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ACMG Studies Provide Lessons in Genetic Test Ordering Management

As use of clinical molecular testing skyrockets, many believe a closer examination of ordering patterns is warranted. Molecular tests have the potential to offer key diagnostic information. However, if not properly ordered these tests can be expensive and still not provide the needed answers to key clinical questions.

A few early studies have shown that misorders are common and can result from clerical errors, misunderstanding of genetics, or inappropriate testing sequences. These misorders can both be costly to the health care system and delay appropriate diagnosis and treatment. As a result of increasing health care costs associated with molecular testing, many institutions are undertaking a review of test orders in formal genetic test utilization management (GTUM) programs.

Three studies presented recently at the American College of Medical Genetics and Genomics (ACMG) Annual Clinical Genetics Meeting (Tampa, Florida; March 8-12) demonstrate the savings associated with improving molecular test ordering practices. The early results of existing GTUM programs can provide guidance to laboratories and medical systems on the benefits of reviewing genetic test orders.

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PSA Screening Cost Effective With 'Smarter' Approach

A combination of conservative screening and selective treatment strategies can make screening for prostate cancer cost effective, according to an economic analysis published March 24 in *JAMA Oncology*. According to testing of various models, less frequent screening and more restrictive biopsy criteria, when accompanied by active surveillance of low-risk cancer, hold the most economic promise.

The incidence of early-stage prostate cancer and rates of prostate-specific antigen (PSA) screening have both declined following the 2012 U.S. Preventive Services Task Force (USPSTF) recommendation against PSA screening as routine primary care for men. While there have been calls for more personalized approaches for prostate cancer screening and management, there is no consensus strategy of how to implement screening that preserves the benefits of detection, while cutting the harm associated with overdiagnosis and overtreatment.

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ACMG Studies Provide Lessons in Genetic Test Ordering Management, *Continued from top of p.1*

Substantial Savings Realized Through Test Review

GTUM led to improved financial management of the testing process, including limiting of patient financial liability and increasing institutional reimbursement, according to an oral presentation at ACMG by Julie Kaylor, a certified genetic counselor at Arkansas Children's Hospital. Additionally, the review of testing orders led to further refinement of the GTUM program by addressing additional sources of ordering errors.

Arkansas Children's Hospital's GTUM program was implemented to assess the potential fiscal and clinical benefits of improved appropriateness of genetic test orders. The program relies upon a laboratory genetic counselor to review genetic tests sent to reference laboratories. In the study presented at ACMG, test order appropriateness was determined through a review of the medical records, literature searches, and cost analysis. The GTUM program required follow up of inappropriate test orders with the ordering physician to discuss potential test modifications.

The initial phase of the GTUM program (from July 2012 through December 2014) reviewed 4,079 tests with 5 percent (n=217) requiring modification. This yielded $353,676 in cost avoidance through genetic test modification with an average cost avoidance of $1,679 per each modification.

With the addition of a revised protocol that included confirmation of insurance prior authorization, the utilization management program yielded $102,335 in savings from January to September 2015. The revised protocol led to a review of 506 tests, with 12 percent requiring modification (n=61) with an average cost avoidance of $1,677 per modification.

Common Genetic Test Misorders

Continuous improvement to the GTUM review process is possible through evaluation of the types of common misorders, according to an oral presentation by David Stevenson, M.D., from Stanford University. While many common ordering mistakes can be easily rectified, orders made for tests without adequate evidence of clinical utility remain a large problem for laboratories.

The GTUM service at Stanford University Medical Center consists of a genetic counselor, a molecular pathologist and a medical geneticist. Beginning in December 2014, all high-complexity, send-out molecular tests were systematically reviewed. Discussions with the ordering clinicians occur, as needed. Five types of genetic test misorders were identified: those with questionable clinical utility (controversial orders), clerical errors, redundant testing, tests with better alternatives available, and uncategorized or miscellaneous misorders. The percent of modifications or cancellations per category were calculated to estimate the impact of the intervention.

Over a 10-month period, 544 genetic test orders were reviewed. These orders primarily included clinical exomes and large gene sequencing panels. Overall, the GTUM intervention reduced misorders by half (from 14 percent or 75 misorders to 6.4 percent or

Case Studies from Arkansas Children's Hospital's GTUM Program

Kaylor provided specific case studies demonstrating the direct clinical impact of the order review process, which considered modifications in testing strategies, laboratory choices, and clinical benefit to the patient.

- An order for PIK3CA sequencing was placed for a patient with a somatic overgrowth syndrome. The order was placed for a reference laboratory that does not routinely perform this test on tissue. The genetic counselor confirmed that with this specific test the lower limit of mosaicism detectable would be 20 percent. The genetic counselor suggested an alternative laboratory and panel test capable of detecting much lower limit of mosaicism. Ultimately, the patient was diagnosed with a PIK3CA mutation at a mosaic level of 5 percent.
- Von-Willebrand Type 2B and Platelet type genetic testing was ordered for a hematology patient at the same time that Type 2 Binding assay was ordered. The genetic counselor suggested the binding assay would be the appropriate first step and that genetic tests could be held pending the binding assay results. The ordering physician agreed and ultimately, the genetic tests were cancelled once the normal binding assay results were received, saving $2,915 in unnecessary testing.

35 misorders). Analysis by misorder type showed the GTUM intervention:

- Completely eliminated clerical errors and redundant testing (2.4 percent and 1.1 percent of the total test orders, respectively, to zero for both misorders).
- Cut tests with better alternatives available from 4.2 percent to 0.9 percent.
- Only slightly impacted controversial misorders (5.7 percent to 5.3 percent).

"Due to the lack of widely accepted and precisely defined guidelines for the utilization of many high-complexity molecular tests (clinical exomes and some large gene panels), a significant portion of our misorders (41 percent) fell under the controversial category, a group that was negligibly affected by the UM intervention," Stevenson explained. "Identification of the types of misorders and classification of tests reviewed accordingly allows for a better evaluation of the impact of a UM intervention. Strategies other than UM may be necessary to reduce misorders of the controversial type."

"Many of these recommended changes not only benefited the institution by decreasing costs but also benefited the patient as they were tested with an expanded panel increasing the chance of identifying the genetic etiology of the patient's condition."

—Laura Fairbrother

Cyto Test Review Yields Smaller Savings Than Molecular Send Outs

Over a one-month period, a team consisting of a pediatric genetic counselor and a pediatric pathologist at Vanderbilt University Medical Center, reviewed molecular tests that were sent to outside vendor labs and cyto tests (karyotypes and microarrays) conducted through our institutional lab. The review assessed whether the test ordered answered the intended clinical question and whether the most appropriate vendor lab was chosen based on methodology, genes included in panels, and price. If multiple tests were ordered together, the possibility of reflex testing was evaluated.

Over the study period, the team reviewed molecular tests for 90 patients (two-thirds were pediatric). The majority (93 percent) was deemed appropriate based on the clinical indication. Changes were recommended to 35.5 percent of tests and cancelling 3.3 percent of tests was recommended. The potential institutional cost savings associated with the recommendations ranged from $31,179 to $45,219. The molecular test review took 23 hours. Over the same study period, cyto tests were reviewed for 68 patients, of which 12 were inpatients at the time of the test order. Again, the majority (95 percent) of tests ordered answered the intended clinical question. The review team recommended changing nine test orders and cancelling four tests with a potential cost savings of $2,248. A total of four hours were spent reviewing the cyto tests.

The recommended test order changes focused on ordering reflex testing versus concurrent testing and identifying comparable tests available at a lower cost through use of an online database.

"Many of these recommended changes not only benefited the institution by decreasing costs but also benefited the patient as they were tested with an expanded panel increasing the chance of identifying the genetic etiology of the patient's condition," writes Laura Fairbrother, from Vanderbilt, in an ACMG poster presentation. "We conclude that review of all genetic tests could be worthwhile based on a cost savings and quality optimization."

Takeaway: Existing GTUM programs show the cost savings and improvements to patient care associated with reviewing the appropriateness of genetic test orders. Evaluation of GTUM programs can also inform further improvements to the test order review process. G2

Inside The Diagnostics Industry

VolitionRx Uses Nucleosome Technology for Early Cancer Diagnosis

Cameron Reynolds, CEO, VolitionRx

Jake Micallef, Ph.D., CSO, VolitionRx

VolitionRx (Belgium) is tackling cancer diagnostics 160 base pairs (BP) at a time. The company's nucleosome-based detection technology is able to detect, measure, and analyze cancer-causing mutations using a drop of blood on widely available enzyme-linked immunosorbent assay (ELISA) platforms. The company will commercially roll out the first of its NuQ tests—for colorectal cancer (CRC) detection—in Europe later this year, while it completes further validation on NuQ tests for prostate, lung, and pancreatic cancer.

The company believes its low-cost, noninvasive test is a solution for the compliance problem associated with other CRC screening methods and could establish itself as an accurate screening test in other cancer areas. *DTET* recently spoke to VolitionRx's CEO Cameron Reynolds and Chief Scientific Officer Jake Micallef, Ph.D., about the company's technology and upcoming commercialization plans.

"We are not talking about a marginal cost difference. We are talking about a completely different ball game."

—Jake Micallef, Ph.D., CSO, VolitionRx

Why is your technology based on nucleosomes?

Micallef: Actually, nucleosomes are the biomarker that everybody is using right now, although they just are not calling it that. Most of the companies involved in new cancer detection technologies in blood look at DNA sequences. As the name suggests, circulating tumor DNA (ctDNA) technologies analyze tumor DNA that is circulating in the blood. If you can find cancer mutations in that ctDNA that means the patient has a tumor somewhere.

If you look at published papers, you will find that ctDNA is very short fragments—less than 200 BP—and with more accurate detection, papers specify 160 BP, exactly the amount of DNA bound to a nucleosome. DNA does not circulate in the blood in the hypothetical double helix. It doesn't exist that way outside of a molecular biologist's test tube. In cells, DNA is always protein bound as a nucleosome.

So, what we're looking at is the same thing everybody is looking at, but the main technical difference is that other companies have a DNA extraction step. They take the DNA off the nucleosome and then it is just the double helix and short pieces of DNA. We are looking at the same target, but instead of looking for just the DNA bit of the target, we are looking at the whole nucleosome. It has been known for 20 years that the whole nucleosome structure is changed in the presence of cancer cells—the epigenetic changes. We look at whole structural changes, as opposed to just the sequence changes.

What are the advantages of using nucleosomes instead of ctDNA?

Micallef: The advantages of doing this are a few-fold. In the GRAIL (Illumina) announcement, it said their aim was to try to reduce the cost of a ctDNA test down to about $1,000. Our test costs dramatically less than this. We are not talking about a marginal cost difference. We are talking about a completely different ball game. That is because we use ELISA, which has been established for 30 years. It is a robust and mature technology.

Inside The Diagnostics Industry

The other advantage is that, in general, in order to find one mutation in a sequence 160 BP long, if you could target the whole 160 BP sequence from the 3 billion BP in the human genome, you are talking about one part in tens of millions. You need quite a lot of blood for that—most need five mL to 10 mL of blood. We use 10 µL, which is equivalent to about a drop of blood. In addition, our test can be run during an annual blood draw, for example, with a cholesterol test.

How do you select the clinical applications for the nucleosome technology?

Reynolds: Basically there are hundreds and hundreds of changes in physical structure on the nucleosome, which are under our proprietary intellectual property portfolio. Each cancer has some of these changes that are similar to other cancers and some that are different from each other. We could have gone with any cancer early on because they all seemed to work very well. We went with CRC first purely opportunistically because it is currently screened for regularly. We could work with very large organizations to collect blood before colonoscopy. Given that colonoscopy is an accurate diagnostic, we had a very good gold standard to measure against. And because there are populations that are screened through the colonoscopy or fecal tests, we could run very large, low-cost trials, where the case has already been made for screening.

In CRC, we have trials that have gone head-to-head with both colonoscopy and fecal tests. As a small company, doing a large trial in lung or pancreatic is a lot more difficult because there are not screening programs in the general population for those cancers.

We are looking at other conditions now. We start with the biggest cancers. Our earliest targets are colorectal, lung, prostate, and pancreatic. We will look at breast and then move into less common cancers.

"We think in terms of Android rather than Apple. We want to get our license used by lots of different people on different platforms."

—Cameron Reynolds, CEO, VolitionRx

Can you tell us about the company's commercialization strategy?

Reynolds: We aim to have a product to sell this year, which is a big milestone, but it will take time. We will have a large amount of data coming out this year and next year too. Our CE mark allows us to sell clinically in Europe, but the size of study to get a CE mark isn't very large. We are doing large studies in Denmark, in symptomatic and screening populations of about 19,000 patients, to convince key opinion leaders and the government that the test is worthy of buying and adopting. We have data from the first 4,800 patients and we are processing the last 14,000 in a symptomatic population study. It is going to take some time to start marketing directly to medical professionals and key opinion leaders and governments in Europe. It will take a few years to get full access, but I think we have a very compelling case. In Europe there are not many colonoscopies given for screening purposes, but fecal tests have a lot of issues with compliance.

In the United States, we are going to apply for a 510k in a symptomatic population, while we are getting a large screening study done in CRC. We will also apply for 510K as quickly as we can in lung and pancreatic cancers. Although these are small-

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> —Cameron Reynolds, CEO, VolitionRx

er markets, we believe we can help the high-risk and symptomatic populations in the short term. Our aim is to start the process with the U.S. Food and Drug Administration (FDA) this year, get a CRC screening trial done next year, and get 510k for lung and pancreatic in 2018. We believe we have something unique. While we are keen to do the large screening PMA trial for FDA approval, it can take several years. We can help other patients in the meantime by going through the 510k process to make the tests available.

We never intended to establish our own CLIA lab, but we have been in discussions to license our technology for laboratory developed test development in parallel course with the FDA. Our main aim is to go the FDA route. We have very solid intellectual property. We have a very simple test. Being on a simple platform we can do lots of different things at the same time. Our aim is to get it out and as broadly as quickly as possible, in as many ways as possible, to help as many people as we can. We think in terms of Android rather than Apple. We want to get our license used by lots of different people on different platforms.

How will cancer screening and diagnostics evolve in the next few years?

Micallef: Currently, most cancer diagnostics are based on scanning—mammography and low-dose CT imaging. The actual diagnosis of cancer will always be based on identifying the lump or lesion and sampling it with histochemical methods. The diagnosis will be the same, but the detection of cancer, within five years, will use far more blood tests. The detection of cancer through blood tests will become the norm. The scanning methods will never become obsolete. If you have a blood test that says lung tumor, the clinician still needs a scan to see how big, where it is, and how to cut it out. If they can't find it on the scanner they will monitor with further blood tests. Scanning methods will be used as part of diagnosis, not detection.

Reynolds: People realize, short of curing cancer, the next best thing is to diagnose early and have it removed. Currently there are real problems with pretty much every diagnostic out there because of compliance or invasiveness. The blood test will become a very important part of the mix. Very few years down the road we plan to have our tests as a primary or adjunct test for three or four major cancers. They will help make the cancer diagnosis early and with highly compliant tests. People forget the accuracy of tests for people who never take the test equals exactly zero. So a low-cost, highly compliant blood test will really help revolutionize how early and how often cancer is found.

VolitionRx By-the-Numbers

- 4 automated machines enable processing of 30,000 assays per month
- 10 ongoing clinical trials
- 11 member core team of scientists
- 27 NuQ blood biomarker assays
- 90% accuracy for colorectal, lung and pancreatic cancers
- 2015 listing in the U.S. NYSE

PSA Screening Cost Effective With 'Smarter' Approach, *Continued from bottom of p.1*

"This study provides, to our knowledge, the first quantitative framework to evaluate the comparative effectiveness of PSA-based screening strategies and selective treatment approaches, and it addresses an urgent need for direction concerning the future of PSA screening in the United States," write the authors led by Joshua Roth, Ph.D., from University of Washington, Seattle. "Our work indicates that strategies with a conservative screening frequency (e.g., quadrennial) and/or a higher PSA biopsy threshold (e.g., 4.0 ng/mL) are potentially cost-effective when combined with the increased use of conservative management for low-risk cases."

The Fred Hutchinson Cancer Center researchers recently undertook modeling of a variety of plausible screening strategies to assess their value. They used a microsimulation model of prostate cancer that links disease progression with individual PSA growth. For each strategy, the model simulated a cohort of men beginning at age 40 and projected prostate cancer outcomes over a lifetime. Each scenario was compared to no screening. The 18 screening strategies varied by start and stop age, screening interval (time or PSA-dependent), and PSA threshold for biopsy referral. The strategies also varied by treatment practice—either contemporary (receipt of curative treatment by all) or selective treatment practices (active surveillance or curative treatment).

Modeling results showed that all screening strategies were associated with increased life-years (LYs; range, 0.03 to 0.06) and costs ($263 to $1,371) versus no screening, yielding a cost per LY of $7,335 to $21,649. With contemporary treatment, the only possibly cost-effective strategy involved screening men between the ages of 55 and 69 years every 4 years and referring them for a biopsy when their PSA levels were higher than 10.0 ng/mL. This yielded a cost per QALY of $92,446.

With selective treatment strategies, in which a Gleason score less than 7 and clinical T2a stage cancer or lower are treated only after clinical progression during active surveillance, all strategies were associated with increased QALYs (0.002 to 0.004) and several strategies were potentially cost-effective in terms of cost per QALY (incremental cost-effectiveness ratio, $70,831 to $136,332). Among the selective treatment practices (PSA less than 10 ng/mL) all strategies increased costs (range, $263-$703); however, in contrast to many strategies under contemporary treatments, all strategies under selective treatment practices increased QALYs (range, 0.002-0.004). All of these screening strategies have screening intervals of 2 to 4 years with PSA biopsy thresholds of 4.0, 3.0, and 3.0 ng/mL, yielding an incremental cost-effectiveness ratio of $89,333, $120,952, and $70,831 per QALY gained, respectively.

"Our findings have clear implications for the future of PSA screening in the United States," write Roth and colleagues. "Rather than stopping PSA screening, as recom-

Little Genomic Diversity Seen in Metastasized Prostate Cancer Within Individual Men

Among men with metastasized prostate cancer there is substantial heterogeneity of genomic alterations between men, but limited diversity among metastases within an individual, according to a study published Feb. 29 in *Nature Medicine*. The researchers say evaluating a single metastasis site provides a "reasonable" assessment of the major oncogenic driver alterations that are present in disseminated tumors within an individual.

The researchers analyzed multiple tumors from men with metastasized prostate cancer using whole-exome sequencing, array comparative genomic hybridization and RNA transcript profiling. The number of somatic mutations, the burden of genomic copy number alterations, and aberrations in known oncogenic drivers were all highly concordant in individuals. Metrics of androgen receptor activity and cell cycle activity were also similar within individuals.

"Although the analysis of a single metastatic tumor site clearly does underestimate the total burden of molecular aberrations found in the totality of all metastases, most drivers and actionable features are either represented as common roots across all tumors or result from convergent evolution conferred by therapeutic pressure, and most molecular differences between metastases do not seem to influence tumor phenotypes," write the authors led by Akashi Kumar, M.D., Ph.D., candidate, from University of Washington, Seattle. "Although there are exceptions, these findings suggest that clinical decision-making on the basis of a biopsy from a single metastatic site is reasonable."

mended by the USPSTF, implementation of strategies that extend the screening interval and/or use higher PSA biopsy thresholds have the potential to preserve substantial benefit while controlling harm and costs."

Takeaway: Concerns over the harms associated from PSA screening are not due to the screening test itself, but rather the action taken from test results. Modeling indicates that modifying screening strategies in terms of age of men screened, interval of screening, and PSA thresholds for biopsy referral may be able to improve identification of higher-risk cases of prostate cancer in a more cost-effective manner. G2

Disease-Causing Variants Often Occur Outside of 'High-Confidence' Sequence Areas

A significant proportion of known genetic disease-causing variants lie outside of regions able to be sequenced with high confidence, according to a study published March 2 in *Genome Medicine*. Nearly 20 percent of many medically important genes may be sequenced inaccurately with current technology. Genomic region, variant type, read depth, and analytical pipeline all affect accuracy of variant calls, the authors say, which highlight the need to improve technical benchmarks in clinical genomics.

> *"The knowledge that nearly one fifth of each gene, for which laboratory directors are recommended to provide clinical reporting for every patient undergoing clinical exome or genome sequencing, would not reach consensus across different chemistries and pipelines, is sobering."*
>
> —Rachel Goldfeder

"We hope by highlighting and scrutinizing the challenging areas of the genome, we can optimize our pipelines for greater consensus and, at the very least, provide transparency regarding our confidence level in every call," write the authors led by Rachel Goldfeder, a Ph.D., candidate at Stanford University. "The good news is that, in this case, 77 percent of the donor's genome was reliably sequenced using current methods. The challenge now is to focus our efforts on the other 23 percent—namely, on regions of the genome that remain elusive. Only then can we realize the full potential of precision medicine."

The researchers used the U.S. National Institute of Standards and Technology reference genome, which had been previously sequenced with five different sequencing technologies. These five technologies were previously combined to identify genomic areas of agreement, but a reliable consensus was achieved for just 77 percent of the genome. In the present study, the researchers assessed how these "high confidence" areas of the donor's genome overlap with 3,300 known disease-causing genes in the ClinVar and OMIM (Online Mendelian Inheritance in Man). Additionally, the researchers mapped the high confidence areas to gene regions of high medical relevance, as designated by American College of Medical Genetics and Genomics (ACMG) list of 56 medically actionable genes.

Overall, only 74.6 percent of the exonic bases in ClinVar and OMIM genes and 82.1 percent of the exonic bases in ACMG-reportable genes are found in high-confidence regions. Of the 3,300 ClinVar/OMIM genes, 593 have less than 50 percent of their total exonic base pairs in high-confidence regions. Similarly, only 990 genes in the genome are found entirely within high-confidence regions.

"The knowledge that nearly one fifth of each gene, for which laboratory directors are recommended to provide clinical reporting for every patient undergoing clinical exome or genome sequencing, would not reach consensus across different chem-

istries and pipelines, is sobering," write the authors. "But it is a call to arms for those interested in clinical grade technical accuracy for genome sequencing. … In contrast with the lack of immediate personal implication of a false call in a discovery cohort study, a false call on a clinical report could have immediate detrimental consequences in the life of an individual, family, or disease community."

The researchers identified 39,301 loci where the benchmark data contain a high-confidence homozygous reference call, but at least one sequencing technology incorrectly called a variant. For whole-exome sequencing (WES), poor read depth primarily drove sensitivity, with 95 percent of false negative variants (FNV) falling within regions having a read coverage of less than 10. Whereas for whole-genome sequencing (WGS), most FNVs resulted from filtering during variant calling due to their presence within difficult-to-sequence or difficult-to-call regions.

The study found that the majority of disease-causing mutations identified to date fall within easy-to-sequence areas, generally defined as stretches of unique DNA or less repetitive regions. More than 90 percent of 35 bp sequences in high-confidence regions are unique to one location in the genome compared to 47.5 percent of 35 bp sequences in low-confidence regions.

"The challenges of repetitive, paralogous sequence and structural variation complicate the analysis of clinical WGS and WES data," explain the authors. "Not only is short-read sequencing prone to false negative or false positive variant calls due to systematic sequencing errors, but the repetitive nature of the genome introduces global mapping and local alignment challenges."

Takeaway: For whole-exome and -genome sequencing to be clinically meaningful, improvement is needed in the technical benchmarks around sequencing. This includes development of better means to characterize more challenging parts of the genome, where a substantial portion of disease-causing variants lie. G2

Methylation Patterns Are New Target for ctDNA Analysis

Tests that measure circulating DNA are not entirely new. Yet, until now such tests have been unable to determine the tissue of origin (other than in cases of noninvasive prenatal testing, in which DNA can be determined to be from fetal or maternal origin).

But, two recent studies highlight the potential for assessment of methylation patterns. One study found a common methylation signature across cancers and another study found that methylation patterns can trace circulating DNA back to the tissue of origin. In both cases, these discoveries may make noninvasive diagnostics possible for detection of a wide number of conditions.

Methylation Patterns May Lead to Pan-Cancer Test

Hypermethylation of the ZNF154 CpG island is common across tumors and may have utility as a generalizable marker for circulating tumor DNA (ctDNA), according to a study published in the March issue of the *Journal of Molecular Diagnostics*.

DNA methylation has been known to control gene expression. Previously, researchers at the National Human Genome Research Institute (NHGRI) identified pan-cancer hypermethylation at the ZNF154 CpG island in 15 solid epithelial tumor types from 13 different organs. In the present study they measured the mag-

nitude and pattern of differential methylation of this region across colon, lung, breast, stomach, and endometrial tumor samples using next-generation bisulfite amplicon sequencing.

The authors previously validated the marker on an Illumina methylation array. But, the authors say bisulfite amplicon sequencing holds the advantage of being time efficient and cost-effective due to the ability to conduct multi-sample sequencing in parallel. Additionally, the approach offers greater resolution of a target region than array methods, showing patterns of methylation.

The researchers confirmed that the ZNF154 amplicon region was significantly hypermethylated in all of these tumor types (n=184 samples), compared to normal samples (n=34). The marker performed best for endometrial and colon tumors. The hypermethylation occurred regardless of subtype, stage of differentiation, age, or sex. In a computational simulation to predict a threshold of ctDNA detection, limited amounts of ctDNA (1 percent tumor to 99 percent normal) yielded areas under the curve of up to 0.79 for detection of cancer-related methylation markers, and led the authors to conclude that the ZNF154 amplicon can distinguish ctDNA in a blood-based test.

"We have laid the groundwork for developing a diagnostic test, which offers the hope of catching cancer earlier and dramatically improving the survival rate of people with many types of cancer," senior author Laura Elnitski, Ph.D., from NHGRI, said in a statement.

"In the long run, we envision a new type of blood test aimed at the sensitive detection of tissue damage, even without a-priori suspicion of disease in a specific organ."

—Benjamin Glaser

Methylation May Identify Multiple Diseases

Tissue-specific methylation patterns in circulating DNA that is released by dying cells can implicate the tissue source involved in cell death, according to a proof-of-concept study published March 14 in *Proceedings of the National Academy of Sciences*. A blood test, based on methylation, can detect multiple pathologies, including diabetes, cancer, traumatic injury and neurodegeneration.

"In the long run, we envision a new type of blood test aimed at the sensitive detection of tissue damage, even without a-priori suspicion of disease in a specific organ," said co-lead author Benjamin Glaser, from Hadassah Medical Center in Israel. "We believe that such a tool will have broad utility in diagnostic medicine and in the study of human biology."

Since the DNA sequence is identical between all normal cells in a body, it has not been possible to determine the tissue of origin of circulating DNA. But DNA contains methylation modifications that are unique to each cell type and are stable in both healthy and disease conditions.

The researchers identified tissue-specific DNA methylation markers and developed a method for sensitive detection of these markers in plasma or serum. They isolated cfDNA from plasma or serum of donors, treated the cfDNA with bisulfite, PCR amplified the cfDNA, and sequenced it to quantify cfDNA carrying the methylation markers of the cell type of interest. They then demonstrated the utility of the method for identification of pancreatic β-cell death in type 1 diabetes, oligodendrocyte death in relapsing multiple sclerosis, neuronal/glial cell death in patients after traumatic or ischemic brain damage, and exocrine pancreas cell death in pancreatic cancer or pancreatitis.

"Methylation patterns are unique to each cell type, are conserved among cells of the same type in the same individual and among individuals, and are highly stable under physiologic or pathologic conditions," the authors write. "Therefore, it is possible to use the DNA methylation pattern of cfDNA to determine its tissue of origin and hence to infer cell death in the source organ."

Takeaway: While it has long been understood that dying cells release DNA fragments into the blood and that methylation patterns are tissue-specific, emerging evidence shows that it is possible to use these methylation patterns to screen for and diagnose a variety of diseases. G2

Disposable, Electrochemical Sensor IDs Wound Infections in Seconds

An inexpensive, disposable electrochemical sensor may be able to detect Pseudomonas aeruginosa (P. aeruginosa) infections in wounds in real-time at the point of care, according to a pilot study published in *Wound Repair and Regeneration*. The new method cuts time to infection detection and characterization from 24 hours to a mere minute, allowing improved antibiotic selection that holds benefits both for patient care and the health system.

"For the field of wound care, there is a compelling need to develop rapid alternatives for bacterial identification in the clinical setting, where it generally takes over 24 hours to receive a positive identification," writes senior author Victoria Shanmugam, M.D., from George Washington University in Washington, D.C. The authors say that even new molecular identification methods require an initial incubation period before analysis is possible. To date, the standard for clinical identification of bacterial infections requires overnight plated cultures.

The new method identifies a metabolite molecule (pyocyanin) produced by the Pseudomonas bacteria, which commonly infects chronic wounds. To test the sensor, researchers used wound fluid (7.5 µL) from 12 patients with chronic wounds enrolled in the WE-HEAL Study. The electrochemical results were compared against 16S rRNA profiling using 454 pyrosequencing.

The researchers found that the sensor technology correctly identified nine sample matches, but had two false negatives and 3 false positives. Overall, the detection method had a sensitivity of 71 percent and specificity of 57 percent for detection of Pseudomonas. The authors say this demonstrates potential for the technology as a rapid, point-of-care diagnostic with "ongoing enhancement." Since the electrochemical detection strategy eliminates sample preparation, results are available in less than a minute.

"Despite the polymicrobial nature of human wound specimens, there do not appear to be other redox-active molecules that would impede the probe performance in a clinical setting," writes Shanmugam and colleagues. "Although still unclear if it is necessary, the detection limit of electrochemical sensors can be improved by switching to micro and nanofabricated electrodes, albeit with increased sensor cost. Nevertheless, future technological advances will lead to reduced costs and more sensitive electrochemical sensors, making this approach a practical option."

Takeaway: With further refinement, a rapid, point-of-care diagnostic that could instantly identify Pseudomonas, a common cause of wound infection, would enable better patient care and stewardship of antibiotics. G2

G2 INSIDER

Adherence Poor for Lynch Syndrome Testing

There is poor adherence to recommended genetic testing and colonoscopy screening among patients at high-risk for Lynch syndrome, according to a study published online Feb. 9 in the *American Journal of Gastroenterology*. Furthermore, only a small percentage of their endoscopists provide these high-risk patients with appropriate screening recommendations, highlighting the need for improved educational interventions targeted to both providers and patients, the authors say.

While Lynch syndrome only accounts for two to four percent of all colorectal cancers (CRCs), those with the autosomal dominant mutation have up to an 80 percent lifetime risk of developing CRC, experts say. Professional guidelines recommend offering genetic counseling to all high-risk individuals and have found that aggressive screening reduces CRC incidence and mortality. But, the researchers say that individuals at risk for Lynch syndrome are "grossly under-recognized, resulting in missed opportunities to capture high-risk patients and their family members for appropriate genetic counseling and colonoscopic screening."

At-risk participants in the Family Health Promotion Project (n=165) were surveyed at baseline and 24 months later to assess their knowledge of risk-appropriate guidelines for genetic counseling and colonoscopy screening. Participants were recruited from both high-risk cancer clinics and population-based registries of unaffected first-degree relatives of CRC patients. Follow-up recommendations made by their endoscopists were also assessed.

The survey revealed that the majority (98 percent) agreed that genetics and family history are important predictors of CRC. Yet, under two-thirds (63 percent) had heard of genetic testing for CRC, only 31 percent reported being advised to undergo genetic counseling by their doctor, and only 7 percent had undergone genetic testing. Just over one-quarter of participants (26 percent) reported that they thought they should have a colonoscopy every one to two years and 30 percent of endoscopists for these participants recommended screening at this interval—a 65 percent concordance between endoscopist recommendations and participant reports regarding screening intervals.

The researchers found that participants who were recruited because their family member was being seen in a high-risk cancer clinic were 20 percent more likely to report that they had heard of genetic testing and had discussed genetic testing with their provider. However, this knowledge did not translate into higher rates of referral for or completion of genetic testing. Similarly, those recruited from high-risk clinics were more likely to have better knowledge of appropriate screening intervals 24 months after enrollment and were more likely to adhere to colonoscopic screening, suggesting that at-risk families in the general population are not getting needed information, the authors say.

"These findings demonstrate a major deficit in guideline-based care for members of hereditary non-polyposis CRC families in the United States," write the authors led by Swati Patel, M.D., from University of Colorado (Aurora). G2

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