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THE PRIMER
34 Fetal molecular diagnostics from maternal peripheral blood
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Screening with HPV-Alone invites more risk into women’s lives than you may think.

One out of 5 cases of cervical cancer were missed with HPV-Alone screening in a recent landmark, retrospective study—the largest ever conducted to evaluate the effectiveness of cervical cancer screening strategies in women ages 30-65. And screening with Pap+HPV Together (co-testing) identified more than 70% of these missed cancers. So is HPV-Alone screening really worth the risk?

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VALIDATING NEW TRENDS IN GENETIC TESTING

There are some controversial testing trends happening in the genetic test market. As clinical laboratory professionals, should we be prepared to embrace these trends? Will some of these online easy, one drop, technologies affect our future? Is anyone out there questioning the science or looking for standards to validate these offerings?

The controversial direct-to-consumer genetics testing company 23andMe, which has had its misadventures with regulators since its founding in 2006, is addressing potential customers via a slick new television advertisement. Soft, pleasant music plays throughout the minute-long ad. Images of people of different ages and races appear on screen (all of them with a genetic predisposition, apparently, to being slim and attractive, with good teeth). The copy is read by a cheery female voice: “This is a story. About you. The incredible you. It starts with your DNA, your twenty-three pairs of chromosomes, that make you unique—your traits, your tastes. This is a story about why you became who you are. 23andMe dot com is the first and only genetic service available directly to you that includes reports that meet FDA standards. You’ll get personalized, detailed reports that provide unique insights into your health, traits, and ancestry. Simply order your DNA kit from 23andMe dot com, provide your saliva sample at home, and mail it back. Then you’ll be notified when your online reports are ready. You’ll be able to explore your reports and use tools to compare your genetics with friends and family. See how your twenty-three pairs of chromosome help tell the story of one, incredible you. Order your DNA kit today at 23andMe dot com.”

In addition to the parade of appealing would-be customers, images include quick shots of reports on “caffeine consumption,” “lactose intolerance,” “eye color,” and “ancestry composition,” a shot of the saliva-collection device, and, finally, a few images of people viewing their “online reports” on laptops. The ad copy is skillfully written; it pushes the right rhetorical buttons. It appeals to narcissism, telling viewers how incredible and special they are. The words you, your, and you’ll are spoken 22 times. It stresses how easy the whole process is, while alluding to the science of genetics that underlies the product as little as it possibly can. It conveys the message that what is being offered is much more than that “ancestry” stuff the viewer might see in other commercials—while at the same time suggesting that it includes that too, for viewers who want that. And, almost as an aside, it mentions “reports that meet FDA standards.”

That seems to suggest a blanket endorsement by the government agency but, in fact, glosses over the checked history of 23andMe. It leaves out the fact that, in 2013, the U.S. Food and Drug Administration ordered the company to stop presenting its product to the public as way of assessing disease risk. The FDA underscored its displeasure by publicly releasing a letter criticizing the company for failing to respond to the agency’s concerns in a prompt manner. After that, 23andMe continued to sell a personal genome test, but without a health component.

Some fence-mending has occurred in the meantime, culminating in the FDA’s decision last February to approve the company’s test for a genetic variant linked to one of the most common forms of cancer, breast cancer. As clinical laboratory professionals, should not make medical decisions based only on information they receive from 23andMe. And clinical lab professionals should watch the story closely: if consumers increasingly use services like this to predict and try to prevent health problems, that has implications for the industry and for public health.
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**Infectious Disease**

Using copper to prevent the spread of respiratory viruses. New research from the University of Southampton has found that copper can effectively help to prevent the spread of respiratory viruses, which are linked to severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS).

Animal coronaviruses that “host jump” to humans, such as SARS and MERS, result in severe infections with high mortality. The Southampton researchers found that a closely-related human coronavirus, 229E, can remain infectious on common surface materials for several days, but is rapidly destroyed on copper.

A newly-published paper in mBio reports that 229E, which produces a range of respiratory symptoms from the common cold to more lethal outcomes such as pneumonia, can survive on surface materials including ceramic tiles, glass, rubber, and stainless steel for at least five days. While human-to-human transmission is important, infections can be contracted by touching surfaces contaminated by respiratory droplets from infected individuals, or hand touching, leading to a wider and more rapid spread.

On copper, and a range of copper alloys—which are collectively termed “antimicrobial copper”—the coronavirus was rapidly inactivated (within a few minutes, for simulated fingertip contamination). Exposure to copper destroyed the virus completely and irreversibly, leading the researchers to conclude that antimicrobial copper surfaces could be employed in communal areas and at any mass gatherings to help reduce the spread of respiratory viruses and protect public health.

**Alzheimer’s Disease**

NIH supports new studies to find Alzheimer’s biomarkers in Down syndrome. The National Institutes of Health (NIH) has launched a new initiative to identify biomarkers and track the progression of Alzheimer’s disease in people with Down syndrome. Many people with Down syndrome have Alzheimer’s-related brain changes in their 30s that can lead to dementia in their 50s and 60s. Little is known about how the disease progresses in this vulnerable group. The NIH Biomarkers of Alzheimer’s Disease in Adults with Down Syndrome Initiative will support two teams of researchers using brain imaging, as well as fluid and tissue biomarkers in research that may one day lead to effective interventions for all people with dementia.

The link between Alzheimer’s and Down syndrome is well-known. People with Down syndrome are born with an extra copy of chromosome 21, which contains the amyloid precursor protein gene. This gene plays a role in the production of harmful amyloid plaque, sticky clumps that build up outside neurons in Alzheimer’s disease. Having three copies of this gene is a known risk factor for early-onset Alzheimer’s that can occur in people in their 30s, 40s and 50s.

The teams will employ an array of biomarkers to identify and track Alzheimer’s-related changes in the brain and cognition for more than 500 Down syndrome volunteers. The measures include blood tests to identify biomarkers in blood, including proteins, lipids and markers of inflammation; and blood tests to collect DNA for genome-wide association studies that identify the genetic factors that may confer risk, or protect against, developing Alzheimer’s.

**Genetics/Genomics**

Researchers identify genes linked to stress-triggered heart disease. Researchers at the Translational Genomics Research Institute (TGen) and Barrow Neurological Institute have identified genetic risk factors that are linked to stress-induced cardiomyopathy (SIC), a rare type of heart disease.

In a study published in the journal Neurosurgery, researchers reported on the identification of new genetic risk factors through the use of genomic sequencing. Knowing which patients harbor the genes associated with SIC could help guide their care and treatment before, and after, they suffer a life-threatening stressor that induces SIC.

Using ultra-high resolution cameras and supercomputers, researchers identified the suspect genes by next generation DNA sequencing, essentially by spelling out the billions of bits of information in the genomes of seven women who exhibited SIC following a brain aneurysm.

Among the gene variants identified in the study as associated with SIC are MYLK2, DSG2, FKTN, and LDB3. All these genes were previously known to play a role in other cardiac diseases, but not in SIC. These variants are extremely rare, but their identification suggests a way to identify patients at risk of SIC.
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Unmet clinical needs in cervical cancer screening
By Jianyu Rao, MD, Luisa Escobar-Hoyos, MSc, and Kenneth R. Shroyer, MD, PhD

The incidence of cervical cancer has dropped dramatically due to the success of the Papanicolaou (Pap) test, the cytologic examination of cervical cells in the detection of high-grade premalignant lesions that can be treated before they progress to invasive cervical carcinoma. Although the Pap test is the most effective tool ever deployed for cancer screening, it has been limited by problems of low sensitivity for high-grade premalignant lesions of the cervical mucosa.

Epidemiologic and molecular studies over the past three decades have firmly established that human papillomavirus (HPV) infection is the etiologic agent for virtually all cases of cervical squamous cell carcinoma (SCC) and also for the vast majority of cases of endocervical adenocarcinoma. Although the development of HPV-based test strategies can be used to enhance sensitivity for the detection of clinically significant lesions, HPV testing has been less effective as a primary screening assay in patient populations that have a high prevalence of HPV infection, including adult women under age 30 in both industrialized nations and many third-world countries. In addition, the incidence of death due to cervical cancer has not changed in many developing countries due to difficulties in introducing cost-effective and highly sensitive and specific cervical cancer screening programs that do not require a large clinical laboratory infrastructure and trained cytotechnologists to analyze results. Hence, there is a significant need for a new generation of molecular tests that can augment the existing Pap test, and potentially replace the HPV or Pap tests as the frontline screen in developing countries.

The biology of cervical cancer
Cervical cancer is highly curable when found early because it is usually a very slow-growing cancer, generally taking two years or more to progress and begin to spread to surrounding tissue. Invasive SCC comprises 75 percent to 77 percent of cases of malignant epithelial neoplasms of the cervix and is the most thoroughly investigated of cervical cancers. In addition to SCC, invasive adenocarcinoma is also caused by HPV-mediated transformation and accounts for nearly 15 percent of cases of cervical carcinoma.

With exceedingly rare exceptions, virtually all cervical SCCs and the great majority of endocervical adenocarcinomas result from persistent HPV infection that has not been cleared by the patient’s immune system. Cervical SCC does not arise de novo but is preceded by premalignant high grade squamous intraepithelial lesions (HSILs) that are composed of immortalized, clonal cell population that have not yet gained the potential to invade the underlying cervical stroma.

Although cervical cancers originate from cells with pre-cancerous changes, epidemiologic studies suggest that only a minority of women with pre-malignant lesions of the cervix will develop cancer (10 percent to 30 percent, depending on regional influences). The prevalence of infection is age-related, with approximately 25 percent of women in their 20s in the United States harboring asymptomatic infections at any one time, but decreasing in prevalence as a woman ages. While most cases of HPV infection spontaneously regress within one to two years, in some cases, a transforming event occurs, frequently as a result of integration of the high risk HPV viral genome into the genome of cervical basal epithelial cells, leading to the loss of normal cell cycle regulatory mechanisms and the development of premalignant lesions of the squamous and/or glandular mucosa.

Based to a large extent on the recognition that most cases of HPV spontaneously clear without treatment, the American College of Obstetrics and Gynecology, the American Cancer Society, and a number of other organizations recently recommended a reduction in the number of screening tests in young women. Recognition that most cases of HPV infection are transient has also highlighted the limited specificity of HPV testing for clinically significant lesions that require therapeutic intervention, reflecting clinically false-positive test results. Although the recent introduction of the HPV vaccine will ultimately reduce the mortality associated with cervical cancer in vaccinated patient populations, early detection of cervical cancer by routine testing is likely to continue to be the most important aspect of cervical cancer management over the next several decades.

Current cervical cancer screening methods and the need for improvements
The Pap test, first developed in the 1940s, has remained the gold-standard screening test for cervical cancer. When it is detected early, cervical cancer is among the most treatable of all cancers. In the U.S., deployment of the Pap test to detect pre-malignant lesions has reduced the incidence of cervical cancer by more than 75 percent over the past 30 years. The American Cancer Society estimates that in 2015 approximately 12,900 new cases of invasive cervical cancer will be diagnosed, and about 4,100 women will die from it. Notably, according to the World Health Organization (WHO), in 2008, there were more than 530,000 new cases of cervical cancer worldwide and 275,000 deaths from cervical cancers. More than 90 percent of them were recorded in developing countries. In Africa, 75,000 new cases were recorded in 2008 and 50,000 women died. Women are dying in the developing world from a disease that has been largely eliminated in women in the U.S.

In recent years, incremental improvements in cervical cancer testing have been made to offset the limitations of the Pap test: the low sensitivity for detecting significant high-grade and above lesions; the need for in-laboratory analysis with trained cytotechnologists; and test inaccessibility in the developing world. To date, more than 100 strains of the HPV virus have been described.
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individuals are newly infected each year. By contrast, the prevalence infected with HPV and approximately 14 million additional individuals with a more expensive cervical cancer screening assay (the HPV test). Conversely, however, HPV-positive test results have limited specificity for clinically significant lesions because the vast majority of HPV infections in young women are transient and do not lead to clinically significant disease. This limitation can be mitigated to some extent by limiting HPV testing to low-risk populations, but positive test results still result in a dilemma regarding the clinical management of patients with normal or minimally abnormal cytology findings. Incremental improvement in test specificity may also be achieved by a focus on detection of E6 and E7 oncogene expression, but the underlying problem of poor positive predictive value of positive test results has not been resolved. Thus, the clinical management of infections by an oncogenic virus (high-risk HPV) of undetermined origin (for a given patient) that cannot be effectively treated and usually resolves without treatment, but can cause HSIL or cancer, drives increased healthcare expenditures and resource utilization for the great majority of patients who are unlikely to benefit from clinical intervention.

In April 2014, the FDA approved the first frontline screen with an HPV-DNA test that could be used in place of the Pap test in women age 25 and older. The agency’s approval came despite objections from several parties, including a coalition of 17 patient and women’s health groups that expressed “grave concerns” that using the HPV test as a primary screen lacked support from evidence-based guidelines and may result in more invasive procedures for clinically insignificant infections. FDA approval required that a positive test for HPV types 16 or 18 be directly referred for colposcopy even though the HPV test does not reliably distinguish between transient versus transforming infections and does not necessarily indicate the presence of disease in multivariate modeling. The FDA approval states that women who test positive for one or more of the other high-risk HPV types should have a Pap test to determine the need for a colposcopy. In following this approach, clinicians would essentially replace the Pap test as a highly effective screening device with a more expensive cervical cancer screening assay (the HPV test) that will increase the cost of cervical screening, in the absence of clear evidence of enhanced overall accuracy.

In the U.S., approximately 79 million individuals are currently infected with HPV and approximately 14 million additional individuals are newly infected each year. By contrast, the prevalence of cervical cancer in the U.S. is approximately 12,900 cases/year. Thus, only about 1.6 in 10,000 women with HPV infection actually have cervical cancer (and only about 0.7 percent of women with HPV infection have underlying HSIL). Thus the vast majority of women with positive HPV test results do not have underlying lesions that require therapeutic intervention. (This general conclusion holds true even when factoring in women with high grade glandular lesions of the cervical mucosa which are also usually caused by HPV infection.) The extraordinarily high prevalence of HPV infection compared to the relatively low prevalence of cervical cancer and HSIL suggests that most cases of HPV infection have limited clinical significance. This is because HPV infection in the vast majority of cases will spontaneously resolve without treatment within two years, and most patients with positive HPV test results do not have lesions that require clinical intervention. HPV testing can also be limited by false-negative test results. Although there are numerous anecdotal reports of false-negative testing, the largest study of its kind, the Quest Diagnostics Health Trends study (based on tests of more than 250,000 women), found that nearly five percent of women with a CIN3+ (severely abnormal cells and cancer cells) Pap test had a negative HPV test. Within five years, nearly 25 percent of these women were found to have cervical cancer. This finding could be due to several factors including transient infection that has cleared after initiation of the oncogenic processes or a false-negative HPV test result. In either case, this result confirms the risk of using HPV as the sole testing in the frontline screening of cervical cancer.

Need for cervical cancer screening in developing countries

In a seminal study, Gakidou et al. investigated cervical cancer screening in 57 developing countries and found that the percentage of women being screened, on average, was 19 percent, compared to 63 percent in developed countries, and ranged from one percent in Bangladesh to 73 percent in Brazil. Effective coverage varied widely across countries, from more than 80 percent in Austria and Luxembourg to one percent or less in Bangladesh, Ethiopia, and Myanmar (Burma). In many countries, a large proportion of women have had pelvic examinations, but the exam was not accompanied by laboratory tests or was not done in the three years preceding the survey. In the nation of Georgia, for example, 67 percent of women have had a pelvic exam, but only 11 percent had had one in the preceding three years, accompanied by a Pap smear; likewise in China, crude coverage (i.e., any past pelvic exam) is 70 percent but effective screening coverage (e.g., a pelvic exam with Pap test sometime in the preceding three years) is only 23 percent. The study analysis points to an acute shortage of cervical cancer prevention services across much of the developing world and striking inequalities in access to these services, highlighting the need for new prevention and treatment strategies. This study also identifies a number of countries where the vast majority of women have never had a pelvic exam. In such settings, where the health system is unable to provide even low levels of crude coverage of this basic intervention, improved screening is urgent, especially for women older than 35.

The burden on healthcare availability and delivery will be exacerbated as world population grows. Significantly, the world population will be aging faster by 2050 than in the U.S., according to a 2014 Pew Research Center analysis of a recent United Nations study: World Population Prospects 2012. By 2050, the report notes, the number of people 65 and older is projected to triple, from 531 million in 2010 to 1.5 billion in 2050. In the U.S., the number of people 65 and older is expected to slightly more than double, from 41 to 86 million. In early 2015, the WHO estimated that the number of new cancer cases is expected to rise by about 70 percent over the next two decades, from 14 million in 2012 to 22 million new cases. The report notes that cancer-causing viral infections such as HBV/HCV and HPV are responsible for up to 20 percent of cancer deaths in low- and middle-income countries and that more than 60 percent of the world’s total new annual cases occur in Africa, Asia and Central and South America. These regions account for 70 percent of the world’s cancer deaths. Clearly, there is a pressing medical need for highly accurate...
INDICATION

TAGRISSO is indicated for the treatment of patients with metastatic epidermal growth factor receptor (EGFR) T790M mutation-positive non-small cell lung cancer (NSCLC), as detected by an FDA-approved test, who have progressed on or after EGFR tyrosine kinase inhibitor therapy.

This indication is approved under accelerated approval based on tumor response rate and duration of response. Continued approval for this indication may be contingent upon verification and description of clinical benefit in confirmatory trials.

IMPORTANT SAFETY INFORMATION

• There are no contraindications for TAGRISSO
• Interstitial Lung Disease (ILD)/Pneumonitis occurred in 3.3% and was fatal in 0.5% of 813 TAGRISSO patients. Withhold TAGRISSO and promptly investigate for ILD in any patient presenting with worsening of respiratory symptoms indicative of ILD (e.g., dyspnea, cough and fever). Permanently discontinue TAGRISSO if ILD is confirmed
• QTc interval prolongation occurred in TAGRISSO patients. Of the 411 patients in two Phase II studies, 0.2% were found to have a QTc greater than 500 msec, and 2.7% had an increase from baseline QTc greater than 60 msec. Conduct periodic monitoring with ECGs and electrolytes in patients with congenital long QTc syndrome, congestive heart failure, electrolyte abnormalities, or those who are taking medications known to prolong the QTc interval. Permanently discontinue TAGRISSO in patients who develop QTc interval prolongation with signs/symptoms of life threatening arrhythmia
• Cardiomyopathy occurred in 1.4% and was fatal in 0.2% of 813 TAGRISSO patients. Left Ventricular Ejection Fraction (LVEF) decline >10% and a drop to <50% occurred in 2.4% of (9/375) TAGRISSO patients. Assess LVEF before initiation and then at 3 month intervals of TAGRISSO treatment. Withhold TAGRISSO if ejection fraction decreases by 10% from pretreatment values and is less than 50%. For symptomatic congestive heart failure or persistent asymptomatic LV dysfunction that does not resolve within 4 weeks, permanently discontinue TAGRISSO
• Advise pregnant women of the potential risk to a fetus. Advise females of reproductive potential to use effective contraception during TAGRISSO treatment and for 6 weeks after the final dose. Advise males with female partners of reproductive potential to use effective contraception for 4 months after the final dose
• The most common adverse reactions (>20%) observed in TAGRISSO patients were diarrhea (42%), rash (41%), dry skin (31%) and nail toxicity (25%)

Please see Brief Summary of complete Prescribing Information.

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TAGRISSO™ (osimertinib) tablet, for oral use

Brief Summary of prescribing information

For complete prescribing information consult official package insert

INDICATIONS AND USAGE

TAGRISSO is indicated for the treatment of patients with metastatic epidermal growth factor receptor (EGFR) T790M mutation-positive non-small cell lung cancer (NSCLC), as detected by an FDA-approved test, who have progressed on or after EGFR tyrosine kinase inhibitor (TKI) therapy. This indication is approved under accelerated approval based on tumor response rate and duration of response [see Clinical Studies (14) in the full Prescribing Information]. Continued approval for this indication may be contingent upon verification and description of clinical benefit in confirmatory trials.

DOSAGE AND ADMINISTRATION

Patient Selection

Confirm the presence of a T790M EGFR mutation in tumor specimens prior to initiation of treatment with TAGRISSO [see Indications and Usage (1) and Clinical Studies (14) in the full Prescribing Information]. Information on FDA-approved tests for the detection of T790M mutations is available at http://www.fda.gov/companiondiagnostics.

Recommended Dosage Regimen

The recommended dose of TAGRISSO is 80 mg tablet once a day until disease progression or unacceptable toxicity. TAGRISSO can be taken with or without food.

Administration to Patients Who Have Difficulty Swallowing Solids

Disperse tablet in 4 tablespoons (approximately 50 mL) of non-carbonated water only. Stir until tablet is completely dispersed and swallow or administer through the nasogastric tube immediately. Do not crush, heat, or ultrasonicate during preparation. Rinse the container with 4 to 8 ounces of water and immediately drink or administer through the nasogastric tube [see Clinical Pharmacology (12.3) in the full Prescribing Information].

Dose Modification for Adverse Reactions

Table 1: Recommended Dose Modifications for TAGRISSO

<table>
<thead>
<tr>
<th>Target Organ</th>
<th>Adverse Reactiona</th>
<th>Dose Modification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary</td>
<td>Interstitial lung disease (ILD)/Pneumonitis</td>
<td>Permanently discontinue TAGRISSO.</td>
</tr>
<tr>
<td></td>
<td>QTc interval greater than 500 msec on at least 2 separate ECGs b</td>
<td>Withhold TAGRISSO until QTc interval is less than 481 msec or recovery to baseline if baseline QTc is greater than or equal to 481 msec, then resume at 40 mg dose.</td>
</tr>
<tr>
<td></td>
<td>QTc interval prolongation with signs/symptoms of life threatening arrhythmia</td>
<td>Permanently discontinue TAGRISSO.</td>
</tr>
<tr>
<td></td>
<td>Asymptomatic, absolute decrease in LVEF of 10% from baseline and below 50%</td>
<td>Withhold TAGRISSO for up to 4 weeks. If improved to baseline LVEF, resume. If not improved to baseline, permanently discontinue.</td>
</tr>
<tr>
<td></td>
<td>Symptomatic congestive heart failure</td>
<td>Permanently discontinue TAGRISSO.</td>
</tr>
<tr>
<td></td>
<td>Grade 3 or higher adverse reaction</td>
<td>Withhold TAGRISSO for up to 3 weeks.</td>
</tr>
<tr>
<td></td>
<td>If improvement to Grade 0-2 within 3 weeks</td>
<td>Resume at 80 mg or 40 mg daily.</td>
</tr>
<tr>
<td></td>
<td>If no improvement within 3 weeks</td>
<td>Permanently discontinue TAGRISSO.</td>
</tr>
</tbody>
</table>

a Adverse reactions reported by the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 (NCI CTCAE v4.0).

b ECGs = Electrocardiograms

TAGRISSO is indicated for the treatment of patients with EGFR T790M mutation-positive non-small cell lung cancer who received prior EGFR TKI therapy, in two single arm pivotal studies, Study 1 and Study 2. Patients with a past medical history of ILD or radiation pneumonitis that required steroid treatment, serious arrhythmia or baseline QTc interval greater than 470 ms were excluded from Study 1 and Study 2. Baseline patient and disease characteristics were: median age 63 years, 13% of patients were ≥75 years old, 63% were female (68%) and White (36%) (see Warnings and Precautions (5.1) in the full Prescribing Information). QTc Interval Prolongation [see Warnings and Precautions (5.2) in the full Prescribing Information].

Cardiomyopathy

Across clinical trials, cardiomyopathy (defined as cardiac failure, pulmonary edema, ejection fraction decrease, or stress cardiomyopathy) occurred in 1.4% (n=11) of TAGRISSO treated patients (n=813); 0.2% (n=2) were fatal. Withhold TAGRISSO and promptly investigate for ILD in any patient who presents with worsening of respiratory symptoms which may be indicative of ILD (e.g., dyspnea, cough and fever). Permanently discontinue TAGRISSO if ILD is confirmed (see Dosage and Administration (2.4) and Adverse Reactions (6) in the full Prescribing Information).

QTc Interval Prolongation

The heart rate-corrected QT (QTc) interval prolongation occurs in patients treated with TAGRISSO. Of the 411 patients in Study 1 and Study 2, one patient (0.2%) was found to have a QTc greater than 500 msec, and 11 patients (2.7%) had an increase from baseline QTc greater than 60 msec [see Clinical Pharmacology (12.2) in the full Prescribing Information]. Continued approval for this indication may be contingent upon verification and description of clinical benefit in confirmatory trials.

Cardiomyopathy

Across clinical trials, cardiomyopathy (defined as cardiac failure, pulmonary edema, ejection fraction decrease, or stress cardiomyopathy) occurred in 1.4% (n=11) of TAGRISSO treated patients (n=813); 0.2% (n=2) were fatal. In Study 1 and Study 2, Left Ventricular Ejection Fraction (LVEF) decline ≥10% and a drop to <50% occurred in 2.4% (n=51) of patients who had baseline and at least one follow up LVEF assessment. Assess LVEF by echocardiogram or multigated acquisition (MUGA) scan before initiation of TAGRISSO and then at 3 month intervals while on treatment. Withhold treatment with TAGRISSO if ejection fraction decreases by 10% from pretreatment values and is less than 50%. For symptomatic congestive heart failure or persistant, asymptomatic LV dysfunction that does not resolve within 4 weeks, permanently discontinue TAGRISSO [see Dosage and Administration (2.4) in the full Prescribing Information].

Embryo-Fetal Toxicity

Based on data from animal studies and its mechanism of action, TAGRISSO can cause fetal harm when administered to a pregnant woman. In animal reproduction studies, osimertinib caused post-implantation fetal loss when administered during early development at a dose exposure 1.5 times the exposure at the recommended human dose. When mares were treated prior to mating with untreated females, there was an increase in preimplantation embryonic loss at plasma exposures of approximately 0.5 times those observed in patients at the 80 mg dose level. Advise pregnant women of the potential risk to a fetus.

Adverse reactions of reproductive potential to use effective contraception during treatment with TAGRISSO for 6 weeks after the final dose. Advise females with partners of reproductive potential to use effective contraception for 4 months after the final dose [see Use in Specific Populations (8.1), (8.3) and Clinical Pharmacology (12.3) in the full Prescribing Information].

Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

The data described below reflect exposure to TAGRISSO (80 mg daily) in 411 patients with EGFR T790M mutation-positive non-small cell lung cancer who received prior EGFR TKI therapy, in two single arm pivotal studies, Study 1 and Study 2. Patients with a past medical history of ILD or radiation pneumonitis that required steroid treatment, serious arrhythmia or baseline QTc interval greater than 470 ms were excluded from Study 1 and Study 2. Baseline patient and disease characteristics were: median age 63 years, 13% of patients were ≥75 years old, female (88%), White (36%), Asian (60%), metastatic (96%), sites of brain metastases (39%), World health organization (WHO) performance status of 0 (37%) or 1 (63%), 1 prior line of therapy (EGFR TKI) treatment only, second line, chemotherapy-naive (31%), 2 or more prior lines of therapy (68%). Of the 411 patients, 333 patients were exposed to TAGRISSO for at least 6 months; 97 patients were exposed for at least 9 months; however no patient was exposed to TAGRISSO for 12 months.

In Studies 1 and 2, the most common (>20%) adverse reactions (all grades) observed in TAGRISSO-treated patients were diarrhea (42%), rash (41%), dry skin (31%), and nail toxicity (25%). Dose reductions occurred in 4.4% of patients treated with TAGRISSO. The most frequent adverse reactions that led to dose reductions or interruptions were: electrocardiogram QTc prolonged (2.2%) and neutropenia (1.9%). Serious adverse reactions reported in ≥2% or more patients were pneumonitis and pulmonary embolus. There were 4 patient deaths (1%) in patients with TAGRISSO, two of which developed fatal adverse reactions of ILD/pneumonitis. Other fatal adverse reactions occurring in more than 1 patient included pneumonia (4 patients) and cerebrovascular accident (2 patients). Discontinuation of therapy due to adverse reactions occurred in 5.6% of patients treated with TAGRISSO. The most frequent adverse reactions that led to discontinuation were ILD/pneumonitis and cerebrovascular accidents/infarctions.

Tables 2 and 3 summarize the common adverse reactions and laboratory abnormalities observed in TAGRISSO-treated patients.

Table 2: Adverse Reactions (>10% for all NCI CTCAE Grades or >2% for Grades 3-4) in Study 1 and Study 2

<table>
<thead>
<tr>
<th>Adverse Reaction</th>
<th>All Grades</th>
<th>Grade 3-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nausea</td>
<td>52.5%</td>
<td>4.3%</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>42%</td>
<td>1.0%</td>
</tr>
<tr>
<td>Constipation</td>
<td>16%</td>
<td>0.7%</td>
</tr>
<tr>
<td>Rash</td>
<td>14%</td>
<td>0.5%</td>
</tr>
<tr>
<td>Mouth ulceration</td>
<td>10%</td>
<td>0.7%</td>
</tr>
<tr>
<td>Fatigue</td>
<td>14%</td>
<td>0.5%</td>
</tr>
<tr>
<td>Anorexia</td>
<td>9.7%</td>
<td>0.5%</td>
</tr>
<tr>
<td>Cough</td>
<td>14%</td>
<td>0.2%</td>
</tr>
<tr>
<td>Vomiting</td>
<td>10%</td>
<td>0.2%</td>
</tr>
<tr>
<td>Headache</td>
<td>10%</td>
<td>0.2%</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>14%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Anemia</td>
<td>14%</td>
<td>0.2%</td>
</tr>
<tr>
<td>Peripheral edema</td>
<td>10%</td>
<td>0.2%</td>
</tr>
<tr>
<td>Blood pressure decrease</td>
<td>10%</td>
<td>0.2%</td>
</tr>
<tr>
<td>Asthenia</td>
<td>10%</td>
<td>0.2%</td>
</tr>
<tr>
<td>Night sweats</td>
<td>14%</td>
<td>0.2%</td>
</tr>
<tr>
<td>Sweating</td>
<td>14%</td>
<td>0.2%</td>
</tr>
<tr>
<td>Sinusitis</td>
<td>14%</td>
<td>0.2%</td>
</tr>
<tr>
<td>Peripheral edema</td>
<td>10%</td>
<td>0.2%</td>
</tr>
<tr>
<td>Asthenia</td>
<td>14%</td>
<td>0.2%</td>
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</tr>
</tbody>
</table>

In Study 1 and Study 2, Left Ventricular Ejection Fraction (LVEF) decline ≥10% and a drop to <50% occurred in 2.4% (n=51) of patients who had baseline and at least one follow up LVEF assessment.
observed in patients at the recommended dose of 80 mg), an equivocal increase in the rate of
of the hard palate (gestation days 6 to 16) at doses of 1 mg/kg/day and above (0.1-times the AUC
organogenesis (gestation days 2-20) at a dose of 20 mg/kg/day, which produced plasma exposures
Animal Data
Data
in clinically-recognized pregnancies is 2% to 4% and 15% to 20%, respectively.
In the U.S. general population, the estimated background risk of major birth defects and miscarriage
reduced fetal growth at plasma exposures 1.5 times the exposure at the recommended human dose
women. Administration of osimertinib to pregnant rats was associated with embryolethality and
Based on data from animal studies and its mechanism of action, TAGRISSO can cause fetal harm
Risk Summary
Drug interaction studies with inhibitors, inducers or substrates of CYP enzymes and transporters
have not been conducted with TAGRISSO.
Effect of Other Drugs on Osimertinib
Strong CYP3A Inhibitors
Avoid concomitant administration of TAGRISSO with strong CYP3A inhibitors, including macrolide
antibiotics (e.g., troleandomycin), antifungals (e.g., itraconazole), antivirals (e.g., ritonavir),
nelfinavir, as concomitant use of strong CYP3A inhibitors may increase osimertinib plasma concentrations.
If no other alternative exists, monitor patients more closely for adverse reactions of TAGRISSO [see Dosage and Administrations (2.4) and Clinical Pharmacology (12.3) in the full Prescribing Information]
Strong CYP3A Inducers
Avoid concomitant administration of TAGRISSO with strong CYP3A inducers (e.g., phenytoin,
rifampin, carbamazepine, St. John’s Wort) as strong CYP3A inducers may decrease osimertinib plasma concentrations [see Clinical Pharmacology (12.3) in the full Prescribing Information]
Effect of Osimertinib on Other Drugs
Avoid concomitant administration of TAGRISSO with drugs that are sensitive substrates of CYP3A,
including but not limited to fentanyl, cyclosporine, quinidine, ergot alkaloids, phenytoin, carbamazepine,
as osimertinib may increase or decrease plasma concentrations of these drugs [see Clinical Pharmacology (12.3) in the full Prescribing Information]
Drug Interactions
Drug interactions studies with inhibitors, inducers or substrates of CYP enzymes and transporters
have not been conducted with TAGRISSO.

### Laboratory Abnormalities (≤20% for all NCI CTCAE Grades in Study 1 and Study 2)

<table>
<thead>
<tr>
<th>Laboratory Abnormality</th>
<th>Change from Baseline All Grades (%)</th>
<th>Change from Baseline to Grade 3 or Grade 4 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Chemistry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anemia</td>
<td>44</td>
<td>0.2</td>
</tr>
<tr>
<td>Hypoglycemia</td>
<td>67</td>
<td>0.7</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>63</td>
<td>3.3</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>90</td>
<td>3.4</td>
</tr>
<tr>
<td>Nephrolithiasis</td>
<td>51</td>
<td>0.7</td>
</tr>
<tr>
<td>Orthostatic hypotension</td>
<td>55</td>
<td>1.1</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>54</td>
<td>1.2</td>
</tr>
<tr>
<td>Hyponatremia</td>
<td>26</td>
<td>3.4</td>
</tr>
<tr>
<td>Hyperkalemia</td>
<td>25</td>
<td>3.4</td>
</tr>
<tr>
<td>Hypermagnesemia</td>
<td>20</td>
<td>0.7</td>
</tr>
</tbody>
</table>

*The only grade 4 laboratory abnormality was 1 patient with grade 4 thrombocytopenia.*

### USE IN SPECIFIC POPULATIONS

#### Pregnancy

**Risk Summary**

Based on data from animal studies and its mechanism of action, TAGRISSO can cause fetal harm when administered to a pregnant woman. There are no available data on TAGRISSO use in pregnant women.

Administration of osimertinib to pregnant rats was associated with embryopathy and reduced fetal growth at plasma exposures 1.5 times the exposure at the recommended human dose (see Data). Advise pregnant women of the potential risk to a fetus.

In the U.S. general population, the estimated background risk of major birth defects and miscarriage is 2% to 4% and 15% to 20%, respectively.

#### Data

**Animal Data**

When administered to pregnant rats prior to embryonic implantation through the end of organogenesis (gestation days 2-20) at a dose of 20 mg/kg/day, which produced plasma exposures of approximately 1.5 times the clinical exposure, osimertinib caused post-implantation loss and early embryonic death. When administered to pregnant rats from implantation through the closure of the hard palate (gestation days 8 to 16) at doses of 1 mg/kg/day and above (0.1-times the AUC observed in patients at the recommended dose of 80 mg), an equinocital increase in the rate of fetal malformations and variations was observed in treated litters relative to those of control groups.

When administered to pregnant dams at doses of 30 mg/kg/day during organogenesis through lactation Day 6, osimertinib caused an increase in total litter loss and postnatal death. At a dose of 20 mg/kg/day, osimertinib administration during the same period resulted in increased postnatal death as well as a slight reduction in mean pup weight at birth that increased in magnitude between lactation days 4 and 6.

#### Lactation

There are no data on the presence of osimertinib in human milk, the effects of osimertinib on the breastfed infant or on milk production. Administration to rats during gestation and early lactation was associated with adverse effects, including reduced growth rates and neonatal death (see Use in Specific Populations (8.1) in the full Prescribing Information).

Because of the potential for serious adverse reactions in breastfeeding infants from osimertinib, advise a lactating woman not to breastfeed during treatment with TAGRISSO and for 2 weeks after the final dose.

#### Females and Males of Reproductive Potential

**Contraception**

- **Females**
  - Advise females of reproductive potential to use effective contraception during treatment with
  - TAGRISSO and for 6 weeks after the final dose [see Use in Specific Populations (8.1) in the full Prescribing Information]
- **Males**
  - Advise males with female partners of reproductive potential to use effective contraception
during and for 6 months following the final dose of TAGRISSO [see Nonclinical Toxicology (13.1) in the full Prescribing Information]

**Fertility**

Based on animal studies, TAGRISSO may impair fertility in females and males of reproductive potential. It is not known if the effects on fertility are reversible [see Nonclinical Toxicology (13.1) in the full Prescribing Information]

#### Pediatric Use

The safety and effectiveness of TAGRISSO in pediatric patients have not been established.

#### Geriatric Use

One hundred eighty-seven (45%) of the 411 patients in clinical trials of TAGRISSO were 65 years of age and older, and 54 patients (13%) were 75 years of age and older. No overall differences in effectiveness were observed based on age. Exploratory analysis suggests a higher incidence of Grade 3 and 4 adverse reactions (32% versus 25%) and more frequent dose modifications for adverse reactions (32% versus 17%) in patients 65 years or older as compared to those younger than 65 years.

#### Renal Impairment

No dedicated clinical studies have been conducted to evaluate the effect of renal impairment on the pharmacokinetics of osimertinib. Based on population pharmacokinetic analysis, no dose adjustment is recommended in patients with mild [creatinine clearance (CLCr) 60-89 mL/min] or moderate [CLCr 30-59 mL/min] renal impairment. There is no recommended dose of TAGRISSO for patients with severe renal impairment [CLCr <30 mL/min] or end-stage renal disease [see Clinical Pharmacology (12.3) in the full Prescribing Information]

#### 17 PATIENT COUNSELING INFORMATION

Advise the patient to read the FDA-approved patient labeling (Patient Information) and Prescribing Information. Advise patients of the risks of severe or fatal ICD, including pneumonitis. Advise patients to contact their healthcare provider immediately to report new or worsening respiratory symptoms [see Warnings and Precautions (5.1) in the full Prescribing Information]

#### QTc Interval Prolongation

Inform patients of symptoms that may be indicative of significant QTc prolongation including diziness, lightheadedness, and syncope. Advise patients to report these symptoms and to inform their physician about the use of any heart or blood pressure medications [see Warnings and Precautions (5.3) in the full Prescribing Information]

#### Cardiomyopathy

- **TAGRISSO** can cause cardiomyopathy. Advise patients to immediately report any signs or symptoms of heart failure to their healthcare provider [see Warnings and Precautions (5.3) in the full Prescribing Information]
- **Emphysema/Fatality**
  - **TAGRISSO** can cause fatal harm if taken during pregnancy. Advise pregnant women of the potential risk to a fetus.
  - Advise females who are pregnant or may become pregnant to avoid treatment with TAGRISSO and for 6 weeks after the final dose [see Use in Specific Populations (8.1) in the full Prescribing Information]
  - Advise females to inform their healthcare provider if they become pregnant or if pregnancy is suspected, while taking TAGRISSO [see Warnings and Precautions (5.3) and Use in Specific Populations (8.1) in the full Prescribing Information]
- **Females and Males of Reproductive Potential**
  - Advise females of reproductive potential to use effective contraception during treatment with TAGRISSO and for 6 weeks after the final dose [see Use in Specific Populations (8.1) in the full Prescribing Information]
  - Advise males to use effective contraception during treatment and for 4 months after the final dose of TAGRISSO [see Use in Specific Populations (8.1) in the full Prescribing Information]

#### Lactation

Advise women not to breastfeed during treatment with TAGRISSO and for 2 weeks after the final dose [see Use in Specific Populations (8.1) in the full Prescribing Information]

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Protein biomarkers in cervical cancer testing

Failure of the immune system to clear HPV may enable persistent infection and increase the risk of HPV viral genome integration into the genome of cervical mucosa basal cells.1,2

HPV viral integration, and possibly other pathways that lead to an HPV-transforming event, disrupt normal cell cycle regulatory mechanisms and result in the degradation of the key cell cycle regulatory proteins p53 and Rb, enabling cells to continue to divide, even in the face of genomic DNA damage. These mechanisms may explain, at least in part, how a persistent infection can ultimately lead to malignant transformation of the cervical mucosa, reflected by changes in the expression of the range of proteins that both have roles in the pathogenesis of cervical cancer and also could serve as biomarkers of premalignant and malignant lesions.

Altered protein expression can be measured using quantitative assays for biomarkers that increase with the disease progression. Although HPV testing generally provides information only on the presence or absence of viral infection, tests based on cervical cancer biomarkers may identify patients that are most likely to benefit from clinical intervention while reducing the number of unnecessary biopsies. Several host and viral molecules are being investigated for their use in the detection of HSIL.22 One study involved testing of 302 biopsy samples against a panel of 13 different potential biomarkers.23

Multivariate modeling completed in this study, as well as additional modeling, strongly demonstrate that a panel of three to five markers can be used to confidently grade cervical intraepithelial lesions. Another study showed that measurement of HPV E6 protein in cervical cytology samples could be used to distinguish HSIL from cytologically normal specimens, improving diagnostic accuracy compared to what can be achieved by traditional HPV DNA tests.23

Most recently, a team of investigators at Stony Brook University Medical Center led by one of the authors of this article, Dr. Kenneth Shroyer, conducted research leading to the identification of unique protein biomarkers that have both the potential to play a role as diagnostic biomarkers and to be prognostic for patient survival. In this lab, mass spectrometry of laser capture-microdissected cervical biopsy specimens enabled the discovery of more than 2,000 proteins that were differentially expressed in cervical cancer and HSIL compared to normal mucosa and productive HPV infections.24 In silico analysis and immunohistochemical evaluation subsequently identified keratin 17 (K17) as a powerful prognostic biomarker for survival of patients with SCC that was independent of tumor grade, stage, or HPV status.24,25 Patients with elevated K17 expression have a very poor prognosis for survival, even in cases that appeared to be confined to the cervix at the time of diagnosis, while patients with advanced-stage disease but low K17 status have excellent long-term survival.24,25 Further investigation led to the surprising discovery that K17 is able to enter the nucleus of cancer cells, where it binds to and ultimately causes the degradation of p27KIP1, an important regulator of early (G1) cell cycle arrest. Thereby, the disruption of normal cell cycle regulatory mechanisms explains, at least in part, why cervical SCCs with high K17 are much more likely to be fatal than SCCs that express only a low level of K17.

These findings support a premise that the measurement of protein biomarkers in cervical cytology samples is a valid approach that not only can provide clinically useful information in identifying high-grade cervical lesions and cervical cancer in developed nations, but could potentially be utilized in developing countries where there are obstacles to deployment of the Pap test for cervical cancer screening. Furthermore, multiplexing of the relevant cervical cancer biomarkers has the potential to both establish disease status and predict cervical cancer progression, providing a valuable diagnostic tool for early detection to enable more focused treatment of high-grade cervical lesions in low-resource settings where colposcopy and histological analyses are less readily available.

Summary

Cancer rates worldwide are expected to increase disproportionally in coming decades relative to the projected increase in population, especially in the developing world. The general unavailability of the Pap test and the cost of the HPV test in the developing world have precluded the deployment of effective cervical cancer screening programs in many developing countries. Recent improvements in testing technology arise from a need to overcome the significant limitations of the Pap test and HPV test, but results require first-world technology and validation.

Developing countries, where cervical cancer remains one of the most important causes of cancer death, have the greatest need for an affordable, easy-to-use, and highly reliable cancer screening method that can return a diagnosis through efficient laboratory analysis or, more easily, at a woman’s point of care.

While research, testing, and vaccine improvements in recent years continue to lower the incidence of cervical cancer in some developed countries such as the U.S., HPV testing research needs to do more than test for the presence of virus. The tests must determine the presence and progression of cervical disease. Tests should be more sensitive and specific than Pap tests and Pap-related tests, and should be accurate in more than 90 percent of cases. Tests also need to be low-cost, objective, and easy to perform so screening programs can be widely implemented in developing countries where the need for a better cervical cancer screening test is highest. Such tests may be available through the recent advances in specific biomarkers of cervical cancer and multiplex detection technologies. Development of the next generation of cervical cancer tests that are more specific, sensitive, and informative than the traditional Pap or HPV test will make a significant impact on the reduction of cervical cancer worldwide.26

Please visit www.mlo-online.com to see references for this article.

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Kenneth R. Shroyer, MD, PhD, serves in the Department of Pathology at Stony Brook Medicine.
1. Identify the screening test which has tremendously brought down the incidence of cervical cancer:
   a. colonoscopy
   b. colposcopy
   c. Papanicolaou (Pap) test
   d. HPV titer

2. What is the main limitation of the Pap test?
   a. low specificity for low-grade premalignant lesions
   b. low sensitivity for low-grade premalignant lesions
   c. low sensitivity for high-grade premalignant lesions
   d. none of the above

3. Studies over the last three decades have defined the carcinomatous agent for almost all instances of cervical squamous cell carcinoma (SCC):
   a. human papillomavirus (HPV)
   b. human immunodeficiency virus (HIV)
   c. herpes simplex virus type 2 (HSV2)
   d. none of the above

4. How long does cervical cancer generally take to progress and spread into surrounding tissue?
   a. ten years
   b. two years
   c. one year
   d. five years

5. Virtually all cases of cervical SCCs and many cases of endocervical adenocarcinomas result from persistent HPV infection that hasn’t been cleared from the immune system.
   a. True
   b. False

6. The mechanism in which HPV can cause cervical lesions is described by:
   a. the integration of the HPV viral genome into the genome of cervical basal epithelial cells.
   b. the formation of HPV antibodies in the blood stream.
   c. both a and b.
   d. none of the above.

7. The goal of the HPV vaccine has been to ultimately replace the Pap test as the most important aspect of cervical cancer management.
   a. True
   b. False

8. Over the last 30 years, the use of the Pap test in the United States has reduced the incidence of cervical cancer by:
   a. 85 percent.
   b. 99 percent.
   c. 15 percent.
   d. 75 percent.

9. The FDA approval of the HPV- DNA test caused concern among many women’s health groups and agencies because the test lacked support from evidence-based guidelines.
   a. True
   b. False

10. What is the main limitation of concern with HPV-DNA testing in patients with clinically significant lesions?
    a. false-negative test results
    b. poor positive predictive value in positive test results
    c. both a and b
    d. neither a nor b

11. The apparent need for prevention and treatment strategies in developing countries can be identified by statistics that demonstrate a high percentage of women dying from cervical cancer in these countries.
    a. True
    b. False

12. Which regions account for the majority of the world’s cancer deaths?
    a. North America, South America, and Asia
    b. South America, Asia, and Europe
    c. Europe and Oceania
    d. Africa, Asia, and Central/South America

13. In order to employ an effective cervical cancer screening test in developing countries, the test must exhibit which characteristic(s)?
    a. affordable
    b. easy to use
    c. highly reliable
    d. all of the above

14. Which factor is most likely to increase one’s risk of developing cervical cancer?
    a. persistent infection with HPV
    b. smoking and drinking habits
    c. number of past sexual partners
    d. none of the above

15. Which type of testing is currently used to evaluate cervical cancer staging and spread into surrounding tissue?
    a. ten years
    b. 99 percent.
    c. 15 percent.
    d. 85 percent.

16. Which protein biomarker(s) can distinguish normal cervical basal epithelial cells.
    a. PSA
    b. K-RAS
    c. E6, K7
    d. P53, RB

17. Current studies demonstrate that the measurement of which protein biomarker(s) can distinguish normal cervical cytology samples from specimens that contain high grade squamous intraepithelial lesions (HSILs):
    a. P53, RB
    b. HPV E6
    c. K-RAS
    d. none of the above

18. Recent studies on keratin 17 have demonstrated that this biomarker can be a useful tool in predicting the prognosis and survival of patients with squamous cell carcinoma independent of tumor grade, stage, or HPV status.
    a. True
    b. False

19. Future testing methods must employ which characteristic(s) in order to make the most impact on the reduction of cervical cancer worldwide?
    a. highly sensitive
    b. highly specific
    c. highly informative
    d. all of the above

20. To what extent was the article well-organized and readable?
    a. Poor
    b. Excellent

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When verifying thermometers in a small clinical lab, does the NIST thermometer that you use to verify the other thermometer have to be NIST-certified (bought with the accompanying calibration report), or is the generic traceability standard enough to meet CLIA guidelines?

Traceability refers to an unbroken chain of documented calibrations that have been compared to a National Institute of Standards and Technology (NIST) set standard. 1 The NIST-certified thermometer was compared to a temperature device that was NIST-validated and deemed accurate, generally using a standard platinum resistance thermometer. 2,3

A “traceable” thermometer is not necessarily calibrated against the NIST reference standard, but verified against a thermometer that can be traced back to a calibrated NIST-certified thermometer. 2 This certified thermometer comes with a certificate validating its accuracy to one-tenth of the smallest division and includes an expiration date that indicates when the thermometer is to be recalibrated if it hasn’t already undergone revalidation. Certification of the NIST thermometer should be within the last five years. 4

There are several types of thermometers including partial immersion, total immersion, liquid-in-glass, dial thermometers, and digital thermometers. 2 Due to the toxicity of mercury-filled thermometers, many laboratories have phased them out. NIST is a resource often used to recalibrate industrial-grade thermometers. National Institute of Standards and Technology. 2015.

REFERENCES


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dures-IGs-for-Labs-Labs-Svcs-Final.pdf.

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The evolution of prenatal testing: how NIPT is changing the landscape in fetal aneuploidy screening

By Adam Wolfberg, MD, MPH, FACOG

The advent of non-invasive prenatal testing (NIPT) for fetal chromosomal aneuploidies has transformed the typical obstetrics practice and the prenatal care experience for many pregnant women. Also known as cell-free DNA (cfDNA) testing, NIPT has demonstrated better accuracy than conventional first-trimester screening and serum tests for the detection of fetal trisomies—aneuploidies that involve an extra chromosome—and its low false-positive rate in particular has reduced the need for more invasive, higher-risk diagnostic procedures, such as amniocentesis and chorionic villus sampling (CVS).

As a result, NIPT screening technologies offer potential benefits in both patient care and cost management. But most studies assessing the performance of NIPT have focused on high-risk patient populations. Recent study data indicate that the benefits of NIPT extend to the normal patient populations as well—and the results are particularly encouraging in the area of reducing false positives.

Fetal aneuploidy testing guidelines

The American College of Obstetricians and Gynecologists (ACOG) recommends that all women be offered aneuploidy screening during the first or second trimester of pregnancy. Most women are offered screening for trisomies 21 (Down syndrome), 18 (Edward syndrome), and 13 (Patau syndrome).

The risk for aneuploidies increases with maternal age. The estimated risks of fetal trisomies 21, 18, and 13 for a 20-year-old woman at 12 weeks of gestation are approximately 1 in 1,000, 1 in 2,500, and 1 in 8,000 respectively. The risks of these aneuploidies for a 35-year-old woman at 12 weeks of gestation are approximately 1 in 250, 1 in 600, and 1 in 1,800.

The most common prenatal screening test for fetal aneuploidy is the quad screen, or quadruple marker test, which is typically performed between 16 and 18 weeks of pregnancy, but occasionally up to week 20. Using a maternal blood draw, the quad screen examines four biochemical analytes: alpha-fetoprotein (AFP), a protein made by the fetus; human chorionic gonadotropin (hCG), a hormone made by the placenta; estriol, a hormone made by the placenta and the liver of the fetus; and inhibin A, another hormone made by the liver. In addition to screening for trisomies 21 and 18, the quad screen evaluates the likelihood of neural tube defects, such as spinal bifida and anencephaly, and abdominal wall defects.

The quad screen is easy to do, noninvasive, and inexpensive, and poses no risk of miscarriage or other pregnancy complications. The results of the quad test are evaluated along with maternal demographic information such as age, weight, gestational age, diabetic status, and race to derive a risk estimate using a mathematical model. The test correctly identifies about 80 percent of women who are carrying a baby with Down syndrome and has a false-positive rate of about five percent.

Another common screening test, the nuchal translucency (NT) scan, uses ultrasound to measure the clear (translucent) space in the tissue at the back of the fetus’ neck. Babies with certain abnormalities tend to accumulate more fluid at the back of their neck during the first trimester, which causes this clear space to be larger than average. That measurement is typically combined with a biochemical test to measure levels of pregnancy-associated plasma protein A (PAPP-A) and human chorionic gonadotropin (hCG) at 11 to 14 weeks. The combined screening test has demonstrated a detection rate of 95 percent for Down syndrome. This combination test is more accurate than the Quad, but the NT scan is more difficult to perform correctly given the challenges inherent in accurately measuring the NT. While it is widely available as the standard of care, it is not available everywhere due to lack of access to NT-certified sonographers. Other screening tests combining maternal serum analytes and the NT ultrasound are available, but are less frequently used.

The impact of false positives

A major drawback of both the quad screen and the NT scan/combined screen is the high false-positive rate. Both of these tests use a statistical modeling algorithm that sets a five percent false-positive rate for trisomy 21. Multiplying the five percent false-positive rate by an estimated four million pregnancies per year in the United States yields 200,000 potential false-positive results for Down syndrome annually.

Screening for fetal aneuploidy using cfDNA testing was introduced in 2011. Using a maternal blood draw, it can be performed as early as 10 weeks and poses no risk of miscarriage or other complications to the pregnancy. Studies using today’s commercially available options, all lab-developed tests, report sensitivity and specificity above 99 percent for the detection of Down syndrome. Some of these tests also measure and report fetal fraction—the percent of fetal DNA in the mothers’ blood—to provide an individualized risk score for each patient. Fetal fraction is the most critical quality control metric that impacts the accuracy of this testing.

However, the high-risk populations used in most studies of cfDNA testing to date are not representative of the normal pregnancy population, and thus they have not provided good statistical samples for the purpose of analyzing false-positive rates.

The NEXT study

That changed in 2015 with the publication of the results of the Noninvasive Examination of Trisomy (NEXT) study in the New England Journal of Medicine. The NEXT study, 18,955 women were enrolled, and results from 15,841 patients were available for analysis. The patients represented a general prenatal screening population (ages 18 to 48) from practices in the U.S., Canada, and Europe. This yielded a real-world demographic that is representative of the way obstetric screening is practiced rather than how it is studied in an academic setting.

The primary focus of the study was to compare the performance of cfDNA testing and standard first-trimester screening (with measurement of nuchal translucency and biochemical analytes) in risk assessment for trisomy 21. The authors concluded that “the performance of cfDNA testing was superior to that of traditional first-trimester screening for the detection of trisomy 21 in a routine prenatal population.” In the study, the cfDNA test demonstrated higher sensitivity (99.5 percent) and higher positive predictive value (80.9 percent) for Down syndrome than did standard screening (78.9 percent and 3.4 percent, respectively).

One of the most significant findings in the study, however, was the difference in the rate of false-positive results. The false-positive rate for Down syndrome with the cfDNA test was 0.06 percent—nearly 100 times lower than the 5.4 percent false-positive rate for standard screening. The implications of this improvement for prenatal care in the U.S. are significant.

Implementation considerations and future directions

The performance of cfDNA testing for the detection of trisomy 21 in a routine prenatal population was clearly superior to that of traditional first-trimester screening in the NEXT study. Having clear answers early in pregnancy regarding chromosomal abnormalities through the use of cfDNA testing can help reduce the frequency of invasive diagnostic procedures in high-risk positives and alleviate a significant amount of patient anxiety.

Visit the references on the MLO website, www.mlo-online.com.
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Beyond conventional cell analysis: the latest science and technology in flow cytometry

By Sharlene Wright

The field of flow cytometry has enabled a greater understanding of cell biology and immune function over the past 50 years. The power of the technology is used in basic cell biology research, pharmaceutical discovery, and routine clinical diagnosis, as well as in agricultural and environmental applications. This progress has been made possible by a higher number of analytical parameters to measure cells in suspension. The first cytometers were systems capable of merely three or four parameters, using a single laser and four detectors, and were the size of a small car. Today, however, flow cytometers (including cell sorters) can analyze more than 30 parameters, and new technology in benchtop analyzers can deliver exponentially better performance in a footprint less than 45 cm².

Shifting paradigms
This paradigm shift, of higher performance in a small, less expensive instrument, is driven by investigators who want to capture the power of flow cytometric analysis, but don’t want to devote a career to learning the instrumentation. The democratization of flow cytometry is enabled by key advances in technology. Prominent concepts in other scientific fields such as the telecommunications industry are being leveraged to allow the subsystems to be miniaturized while at the same time providing even better performance. These compact high-performance systems not only deliver better performance than historically expensive systems, but they are also easy to set up, operate, and maintain, enabling a greater number of laboratory scientists to leverage the power of flow cytometry.

Seeing the light
Performance of flow cytometers is typically measured in their capacity to resolve and sensitivity to detect dim and/or rare populations. In this regard, efficient light management for optimal excitation and emission of fluorochrome-tagged cells is critical to performance.

With conventional analyzers, laser excitation sources are optimized by shaping and focusing light through a series of lenses and filters onto a flow cell where cells are hydrodynamically focused. However, newer systems use unique laser designs that are focused onto a flow cell with integrated optics. These systems can ensure both maximum excitation of the dyes not only on (and within) cells, but also maximum collection of the emitted light for integration and measurement. When designing a compact flow cytometer, the use of fiber optics to carry light is an efficient way of transmission, providing flexibility in laying out system components. These cables capture emitted light to deliver it onto a unique detector array, reducing cross talk between channels, which improves performance.

Another recent development is a key concept borrowed from the telecommunications industry, the wavelength division multiplexor (WDM), which is used for light detection and measurement. The WDM is the method used to deconstruct and measure multiple wavelengths of light as signals that relate to analytical parameters. These detectors are highly sensitive semiconductor devices used to measure each parameter. By contrast, conventional cytometers to date have (and continue to use), photomultiplier tubes (PMTs). The major advantages of the use of alpha photodiodes (APDs) over PMTs include but are not limited to 1) perfect linearity; 2) 4-5x the quantum efficiency; 3) higher dynamic range, $10^9$ vs $10^7$; and 4) significantly smaller size and about one-tenth the cost.

The WDM’s innovative and simple design uses a single bandpass filter to select the various colors of light. This contrasts with traditional systems, which use a series of dichroic steering- and bandpass-filters that bounce the light along an array leading to successively less available light, resulting in diminishing light collection efficiency and ultimately compromising fluorescence sensitivity and resolution.

Simplifying the complex
Leveraging the linearity of detection systems that use APDs in the operation of the cytometer can be dramatically simplified due to the predictability of the signals. The linear gain and the normalization performed during the daily QC routine takes care of the relative variations during instrument setup typically seen in instruments. Further, setting up a multicolor assay is simplified by using a software gain-only adjustment. The linearity of gain adjustment also simplifies the typically arduous task of spectral compensation which has been the barrier for many to push to higher number of colors/parameters. Leveraging the APD linearity, new software algorithms have been developed to facilitate setup and analysis of multi-color experiments by simplifying compensation.

It is now possible to create a compensation library which stores the APD gain settings and spectral spillover coefficients for every parameter and multicolor combination. This allows users to make a virtual spectral compensation matrix selecting various single colors from the library. In addition, the library can intelligently adjust the compensation values when gains are adjusted due to the predictive responses of linear APDs. The result is a dramatically simplified and intuitive method of setting up multicolor applications.

continued on page 22
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The size factor

Along with multicolor performance, flow cytometry has proven to be a valuable tool in small particle research, which is growing exponentially along with the medical research field’s understanding of microvesicle and exosome native biology and their potential applications in diagnostics and therapeutics. These extracellular nanoparticles are heterogeneous cell-derived particle populations in a size range between 50 nm and 500 nm. Flow cytometry is unique in that it is not only able to measure size but can deliver much more information about the characteristics and function of these nanoparticles using multiple fluorescent markers.

For most cytometers, however, measuring less than 300 nm is difficult if not impossible because of the fact that they deliver relative sizing information using forward scattered light off of the 488 nm Blue laser. For these systems, particles of less than 1 mm (1,000 nm) usually fall below the noise threshold of the laser and detector sub-systems. In contrast, newer systems use principles of Mie scattering which predicts that with lower wavelengths of excitation there will be an increased amount of scattered light and improved resolution. Therefore, measuring scattered light from a shorter-wavelength 405 nm Violet laser versus a longer-wavelength 488 nm Blue laser will allow the system to resolve smaller particles. The use of the Violet Side Scatter parameter enables systems to detect particles of less than 0.2 mm (200 nm) in size, enabling excellent resolution of microparticles.

In summary

Combining powerful performance and innovative design and technology, it is possible to deliver a compact, easy-to-use flow cytometry system. Pushing the ‘norms’ of conventional flow cytometry, today’s—and tomorrow’s—systems enable complex research into high-content applications in cell biology, as well as a deeper understanding of the advantages gained from the emerging nanoparticle frontier. Flow cytometry is a powerful tool for interrogating complex biological questions at the forefront of biomedical and life science research and increasingly for clinical laboratory applications. Today’s investigators want to harness that power and are demanding smaller and more powerful instruments that are more affordable and easier to use. Using innovation, engineers are able to deliver solutions to meet the challenge.

Sharlene Wright serves as a Director of Marketing for Flow Cytometry for Beckman Coulter Life Sciences. She has more than 23 years in the flow industry in various roles including R&D, Field Support, and Marketing. She has led the development effort and launch of several successful innovative Flow Cytometry Solutions, including most recently the CytoFlex Flow Cytometer.

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Liquid biopsy: the time is even more right!

By Lyle Arnold, PhD, and Raaj Trivedi

Editor’s note: Almost one year ago, in the February 2015 issue of MLO, Dr. Arnold and Mr. Trivedi published the article, “Liquid biopsy: the time is right.” We asked them for an update for publication in this issue, and we are pleased to share their response with readers.

Precision therapies that target alterations around the EGFR and ALK gene have radically impacted the way we care for a large subset of lung cancer patients. The movement in personalized medicine is well on the way to transforming many cases of cancer into a relatively manageable chronic disease. However, due to challenges related to obtaining sufficient biopsy material, clinicians who adhere to conventional diagnostics cannot know the molecular status of a large number of their patients with a lung cancer diagnosis. They are not able to monitor the effectiveness of chemotherapy, and they do not have the tools to determine whether the patients are eligible for clinical trials. Liquid biopsy is primed to change that.

Limited tissue availability

Today, there are a number of drug therapies for lung cancer, either on the market or in clinical trials, which require a companion diagnostic test to identify eligible patients. These biomarkers include EGFR, ALK, ROS, BRAF, and PDL-1 for lung cancer patients. The challenge is to identify patients who qualify for these companion therapies when tissue is available in limited abundance. And therein lies the problem: tissue availability is very often limited. Some recent examples:

- In 2010, the International Working Group on Multidisciplinary Lung Adenocarcinoma Classification estimated that only 57 percent of such biopsies had sufficient tissue for genomic analysis after initial pathology diagnosis and staining. (Please visit www.mlo-online.com to read references for this article.)
- In the Lung Cancer Mutation Consortium study of 14 U.S. academic centers, one oncogenic driver mutation could be tested in 91 percent of tissue specimens, but the ten desired target gene alterations could only be measured in 66 percent of them.2
- In a study of advanced breast cancer, yield for molecular testing was only 36 percent for biopsy samples from bone, the most common site of breast cancer metastasis.3

When tissue biopsies have insufficient tumor cell content or are QNS (quantity not sufficient) for genomic analysis, formalin-fixed paraffin embedded (FFPE) material or slides for tissue-based next generation sequencing are often unavailable or unobtainable, or maybe outdated because they no longer reflect the current genomic status of the tumor.

Liquid biopsy as a feasible strategy

It is in this context that interest in the kind of diagnostic now commonly known as “liquid biopsy” has been growing substantially in recent years—and, indeed, during the year since we last addressed readers of MLO. What most recommends liquid biopsy is that it circumvents the problem of tissue availability. A liquid biopsy has the ability to detect solid tumor material from a simple blood draw. This is accomplished by either evaluating circulating tumor cells (CTCs) that are derived from lesions in the body that enter the blood stream or by evaluating circulating tumor DNA, where fragments of DNA from a tumor cell are shed into the blood stream. Either approach can give clinicians the ability to interrogate tumor material that may not otherwise be accessible for analysis. The good news for patients is that it is not an either/or scenario. CTCs and ctDNA can be complementary to one another, and both of these components may allow testing of a patient’s eligibility for targeted therapies.

This technique has generated significant interest and excitement—particularly in the clinical realm. Until recently, evaluating CTCs and ctDNA was seen primarily as an academic endeavor and not something ready for mainstream commercialization. Part of the challenge is that tumor material in the blood might be present only in small quantities when compared to the total genomic content produced by normal blood cell populations. Therefore, effectively capturing and assaying these rare components has been seen as a major obstacle. But recent progress in this area has indicated that tumor cells even in minute proportion can provide a noninvasive, ongoing picture of a patient’s cancer. Clinical oncologists are now using liquid biopsy to gain real-time insight into how best to treat a patient’s cancer.

Lung cancer applications

Lung cancer is one of the diseases that most requires a liquid biopsy approach. According to the National Cancer Institute, it is estimated that 220,000 patients are diagnosed with lung cancer each year, and approximately 20 percent to 25 percent may not have enough tissue to interrogate for one of the many targeted therapies available today.

The recent approval of Tagrisso (osimertinib), a new treatment option for metastatic lung cancer patients whose tumors have a specific epidermal growth factor receptor (EGFR) mutation (T790M) and whose disease has gotten worse after treatment with other EGFR-blocking therapy, is a case in point. Currently, to qualify for this breakthrough drug, patients would need repeat biopsies as they are progressing on current therapy. That means that the tumor burden has substantially increased, and the performance status of the patient may put them at high risk for side effects and co-morbidities if a repeat biopsy is obtained during a surgical procedure. However, a simple blood draw consisting of 10ML of fluid can identify the presence of the tumor alteration. In this case, a liquid biopsy can address a growing unmet need for patients who need re-biopsies because their cancer is starting to progress as they are not responding to their current therapy.

Liquid biopsy is a drastic change from prior technologies that have been available on the market. But liquid biopsy is not just about counting the amount of tumor material in the blood (enumeration). Although other available systems evaluate and measure intact CTC to give a prognosis of overall survival, the potential predictive value of biomarker analysis on this sample type creates a much larger value proposition for qualifying patients for targeted therapy options. Evaluating biomarkers as “predictors” has the potential to help guide treatment decisions. In a sense, the ultimate potential of a liquid biopsy will allow us to understand specifically what kind of molecular changes are happening in the tumor in real time—a very big step beyond where CTCs are today, clinically.

Lyle Arnold, PhD, serves as Senior Vice-President of Research & Development and Chief Scientific Officer for Biocept, Inc.

Raaj Trivedi serves as Vice President, Commercial Operations, for Biocept, Inc.
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In the news: antibiotic resistance

By Alan Lenhoff, Editor

We don’t know what will be remembered as the most important healthcare stories of 2015, but almost certainly one of them will be the heightened awareness of the threat posed by methicillin-resistant *Staphylococcus aureus* (MRSA) and other antibiotic-resistant bacteria, and the ramped-up efforts to reverse the alarming increase in those potential threats to public health. Both within the medical community and among the general public, concerns have grown about bacterial infections that have long been treatable—but may have to be dealt with without established drug therapies. Public health experts have come together to devise strategies and best practices to slow the development of antibiotic-resistant organisms by reducing over-prescription, and to develop new therapies as well.

The battle against antibiotic-resistant bacteria gained momentum last June, when the Obama Administration hosted the White House Forum on Antibiotic Stewardship. Scientists, clinicians, leaders of the diagnostic and pharmaceutical industries, and healthcare policymakers met to raise awareness and agree on broad strategies. Those consensus strategies include avoiding the unnecessary prescription of antibiotics; obtaining data to gauge the unnecessary prescription of antibiotics; employing diagnostic advances; and agree on broad strategies. Those consensus strategies include avoiding the unnecessary prescription of antibiotics; employing diagnostic advances; and agree on broad strategies.

The Joint Commission also has a Speak Up campaign to help with educating patients about antibiotics, focusing on the do’s and don’ts of antibiotic use. The Joint Commission also has a Speak Up campaign to help with educating patients about antibiotics, focusing on the do’s and don’ts of antibiotic use. The Joint Commission also has a Speak Up campaign to help with educating patients about antibiotics, focusing on the do’s and don’ts of antibiotic use.

In the meantime, news came across the healthcare wires on this topic with regularity, including scientific advances, policy proposals, and public awareness issues. Here are a few of the items that have collected in my in-box.

Public misunderstanding

As the World Health Organization (WHO) ramps up its fight against antibiotic resistance, a new multi-country survey shows that people are confused about this major threat to public health and do not understand how to prevent it from growing.

Antibiotic resistance happens when bacteria change and become resistant to the antibiotics used to treat the infections they cause. Overuse and misuse of antibiotics increase the development of antibiotic-resistant bacteria, and this survey points out some of the practices, gaps in understanding, and misconceptions which contribute to this phenomenon.

“The findings of this survey point to the urgent need to improve understanding around antibiotic resistance,” says Dr. Keiji Fukuda, Special Representative of the WHO Director-General for Antimicrobial Resistance. “One of the biggest health challenges of the 21st century will require global behavior change by individuals and societies.”

The multi-country survey included 14 questions on the knowledge and use of antibiotics and on antibiotic resistance, and it used a mix of online and face-to-face interviews. It was conducted in 12 countries: Barbados, China, Egypt, India, Indonesia, Mexico, Nigeria, the Russian Federation, Serbia, South Africa, Sudan, and Vietnam. While not claiming to be exhaustive, this and other surveys will help the WHO and its partners to determine and address key gaps in public understanding of the problem and misconceptions about how to use antibiotics.

Some common misconceptions revealed by the survey include:

- Three-quarters (76 percent) of respondents think that antibiotic resistance happens when the body becomes resistant to antibiotics; obtaining data to gauge the unnecessary prescription of antibiotics; employing diagnostic advances; and agree on broad strategies. Those consensus strategies include avoiding the unnecessary prescription of antibiotics; employing diagnostic advances; and agree on broad strategies.
- More than half (57 percent) of respondents believe that individuals are not at risk of a drug-resistant infection if they personally take their antibiotics as prescribed. More than four in ten (44 percent) of people surveyed think antibiotic resistance is only a problem for people who take antibiotics regularly. In fact, anyone, of any age, in any country can get an antibiotic-resistant infection.
- Two-thirds (66 percent) of respondents believe that antibiotic resistance is only a problem for people who take antibiotics regularly. In fact, anyone, of any age, in any country can get an antibiotic-resistant infection. Two-thirds (66 percent) of respondents believe that antibiotic resistance is only a problem for people who take antibiotics regularly. In fact, anyone, of any age, in any country can get an antibiotic-resistant infection. Two-thirds (66 percent) of respondents believe that antibiotic resistance is only a problem for people who take antibiotics regularly. In fact, anyone, of any age, in any country can get an antibiotic-resistant infection.

Questions about MRSA therapy

A new study sheds light on how treatment of MRSA with certain antibiotics can potentially make patients sicker. The findings by Cedars-Sinai scientists could have implications for managing the virulent form of the common staph infection.

MRSA causes more than 80,000 invasive infections and 11,000 related deaths per year, according to 2011 figures from the CDC. Although many studies have established that MRSA infections cause more severe diseases compared with normal staph infections, what makes MRSA so pathogenic is not entirely clear. The Cedars-Sinai researchers suggest that one answer lies in part of the very name of the superbug—methicillin-resistant. In laboratory mice, treatment with antibiotics similar to methicillin, called beta lactams, caused the MRSA...
bacteria to build cell walls that are highly inflammatory and damaging to tissues.

The Cedars-Sinai scientists explain that beta-lactam antibiotics kill normal staph bacteria by inactivating their cell-wall-making enzymes. But one of these enzymes, PBP2A, which is induced when MRSA is exposed to beta-lactam antibiotics, is not rendered inactive by the antibiotics. In fact, PBP2A allows the superbug to continue making its cell wall. Further, the cell wall’s structure differs from that of normal staph.

“This altered cell wall induces a powerful inflammatory response,” says study co–senior author, David Underhill, PhD. “In mice infected with MRSA, infection of PBP2A with methicillin led to more inflammation and pathology.”

The authors say their study findings in mice raise the possibility that prescribing beta-lactam antibiotics to treat infections in humans may worsen the infection, should the source prove to be MRSA. The potential dilemma for physicians is that beta-lactam antibiotics’ overall effectiveness often makes them the first line of defense when the origin of a severe infection is unknown. In addition, the time it takes to culture MRSA can make early diagnosis difficult.

**Using diagnostic tests to stop antibiotic overuse**

Doctors should use diagnostic tests for infection more often to prevent overprescribing of antibiotics that are responsible for the rise of drug-resistant bacteria, says the British government’s so-called superbug tsar.

Lord Jim O’Neill, who chairs the Review on Antimicrobial Resistance, says it often seems cheaper and easier to give patients antibiotics rather than using tests to find out if they really need them. He also calls for more investment to make tests faster and more accurate.

Unnecessary over-prescribing of antibiotics has helped drive the rise of drug-resistant bacteria, and Lord O’Neill’s latest report asserts that the medical profession has been slow to embrace the use of diagnostic testing before antibiotics are prescribed. Most prescriptions are still based on so-called “empirical” diagnosis: physicians use their expertise, intuition, and professional judgement to guess whether an infection is present and what is likely to be causing it.

“For far too long we haven’t recognized the huge cost to society of increasing resistance when we use antibiotics that we don’t need—such as antibiotics for flu which have no effect except to increase the chances of superbugs developing,” says Lord O’Neill. “To avoid the tragedy of 10 million people dying every year by 2050, the world needs rapid diagnostics to improve our use of antibiotics.”

Lord O’Neill is expected to deliver a comprehensive plan of action in the late spring of 2016. His interim report calls for:

• Subsidizing the cost of diagnostic tests to make their use more competitive than issuing on-demand prescriptions of antibiotics
• Providing funding for developing new diagnostic tests
• Building a long-term economic case for rapid diagnostics.

His “wish-list” also includes a rapid diagnostic test that could be used by patients at home to indicate if they had a bacterial infection that might be treatable with antibiotics or a viral infection that would not.

**Drug-resistant E. coli in community hospitals**

Drug-resistant *E. coli* infections are on the rise in community hospitals, where more than half of United States patients receive their healthcare, according to a new study from researchers at Duke Medicine.

The study reviewed patient records at 26 hospitals in the Southeast. By examining demographic information, admission dates, and tests, the researchers also found increased antibiotic-resistant infections among community members who had limited exposure to healthcare settings, but who may have acquired the bugs through some other environmental factors.

The study data were gathered through the Duke Infection Control Network (DICON), which helps community hospitals and surgery centers across the U.S. prevent infections, using education and evidence-based strategies. The data showed that between 2009 and 2014, the incidence of drug-resistant extended-spectrum beta-lactamase (ESBL)-producing *E. coli* doubled from a rate of 5.28 infections per 100,000 patients to a rate of 10.5 infections per 100,000. The median age of patients infected with *E. coli* was 72 years.

“Overall, the majority of *E. coli* infections occurred after healthcare exposure, which makes all hospitals, big and small, important areas of focus to reduce transmission,” says lead author Joshua Thaden, MD. “It’s important to consider that a patient’s skin may be colonized with drug-resistant bacteria, but because the patient does not display symptoms, providers may not test him or her use extensive contact precautions during care.

I think we may be close to a point where it becomes worth the cost and effort to actively screen patients for resistant *E. coli*.”

Looking at the timing of patients’ infections and when they were last in contact with a healthcare setting, the researchers also discovered that people with infrequent healthcare contact were acquiring the superbug at an even faster rate than patients who have regular contact with hospitals or nursing homes. The data showed a greater than three-fold increase in community-associated infections between 2009 and 2014.

**Three-drug combination effective**

Three antibiotics that, individually, are not effective against MRSA, “kill the deadly pathogen when combined as a trio, according to new research. The researchers, at Washington University School of Medicine in St. Louis, have killed MRSA in test tubes and laboratory mice, and believe the same three-drug strategy may work in people.

The trio of antibiotics are meropenem, piperacillin, and tazobactam. They are beta-lactams (as in the Cedars-Sinai study described on page 26), and they have not been effective against MRSA for decades.

Working with collaborators at Barnes-Jewish Hospital, investigator Gautam Dantas, PhD, and his team tested and genetically analyzed 73 different variants of the MRSA microbe to represent a range of hospital-acquired and community-acquired forms of the pathogen. The researchers treated the various MRSA bugs with the three-drug combination and found that the treatments worked in every case.

Then, in experiments conducted by collaborators at the University of Notre Dame, the team found that the drug combination cured MRSA-infected mice and was as effective against the pathogen as one of the strongest antibiotics on the market.

Dantas explained that the drugs, which attack the cell wall of bacteria, work in a synergistic manner, meaning they are more effective combined than each alone.

The researchers also found that the drugs didn’t produce resistance in MRSA bacteria—an important finding, since more and more bacteria are developing resistance to available drugs.

“This three-drug combination appears to prevent MRSA from becoming resistant to it,” Dantas says. “We know all bacteria eventually develop resistance to antibiotics, but this trio buys us some time—potentially a significant amount of time.”

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Co-creating critical limits for enhanced acute care: proven need and web knowledge base

Part 2: Standard of care, what it means and how it is applied

By Gerald J. Kost, MD, PhD, MS, FACP

Editor’s note: A few months ago, I sent Dr. Kost, who serves as the reviewer of the Table of Critical Limits that appears in MLO’s annual publication Clinical Laboratory Reference (CLR), a question from a reader, in the hopes that he would respond to it for MLO’s recurring “Tips from the Clinical Experts” column. The question was this: “What are critical values for routine coagulation testing (i.e., PT and PTT) for therapeutic and diagnostic testing?”

Dr. Kost responded with a discussion of a scope far beyond that of typical “Tips” answers: A two-part discussion—and call to action—on the broader topic of standardized critical limits for enhanced acute care. I enthusiastically took Dr. Kost up on his offer to address this important topic for MLO once again, as he did for the magazine with a colleague more than 20 years ago. MLO ran Part 1 in the December 2015 issue, and is pleased to present Part 2 in this January 2016 issue.

In Part 1 in the December 2015 issue of MLO, I focused primarily on coagulation critical limits and showed that we could use the web productively. Now I move on to discuss some important legal aspects of the definition and application of critical limits. These legal aspects apply broadly. They are not limited to any one category of analytes.

The fundamental concept of standard of care is based on the legal case, Vaughan vs. Menlove, 3 Bing NC 468, 132 ER 490 (CP 1837), wherein the judge instructed the jury to reason whether the defendant “proceeded with such reasonable caution as a prudent man would have exercised under such circumstances.” In simple terms, it is the level at which the average, prudent provider in a given community would practice and how similarly qualified practitioners would have managed the patient’s care under the same or similar circumstances.

When under oath, whether during a deposition or in a court of law, including arbitration proceedings, one must be able to recite this definition of the standard of care and explain whether or not the medical actions being challenged met the standard of care under the same or similar circumstances.

Legal Principles. The easiest way to appreciate the legal principles that unfold when there is an adverse outcome related to critical limits is to consider that you, the reader, could be called to task and required to explain during deposition or to a jury: a) each and every detail of your critical values policy and each entry on your critical limit list; b) the rationale and consensus process by which you selected them; and c) the manner in which they were originally derived, implemented, updated, changed, and practiced in your hospital setting or in point-of-contact areas. The last could include even primary care sites and homes in the community of patients who self-test.

Application. Specifically, you will be interrogated intensively regarding the selection process and, under cross-examination, must explain any and all variances from published national survey critical limits deemed admissible for comparison by the court. Usually, admissibility means you can use the data you find online in the MLO CLR (Clinical Laboratory Reference) tables, in the publications cited herein, or in other pertinent factual sources revealed during discovery by either side.

Sometimes attorneys will refer to the mean as indicative of the standard of care, and at other times the median. So a basic appreciation of the differences in parametric versus non-parametric statistics is helpful. All levels of personnel can be subjected to these legal interrogations—the clinical laboratory professional, nurse, laboratory director, IQCP planner, physician, administrator, information technology staff, and others. You might find yourself on the stand in court, so it is important to understand the court’s viewpoint!

How surveys are treated by attorneys and courts

Attorneys and the courts are interested in establishing the standard of care and whether it was breached in a particular case. In some instances, a court has thrown out critical values publications based on surveys of professional members/subscribers, because the data were deemed “heresy,” defined as member/subscriber opinions potentially at variance and not reflecting the broad national standard of care. On the other hand, legal authorities accept results based on objectively defined and statistically sampled surveys with an adequate response rate, typically in the order of 70 percent (e.g., references 1-3 at the end of this article).

Various professionals with proven credentials, typically demonstrated by published track records in critical values innovation, research, and practice, are called upon as expert witnesses in litigated controversies involving inappropriate selection of quantitative critical limits or qualitative critical values, failure to follow hospital written policies, lack of timely notification of critical results, instrument measurement errors leading to missed critical test result findings, and other factors, such as results overlooked at the point of care, that may be considered medical malpractice.

The impact of legal outcomes

Settlements, which depend on both adverse outcomes and their causality, such as the death of an adult from a missed critical cardiac biomarker result in the clinical laboratory or at the point of care, or the lifelong disastrous mental impairment of an infant from kernicterus following an overlooked elevated bilirubin level not treated promptly with phototherapy, can be in the range of millions of dollars.

Patient harm and the dysfunctional consequences of sloppy communication practices motivated The Joint Commission (TJC) to integrate urgency rules for critical results notifications in the National Patient Safety Goals (NPSGs) several years ago. These derisking measures currently appear in the 2015 NPSGs. The reader can find the NPSGs online.
However, TJC offers no precise guidance for selecting critical limits nor justifying them medically. That part is up to the individual hospital team, and in this article, we are positing that the time has come for hospitals everywhere in America to share their critical values experience in the web-public domain in order to improve collective practice and the standard of care in this area.

Public domain listings—mostly pros and hardly cons
What has not happened, and should, is proactive action by hospitals and their laboratory professionals to make critical values lists and policies freely and easily available for harmonization, while at the same time mitigating risk from inconsistent practices, clumsy thresholds, or just plain confusion, such as our current survey found for PT and aPTT. This article calls to action transparency, consistency, cost-effectiveness, and ultimately, harmonization. Further, this approach is not based on guidelines written from professional opinions, the lowest form of evidence, but instead on actual critical limits used every day in American hospitals—that is, raw data.

Note that since the publication of the JAMA and Pediatrics papers about 25 years ago,1,2 we have not observed a single situation in which attorneys have been unable to produce lists and policies, no matter how well cloistered, from individual hospitals during the discovery phase of litigation and then present them as evidence for or against the legal action, as the case might be. I have seen six-foot tall poster boards of critical limits lists presented to juries, and unbeknownst, even from my own institution!

Therefore, especially now in the current e-world there is nowhere to hide, and hospitals have little to be concerned about in regard to proactively making critical limits lists public and much to gain if they do so. National practices will become transparent, including inadvertent outliers, omitted analytes deemed essential, forgotten points of care, deficiencies in identifying age-related limits, confused therapeutic constraints, or simply listing too many analytes and reducing impact.

Establishing a public domain knowledge base using the web
Individual hospitals, clinics, and primary care sites should first originate or confirm frequently critical values based on their own consensus process among laboratory, clinical, and other stakeholders. Operators and patients performing testing at points of need also need guidance. In fact, this study may be the first web-based documentation of an INR of 5.0 as a reasonable critical limit for the standard of care in coagulation. Besides being well confirmed by other studies, its novelty lies in proving the feasibility of using the web as a suitable media in the worldwide call to action (In this regard, the reader might return to the sidebar in Part 1, MLO 47(12):36.)

Therefore, it is recommended that all hospitals publish their list(s) of critical limit list(s) and associated policy(ies) on the web, in order to co-create an actionable public domain knowledge base with unburdened free access for practitioners, researchers, point-of-care coordinators, patients, and even attorneys.

Care must be taken to assure that point-of-care results, wherever and by whomever they are produced along patient spatial care paths (SCP), starting in the home and possibly communicated to the clinical team, follow the same uniform practice standard of care, including rapid bidirectional notifications and clear documentation of how long it takes for the decision maker to receive critical test results and take appropriate action.

Conclusions and recommendations
- The selection and design of critical limits, qualitative critical values, and attendant policies should be evidence-based. The WorldWide Web presents collective groups of medical professionals with a unique opportunity to proactively contribute to a publicly shared knowledge base by posting their hospital documents and then extracting from the web “colligative” properties that harmonize the practice of urgent critical test result notifications.
- The key is getting adequate numbers of postings on the web and achieving fairly even geographic representation of states throughout the nation and of countries globally, so that researchers can sample from various sets of raw data without bias error-free, and also be assured of an immediately adequate response rate, which is more or less automatic if access is freely available and enough primary documents are available. Indeed, the primary documents should be collected, that is, extracted from websites, to avoid transcription errors and flaws resulting from truncation as described above.
- With such a knowledge base resource, we will be able to answer important questions, such as whether there has been “drift” in quantitative critical limits since the first national surveys were conducted 25 years ago, why so many critical values are now listed, whether they are necessary, and which critical values reflect the “core” essential ones for patient survival.
- Additional questions include, but are not limited to, the following: Has that core been altered by the implementation of POCT? Can practice be more impactful for outcomes? How can inconsistencies be eliminated to restore original purpose, treating life-threatening conditions immediately upon their detection? This call to action for a freely shared web knowledge base and global harmonization seeks and suggests the means to facilitate high quality patient-focused decision making at the speed of life.

Disclaimer
The results presented in this article are preliminary, subject to change upon gathering of more data during this new e-round of national surveys, and likewise, interpretations are tentative. The small sample for POCT may not be representative of the larger population who use POC critical limits, and here, only INR is reported, while actually other POC instrument analytes, such as glucose, potassium, and troponin I, were posted in the lists we gathered online—so beware this new facet of the standard of care!

REFERENCES

Gerald J. Kost, MD, PhD, MS, FACP, serves as Founding Director of the Point-of-Care Testing Center for Teaching and Research (POCTCTR) and as Emeritus Faculty in the School of Medicine, UC Davis. He is also founder of Knowledge Optimization in Davis, CA.
What’s the buzz in drug testing?

Compiled by MLO staff

**Novel psychoactive substances (NPS)**

One of the current and most challenging areas for drug-testing in both clinical and forensic laboratories is that of novel psychoactive substances (NPS). The chemical make-up of these substances is constantly evolving, which means that laboratories are facing a serious problem when it comes to detection. Synthetic cannabinoids, more commonly known as “spice,” are one of the most prevalent NPS and have been marketed as legal versions of cannabis since around 2008—so they are not a new problem. NPS compounds are designed to circumvent government legislation as suppliers continually tweak their products. As active ingredients are synthesized in unregulated laboratories, the drug quality also varies considerably.

There has been a rise in the international prevalence of NPS. In the United Kingdom, call-outs to paramedics for treatment of adverse effects from NPS have increased significantly in recent years. Scotland now has an average of six emergency NPS call-outs per day. Adverse effects may include confusion, agitation, and drowsiness, and a small number of deaths have been linked to these compounds. There have also been reports of patients demonstrating extreme aggression and paranoia.

Due to the novel nature of these compounds, the metabolic pathway is often not known, and that makes it more difficult to devise tests for their detection. As some drug users are moving away from the typical drugs of abuse that fall into the standard testing profiles, it is important for the clinical testing laboratory to keep up with the evolving nature of NPS.

**The future of toxicology testing in the clinical lab**

2016 will usher in changes in the way laboratories are reimbursed for their toxicology testing. For mid-size to large laboratories, the changes will not greatly impact total revenue.

CMS (Centers for Medicare & Medicaid Services, previously known as the Health Care Financing Administration) published the Clinical Laboratory Fee Schedule (CLFS), which mandated a lower reimbursement for presumptive testing starting January 1, 2016. Familiar codes, such as Codes G0431 and G0434, were eliminated, and three new codes are in place for presumptive testing. For the presumptive instrumented testing the complexity no longer impacts reimbursement.

CMS has added four additional codes that will be implemented for the definitive testing in 2016. The reimbursement for this definitive testing has also been decreased, which could make it more difficult for small and medium-sized laboratories to cost-effectively implement new definitive methodologies. Laboratory leaders might choose to consult with a coding specialist, to better understand how these changes may impact their individual lab.

Overall, laboratories that perform toxicology testing will become more focused on what is needed as a STAT test and what testing should be sent to an outside laboratory for testing.

**Industrial Drug Testing**

The year was 1987. Disposable contact lenses became available for commercial distribution; the FDA approved the anti-AIDS drug AZT; and Prozac made its debut in the United States. Nineteen eighty-seven was also the year Partnership for a Drug-Free America debuted the famous anti-drug campaign, “This Is Your Brain on Drugs.” You know the one…the egg frying in the pan.

 Appropriately enough, the cover story for MLO in October 1987 was, “Drug Abuse Screening: Should your lab enter this booming market?” The magazine cover art—a colorful montage of narcotics in their “natural” state, including green marijuana leaves, red opium poppies, and yellow coca blooms—attracted readers’ attention.

In 1987, industrial drug testing was a burgeoning specialty. Essential elements of the article included the importance of skilled specimen collection, test accuracy, and confidentiality, (reminding laboratories that they can be subject to defamation suits) and, of course, the potential to increase laboratory revenue.

As the saying goes, "the more things change, the more they stay the same." Today, 28 years later, employee drug screening is more necessary than ever thanks to greater employment and sky-rocketing drug and substance abuse. The demand for toxicology labs is high, with estimates of a three-billion dollar industry this year, alone.

However, industry experts say the soaring cost of drug screening is not just a reflection of the increasing use of narcotics, but of unnecessary testing, overbilling, and sometimes even outright fraud by doctors and drug screening companies.

And although industrial drug screening has changed over the years in terms of methods, protocols, and demand, the final result of a positive drug test has not: denial of employment or dismissal.
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**MedTest distributes Mindray’s BC-3600 Auto Hematology Analyzer**

Mindray and MedTest received 510(k) clearance from the U.S. Food and Drug Administration to market the Mindray BC-3600 Auto Hematology Analyzer. This analyzer meets the testing needs of small- and mid-volume hematology laboratories while offering features commonly found on large-volume analyzers. Features include: closed-tube sampling; a large 10.4 inch color touchscreen; bar-code reader; LIS connectivity; 3-part difference; cyanide-free reagents; throughput of up to 60 samples per hour; and storage capacity up to 40,000 results with histograms. Nearly all scheduled maintenance procedures are automated by touch buttons. The intuitive software enhances workflow efficiency and offers a pleasant user experience.

For more information, please visit: http://www.medtestdx.com/product/1225

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The Freelite assay is composed of two sensitive and specific immuno-diagnostic tests to measure kappa (\(\kappa\)) and lambda (\(\lambda\)) free light chains. The assays are based on affinity purified polyclonal antibodies that are coated onto latex particles which are used to produce kits specific for \(\kappa\) FLCs and \(\lambda\) FLCs for nephelometric and turbidimetric platforms. Freelite reacts only with exposed free light chain epitopes which are hidden when the light chain is bound to the heavy chain. Freelite is recommended for use in patients with monoclonal gammapathies. It is the only free light chain assay recommended by national and international guidelines.

For more information, please visit: http://www.us.bindingsite.com/en/discover/freelite-and-hevylite/freelite/overview/freelite#

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For more information, please visit: http://www.streck.com/product.aspx?p=ESR-Chex

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Sysmex America, Inc. recently introduced the TS-10 Integrated Tube Sorter and Archiver for pre- and post-analytic tube management, available for the Sysmex XN-9000 Automation system. The TS-10 supports hands-free sorting, archiving, and sample loading and unloading flexibilities with XN-9000 automation system. With the Ethylenediaminetetraacetic acid (EDTA) automation system, the laboratory can realize efficiencies with the TS-10 by significantly reducing the number of tube touches from pre-analytical through post-analytical testing, for virtually 100 percent of EDTA samples. The system has the capability to test and sort >90 percent of the EDTA tests (CBC, Diff, retic, HbA1c), allowing the laboratory to promote single tube draws. Sysmex America’s integrated, automated sorting and archiving system can improve patient care by reducing test turnaround time and supporting efficient labor utilization.

For more information, please visit: https://www.sysmex.com/us/en/Products/Hematology/XNSeries/Pages/XN-9000-Hematology-Analyzer.aspx

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A major subset of laboratory diagnostics is devoted to the prenatal detection of a range of fetal abnormalities. Best known among these is probably trisomy 21 (Down syndrome) due to its high prevalence of roughly one in 800 live births. Some other aneuploidies (conditions where an abnormal chromosome number is present) are also common targets for prenatal detection, including trisomy for chromosomes 18 and 13 or abnormal numbers of sex chromosomes. These abnormalities are genetic in nature, but their testing predates the molecular diagnostics lab era through the application of historical methods relying on analysis of various maternal serum markers and/or ultrasound imaging methods. While these approaches have been and are useful, they have limitations in accuracy and technical challenges which make them better suited to a screening role than a definitive diagnostic role.

Samples flagged by these methods as being high-risk have thus generally been forwarded for examination by the oldest of the genetic testing methods, cytogenetics, through the direct examination of fetal cells. Cytogenetic methods are good at detecting gross genetic changes such as aneuploidies and some translocations, although less (or un-) able to detect smaller-scale mutations. Further, cytogenetic methods suffer from their underlying need for fetal cells to examine, which by nature remains too costly, time consuming, and low-yield to be ideal for routine use.

A simpler route to obtaining fetal DNA became apparent in 1997 when research was published indicating that cell-free circulating DNA of fetal origin had been detected in maternal peripheral blood. While this too occurs in a background of maternally derived cell-free DNA, the proportion of fetal to maternal contribution is much more favorable, with estimates suggesting it represents three percent of total free circulating DNA in the first trimester, increasing to possibly in excess of 10 percent by end of term. While this has gone a great way toward improving on the amount of fetal DNA available over that in isolated fetal cells, it has also presented the challenge of how one goes about distinguishing which bits of free-floating DNA originated from the mother as opposed to the child.

One clever approach to detection of aneuploidies simply ignores this problem. Imagine, for example, that we have a mother with a trisomy 21 fetus, and we take the circulating free DNA from maternal peripheral blood and just throw all of it on an NGS platform. These platforms all work, in effect, by sequencing millions of short sequence elements in parallel, and since we have full human reference genomes to compare against, many of these “reads” can be uniquely mapped back to a specific
chromosome and position. Barring intricacies of differential detection of chromosomal regions (which can be compensated for with bioinformatics data processing to some degree), the number of “reads” mapping to a chromosome relates to the physical linear amount of each chromosome present in the sample. That is, more reads are generated from a long chromosome than a short one—but also, more reads are generated from three copies of a chromosome, than two. Since our hypothetical sample here has a very small fractional excess of chromosome 21 (50 percent extra in the small total fraction of DNA arising from the fetus), if we do a large enough number of reads, eventually this excess will give rise to a small but statistically relevant overabundance of chromosome 21 reads compared to all the other chromosome reads in the sample.

As described, this method can detect essentially any aneuploidy condition; however, it requires an enormous number of reads per sample, which translates to high cost and low throughput. An improvement on this is to carry out a targeted NGS, whereby, for instance, only representative regions of the highest-risk chromosomes X, 13, 18, and 21 might be read. This vastly reduces the total number of reads needed per sample and allows cheaper assays with higher throughputs (as many more samples can be included on a single NGS run). The trade-off is that only aneuploidies impacting the queried chromosomes will be detected; however, in most cases this is an acceptable balance.

A still simpler approach to this basic method has been suggested through the approach of digital PCR, targeted to a similar set of informative chromosomal markers. This approach promises simplicity both of performance and data interpretation, as it is inherently highly accurate for quantitation.

What if we wanted to take advantage of the (relative) prevalence of free fetal DNA in maternal circulation, but still wanted some way to identify and examine it as distinct from maternal? That is, can we have our proverbial cake and eat it too? It turns out that we can, at least sometimes. The simplest and most immediately obvious case would be when we’re interested in a holandric (Y-chromosome) fetal locus. If we assume that any circulating Y-chromosome DNA in the maternal circulation is fetal in origin, then all we need do is apply PCR, Sanger sequencing, or whatever our method of choice is to the locus of interest to get our desired information (more about the validity of this assumption, below).

Unfortunately, not that many loci of interest reside on the Y chromosome. Recall, however, that every person has a unique set of single nucleotide polymorphisms (SNPs) scattered across their genome, meaning that for every SNP locus there is a finite chance that the father and mother will have a differentiable marker. If we know the SNP genotypes of both parents and they differ at a specific locus, it is then possible by analogy to the Y chromosome example to identify the fetal genotype for that locus. (This is probably most intuitively obvious in the case where a read contains a uniquely paternal-derived SNP; it’s presumably from the fetus, and allows us to make assumptions about fetal carriearship of paternal gene alleles closely linked to the SNP.) Note that it’s not even a requirement that the parents each be homozygous (and different) at the associated SNP; for example, if the maternal SNP is “A/A” homozygous and the paternal SNP is “A/C” heterozygous, then with deep enough sequencing, if no “C” SNP reads are found, one can assume the fetus to have gotten the paternal “A” allele.

The exact amount of information which can be obtained from SNP linkages in this sort of analysis will vary from case to case, but with proper context and bioinformatic analysis it can be helpful in determining risk of particular Mendelian inherited conditions. Mass readout of SNP genotype can be done by NGS or array-based methods, or for a smaller selected allele set of interest by classical PCR approaches.

In addition to the SNP-based methods for selectively identifying non-maternal (and thus presumably fetal) content in circulating peripheral free DNA, DNA methylation state (that is, imprinting) may have some current or future applicability as well.

In all of the above, we’ve been working with the tacit assumption that if DNA (cellular or free-floating) in maternal peripheral circulation isn’t maternal, it’s from a current fetus. Is this an unassailable truth? Probably not. One case study has detected fetal cells in maternal peripheral circulation up to an astounding 27 years postpartum (suggestive that during pregnancy, some of the fetal cells have actually engrafted within the mother and continued to propagate in a form of microchimerism). Additionally, examples of male microchimerism (that is, trace amounts of male DNA) have been reported in women without a known history of pregnancy with a male fetus, suggesting other possible sources such as an in-utero absorbed fraternal (male) twin, or possibly even from a mother’s male older sibling. While these are intriguing, it’s important to note that these anomalies generally have been found at very low levels—much lower than the 3%+ of total circulating free DNA which is well associated with a current fetus. For the types of applications described here, these vanishingly small template amounts from “other sources” will generally not alter the results obtained, although they present a warning not to rely solely on an ultrasensitive qualitative detection of any marker as irrefutable evidence of fetal genotype.

In the context of proper bioinformatics analysis and genetic counselling, prenatal genetic diagnosis from maternal peripheral blood is a useful tool and likely to become increasingly commonly applied as NGS methods become cheaper and more widely available in clinical settings.

John Brunstein, PhD, is a member of the MLO Editorial Advisory Board. He serves as President and Chief Science Officer for British Columbia-based PathoID, Inc., which provides consulting for development and validation of molecular assays.
CMO of Exosome Diagnostics focuses on personalized, precision healthcare

If you were explaining Exosome Diagnostics to someone who is not familiar with the organization, how would you characterize its primary areas of expertise? What are the major categories of solutions that Exosome provides for its customers? The company was founded on the discovery that exosomes, small vesicles found in blood and other biofluids, contain abundant RNA, as well as DNA and protein, making them ideal for diagnostic purposes. Our expertise is therefore in the isolation and downstream analysis of the exosomes and their contents. To this end, we have two isolation platforms: one for isolation of RNA, which has applications in oncology and non-oncology areas, like neurodegenerative disease and cardiovascular disease; and one for simultaneous isolation of RNA and cfDNA, designed to maximize sensitivity for non-invasive genotyping, principally in oncology.

What would be a good thumbnail definition of “exosome biology”? Exosomes are packages of information used by cells for communication in both healthy and disease states. Therefore, analyzing their contents provides a real-time window into what’s happening in cells and their surrounding microenvironment, with very immediate diagnostic relevance.

Professional
I joined Exosome Diagnostics in June of 2013. Prior to that, I founded and headed the Personalized Medicine Group at Sanofi, where I worked for two years. Before this, I worked at Roche/Genentech for many years, leading the development of a number of new therapeutics with their associated biomarkers and companion diagnostics. So my expertise is a blend of translational science, therapeutics, and diagnostic product development.

Education
I attended the University of Glasgow, UK, where I obtained my BSc in Pathology, MBCS, and MD. In the UK an MD is a research degree, requiring a full-time lab commitment. In addition, I’m a Member of the Royal College of Physicians.

Personal
I enjoy cycling and hill walking, but my main interests are bookish; I read voraciously, typically modern literature, and try to maintain my long-time interest in Classical languages and culture.

Your company’s Solid Tumor Mutation Panel isolates both cell-free DNA and exosomal RNA. What are the advantages of this approach? This is quite revolutionary. Nucleic acid in biofluids comes from two sources: cfDNA from dying cells through cellular necrosis, and apoptosis; and exosomal RNA (plus some DNA) from living, dividing cells. Combining these two sources captures all of the available mutation signal, and so markedly increases the sensitivity of the assay. This is very relevant for rare mutation detection, where the mutations are infrequent (typically less than one percent) on a background of normal, wild-type genes.

How and where will that panel be launched, and what are the prospects for clinical laboratory applications in the future? We will launch two separate blood-based panels in the near future: a 26-gene panel of actionable mutations, with a turnaround time (TAT) of five working days; and a more comprehensive 88-gene panel, with a typical TAT of two to three weeks. The smaller, focused panel will be launched as a laboratory developed test (LDT) in our CLIA lab and will be available to pharmaceutical companies by the end of the year.

Your company is active in companion diagnostics. How is this collaboration between diagnostics and pharmaceutical companies going to affect the clinical lab? Consistent with the personalized medicine mantra “the right drug for the right patient at the right time,” most oncology clinical trials today require patient stratification or selection based on a molecular marker(s). This stratification, however, is currently based on tissue diagnostics, often requiring a new tissue biopsy procedure. It’s acknowledged that this requirement reduces clinical trial accrual substantially, since many patients are unwilling to undergo such a procedure. The logical next step is to move to stratifying patients by liquid (e.g., blood) diagnostics, and to monitor patients for resistance mechanisms non-invasively. I think the testing paradigm for solid tumors is therefore changing, and beginning to look more like standard practice in heme-oncology. This is an exciting time in oncology.

Before assuming your current position, you held research leadership positions at Sanofi, Genentech, and GlaxoSmithKline. How has your past work prepared you for your present responsibilities? My prior experience is a blend of therapeutics and biomarker/diagnostic development. Given the emphasis now on developing the right drug for the right patient, in other words the precision medicine paradigm, there is a very clear need for molecular, non-invasive, liquid-biopsy approaches to facilitate our understanding of the drug target and mechanisms of resistance to the drug. Exosome Diagnostics is at the forefront of this revolution in noninvasive testing. So ultimately I think my prior positions and experience are perfectly suited to the job at hand. With this experience, my ultimate focus is on creating smarter diagnostic and testing approaches that will advance healthcare and improve the lives of patients.
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