Article Title: Liquid Biopsy Based on Cell-Free DNA and RNA

Abstract: This review delves into the rapidly evolving landscape of liquid biopsy technologies based on cell-free DNA (cfDNA) and cell-free RNA (cfRNA) and their increasingly prominent role in precision medicine. With the advent of high-throughput DNA sequencing, the use of cfDNA and cfRNA has revolutionized noninvasive clinical testing. Here, we explore the physical characteristics of cfDNA and cfRNA, present an overview of the essential engineering tools used by the field, and highlight clinical applications, including noninvasive prenatal testing, cancer testing, organ transplantation surveillance, and infectious disease testing. Finally, we discuss emerging technologies and the broadening scope of liquid biopsies to new areas of diagnostic medicine.

Article Title: Quantification of Circulating Cell-Free DNA in Idiopathic Parkinson's Disease Patients

Abstract: Parkinson's disease (PD) is one of the most common neurodegenerative disorders globally and leads to an excessive loss of dopaminergic neurons in the substantia nigra of the brain. Circulating cell-free DNA (ccf-DNA) are double-stranded DNA fragments of different sizes and origins that are released into the serum and cerebrospinal fluid (CSF) due to cell death (i.e., necrosis and apoptosis) or are actively released by viable cells via exocytosis and NETosis. Using droplet digital polymerase chain reaction (ddPCR), we comprehensively analyzed and distinguished circulating cell-free mitochondrial DNA (ccf mtDNA) and circulating cell-free nuclear DNA (ccfDNA) in the serum and CSF of PD and control patients. The quantitative analysis of serum ccf-DNA in PD patients demonstrated a significant increase in ccf mtDNA and ccfDNA compared to that in healthy control patients and a significantly higher copy of ccf mtDNA when compared to ccfDNA. Next, the serum ccf mtDNA levels significantly increased in male PD patients compared to those in healthy male controls. Furthermore, CSF ccf mtDNA in PD patients increased significantly compared to ccfDNA, and ccf mtDNA decreased in PD patients more than it did in healthy controls. These decreases were not statistically significant but were in agreement with previous data. Interestingly, ccf mtDNA increased in healthy control patients in both serum and CSF as compared to ccfDNA. The small sample size of serum and CSF were the main limitations of this study. To the best of our knowledge, this is the first comprehensive study on serum and CSF of PD patients using ddPCR to indicate the distribution of the copy number of ccf mtDNA as well as ccfDNA. If validated, we suggest that ccf mtDNA has greater potential than ccfDNA to lead the development of novel treatments for PD patients.

Article Title: Cell-Free DNA Fragmentomics: The Novel Promising Biomarker

Abstract: Cell-free DNA molecules are released into the plasma via apoptotic or necrotic events and active release mechanisms, which carry the genetic and epigenetic information of its origin tissues. However, cfDNA is the mixture of various cell fragments, and the efficient enrichment of cfDNA fragments with diagnostic value remains a great challenge for application in the clinical setting. Evidence from recent years shows that cfDNA fragmentomics' characteristics differ in normal and diseased individuals without the need to distinguish the source of the cfDNA fragments, which makes it a promising novel biomarker. Moreover, cfDNA fragmentomics can identify tissue origins by inferring epigenetic information. Thus, further insights into the fragmentomics of plasma cfDNA shed light on the origin and fragmentation mechanisms of cfDNA during physiological and pathological processes in diseases and enhance our ability to take the advantage of plasma cfDNA as a molecular diagnostic tool. In this review, we focus on the cfDNA fragment characteristics and its potential application, such as fragment length, end motifs, jagged ends, preferred end coordinates, as well as nucleosome footprints, open chromatin region, and gene expression inferred by the cfDNA fragmentation pattern across the genome. Furthermore, we summarize the methods for deducing the tissue of origin by cfDNA fragmentomics.

Article Title: Single-molecule methylation profiles of cell-free DNA in cancer with nanopore sequencing

Abstract: Epigenetic characterization of cell-free DNA (cfDNA) is an emerging approach for detecting and characterizing diseases such as cancer. We developed a strategy using nanopore-based single-molecule sequencing to measure cfDNA methylomes. This approach generated up to 200 million reads for a single cfDNA sample from cancer patients, an order of magnitude improvement over existing nanopore sequencing methods. We developed a single-molecule classifier to determine whether individual reads originated from a tumor or immune cells. Leveraging methylomes of matched tumors and immune cells, we characterized cfDNA methylomes of cancer patients for longitudinal monitoring during treatment.

Article Title: Ovarian Cancer Diagnosis and Prognosis Based on Cell-Free DNA Methylation

Abstract: Background: Ovarian cancer stands as the deadliest malignant tumor within the female reproductive tract. As a result of the absence of effective diagnostic and monitoring markers, 75% of ovarian cancer cases are diagnosed at a late stage, leading to a mere 50% survival rate within five years. The advancement of molecular biology is essential for accurate diagnosis and treatment of ovarian cancer. Methods: A review of several randomized clinical trials, focusing on the ovarian cancer, was undertaken. The advancement of molecular biology and diagnostic methods related to accurate diagnosis and treatment of ovarian cancer were examined. Results: Liquid biopsy is an innovative method of detecting malignant tumors that has gained increasing attention over the past few years. Cell-free DNA assay-based liquid biopsies show potential in delineating tumor status heterogeneity and tracking tumor recurrence. DNA methylation influences a multitude of biological functions and diseases, especially during the initial phases of cancer. The cell-free DNA methylation profiling system has emerged as a sensitive and non-invasive technique for identifying and detecting the biological origins of cancer. It holds promise as a biomarker, enabling early screening, recurrence monitoring, and prognostic evaluation of cancer. Conclusions: This review evaluates recent advancements and challenges associated with cell-free DNA methylation analysis for the diagnosis, prognosis monitoring, and assessment of therapeutic responses in the management of ovarian cancers, aiming to offer guidance for precise diagnosis and treatment of this disease. Ovarian cancer stands as the deadliest malignant tumor within the female reproductive tract. As a result of the absence of effective diagnostic and monitoring markers, 75% of ovarian cancer cases are diagnosed at a late stage, leading to a mere 50% survival rate within five years. Nearly 80% of advanced stages have a poor prognosis or recurrence within five years. Ovarian cancer is linked to a grim long-term prognosis attributable to its elevated mortality and recurrence rates. The advancement of molecular biology and diagnostic methods is essential for accurate diagnosis and treatment of ovarian cancer. Liquid biopsy is an innovative method of detecting malignant tumors that has gained increasing attention over the past few years. Cell-free DNA assay-based liquid biopsies show potential in delineating tumor status heterogeneity and tracking tumor recurrence. DNA methylation represents a prevalent epigenetic modification. DNA methylation influences a multitude of biological functions and diseases, especially during the initial phases of cancer. The cell-free DNA methylation profiling system has emerged as a sensitive and non-invasive technique for identifying and detecting the biological origins of cancer. This review assesses recent progress and obstacles linked to cell-free DNA methylation analysis for diagnosing, prognostic monitoring, and evaluating therapeutic responses in managing ovarian cancers.

Article Title: Cell-Free DNA Maps Tissue Injury and Correlates with Disease Severity in Lung Transplant Candidates

Abstract: Rationale: Plasma cell-free DNA levels correlate with disease severity in many conditions. Pretransplant cell-free DNA may risk stratify lung transplant candidates for post-transplant complications. Objectives: To evaluate if pretransplant cell-free DNA levels and tissue sources identify patients at high risk of primary graft dysfunction and other pre- and post-transplant outcomes. Methods: This multicenter, prospective cohort study recruited 186 lung transplant candidates. Pretransplant plasma samples were collected to measure cell-free DNA. Bisulfite sequencing was performed to identify the tissue sources of cell-free DNA. Multivariable regression models determined the association

between cell-free DNA levels and the primary outcome of primary graft dysfunction and other transplant outcomes, including Lung Allocation Score, chronic lung allograft dysfunction, and death. Measurements and Main Results: Transplant candidates had twofold greater cell-free DNA levels than healthy control patients (median [interquartile range], 23.7 ng/ml [15.1-35.6] vs. 12.9 ng/ml [9.9-18.4]; P < 0.0001), primarily originating from inflammatory innate immune cells. Cell-free DNA levels and tissue sources differed by native lung disease category and correlated with the Lung Allocation Score (P, 0.001). High pretransplant cell-free DNA increased the risk of primary graft dysfunction (odds ratio, 1.60; 95% confidence interval [CI], 1.09-2.46; P = 0.0220), and death (hazard ratio, 1.43; 95% CI, 1.07-1.92; P = 0.0171) but not chronic lung allograft dysfunction (hazard ratio, 1.37; 95% CI, 0.97-1.94; P = 0.0767). Conclusions: Lung transplant candidates demonstrate a heightened degree of tissue injury with elevated cell-free DNA, primarily originating from innate immune cells. Pretransplant plasma cell-free DNA levels predict post-transplant complications.

Article Title: Cell-Free DNA Analysis of Fetal Aneuploidies in Early Pregnancy Loss

Abstract: Background: Products of conception samples are often collected and analyzed to try to determine the cause of an early pregnancy loss. However, sample collection may not always be possible, and maternal cell contamination and culture failure can affect the analysis. Cell-free DNA-based analysis of a blood sample could be used as an alternative method in early pregnancy loss cases to detect if aneuploidies were present in the fetus. Methods: In this prospective study, blood samples from early pregnancy loss patients were analyzed for the presence of fetal aneuploidies using a modified version of a noninvasive prenatal testing assay for cell-free DNA analysis. Results from cell-free DNA analysis were compared against the gold standard, microarray analysis of products of conception samples. This study was registered with ClinicalTrials.gov, identifier: NCT04935138. Results: Of the 76 patient samples included in the final study cohort, 11 were excluded from performance calculations. The 65 patient samples included in the final analysis included 49 with an abnormal microarray result and 16 with a normal microarray result. Based on results from these 65 samples, the study found that genome-wide cell-free DNA analysis had a sensitivity of 73.5% with a specificity of 100% for the detection of fetal aneuploidies in early pregnancy loss cases. Conclusions: This prospective study provides further support for the utility of cell-free DNA analysis in detecting fetal aneuploidies in early pregnancy loss cases. This approach could allow for a noninvasive method of investigating the etiology of miscarriages to be made available clinically.

Article Title: Investigation of Extracted Plasma Cell-Free DNA as a Biomarker in Foals with Sepsis

Abstract: Simple Summary Cell-free DNA (cfDNA) are pieces of DNA released from cells into body fluids. Previous studies in adult horses found that plasma cfDNA concentrations differed significantly between healthy horses and those with emergencies like colic. These studies also showed that accurate measurement of plasma cfDNA in adult horses required extraction, due to matrix effects of equine plasma. It's unclear if similar issues exist in foal plasma. In our study, we aimed to determine if foal plasma has similar interference, and if there are differences in cfDNA levels between healthy, sick non-septic (SNS), and septic foals. Cell-free DNA was measured directly in plasma and after extracting it using a kit in 60 foals. Direct measurement of cfDNA in foal plasma was found to be inaccurate due to matrix effect. However, even after cfDNA extraction, there were no significant differences in plasma cfDNA levels or the ratio of cfDNA to neutrophils between healthy foals, SNS foals, and septic foals. Future research should focus on understanding how neutrophils function during foal sepsis.Abstract Cell-free DNA (cfDNA) is fragmented extracellular DNA that is released from cells into various body fluids. Previously published data from adult horses supports cfDNA as a potential disease biomarker, but also shows that direct measurement in plasma is inaccurate due to matrix effect. It is currently unknown whether a similar matrix effect exists in foal plasma. Given this, the objectives of the current study were to investigate foal plasma for potential matrix effect during fluorescence measurement of cfDNA using a Qubit fluorometer, and to determine whether neat and/or extracted plasma cfDNA concentrations are significantly different in healthy, sick non-septic (SNS) or septic foals. We

hypothesized that matrix effect would interfere with accurate fluorescent measurement of cfDNA in foal plasma. Further, we hypothesized that mean extracted cfDNA concentrations, and/or extracted cfDNA:neutrophil ratio, would be elevated in plasma of septic foals compared to healthy or SNS foals. Cell-free DNA was measured in neat plasma, and following DNA extraction with a commercial kit, from 60 foals. Foal plasma exhibited both autofluorescence and non-specific dye binding, confirming matrix effect. However, even with extraction, no significant difference was found in cfDNA concentrations, or cfDNA:neutrophil ratios, between healthy (sepsis score <= 5), SNS (sepsis score 6-11 and negative blood culture), or septic (sepsis score >= 12 +/- positive blood culture) foals. Our data show that matrix effect interferes with accurate Qubit measurement of cfDNA in foal plasma and supports previous findings that plasma cfDNA concentrations are not associated with sepsis diagnosis in foals. Further research is needed to better understand neutrophil function and dysfunction in foal sepsis.

Article Title: Characterization of Mitochondrial DNA Methylation of Alzheimer's Disease in Plasma Cell-Free DNA

Abstract: Noninvasive diagnosis of Alzheimer's disease (AD) is important for patients. Significant differences in the methylation of mitochondrial DNA (mtDNA) were found in AD brain tissue. Cell-free DNA (cfDNA) is a noninvasive and economical diagnostic tool. We aimed to characterize mtDNA methylation alterations in the plasma cfDNA of 31 AD patients and 26 age- and sex-matched cognitively normal control subjects. We found that the mtDNA methylation patterns differed between AD patients and control subjects. The mtDNA was predominantly hypomethylated in the plasma cfDNA of AD patients. The hypomethylation sites or regions were mainly located in mt-rRNA, mt-tRNA, and D-Loop regions. The hypomethylation of the D-Loop region in plasma cfDNA of AD patients was consistent with that in previous studies. This study presents evidence that hypomethylation in the non-protein coding region of mtDNA may contribute to the pathogenesis of AD and potential application for the diagnosis of AD.

Article Title: Circulating Cell Free DNA and DNA Double-Strand Breakage in Alzheimer's Disease

Abstract: Alzheimer's disease (AD) is an age-related neurodegenerative disease that is characterized by memory loss and multiple cognitive impairments. AD is pathologically characterized by age-dependent accumulation of amyloid-beta protein and the phosphorylation of tau protein in the brains of patients with AD. Clinically, manifestations of AD include cognitive decline, dementia, alterations of high-order brain functions, and movement disorders. Double-stranded DNA breaks are a lethal form of DNA damage and are typically repaired via non-homologous end joining and homologous recombination. However, in AD brain, repair mechanism is disrupted, leading to a cascade of events, cognitive dysfunction, organ failure and reduced lifespan. Increased circulating cell-free DNA in the blood, cerebrospinal fluid, and urine in patients with AD, can be used as early detectable biomarkers for AD. The purpose of our article is to explore the potential uses of cell-free DNA and double-stranded DNA breaks as prognostic markers for AD and examine the recent research on the application of these markers in studies.

Article Title: Individualized Cell-Free DNA Monitoring With Chromosomal Junctions for Mesothelioma

Abstract: Introduction: The spatially complex nature of mesothelioma and interventions like pleurodesis, surgery, and radiation often complicate imaging-based assessment. Further, cell-free DNA (cfDNA) based monitoring strategies are inadequate for mesothelioma, given the presence of a few recurring nonsynonymous somatic variants. However, patient-specific chromosomal rearrangements are commonly found in mesothelioma. Our study objective was to develop an individualized cfDNA assay to enable blood-based monitoring using circulating tumor DNA (ctDNA) in mesothelioma. We hypothesized that the unique chromosomal rearrangement junctions found in mesothelioma could be employed for individualized ctDNA detection and disease monitoring. Methods: DNA was extracted from tumor specimens for whole genome sequencing. Chromosomal junctions, prioritized by highest

allele frequency and low homology to the rest of the genome, were selected for detection. Primers and Taqman probes were designed to span the junctions, forming personalized junction panels. Patient plasma obtained before therapy and at response assessment was tested for the presence of personalized junctions via quantitative polymerase chain reaction. Results: Our study included nine patients, four with peritoneal and five with pleural mesothelioma. 763 chromosomal junctions were identified in the tumors of all cases. We selected three to five junctions per sample for quantitative polymerase chain reaction. We detected 25/30 (83%) of selected junctions in the plasma of seven out of nine patients (78%). Cell-free junction detection at follow-up was concordant with disease status: cfDNA junctions were detected in three patients with persistent disease, and not detected in a patient with no evidence of disease after surgery. Conclusions: With further validation, individualized ctDNA junction assays could supplement imaging for disease monitoring in mesothelioma.

Article Title: Detection of HBV DNA integration in plasma cell-free DNA of different HBV diseases utilizing DNA capture strategy

Abstract: The landscape of hepatitis B virus (HBV) integration in the plasma cell-free DNA (cfDNA) of HBV-infected patients with different stages of liver diseases [chronic hepatitis B (CHB), liver cirrhosis (LC), and hepatocellular carcinoma (HCC)] remains unclear. In this study, we developed an improved strategy for detecting HBV DNA integration in plasma cfDNA, based on DNA probe capture and next-generation sequencing. Using this optimized strategy, we successfully detected HBV integration events in chimeric artificial DNA samples and HBV-infected HepG2-NTCP cells at day one post infection, with high sensitivity and accuracy. The characteristics of HBV integration events in the HBV-infected HepG2-NTCP cells and plasma cfDNA from HBV-infected individuals (CHB, LC, and HCC) were further investigated. A total of 112 and 333 integration breakpoints were detected in the HepG2-NTCP cells and 22 out of 25 (88%) clinical HBV-infected samples, respectively. In vivo analysis showed that the normalized number of support unique sequences (nnsus) in HCC was significantly higher than in CHB or LC patients (P values < 0.05). All integration breakpoints are randomly distributed on human chromosomes and are enriched in the HBV genome around nt 1800. The majority of integration breakpoints (61.86%) are located in the gene-coding region. Both non-homologous end-joining (NHEJ) and microhomology-mediated end-joining (MMEJ) interactions occurred during HBV integration across the three different stages of liver diseases. Our study provides evidence that HBV DNA integration can be detected in the plasma cfDNA of HBV-infected patients, including those with CHB, LC, or HCC, using this optimized strategy.

Article Title: LABS: linear amplification-based bisulfite sequencing for ultrasensitive cancer detection from cell-free DNA

Abstract: Methylation-based liquid biopsies show promises in detecting cancer using circulating cell-free DNA; however, current limitations impede clinical application. Most assays necessitate substantial DNA inputs, posing challenges. Additionally, underrepresented tumor DNA fragments may go undetected during exponential amplification steps of traditional sequencing methods. Here, we report linear amplification-based bisulfite sequencing (LABS), enabling linear amplification of bisulfite-treated DNA fragments in a genome-wide, unbiased fashion, detecting cancer abnormalities with sub-nanogram inputs. Applying LABS to 100 patient samples revealed cancer-specific patterns, copy number alterations, and enhanced cancer detection accuracy by identifying tissue-of-origin and immune cell composition.

Article Title: Plasma microbial cell-free DNA following chimeric antigen receptor T cell therapy in pediatric patients with relapsed/refractory leukemia

Abstract: Chimeric antigen receptor (CAR) T cell therapy is a promising treatment for pediatric patients with relapsed or refractory B cell acute lymphoblastic leukemia (R/R B ALL). Cytokine release syndrome (CRS) is a common toxicity after CAR T cell therapy and fever is often the first symptom. Differentiating CRS from infection after CAR T cell therapy can be challenging. Plasma microbial cell free DNA (mcfDNA) is a novel diagnostic tool which allows for qualitative and quantitative assessment of over 1000 organisms. This pilot study sought to characterize mcfDNA results in pediatric patients with R/R B ALL in the first 2 months after CAR T cell therapy.

Article Title: Emerging Role of Plasma Microbial Cell-free DNA in the Diagnosis of Pediatric Mucormycosis

Abstract: Mucormycosis is a rare and devastating angioinvasive infection that can be challenging to diagnose due to the low sensitivity of current noninvasive diagnostics and the lack of a gold standard reference test. We describe a retrospective case series of children with suspected mucormycosis where plasma microbial cell-free DNA testing was utilized in the diagnostic evaluation to illustrate the ways in which microbial cell-free DNA testing can noninvasively contribute to the evaluation and management of at-risk, immunosuppressed patients suspected of mucormycosis.

Article Title: Development of a media cell-free DNA direct digital PCR method for cell viability estimation

Abstract: Background: Accurate estimation of cell viability is crucial in various applications such as cytotoxicity testing and routine cell culture on both industrial and laboratory scales. For this, the real-time monitoring of cell status would be beneficial. Conventional cell-based assays for cell viability have limitations in sensitivity and time- effectiveness. Analysis of cell-free DNA (cfDNA) in (culture) media is a good alternative as cfDNA release are a well-known phenomenon during cell death. Results: We demonstrate a direct digital PCR (dPCR) method to estimate cell viability by analyzing cfDNA in media during induced cell death. After validating the duplex dPCR method for short and long amplicons of the SMAD4 and RPP30 loci, we determined that a media volume of 2 mu L is feasible to measure the target DNA copy number with minimal negative effects on amplification. dPCR inhibition was evident with a higher media volume per reaction targeting long amplicons. Next, we applied our dPCR method using media cfDNA and other conventional methods to the monitoring of camptothecin (CPT)-induced cell death. Copy numbers increased significantly after 4 h of CPT treatment, showing a fold change of approximately 4-6 compared to the controls. Cell-based assays such as the 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay and annexin V/7-AAD assay also indicated increased cell death at 4 h, but the trypan blue exclusion assay did not. Significance: The developed media cfDNA direct dPCR method allows for efficient measurements of the degree of cell viability. Unlike other conventional cell-based assays, our method has advantages of no loss of cultured cells and the ability to implement online analysis. Accurate and sensitive media cfDNA analysis using dPCR can be adopted in various applications such as determining cytotoxicity levels in large-scale bioreactors or screening for effective anticancer drugs.

Article Title: Cell-Free DNA As Peripheral Biomarker of Alzheimer's Disease

Abstract: Alzheimer's disease (AD) and Alzheimer's disease-related disorders (ADRD) are progressive neurodegenerative diseases without cure. Alzheimer's disease occurs in 2 forms, early-onset familial AD and lateonset sporadic AD. Early-onset AD is a rare (similar to 1%), autosomal dominant, caused by mutations in presenilin-1, presenilin-2, and amyloid precursor protein genes and the other is a late-onset, prevalent and is evolved due to ageassociated complex interactions between environmental and genetic factors, in addition to apolipoprotein E4 polymorphism. Cellular senescence, promoting the impairment of physical and mental functions is constituted to be the main cause of aging, the primary risk factor for AD, which results in progressive loss of cognitive function, memory, and visual-spatial skills for an individual to live or act independently. Despite significant progress in the understanding of

the biology and pathophysiology of AD, we continue to lack definitive early detectable biomarkers and/or drug targets that can be used to delay the development of AD and ADRD in elderly populations. However, recent developments in the studies of DNA double-strand breaks result in the release of fragmented DNA into the bloodstream and contribute to higher levels of cell-free DNA (cf-DNA). This fragmented cf-DNA can be released into the bloodstream from various cell types, including normal cells and cells undergoing apoptosis or necrosis and elevated levels of cf-DNA in the blood have the potential to serve as blood blood-based biomarker for early detection of AD and ADRD. The overall goal of our study is to discuss the latest developments in circulating cell-free DNA into the blood in the progression of AD and ADRD. Our article summarized the status of research on double-strand breaks and circulating cell-free DNA in both healthy and disease states and how these recent developments can be used to develop early detectable biomarkers for AD and ADRD. Our article also discussed the impact of lifestyle and epigenetic factors that are involved in DNA double-strand breaks and circulating cell-free DNA in AD and ADRD.

Article Title: Life and death of circulating cell-free DNA

Abstract: Tumor-specific, circulating cell-free DNA in liquid biopsies is a promising source of biomarkers for minimally invasive serial monitoring of treatment responses in cancer management. We will review the current understanding of the origin of circulating cell-free DNA and different forms of DNA release (including various types of cell death and active secretion processes) and clearance routes. The dynamics of extracellular DNA in blood during therapy and the role of circulating DNA in pathophysiological processes (tumor-associated inflammation, NETosis, and pre-metastatic niche development) provide insights into the mechanisms that contribute to tumor development and metastases formation. Better knowledge of circulating tumor-specific cell-free DNA could facilitate the development of new therapeutic and diagnostic options for cancer management.

Article Title: New Perspectives on the Importance of Cell-Free DNA Biology

Abstract: Body fluids are constantly replenished with a population of genetically diverse cell-free DNA (cfDNA) fragments, representing a vast reservoir of information reflecting real-time changes in the host and metagenome. As many body fluids can be collected non-invasively in a one-off and serial fashion, this reservoir can be tapped to develop assays for the diagnosis, prognosis, and monitoring of wide-ranging pathologies, such as solid tumors, fetal genetic abnormalities, rejected organ transplants, infections, and potentially many others. The translation of cfDNA research into useful clinical tests is gaining momentum, with recent progress being driven by rapidly evolving preanalytical and analytical procedures, integrated bioinformatics, and machine learning algorithms. Yet, despite these spectacular advances, cfDNA remains a very challenging analyte due to its immense heterogeneity and fluctuation in vivo. It is increasingly recognized that high-fidelity reconstruction of the information stored in cfDNA, and in turn the development of tests that are fit for clinical roll-out, requires a much deeper understanding of both the physico-chemical features of cfDNA and the biological, physiological, lifestyle, and environmental factors that modulate it. This is a daunting task, but with significant upsides. In this review we showed how expanded knowledge on cfDNA biology and faithful reverse-engineering of cfDNA samples promises to (i) augment the sensitivity and specificity of existing cfDNA assays; (ii) expand the repertoire of disease-specific cfDNA markers, thereby leading to the development of increasingly powerful assays; (iii) reshape personal molecular medicine; and (iv) have an unprecedented impact on genetics research.

Article Title: Cell-free DNA as a solid-organ transplant biomarker: technologies and approaches

Abstract: High-quality biomarkers that detect emergent graft damage and/or rejection after solid-organ transplantation offer new opportunities to improve post-transplant monitoring, allow early therapeutic intervention and facilitate personalized patient management. Donor-derived cell-free DNA (DD-cfDNA) is a particularly exciting minimally invasive biomarker because it has the potential to be quantitative,

time-sensitive and cost-effective. Increased DD-cfDNA has been associated with graft damage and rejection episodes. Efforts are underway to further improve sensitivity and specificity. This review summarizes the procedures used to process and detect DD-cfDNA, measurement of DD-cfDNA in clinical transplantation, approaches for improving sensitivity and specificity and long-term prospects as a transplant biomarker to supplement traditional organ monitoring and invasive biopsies.