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HIV Screening Underutilized, Particularly in Physicians' Offices

Universal HIV screening has been endorsed by the U.S. Centers for Disease Control and Prevention (CDC) and other groups, but has not been widely adopted by hospitals and outpatient health care providers. New data shows that the vast majority of young men seeking care in physicians' offices are not screened. Experts are simultaneously exploring barriers to screening and possible mechanisms to routinize HIV testing and improve testing coverage.

In a clinical review and education piece published July 12 in the *Journal of the American Medical Association (JAMA)*, infectious disease experts cite insurance barriers, difficulty in assessing risk factors, and provider uncertainty regarding best practices and/or national testing recommendations as possible explanations for why HIV screening is not routinely conducted. This confusion could be due to the fact that in 2006, the CDC recommended HIV testing of adults and adolescents, but a systematic literature review conducted as part of the 2013 U.S. Preventive Services Task Force HIV screening recommendations found inconclusive benefit of universal screening versus targeted screening. Additionally, no single universal screening strategy (opt in versus opt out; standard laboratory testing versus point-of-care testing) proved superior.

"Clinical and risk factor–based testing is inferior to routine testing for identifying infected patients unaware of their diagnosis," writes co-author Moira McNulty, M.D., from University of Chicago, in *JAMA*. "Automatic testing with voluntary opting out is a strategy that should be more seriously considered. It is important to realize that the clinical and public health ben-

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Refined PSA Screening Strategies May Catch More Aggressive Cases

The number of new cases of metastatic prostate cancer climbed 72 percent from 2004 to 2013, according to a controversial new study published July 19 in *Prostate Cancer and Prostatic Diseases*. The investigators say they can't definitively link the increased cases to reduced prostate cancer screening and the rise could reflect the disease becoming more aggressive. However, the largest increase in new cases was among men 55 to 69 years old, which rose 92 percent.

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efits of screening are achieved only by ensuring engagement in care at other points in the continuum of care cascade."

Opportunity to Increase HIV Testing in Physicians' Offices

Young men aged 15 to 39 years frequently visited physicians' offices from 2009 to 2012, but HIV testing was only performed at one percent of those visits, according to a study published June 24 in *Morbidity and Mortality Weekly Report (MWWR)*. Opportunities such as opt-out testing, standing laboratory orders for HIV testing, and electronic medical record reminders need to be explored to increase HIV testing coverage at visits to physicians' offices, the CDC researchers say.

> *"A systems-level approach to increase HIV testing rates that does not rely on individual providers could use interventions to routinize HIV testing such as electronic medical records reminders, opt-out testing policies, provider education campaigns, and removal of barriers to HIV testing (i.e., special consent forms)."*
>
> — D. Cal Ham, M.D.

Young men (aged 20 to 29 years of age) accounted for the highest number of new HIV infection diagnoses in 2014, with even higher rates seen among young, male racial and ethnic minorities. To estimate the rates of health care visits among young men (aged 15 to 39 years) and the rates of HIV testing at these encounters, the CDC researchers analyzed data from the 2009–2012 National Ambulatory Medical Care Survey (NAMCS) and U.S. Census data.

The researchers found that overall, from 2009 to 2012, young males had an average of 1.35 visits to physicians' offices each year, but only one percent of the visits included an HIV test. While black males (2.7 percent) and Hispanic males (1.4 percent) had higher testing rates among compared with white males (0.7 percent), minority males had fewer office visits overall (0.9 and 0.8 visits per person among black and Hispanic males, respectively, versus 1.6 visits per person for white males).

"CDC recommends repeat testing at least annually for persons at high risk for HIV infection, and although the optimal annual percentage of visits with an HIV test to achieve universal testing is unknown, these results indicate there are opportunities to improve HIV testing rates," writes lead author D. Cal Ham, M.D., in *MWWR*. "A systems-level approach to increase HIV testing rates that does not rely on individual providers could use interventions to routinize HIV testing such as electronic medical records reminders, opt-out testing policies, provider education campaigns, and removal of barriers to HIV testing (i.e., special consent forms)."

In *JAMA*, McNulty similarly calls for identifying new research to inform future testing strategies, including determining the optimal screening interval, different testing methods (standard laboratory testing versus point-of-care testing), different processes for obtaining patient consent (opt in or opt out), and different strategies for test result notification and linkage to care for HIV-infected patients.

"There is little evidence to suggest that one universal screening strategy is superior to another," McNulty writes. "While the cost-effectiveness and sustainability of disparate models is unknown, parallel programs with dedicated point-of-care testers and patient navigators are likely to be more costly than incorporating HIV testing and linkage to care into routine medical care."

Takeaway: New strategies are needed to improve rates of HIV screening, particularly among young men and in physicians' offices.

Molecular Autopsy Becoming Viable to Implement

Molecular autopsies are becoming technically and financially feasible for cases of sudden death (SD) with inconclusive autopsy results, according to a study published July 19 in *Genome Research* by the Harris County (Texas) medical examiner's office. Experts say molecular tools will become the standard of care for medical examiners and coroner's offices investigating SD in infants, children, and young adults.

"A crucially important and unique aspect of this approach was the development of a multidisciplinary and multi-institutional panel of experts with expertise in clinical and basic science cardiology, genetics and pathology to review each case for putatively significant genetic variants."

— D. Nicole R. Methner, Ph.D.

Heritable genetic variants, including cardiac channel-associated gene variants, are thought to be a cause of up to one-third of SD in young people, but thousands of these cases are assigned an undetermined cause of death due to a lack of definitive autopsy findings. Elucidation of genetic variants responsible for SD can help to establish cause of death and to determine whether familial genetic testing should be considered. However, up until now, postmortem screenings have not been technically or financially viable for most county medical examiners and coroners.

"The work presented here highlights a transition between research and specific clinical cases to implementation of the molecular autopsy as a cost-effective standard of care in postmortem examinations," writes D. Nicole R. Methner, Ph.D., from the Harris County Institute of Forensic Science in Houston. "By targeting selected exons, cost was kept below $600 per sample."

The researchers sequenced full exons of 64 genes identified through the literature to be associated with SD (cardiac and non-cardiac causes) in 351 infant and young SD decedents (80.7 percent under one year of age). Mass parallel sequencing relied on a custom gene target exon capture array. The researchers acknowledge that targeted sequencing (versus whole exome sequencing) may miss some potentially deleterious variations, but can yield high coverage while remaining cost-effective.

The researchers found that 77 unique single nucleotide variants were detected in 29 genes. Thirteen individuals (eight infants and five children/young adults) had a reportable genetic variant—likely contributing to the cause of death. This yield (3.7 percent) was substantially lower than previously published reports that found up to one-third of SD cases were due to genetic variations. The authors attribute this difference in diagnostic yield to cohort composition, genetic screening method, or interpretation of genetic testing results. They acknowledge more "restrictive" cohort selection—phenotype- or family history-guided testing —may affect results.

"A crucially important and unique aspect of this approach was the development of a multidisciplinary and multi-institutional panel of experts with expertise in clinical and basic science cardiology, genetics and pathology to review each case for putatively significant genetic variants," the authors write. "This approach can also be adopted by other medical examiner offices as a tool to initiate molecular autopsy programs as NGS becomes more widely available and the field continually evolves."

Takeaway: Molecular autopsies are expected to become routine in certain death investigations. However, the benefits of targeted versus broader sequencing approaches should be evaluated in the development of molecular autopsies by weighing the tradeoff between costs and diagnostic yield.

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Genomic Data Changing Design of Oncology Clinical Trials

Drug discovery is known to be a long, expensive process. On average it can take decades and cost more than $1 billion to bring a drug to market. Yet, despite the large investment in time and money, less than 10 percent of drugs succeed in obtaining U.S. Food and Drug Administration (FDA) approval. Two factors are driving significant changes to the drug discovery and approval process—the sharp drop in the cost of high-throughput genomic sequencing and the growing recognition that molecularly targeting therapies to a smaller subset of patients increases effectiveness.

Randomized controlled trials (RCTs) have been the gold standard for the design of clinical trials needed to obtain drug approval. But in the era of personalized medicine RCTs may not be the best fit. RCTs require enrolling hundreds or often thousands of patient participants. After the fact, post-hoc analysis identifies if there is a population subset that particularly benefited from the therapy. However, personalized medicine focuses on identifying baseline predictive markers that will reduce the trial and error effect in therapy selection. Increasingly, these baseline predictive markers are being incorporated early in the drug discovery process. So, experts say that further along the clinical trial spectrum—in phase II and phase III trials—it does not make sense to test drugs in patients who are known to not have the markers associated with therapeutic benefit. Targeted drug trials will naturally require fewer participants.

"The development in medical research motivated the invention of new clinical trial designs, and the revolutionary trials can help the medical research move forward to better understand the disease mechanism and eventually lead to better treatment to serve the patients."

— Ja-An Lin

There is a lot of interest in innovative trial design for targeted therapies to address the challenges created by the increasing molecular fragmentation of diagnosis, particularly for cancer.

"Due to better understanding of genetic effect in disease or disease caused by different molecular features, contemporary trial designs are used not only in oncology but also in psychiatry and neurology and might be extended to all other medical fields," writes co-author Ja-An Lin, from University of North Carolina at Chapel Hill, in a review of innovative biomarker trial designs in the *British Medical Bulletin* last year. "The development in medical research motivated the invention of new clinical trial designs, and the revolutionary trials can help the medical research move forward to better understand the disease mechanism and eventually lead to better treatment to serve the patients."

New Models for Clinical Trials

Enriched models first screen patients to identify those who have the mutation targeted by the treatment in the trial. The trial sample is "enriched" with those patients expected to have a treatment response because of a specific biomarker. The targeted population provides the advantage of more likely validating the effectiveness of a particular treatment in a smaller, cheaper trial, compared to all-comer trials, but runs the disadvantage of not knowing the effect of treatment in a general population. Some enrichment models with a hybrid design allow those patients who do not have the responsive biomarker to serve as a control arm, receiving the standard of care.

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Unlike enriched models, which are still testing a single drug response against a single therapy, in **adaptive trials** researchers can essentially conduct several trials in parallel. Adaptive models have multiple arms and use Bayesian approaches to incorporate information to identify the cohort which is most benefited by intervention treatment gained early in the study to modify the trial implementation later on.

Last year researchers from Memorial Sloan Kettering Cancer Center (New York) published the first results showing the promise of basket studies for studying cancer drugs. **Basket studies** focus on a specific gene mutation, regardless of cancer type. The histology-independent, biomarker-selected, early phase 2 basket study led by Memorial Sloan Kettering enrolled 122 patients from 23 centers around the world. All patients had BRAF V600 mutations, but across a total of more than six nonmelanoma cancer types. Interestingly, the basket trial design enables mutation-positive people with rare tumors to participate in trials that traditional histology-based studies did not.

"We have no fantasies that blocking one pathway will do the trick in most cases," explains Memorial Sloan Kettering physician-in-chief José Baselga, M.D., Ph.D., the study's senior author, in a statement. "But the repertoire of pathways that these tumors rely on is not endless—it's finite. The second wave of these [basket] trials will be appropriate combinations, and this trial is a pioneer for that as well. It's the way forward."

By contrast, an **umbrella study** enrolls patients with a single tumor type or histology but contains multiple sub-trials, each testing a targeted therapy within a molecularly defined subset. This approach can test multiple therapies and multiple markers simultaneously. Also using Bayesian approaches, umbrella studies can add, modify, or drop subtrials based on data generated from the ongoing trial or evolving cancer research field. Umbrella trials usually are performed nationally at multiple clinical sites using a common genomic screening platform.

Biomarkers Increase Trial Success

The biotech trade association Biotechnology Innovation Organization (BIO; Washington, D.C.) partnered with the contract research organization Amplion (Bend, Oregon) to analyze the effects of biomarkers in clinical trial success. To inform the newly released report, *Clinical Development Success Rates, 2006-2015*, the organizations used data from their respective subscription-based databases, Biomedtracker, which tracks the clinical development and regulatory history of investigational drugs, and BiomarkerBase that tracks biomarker usage in clinical trials, drug labels, and tests (including laboratory-developed, FDA-cleared, and FDA-approved tests).

From 2006-2015, the study identified a total of 9,985 clinical and regulatory phase transitions from 7,455 development programs, across 1,103 companies. The success rate at each of the four phases of development—Phase I, II, III, and regulatory filing—was assessed for 14 disease areas.

Analysis found that the overall likelihood of approval (LOA) across all stages of development was 9.6 percent. Phase II clinical programs continued to have the lowest success rate, with only 30.7 percent of developmental candidates advancing from Phase II to Phase III.

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The use of "selection" biomarkers to establish inclusion or exclusion criteria for enrolling patients into clinical studies has increased "dramatically," the report says, but still remains small. Out of all 9,985-phase transitions, 512, or five percent, of transitions incorporated a selection biomarker for patient stratification. However, programs that utilized selection biomarkers had higher success rates at each phase of development, compared to the overall dataset.

"The large differences in Phase II and III transition success rates are quite convincing, quantitatively, of what many drug developers have long argued anecdotally—enrichment of patient enrollment at the molecular level is a more successful strategy than heterogeneous enrollment," the report says.

Use of a selection biomarker raised the LOA from Phase I to one in four versus less than one in 10 when no selection biomarker was used. The largest percentage difference among the four phases of development by selection biomarker use was seen in Phase III, where transition success rates for selection biomarker programs were 76.5 percent (n=132), compared to 55.0 percent (n=1,254) for programs not using a selection biomarker.

"The higher success rates for trials run with biomarker-selected patients suggests that the broader industry is already on the right path," the report says. "Experiencing one in four failures (with selection biomarkers) versus two in four Phase III failures (without biomarkers) could have significant cost implications."

Launch of New Trial Models

With positive proof-of-principle results from these innovative trials demonstrating early successes, the number of active innovative trials is expected to continue to grow. Below is a sampling of current trials.

This fall, the European trial **AcSé eSMART** will launch in collaboration with the Innovative Therapies for Children with Cancer consortium. eSMART is a phase I/II basket-and-umbrella trial for children with relapsed or refractory disease that will explore the effectiveness of 10 investigational oncology drugs from at least three different pharmaceutical companies—as single agents and in combination—based on the results of pangenomic tumor profiling.

The French National Cancer Institute originally launched AcSé (Secured Access to Innovative Therapies) in 2013 to promote safe access to targeted therapies outside of their approved indications for patients lacking treatment options. Through this program, patients undergo molecular testing and, if appropriate, receive targeted therapy within the oversight of a phase II trial, if there are no other trials for which they are eligible. The 2013 proof-of-concept basket study investigated single-agent crizotinib in adults and children with an advanced-stage, incurable malignancy harboring ALK, MET, or ROS1 alterations. In France, crizotinib is only indicated for adults with ALK-positive nonsmall cell lung cancer. However, research evidence shows that more than 15 different malignancies in adults and children feature molecular alterations responsive to crizotinib treatment.

In the AcSé trial, 107 pediatric patients were tested for structural genomic alterations in ALK, MET, or ROS1 using either biomarker tests or pangenomic tumor profiling. Seventeen patients were identified that harbored positive tumors. Five of these patients met

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the eligibility criteria to enroll in a phase I trial of ceritinib, while 11 patients entered into the AcSé trial. The objective response rate reached 45 percent.

The **MyPathway** study is a basket-and-umbrella trial conducted at 39 U.S. treatment centers. It is designed to evaluate agents targeting the HER2, BRAF, Hedgehog, and EGFR pathways in tumor types outside of the approved indications.

As of December 2015, 129 patients with tumors from 25 different primary sites received targeted treatment based on their tumor's molecular profile. Patients had to have exhausted all standard treatment options and potential clinical trials. Across all tumors, 64 percent had HER2 alterations, 26 percent had BRAF mutations, 6 percent had Hedgehog pathway mutations, and 5 percent EGFR mutations. The overall clinical benefit rate was 34 percent.

Sixteen U.S. cancer centers partnered to form the **Lung Cancer Mutation Consortium** to prospectively examine lung adenocarcinomas for genetic and molecular abnormalities and use that information to match patients to the best possible therapies. Tumor tissue from 875 of 1,020 eligible individuals with confirmed stage IV lung adenocarcinomas was probed for 14 different genetic and molecular abnormalities. More than half (54 percent) had a genetic abnormality. The most common mutations were KRAS (25 percent) and EGFR (12 percent) genes, followed distantly by ALK rearrangements (4 percent), MET amplification (3 percent), and BRAF mutations (2.5 percent). Twenty-eight percent of the total cohort (n=242) had a driver alteration. Of these patients, more than one-half (n=131) received targeted therapy. Patients with a driver mutation who received targeted treatment achieved a median overall survival of 2.7 years—an improvement of 1.2 years over patients with driver mutations who did not receive targeted therapy and 1.0 years over patients without targetable driver mutations.

Takeaway: The increasing ease of access to genomic data is reshaping the drug development and clinical trial processes. Innovative new models will increasingly test the effects of more markers and treatment candidates simultaneously.

Efforts to Improve Clinical Trial Recruitment

As the medical field gains a greater understanding of genomics, diseases are increasingly stratified into genetically defined subtypes, in addition to other inclusion criteria such as age, treatment history, or tumor stage. Finding patients for trials involving each subtype becomes more complex, causing enrollment to possibly drag out. As it already stands, experts say that less than five percent of cancer patients participate in clinical trials. Data companies are partnering with pharmaceutical companies and medical centers to try to improve trial recruitment through the use of advanced data-mining and analytical software.

One example of this is IBM's Watson. In late 2014, IBM initiated its Patient Matching proof of concept pilot with the Mayo Clinic. More recently IBM has announced two additional partnerships aimed at expanding matching cancer patients with clinical trials. In June, Froedtert & the Medical College of Wisconsin Cancer Network (Milwaukee) said it is adopting Watson cognitive computing software to help match a patient's unique health profile with the best-suited cancer clinical trial in an attempt to further personalize approaches to cancer treatment. The matching program is slated to begin this fall.

In the fall of 2015, clinical research organization ICON (Ireland) began applying Watson Clinical Trial Matching to 25 breast, lung, colon and rectal cancer trials. "Recruiting the required number of patients for clinical trials is a constant challenge for our customers and can represent more than 30% of total study costs," said ICON's chief operating officer, Steve Cutler, Ph.D. at the time of the announcement. "By applying IBM Watson to our clinical trials, we have the potential to revolutionize clinical trial feasibility, patient recruitment and study start-up timelines which will help our customers take significant time and cost from their development programs."

Refined PSA Screening Strategies May Catch More Aggressive Cases, *Continued from bottom of p.1*

While data shows metastatic disease began rising in 2008, before the 2012 change in screening recommendations by the U.S. Preventive Services Task Force, the authors say the sharp rise in advanced disease in 55- to 69-year old men is "particularly troubling" because these men may be most affected by changing screening guidelines and may benefit most from screening and early treatment.

"The results indicate that screening guidelines and treatment need to be refined based on individual patient risk factors," said lead author Adam Weiner, M.D., a Northwestern University urology resident. "This may help prevent the growing occurrence of metastatic prostate cancer and potential deaths associated with the disease. This also can help minimize overdiagnosing and overtreating men with low-risk prostate cancer who do not need treatment."

Separate studies are beginning to assess new screening strategies.

Single mid-life measurement

A single midlife prostate-specific antigen (PSA) measurement may be a good predictor of subsequent development of lethal prostate cancer, according to a study published online June 13 in the *Journal of Clinical Oncology*. Since men with PSA below the median at age 60 years are unlikely to develop lethal disease, the authors recommend subsequent risk-stratified screening on the basis of results of a midlife PSA test between the age of 45 and 59 years.

The researchers analyzed findings from men (mean age, 55 years) who gave blood before participation in the Physicians' Health Study, initiated in 1982. Baseline PSA levels were available for 234 patients with prostate cancer and 711 age-matched controls. The 71 participants who developed lethal prostate cancer were rematched to 213 controls. The median follow-up time from blood draw to cancer diagnosis was 9.0 years overall and 8.6 years for lethal cases.

The researchers found that men with PSA above the age-specific median had consistently and significantly increased risk of total prostate cancer across all age groups versus men with PSA at or below the median. The majority of lethal cases occurred in men with PSA above the median at all ages (82 percent lethal cases seen in men above the median at ages 40 to 49 years; 71 percent at 50 to 54 years; and 86 percent at 55 to 59 years).

Germline Testing Should Be Offered With Metastatic Prostate Cancer

Germline genetic testing should be offered to all men with metastatic prostate cancer, regardless of age at diagnosis or familial prostate cancer history, according to a study published July 6 in the *New England Journal of Medicine*. Not only did the authors identify a significant association between advanced prostate cancer and mutations in germline DNA repair genes, but these mutations also may identify families with a predisposition to other cancer types.

The multi-institutional group of researchers sequenced germline DNA exomes from 692 men with documented metastatic prostate cancer. Multiplex sequencing assays assessed mutations in 20 DNA-repair genes associated with autosomal dominant cancer-predisposition syndromes. They found 84 germline DNA-repair gene mutations (presumed to be deleterious) in 11.8 percent of the men (n=82). Mutations were identified in 16 genes including BRCA2, CHEK2, ATM, BRCA1, RAD51D, and PALB2. Frequency of mutations was not affected by a family history of prostate cancer or the patient's age at diagnosis. The authors say the frequency of germline mutations was "substantially" higher than either the known incidence of BRCA2 mutations in localized prostate cancer or the incidence of mutations in 22 tumor-suppressor genes identified in familial prostate cancer.

Unexpectedly, the researchers found that 51 of the 82 patients with DNA-repair gene mutations had a first-degree relative with another cancer type (most commonly breast, ovarian, and pancreatic, all with known associations to DNA repair gene mutations). This rate of familial cancer was significantly higher than in the 270 patients without the germline DNA-repair gene mutations.

"These findings potentially change clinical practice because we now show that testing for these DNA repair genes should be offered to all men with advanced prostate cancer," said co-senior author Kenneth Offit, M.D., from Memorial Sloan Kettering Cancer Center, in a statement. "By thinking beyond the present and looking for opportunities to prevent cancer in the next generation, this work will create a large paradigm shift. The identification of a germline DNA repair gene mutation provides critical information for relatives as well as for the patient, prompting a cascade of counseling to identify cancer predisposition and deploy risk-reduction strategies in family members."

As a next step, the authors say, prospective studies are needed to determine if DNA repair gene mutations are predictive of clinical outcomes.

"We found that risk of developing lethal prostate cancer in the next 30 years among those with baseline PSA levels below the median was less than 2 percent at ages 40 to 59 years. Although risk was small, it remained present, and so screening should be continued, albeit with longer intervals."

— Mark Preston, M.D.

The authors sought to investigate how these findings should inform screening strategies. Given the nine cases of lethal prostate cancer among men with the lowest quartile of baseline PSA across all age groups, the authors say it would be prudent to conduct another PSA test during the lifetime of men age 40 to 49 years, even if the first measure is exceptionally low, because of the lingering small risk of prostate cancer-related death. Additionally, the researchers sought to determine whether it is safe to stop PSA screening at age 60 years. They found that men aged 55 to 59 years with PSA levels below the median (0.96 ng/mL) were at low risk of lethal prostate cancer, with a cumulative incidence of lethal cases in this age group of 0.59 percent over 30 years. Thus, the authors recommend white men with PSA level below 1.0 ng/mL at age 60 years could "reasonably forgo" further PSA screening.

"We found that risk of developing lethal prostate cancer in the next 30 years among those with baseline PSA levels below the median was less than 2 percent at ages 40 to 59 years. Although risk was small, it remained present, and so screening should be continued, albeit with longer intervals," writes lead author Mark Preston, M.D., from Brigham and Women's Hospital in Boston. "It seems that baseline PSA level below the median at age 45 years followed by repeat measurements at 5-year intervals would capture most lethal cases, given that the 15-year cumulative incidence for lethal prostate cancer at age 40 to 44 years is zero and in the 45 to 49 year age group only 0.07 percent."

Takeaway: A single midlife PSA screening for prostate cancer may be adequate to identify men at risk for lethal prostate cancer later in life. The midlife measurement can inform the need for subsequent personalized, risk-based screening.

Sorting Cell-Free DNA by Length May Increase Tumor Detection

There are "subtle but distinct" differences in the length between normal cell-free DNA (cfDNA) and circulating tumor DNA (ctDNA), according to a study published July 18 in *PLoS Genetics*. Exploiting the consistently shorter fragment length of ctDNA may improve the sensitivity of liquid biopsy testing, the authors say.

"This development has the potential to enable earlier detection of solid tumors through a simple blood draw by substantially improving our ability to detect very low quantities of circulating DNA derived from tumor cells," says lead author Hunter Underhill, M.D., Ph.D., in a statement. "It's possible that jump in sensitivity could make the difference between being able to detect a cancer, and not."

While the prospect for noninvasively detecting and monitoring cancer is exciting, the clinical utility of liquid biopsies has been limited by its sensitivity, particularly in detecting ctDNA from nonmetasticized solid tumors.

Detecting ctDNA against the abundant backdrop of normally occurring cfDNA derived from healthy cells has been likened to detecting a needle in a haystack. However, researchers are working on developing novel approaches to improve detection of ctDNA, including assessing differences in fragment length between healthy cfDNA and ctDNA.

"These results provide compelling evidence that development of techniques to isolate a subset of cfDNA consistent with the ctDNA fragment lengths described in our study may substantially improve detection of non-metastatic solid tumors. As such, our findings may have a direct impact on the clinical utility of ctDNA for the non-invasive detection and diagnosis of solid tumors (i.e., the "liquid biopsy"), monitoring tumor recurrence, and evaluating tumor response to therapy."

— Hunter Underhill, M.D., Ph.D.

Underhill and colleagues utilized massively parallel sequencing to define these fragment length differences. First, animal models of glioblastoma and hepatocellular carcinoma showed that the most common fragment lengths of ctDNA were 134 and 144 bp, compared to the 167 bp fragment length most seen in normal cfDNA.

As a next step, the researchers found similar differences in the fragment length of ctDNA in humans with melanoma and lung cancer, compared to healthy controls. The most common fragment length for the BRAF V600E mutant allele in the melanoma patient was shorter than the most common fragment length of the wild-type allele (132–145 bp versus 165 bp, respectively). Similarly, size selecting for shorter cfDNA fragment lengths substantially increased the EGFR T790M mutant allele frequency in human lung cancer. Overall, the authors say that fractional selection of cfDNA that is 20 to 50 bp shorter than the size of normal healthy cfDNA may "substantially enrich" samples for ctDNA in human cancer testing.

"Size-selection for shorter cfDNA fragments increased the proportion of ctDNA within a sample," writes Underhill and colleagues. "These results provide compelling evidence that development of techniques to isolate a subset of cfDNA consistent with the ctDNA fragment lengths described in our study may substantially improve detection of non-metastatic solid tumors. As such, our findings may have a direct impact on the clinical utility of ctDNA for the non-invasive detection and diagnosis of solid tumors (i.e., the "liquid biopsy"), monitoring tumor recurrence, and evaluating tumor response to therapy."

Takeaway: Using ctDNA tumor length to segregate cfDNA may improve the clinical utility of liquid biopsy technology for the detection of non-metastatic, solid tumors.

Embryo Mitochondrial DNA Levels May Aid IVF Selection

Better methods of embryo selection are needed to improve in vitro fertilization (IVF) efficiency. New research shows that high quantities of mitochondrial DNA (mtDNA) may be a meaningful marker for embryo viability beyond current measures, as normal chromosome counts and high morphological grade have not guaranteed successful pregnancy of embryos.

mtDNA is a useful marker of embryonic implantation potential, independent of embryo quality and female age, according to an abstract presented at the European Society of Human Reproduction and Embryology annual meeting (Helsinki, Finland; July 3-6). The authors, from 9.baby Family and Fertility Center in Italy, say that incorporation of mtDNA copy number analysis into the routine preimplantation genetic screening (PGS) may aid selection of the euploid blastocysts with the best chances to implant.

The researchers analyzed data from 54 euploid blastocysts obtained from 26 patients (average age 35.5 years) following 29 PGS cycles. Blastocysts quality was assessed and categorized as good or bad quality based on morphology and expansion. Biopsy was performed on Day 5-6 and comprehensive chromosome screening was conducted using array comparative genomic hybridization. mtDNA content for each of 54 eu-

ploid blastocysts was quantified using real-time polymerase chain reaction (PCR) and blastocysts were categorized based on a relative mtDNA threshold value of 0.003.

The researchers found that 12 of 54 euploid blastocysts showed high mtDNA levels (22.2 percent). There were 11 successful pregnancies with healthy live births out of 43 transferred blastocysts. All pregnancies resulted from blastocysts with low mtDNA levels. No blastocysts with high mtDNA resulted in pregnancy. Overall, there was no correlation between embryo quality and mtDNA content for either the poor quality or the high quality group. Similarly, there was no statistical significant difference between female age and blastocysts' level of mtDNA content.

In a second abstract presented at the European Society of Human Reproduction and Embryology annual meeting researchers were able to prospectively evaluate the predictive power of mtDNA quantification for the first time.

The researchers quantified mtDNA in 280 blastocysts from 143 couples, previously biopsied and found to be chromosomally normal using PGS. The study took place in a blinded manner in which mtDNA quantity was not known at the time of single embryo transfer. Embryos were biopsied at the blastocyst stage and the samples were analyzed using comprehensive chromosome analysis by next generation sequencing. The same biopsy specimens were also tested using quantitative PCR.

The researchers, funded by Reprogenetics (Livingston, N.Y.), found that 15 of the 280 blastocysts (5.4 percent) had unusually high levels of mtDNA. At the time of abstract presentation, outcome data was available for 111 of the blastocysts, transferred after PGS results were known, but before mtDNA levels were known. All transfers involved a single chromosomally normal blastocyst of good morphology. Of these, 78 (70 percent) led to ongoing pregnancies, and all (100 percent) had mtDNA levels that were normal/low. Just under one-third of blastocysts (n=33) failed to implant. Among these, seven (21 percent) had unusually high levels of mtDNA. Using mtDNA data would have led to an ongoing pregnancy rate for morphologically good, euploid blastocysts, with normal/low levels of mtDNA of 76 percent, while the ongoing pregnancy rate for morphologically good, euploid blastocysts with elevated mtDNA levels was zero.

Takeaway: mtDNA levels appear to be predictive of embryo potential. Chromosomally normal embryos with good morphology, but high mtDNA fail to result in pregnancy.

G2 INSIDER

Diabetes Screening in ER Improves Detection in High Prevalence Areas

Routine glycated hemoglobin (HbA1c) testing in urban emergency departments identifies a large number of people with undiagnosed diabetes and prediabetes, according to a study published June 3 in *BMJ Open Diabetes Research & Care*. The authors say that emergency department screening is both feasible and effective in finding previously undiagnosed cases of diabetes and prediabetes in high prevalence areas.

Diabetes has traditionally been diagnosed by fasting blood glucose tests or the oral glucose tolerance test. However, recent adoption of HbA1c simplifies the diagnostic process, by eliminating pre-test preparation, and enables wider screening. It may be key to targeting screening in high-risk populations where diabetes-related complications resulting from underdetection remain a problem.

"Rapidly identifying admitted patients with poor glycemic control utilizing a test on a single blood sample that does not require any pretest preparation provides an ideal opportunity for intervention by hospital diabetes services," write the authors led by Tien-Ming Hng, M.B.B.S., Ph.D., from Blacktown Hospital in Australia. "The visit to [the] emergency department is an opportunity for us to detect diabetes in individuals who infrequently seek routine medical care, and who may otherwise go undetected."

Australian researchers conducted random glucose testing in patients requiring blood sampling in an emergency department serving an ethnically diverse, low socioeconomic, urban population, regardless of the presenting problem. HbA1c was automatically measured if the random glucose levels were above 5.5 mmol/L. Consistent with the American Diabetes Association definition HbA1c levels of 6.5 percent or higher were diagnosed for diabetes and levels of 5.7 to 6.4 percent were diagnosed as prediabetes. Hospital records were used to identify patients with previously diagnosed diabetes.

Among 4,580 emergency department cases, 2,652 had blood sampled. HbA1c was in 1,267 of these samples. More than one-third (38.4 percent) had diabetes and an additional 27.4 percent had prediabetes. Among those with diabetes, just under one-third (32.2 percent) were previously undiagnosed and unaware of their condition. Cases of diabetes were not confined to the mild cases. A severely elevated HbA1c level (over 9 percent) was seen in 7.3 percent of the entire cohort, and in 10.2 percent of newly diagnosed patients.

"Almost a third of the individuals diagnosed as having diabetes were not aware of their diagnosis, reflecting the hidden burden of diabetes in our community," the authors write. "This may indicate that current screening practices in primary care are insufficient and further supports opportunistic HbA1c testing in individuals presenting to hospital."

As a result of this pilot study, Blacktown and Mount Druitt became the first in Australia to implement routine diabetes screening in emergency department patients requiring a blood test.

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