer SEEK was able to detect eight common types of cancer by assessing 8 protein markers and tumor-specific mutations in circulating DNA found in the blood samples.[6] Foundation One CDx came into existence which is used for in vitro in the detection of substitutions, deletion alterations, insertions, and copy number alterations in 324 genes and select gene re-arrangements, as well as genomic signatures including microsatellite instability (MSI) and tumor mutational burden (TMB) using DNA isolated from formalin fixed paraffin embedded (FFPE) tumor tissue specimens. This test is also used to detect the genomic loss of heterozygosity (LOH) from FFPE ovarian tumor tissue.[7]

METABOLOMICS

The molecular diagnostics have been developed in fields of proteomics, genomics, transcriptomics in the past 10 years for the cancer diagnostics. Nowadays there is increased dedication in the area of cancer metabolism and cancer metabolomics.[8] The changes in the phenotype of genes and molecular physiology are represented by metabolomics. The metabolomics is one of the types of omic technologies which is being used nowadays to

identify metabolomic pathways and biomarkers that are altered in cancerous condition and also to evaluate the efficacy of therapy that is administered to cancerous patients during the therapy. Cancer alters the cellular metabolism of any tissue in the body, so metabolomics is used to identify cancer priorly and evaluate the interventions and therapies utilized in cancer. The analytical technologies and statistical methods invented in the recent years provides metabolomics with an ability to invest more in the cancer metabolism and gives us the understanding of the usage of glycolysis to produce amino acids, lipids, nucleotides that are utilized by cancer cell to proliferate. The genetic and lifestyle factors which include diet, drugs, age, health-disease status, hormonal homeostasis, exercise, gut microbiota, affect the metabolites detected in the body fluids. Lipids are useful in invasion, migration and proliferation of cancer cells. These types of lipids can be identified by other types of metabolomics known as Lipidomics. The biomarkers can also be detected using metabolomics and labelled substrates like 13C labelled glucose. These substrates provide the utilization of glucose in biological pathways which can be used for understanding the pathways in diseased stages or states and the response of drug(s).[1]

There are no methods that are available for measuring the concentration of metabolites on a wider range. The metabolomics uses the technology in which the Nuclear Magnetic Resonance (NMR) and Mass Spectroscopy (MS) are predominant. NMR can measure metabolites in a wider range with a little sample or without any sample preparation and gives us the structural and quantitative information. The sensitivity is low, so they cannot detect a greater number of metabolites which becomes the limitation for this technique. However, high-resolution NMR and high-resolution magic angle spinning NMR (HR-MAS-NMR) have been used to detect metabolites of cancerous cells in biofluids and samples from tissues. The metabolite levels can be detected in-vivo using hyperpolarized NMR.

On the other hand, MS has high sensitivity and gives us semi-quantitative information and analyzes the low-abundant metabolites. Sample preparation is needed in larger quantities and this can only detect a small and specific number of metabolites. There are many types of MS techniques like direct infusion MS and direct analysis in real-time MS (DART-MS), gas chromatography-MS, liquid chromatography-MS (LC-MS) and capillary electrophoresis-MS (CE-MS).[9]

APPLICATIONS OF METABOLOMICS

Biomarkers and Diagnostic Techniques

The biomarkers are the diagnostics which are ideal to metabolites. A study was conducted in which the metabolites were analyzed for hepatocellular carcinoma. There were around 70 metabolites which showed significant differences between the healthy control group and cancer samples. The novel biomarkers are being identified for detecting kidney cancer using urine samples. Many studies have utilized metabolomics to differentiate between healthy and cancerous urine samples. In breast cancer patients, the urine and serum samples can be used to detect cancer stage earlier and breast tissue biopsy can be performed to confirm the disease secondarily. If the ovarian cancer is detected in later stages the survival rate is decreased when compared to early detection (which have >90% survival rate in case of 5-year survival). So, the usage of metabolomics increased for prior detection of this cancer. The DART-MS technique was used in a study conducted on 44 ovarian patients and 50 healthy patients by collecting serum samples, which gave 99% separation accuracy using an algorithm. A study was conducted in colorectal cancer (CRC) patients which identified nearly 30 biomarker metabolites that are significantly different in statistics when compared between CRC samples from tissue and normal mucosae samples. The glycolysis, lipid metabolism and biosynthesis of nucleotides were the commonly known pathways for cancer occurrence.[9] The HR-MAS-NMR has an advantage of directly relating to MRS imaging studies. The HR-MAS-NMR and GC/MS are used to detect the glycolysis, hypoxia, lipid metabolism and inflammation alterations in CRC. The cancer diagnosis can be improved clinically by translating ex vivo MAS to in vivo MRS.

A study included elevated plasma levels of trimethyl-amine-N-oxide were increased among the people who include red meat in their diet, the composition of the microbiome of the gut and risk of cardiac events which was diagnosed using metabolite profiling.[3] There will be altered cellular metabolism in cancerous patients. A pilot study has been conducted utilizing serum metabolomics to assess stages of pancreatic cancer.

Imaging Metabolic Biomarkers

Positron-emission tomography (PET), single positron-emission computed tomography (SPECT), magnetic resonance imaging (MRI), X-ray, magnetic resonance spectroscopy imaging (MRSI) have being used for diagnosing cancer. The glucose uptake by cancerous cells can be detected by using PET. To diagnose the breast cancer MRI is used along with

mammography which is accurate than the mammography alone. The metabolic changes in the tumors can be detected other than glucose uptake using MRSI which uses NMR spectrum from 3-Dimensional voxel.

Cancer Drug Therapy

The anti-metabolites were invented first to treat the cancer as they interfere with normal metabolic pathway. Pharmaco-metabolomics is the emerging field of study used to develop the specific drug of action for specific cancer. A study predicted the use of docetaxel in breast cancer patients which showed dose and time dependent response in glutathione metabolism and phospholipid metabolism.[1] The study of chemotherapy in breast cancer patients evaluated that metabolic profiling of serum before and during the chemotherapy showed human epidermal growth factor receptor 2 (HER2) during metabolite profiling. Lipoprotein-derived lipid levels were identified with intensity of toxicity while studying the toxicity effects of capecitabine on CRC patients.[9]

LIQUID BIOPSY IN CANCER

The tumour derived components like circulating tumours cells (CTCs) are isolated from the peripheral blood smear. The genomic and proteomic assessment is done for these components which are represented through a novel diagnostic tool known as 'Liquid biopsy'. This diagnostic tool utilizes the blood samples to detect the normal and tumoral nucleic acids present in the cells or tissues. This procedure is not being routinely used clinically but used to diagnose response or resistance to the systemic treatments.[4] The CTCs are one of the sources of metastasis which migrate into the bloodstream when they shed from primary and secondary tumours sites. CTCs are very low in number in earlier stages of the disease. Liquid biopsies analyses and target CTCs, circulating tumours DNA (ctDNA), and tumours-derived extracellular vesicles.[5] The liquid biopsy can detect 8 common types of cancer (esophagus, stomach, liver, colorectal, lung or breast cancer.[6] If the thyroid, prostate and breast cancers were identified prior, there will not be any proven decline in cancer progression or increase in the survival rate in patients suffering from those cancers. The detection of tumours earlier should be less than 2cm in size, but this cannot be done due to the poor prior diagnosis. The mutations can be detected if there are millions of cells present in the blood.[5] Cancer SEEK is a new diagnostic tool which can detect up to 8 types of cancer (oral, stomach, lung, breast, colorectal, ovarian, esophagus, pancreatic cancers) with 69% of sensitivity and 89% specificity.[6]

Applications of Liquid Biopsies

In Oral Cancer

As a type of liquid biopsy, saliva is also being used as a biomarker which is a non-invasive and novel method in the detection of cancer. Many several salivary molecules could be used as biomarkers for cancer diagnosis, prognosis, therapy and monitoring. Proteomes, micro-RNAs, microbiome, metabolome, transcriptomics are five diagnostic tools in salivary biomarker. A study stated that there was increased levels of mitochondrial DNA (mtDNA) content in head and neck cancer patients in comparison with a healthy control group. The factors influencing mtDNA content were smoking and postoperative radiation. In a study conducted between 25 healthy controls and 25 oral squamous cell carcinoma (OSCC) to identify oxidative stress-related parameters and anti-oxidant salivary profiles, the DNA and proteins in saliva were found to be highly oxidized, nitrosamines in saliva were increased and antioxidants were found to be decreased. This explains the relationship between antioxidants and free radicals in saliva of patients with OSCC. The alternative diagnosis for salivary biomarkers can be salivary-tumour-derived exosomes. A study evaluated the morphology of exosome and CD63 which is a surface receptor in salivary samples of patients with oral cancer. There were miRNA exosomes involved in oral cancer. The secretion of these exosomes was altered when compared with diseased control groups. So, these could be developed as biomarkers for the detection of oral cancer.[10]

In lung cancer

The liquid biopsy is also used to detect lung cancer in earlier stages. In a study, the patients who had been suspected with COPD were included and the low dose spiral CT was done annually. This showed positive CTCs but normal CT results. By annual monitoring of these patients revealed nodules in lungs after 1-4 years of initial detection of CTCs which lead to surgery and diagnosis based on histological patterns. The CTCs were increased unless the patients were smokers or not. This explains that CTCs can be a diagnostic tool for detecting lung cancer earlier. When compared between CTCs and ctDNA, the CTCs concentration is rare in blood and ctDNA is rich in blood. The detection specificity for CTCs is higher than ctDNA and lower than CTCs in case of ctDNA. Mutation, prognosis, disease status, treatment response, drug resistance prediction can be detected using both of them. CTCs are difficult to get detected in CSF but possible in case of ctDNA. Invitro culture can be done for CTCs but cannot be done in ctDNA. The ctDNA detected in necrotic tumours cells have variable characteristics when compared to apoptotic healthy cells. Exosomes are other biomarkers used in the detection of

lung cancer. The secretome is the protein secreted by Bacillus subtilis. Based on this the proteins secreted by the cell, tissue or organism began to be studied including in cancer conditions. The proteins secreted are related to differentiation, invasion, angiogenesis and metastasis. The patients suffering from non-small cell lung cancer (NSCLC) suffer from pleural effusion which became the source of biomarkers in lung cancer patients. The secretome present in the pleural effusion of lung cancer patients and specific proteins if present in the pleural effusion can also be detected in circulation.[11]

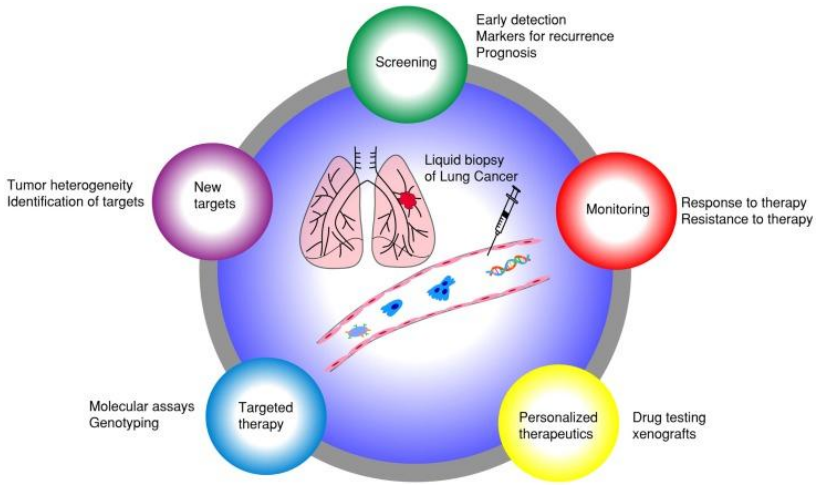


Figure 1: It depicts the uses and applications of liquid biopsy in lung cancer.

Novel Biomarker

Breath biomarkers are novel biomarkers used for early detection of cancer. According to the Warburg effect, the cancerous cells survive in the oxygen-deprived environment that results from changes in cellular metabolism for the survival of cancer cell and altered metabolic intermediates serve as building blocks for new cells. This leads to alteration in the volatile organic components (VOCs) in the breath of an individual. As these changes are related to cancer cell survival, the VOCs can be excellent biomarkers for early detection of cancer. The breath collection device was being introduced in collaboration with 100 breath diagnostic researchers which is the world's largest clinical trial based on breath biopsy for early detection of cancer. The UK NHS funded this research (LuCID trial) by recruiting up to 4000 patients who are suspected with lung cancer and include the VOC breath biomarker as a diagnostic aid which can differentiate presence or absence of health benefits lung cancer in those patients. The breath biopsy can avoid unnecessary CT scans which give false-positive results. This, in turn, can reduce screening costs and improve quality of life of patients.[12]

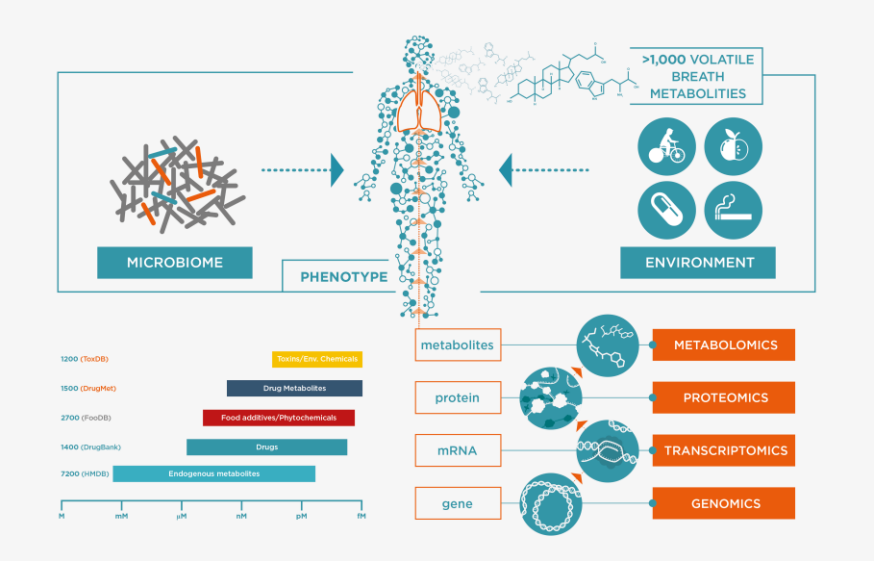


Figure 2: It depicts the alterations in the body and how breath biopsy is used to detect those alterations.

In Bladder Cancer

The CTCs were first identified in bladder cancer in the year 2000 among the patients with urothelial carcinoma. These CTCs were identified using a nested reverse transcription-PCR assay for Uroplakin 2(Upk-2). The metastatic bladder cancer can be detected up to 50% by Cell search technology which is 15% in case of localized bladder cancer. A study proved that there is a strong correlation between CTC and progression upon the recurrence of bladder cancer. The number of CTCs in the circulatory system is correlated with tumours stage, histology of the tumours, metastasis of lymph node. Due to the limited specificity for the diagnosis of bladder cancer, the CTC detection may not be accurate as of the initial screening test but can be used for confirming the bladder cancer diagnosis. On the other hand, the presence of ctDNA in urine samples of bladder cancer patients with progressive disease even ctDNA is not found in the blood. The ctDNA mutations are observed in bladder cancer like TP53, KRAS, BRAF, FGFR3, P1K3CA, CTNNB1. The presence of these mutations suggested a relation to disease progression and recurrence. The alteration in chromosomes 4, 8, 9, 1, 17 leads to microsatellite instability and loss of heterozygosity (LOH) which can be detected using biomarkers. The methylation-specific polymerase chain reaction (MSP) has the ability to detect epigenetic alterations in bladder cancer patients. So, these methylation markers have been proposed for the prevention of bladder cancer. Urine and serum samples can be used for diagnosis and prognosis where urine can also use for recurrence surveillance in bladder cancer.[13]

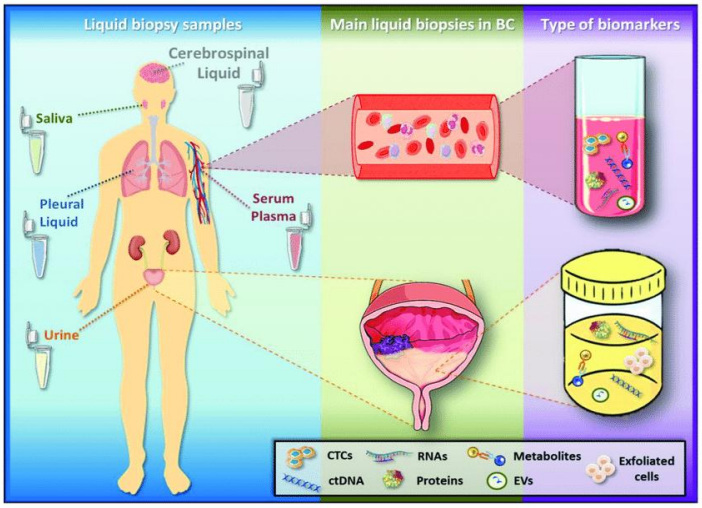


Figure 3: It depicts biomarkers and samples used in liquid biopsy. The picture exhibits total number of biomarkers and targets. It specifies the biomarkers used in Bladder cancer (urine and blood).

In Colorectal Cancer

Worldwide, it is the 2nd most-affected cancer in women and 3rd most in men. The diagnosis used currently is colonoscopy which is the most reliable method for early detection of CRC. This test is expensive, time-consuming and discomfort for the patient. The changes in microbial flora can be related to CRC. In a study which included 39 patients with CRC, 135 patients with a low and high stage of dysplasia, 66 were a control group. Here, the stool biopsy was done by analyzing stool with quantitative PCR technique for identifying clbA gene and afaC gene. To detect pks + bacteria, Escherichia. coli Nissle 1917 was used as a positive control. The bacteria which is positive for clbA gene was used for the detection of CRC as they were more observed in the stools of CRC patients. As there is the usage of microbes for the detection of CRC, these markers are known as microbial markers identified using stool biopsy. The microbial markers are an efficient, cost-effective and noninvasive procedure. These biomarkers can be used for the early detection of CRC as the microbes appear before the occurrence of disease.[14]

In Glioma

Glioma is the most common type of brain cancer. The tumours DNA was first identified by Harker and Rhodes et al in the year 1995. They used specific PCR technique to detect p53 DNA in CSF of glioma patient. The technique shallow whole-genome sequencing of CSF-ctDNA is used to diagnose the alterations in glioma-derived ctDNA. This can be cost-effective for patients with glioma.[15]

CSF as a liquid biopsy in CNS metastases – In CNS metastases several tools were improving from which the CSF is now being used as a biomarker for earlier detection. The FDA approved Veridex Cell search Assay was originally developed to detect CTCs in blood but it is now being used to detect and quantify tumours cells in CSF. Using CSF, the diagnosis of leptomeningitis, brain metastasis, tumours progression, tumours relapse, tumours response to cytotoxic or targeted agents can be done. The resistance to a given medication, monitoring of therapy for resistance mutations with specific targeted drugs can be identified. The CTC can be detected using CSF analysis. During the collection of CSF in post-operative patients, caution must be taken as there will be mechanical tumours spill in 2-3 weeks of post-operation.[16] The ctDNA evaluation in postoperative follow-up of patients and during the recurrence can help to deliver the precision therapies to those patients.[17] In future, the liquid biopsy can be a better non-invasive tool for prognosis and diagnosis in patients with cancer or infectious diseases.[15]

FOUNDATION ONE CDx (F1CDx)

Foundation one CDx which is recently approved by FDA which is next generation sequencing based on invitro diagnostic device used to detect substitutions, insertion and deletion alterations and copy number alterations in 324 genes and genetic re-arrangements including microsatellite instability (MSI) and tumor mutational burden (TMB). This can be performed using DNA isolated from formalin-fixed paraffin embedded (FFPE) tumor tissue specimens. The test is useful as companion diagnostic tool to identify patients who may benefit from targeted therapy. 5 types of cancerous alterations can be found using this diagnostic tool.

The Indications Include

Tumor type	Biomarkers detected	Therapy
Non-small cell lung cancer (NSCLC)	EGFR exon 19 deletions and EGFR exon 21 L858R alterations EGFR exon 20 T790M alterations ALK re-arrangements BRAF V600E	AFATINIB, GEFITINIB OSIMERTINIB ALECTINIB, CRIZOTINIB DABRAFENIB, TRAMETINIB
Melanoma	BRAF V600E BRAF V600E and V600K	DABRAFENIB VEMURAFENIB
Breast cancer	ERBB2 (HER2) amplification	TRASTUZUMAB, PERTUZUMAB

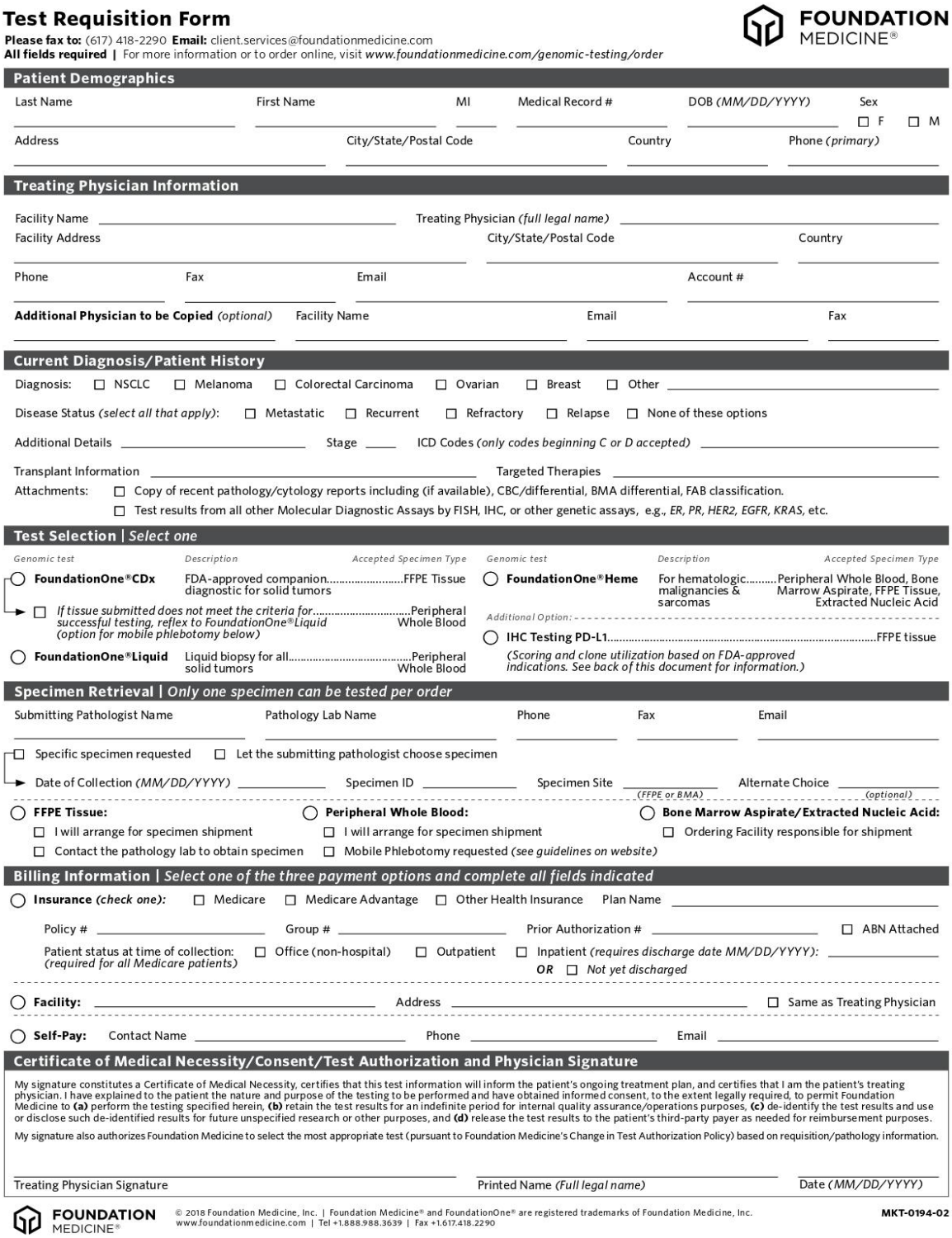
Colorectal cancer	KRAS wild-type (absence of mutations in codons 12 and 13) KRAS wild type (absence of mutations in exons 2,34) and NRAS wild type (absence of mutations in exons 2,3,4)	ERBITUX VECTIBIX
Ovarian cancer	BRCA 1/2 alterations	OLAPARIB or RUBRACA

This test is also used to detect genomic loss of heterozygosity (LOH) from formalin-fixed, paraffin-embedded (FFPE) ovarian tumor tissue. There are no known contraindications.

Warnings and Precautions: There can be somatic or germ line alterations, but this test does not differentiate these alterations. The physician must decide if the patient is eligible for biopsy when archival tissue is not available for use with assay. If the patient has ERBB2 amplification the reflex testing needs to be performed.

Limitations: It is only for invitro diagnosis and prescription use only. A negative result does not diagnose presence of mutation below the limits of detection. Samples which have <25% tumor will have decreased sensitivity for detection of CANs including ERBB2. The decisions on patient treatment and care should be based on independent medical judgement of treating physician, taking into consideration all applicable information concerning the patient's condition. The test must be performed based on specific serial number-controlled instruments by foundation medicine, Inc.[7]

The application form for testing foundation one CDx: (figure 4 & 5) (7).



Test Requisition Form

FOUNDATION MEDICINE®

Please fax to: (617) 418-2290 **Email:** client.services@foundationmedicine.com
All fields required | For more information or to order online, visit *www.foundationmedicine.com/genomic-testing/order*

Patient Demographics

Last Name | First Name | MI | Medical Record # | DOB *(MM/DD/YYYY)* | Sex ☐ F ☐ M

Address | City/State/Postal Code | Country | Phone *(primary)*

Treating Physician Information

Facility Name | Treating Physician *(full legal name)*

Facility Address | City/State/Postal Code | Country

Phone | Fax | Email | Account #

Additional Physician to be Copied *(optional)* | Facility Name | Email | Fax

Current Diagnosis/Patient History

Diagnosis: ☐ NSCLC ☐ Melanoma ☐ Colorectal Carcinoma ☐ Ovarian ☐ Breast ☐ Other

Disease Status *(select all that apply)*: ☐ Metastatic ☐ Recurrent ☐ Refractory ☐ Relapse ☐ None of these options

Additional Details | Stage | ICD Codes *(only codes beginning C or D accepted)*

Transplant Information | Targeted Therapies

Attachments: ☐ Copy of recent pathology/cytology reports including (if available), CBC/differential, BMA differential, FAB classification.
☐ Test results from all other Molecular Diagnostic Assays by FISH, IHC, or other genetic assays, e.g., *ER, PR, HER2, EGFR, KRAS*, etc.

Test Selection | *Select one*

Genomic test	*Description*	*Accepted Specimen Type*
○ **FoundationOne®CDx**	FDA-approved companion diagnostic for solid tumors	FFPE Tissue
☐ *If tissue submitted does not meet the criteria for successful testing, reflex to FoundationOne®Liquid (option for mobile phlebotomy below)*		Peripheral Whole Blood
○ **FoundationOne®Liquid**	Liquid biopsy for all solid tumors	Peripheral Whole Blood
○ **FoundationOne®Heme**	For hematologic malignancies & sarcomas	Peripheral Whole Blood, Bone Marrow Aspirate, FFPE Tissue, Extracted Nucleic Acid
Additional Option: ○ **IHC Testing PD-L1** *(Scoring and clone utilization based on FDA-approved indications. See back of this document for information.)*		FFPE tissue

Specimen Retrieval | *Only one specimen can be tested per order*

Submitting Pathologist Name | Pathology Lab Name | Phone | Fax | Email

☐ Specific specimen requested ☐ Let the submitting pathologist choose specimen

Date of Collection *(MM/DD/YYYY)* | Specimen ID | Specimen Site *(FFPE or BMA)* | Alternate Choice *(optional)*

○ **FFPE Tissue:**
☐ I will arrange for specimen shipment
☐ Contact the pathology lab to obtain specimen

○ **Peripheral Whole Blood:**
☐ I will arrange for specimen shipment
☐ Mobile Phlebotomy requested *(see guidelines on website)*

○ **Bone Marrow Aspirate/Extracted Nucleic Acid:**
☐ Ordering Facility responsible for shipment

Billing Information | *Select one of the three payment options and complete all fields indicated*

○ **Insurance** *(check one):* ☐ Medicare ☐ Medicare Advantage ☐ Other Health Insurance Plan Name

Policy # | Group # | Prior Authorization # ☐ ABN Attached

Patient status at time of collection: *(required for all Medicare patients)* ☐ Office (non-hospital) ☐ Outpatient ☐ Inpatient *(requires discharge date MM/DD/YYYY)*: **OR** ☐ *Not yet discharged*

○ **Facility:** | Address ☐ Same as Treating Physician

○ **Self-Pay:** Contact Name | Phone | Email

Certificate of Medical Necessity/Consent/Test Authorization and Physician Signature

My signature constitutes a Certificate of Medical Necessity, certifies that this test information will inform the patient's ongoing treatment plan, and certifies that I am the patient's treating physician. I have explained to the patient the nature and purpose of the testing to be performed and have obtained informed consent, to the extent legally required, to permit Foundation Medicine to **(a)** perform the testing specified herein, **(b)** retain the test results for an indefinite period for internal quality assurance/operations purposes, **(c)** de-identify the test results and use or disclose such de-identified results for future unspecified research or other purposes, and **(d)** release the test results to the patient's third-party payer as needed for reimbursement purposes.

My signature also authorizes Foundation Medicine to select the most appropriate test (pursuant to Foundation Medicine's Change in Test Authorization Policy) based on requisition/pathology information.

Treating Physician Signature | Printed Name *(Full legal name)* | Date *(MM/DD/YYYY)*

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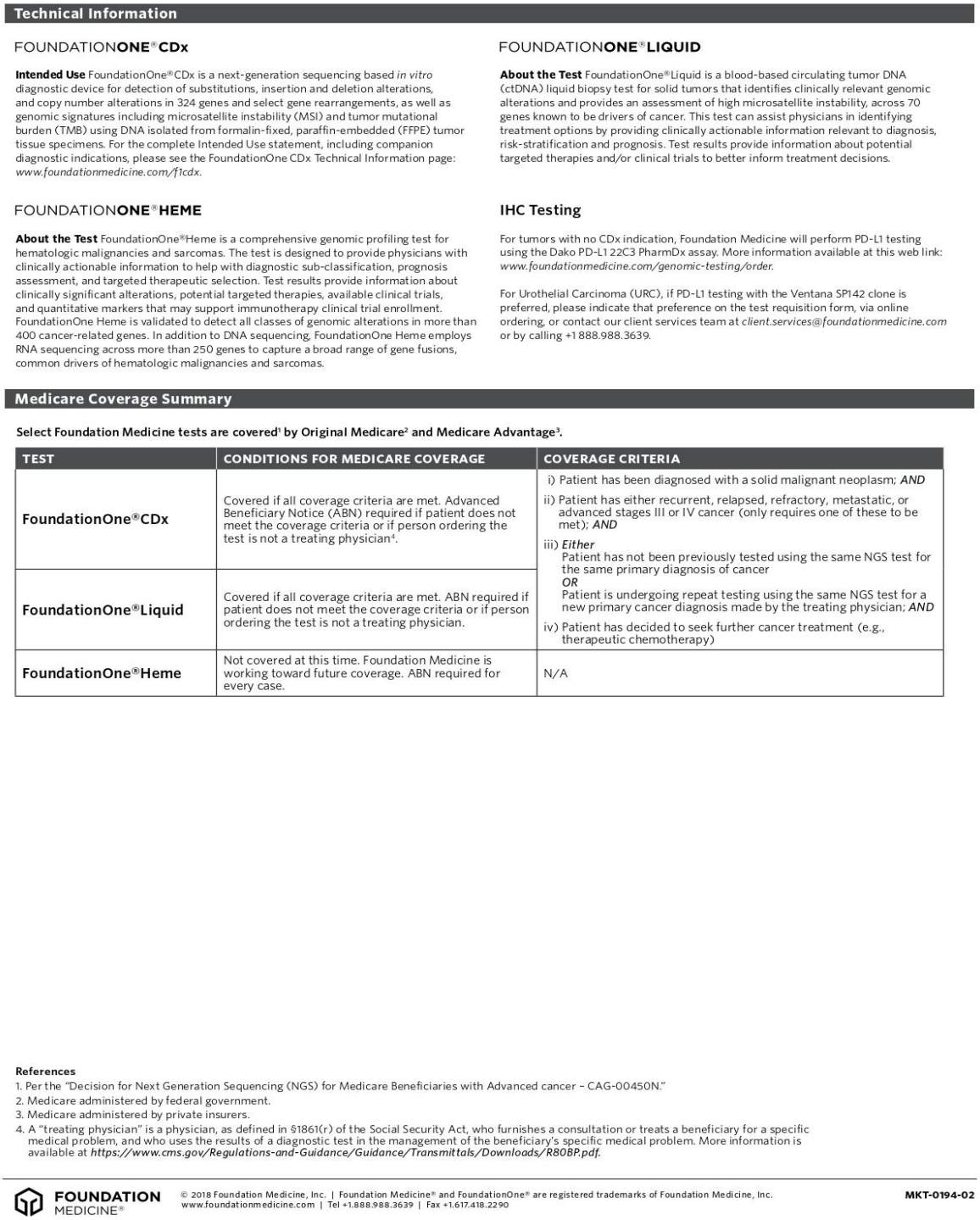
MKT-0194-02

Figure 4



Technical Information

FOUNDATIONONE® CDx

Intended Use FoundationOne® CDx is a next-generation sequencing based *in vitro* diagnostic device for detection of substitutions, insertion and deletion alterations, and copy number alterations in 324 genes and select gene rearrangements, as well as genomic signatures including microsatellite instability (MSI) and tumor mutational burden (TMB) using DNA isolated from formalin-fixed, paraffin-embedded (FFPE) tumor tissue specimens. For the complete Intended Use statement, including companion diagnostic indications, please see the FoundationOne CDx Technical Information page: *www.foundationmedicine.com/f1cdx*.

FOUNDATIONONE® HEME

About the Test FoundationOne® Heme is a comprehensive genomic profiling test for hematologic malignancies and sarcomas. The test is designed to provide physicians with clinically actionable information to help with diagnostic sub-classification, prognosis assessment, and targeted therapeutic selection. Test results provide information about clinically significant alterations, potential targeted therapies, available clinical trials, and quantitative markers that may support immunotherapy clinical trial enrollment. FoundationOne Heme is validated to detect all classes of genomic alterations in more than 400 cancer-related genes. In addition to DNA sequencing, FoundationOne Heme employs RNA sequencing across more than 250 genes to capture a broad range of gene fusions, common drivers of hematologic malignancies and sarcomas.

FOUNDATIONONE® LIQUID

About the Test FoundationOne® Liquid is a blood-based circulating tumor DNA (ctDNA) liquid biopsy test for solid tumors that identifies clinically relevant genomic alterations and provides an assessment of high microsatellite instability, across 70 genes known to be drivers of cancer. This test can assist physicians in identifying treatment options by providing clinically actionable information relevant to diagnosis, risk-stratification and prognosis. Test results provide information about potential targeted therapies and/or clinical trials to better inform treatment decisions.

IHC Testing

For tumors with no CDx indication, Foundation Medicine will perform PD-L1 testing using the Dako PD-L1 22C3 PharmDx assay. More information available at this web link: *www.foundationmedicine.com/genomic-testing/order*.

For Urothelial Carcinoma (URC), if PD-L1 testing with the Ventana SP142 clone is preferred, please indicate that preference on the test requisition form, via online ordering, or contact our client services team at *client.services@foundationmedicine.com* or by calling +1 888.988.3639.

Medicare Coverage Summary

Select Foundation Medicine tests are covered[1] by Original Medicare[2] and Medicare Advantage[3].

TEST	CONDITIONS FOR MEDICARE COVERAGE	COVERAGE CRITERIA
FoundationOne® CDx	Covered if all coverage criteria are met. Advanced Beneficiary Notice (ABN) required if patient does not meet the coverage criteria or if person ordering the test is not a treating physician[4].	i) Patient has been diagnosed with a solid malignant neoplasm; *AND* ii) Patient has either recurrent, relapsed, refractory, metastatic, or advanced stages III or IV cancer (only requires one of these to be met); *AND* iii) *Either* Patient has not been previously tested using the same NGS test for the same primary diagnosis of cancer *OR* Patient is undergoing repeat testing using the same NGS test for a new primary cancer diagnosis made by the treating physician; *AND* iv) Patient has decided to seek further cancer treatment (e.g., therapeutic chemotherapy)
FoundationOne® Liquid	Covered if all coverage criteria are met. ABN required if patient does not meet the coverage criteria or if person ordering the test is not a treating physician.	
FoundationOne® Heme	Not covered at this time. Foundation Medicine is working toward future coverage. ABN required for every case.	N/A

References

1. Per the "Decision for Next Generation Sequencing (NGS) for Medicare Beneficiaries with Advanced cancer – CAG-00450N."
2. Medicare administered by federal government.
3. Medicare administered by private insurers.
4. A "treating physician" is a physician, as defined in §1861(r) of the Social Security Act, who furnishes a consultation or treats a beneficiary for a specific medical problem, and who uses the results of a diagnostic test in the management of the beneficiary's specific medical problem. More information is available at *https://www.cms.gov/Regulations-and-Guidance/Guidance/Transmittals/Downloads/R80BP.pdf*.

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Figure 5

CONCLUSION

Cancer is a devastating disease that alters metabolism of cells in the body. The metabolomics are better employed to understand the changes in cancer metabolism. Along with imaging techniques, metabolomics can be used for both diagnostic and prognostic purposes. The

metabolomics can be preferred in era of precision medicine where there can be personalized medicine for every individual patient. However, there should be complete implementation of these diagnostic biomarkers to reach its potential. The challenges to overcome are quality control and validation standards, interpretation of clinical results for metabolomics data, implementation of MS and NMR in clinical studies, sharing of clinical metabolomics data. The future evaluations of metabolomics include, diagnostic biomarkers, metabolic responses from patients receiving the therapy in terms of efficacy and resistance, microbiome changes during and after surgery and therapy. The combined approaches by studying biology information like miRNAs, transcription of proteins will develop metabolomics complimentary to other omics technologies.[1] The clinical trials should be conducted where the sample size should be more for validation purposes. It is also to be proven and detected that how effective are the metabolites to detect the cancer non-invasively. Challenges ahead, metabolomics could be the future pathway for non-invasive detection of cancer.[9]

The protein-based studies have not been significant in clinics. Recent advances including liquid biopsy have been a novel approach. The early stage detection of pancreatic cancer with Gypian-1 which is a membrane-anchored protein was detected using circulating exosomes. The exosome-based studies were developed for many types of cancers in detection, e.g., prostate cancer detection with prostate specific antigen levels using biomarkers. There is no test which detects all types of cancers.[5] The appropriateness of liquid biopsy is increasing due to the evolution of new generation sequencing (NGS) techniques. The results from large scale trials can be beneficial in implementing the liquid biopsy in clinical practice and precision medicine in clinical oncology.[4] Among body fluids saliva and urine can be the most significant fluids in liquid biopsy as they are cost-effective, non-invasive. Some studies included detection of potential biomarker that detect bladder cancer in earlier stages. Despite the difficulties and limitations in liquid biopsy, there is a futuristic view in which it replaces the tissue biopsy in all solid tumors.[13]

The recently approved FD1 CDx is used to detect the deletions, alterations, insertions, substitutions in genes of tumor cells which can provide effective therapy in the era of precision medicine. This tool detects 5 types of genetic alterations which include melanoma, non-small cell lung cancer, breast cancer, colorectal cancer, ovarian cancer. All these diagnostic tools (metabolomics, liquid biopsy, FD1 CDx) which were included in this study conclude to one end of earlier diagnosis, precision medicine and resistance to the given therapy. Every

invention and discovery have its own limitations, but if we overcome those limitations, metabolomics, liquid biopsy, FD1 CDx can be an era in clinical oncology for the prior detection of stages in cancer patients.

ACKNOWLEDGEMENT

We are gratefully acknowledged Dr. Padmalatha Kantamneni, Principal, Vijaya Institute of Pharmaceutical Sciences for Women for support and valuable guidance.

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