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Feature Story

Cytochrome P450 2C19 Genotyping for Predicting Metabolism of Clopidogrel and Other Drugs p 2

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Cytochrome P450 2C19 Genotyping for Predicting Metabolism of Clopidogrel and Other Drugs

By Joyce Heesun Rogers, MD, PhD

The antiplatelet therapeutic agent clopidogrel (Plavix®, Bristol-Myers Squibb, New York), requires metabolic conversion into the active drug by the cytochrome P450 (CYP) family of liver enzymes, primarily by CYP2C19. The gene encoding the CYP2C19 protein is highly polymorphic, resulting in altered enzyme activity and wide variability among patients taking clopidogrel. Such varied response also can be associated with other drugs metabolized by CYP2C19 causing adverse drug events. A therapeutic approach that factors in the patient's *CYP2C19* gene polymorphisms to individualize drug options will enhance treatment efficacy and reduce adverse side effects.

Genetic testing of *CYP2C19* as a companion diagnostic can identify patients predisposed to varying efficacies of clopidogrel and other drugs metabolized by CYP2C19. Detection of three common variants in the *CYP2C19* gene, including two non-functional alleles (*2 and *3) and one increased-function allele (*17) will facilitate tailoring individualized antithrombotic therapy such as an alternate dosage or alternate treatment. Other less frequent CYP2C19 non-function alleles (*4 to*8) and decreased-function alleles (*9 and *10) also may be associated with absent or reduced metabolism of clopidogrel and other drugs.

Current Status of Clopidogrel Therapy

Clopidogrel is an oral antiplatelet drug used to treat acute coronary syndromes, including patients undergoing percutaneous coronary intervention (PCI), myocardial infarction (MI), cerebrovascular disease and peripheral arterial disease. Clopidogrel is typically prescribed at a daily dosage of 75 mg with or without an initial loading dose of 300-600 mg, as needed. In combination with a daily dose of 75-325 mg aspirin, clopidogrel is used at the 75 mg dosage to prevent stent thrombosis following PCI.

The antithrombotic effect of clopidogrel is not optimal in all patients, and up to 30% of the patients receiving clopidogrel do not benefit from the therapy as determined from measurement of residual platelet reactivity. Non-response or sub-optimal response to therapy can stem from pharmacokinetics and pharmacodynamics, co-administration of other drugs and predisposition due to genetic, clinical and cellular factors.

Genetically, certain polymorphisms in the gene encoding the CYP2C19 enzyme have been associated with poor metabolism of clopidogrel and subsequent decreased formation of active metabolite. The U.S. Food and Drug Administration (FDA) has issued a black box warning about the reduced effectiveness of clopidogrel in patients who metabolize the drug poorly. The FDA has indicated the availability of tests to identify genetic differences in CYP2C19 function and advised healthcare professionals to consider alternate therapy or dosing strategies for poor metabolizers.

Clopidogrel Metabolism and Mechanism of Action

Clopidogrel is metabolized in the liver by several CYP enzymes, including CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP3A4 and CYP3A5. Clopidogrel is largely (~85%) hydrolyzed by esterases into inactive metabolites, and only about 15% is converted into the active form by CYP enzymes. Of these, CYP2C19 plays a major role in the generation of the active metabolite. The active metabolite, an analogue of adenosine diphosphate (ADP), binds irreversibly to platelet ADP receptor $P2RY_{12}$ to inhibit platelet aggregation and consequent thrombosis. The pharmacogenetics and mechanism of action of clopidogrel and other drugs metabolized by CYP2C19 are shown in Figure 1.

Response to clopidogrel therapy is widely variable, which is attributed to at least three common variants in the *CYP2C19* gene. The wild-type form of the *CYP2C19* gene (*1) encodes an enzyme with normal activity (extensive metabolizer, EM) on which current drug dosing recommendations are based. Two other variants (*2 and *3) encode non-functional enzymes (poor metabolizer, PM). In contrast, a third variant (*17) encodes an enzyme with increased function (ultra-metabolizer, UM). Individuals can inherit one wild-type allele and one non-functional allele (intermediate metabolizer, IM). As a result, *CYP2C19*2* and *CYP2C19*3* variants confer

On the Cover: PLMI Chair Kandice Kottke-Marchant, MD, PhD, (seated) with Joyce Heesun Rogers, MD, PhD, and Gurunathan Murugesan, PhD, who collaborated on the development of an assay to identify genetic variants of *CYP2C19* that impact drug metabolism.

non-functional or markedly decreased metabolic status and have been associated with non-responsiveness to clopidogrel. Non-responsiveness is reflected in reduced platelet inhibition and poor outcomes, including increased risk for stent thrombosis, MI, stroke and death. On the other hand, *CYP2C19*17* is associated with increased response to clopidogrel and risk for bleeding.

Accordingly, while suboptimal response to clopidogrel can lead to thrombosis, super-optimal response can result in bleeding. Thus, the genetic testing of patients to determine *CYP2C19* gene variants can enable individualized antithrombotic therapy, including alternate dosing or alternate therapy with an anti-platelet agent such as prasugrel (Effient®, Eli Lilly & Co., Indianapolis, Ind.). However, this genotyping assay should be used in conjunction with the aspirin/clopidogrel aggregation assay to determine platelet response to clopidogrel.

Clinical Significance of *CYP2C19* Genetic Testing

The CYP2C19 isoenzyme metabolizes 5-10% of all prescription drugs, and poor metabolizers taking those drugs may be predisposed to adverse drug events due to underdosing or overdosing, depending on whether it is a pro-drug or active drug, respectively. It is estimated that 2-14% of the U.S. population are poor metabolizers. Pharmacokinetics of drugs and consequent therapeutic efficacy depend on the rate of activation or elimination. Poor metabolizers are likely to have reduced or no conversion rate of pro-drugs (e.g. clopidogrel, tamoxifen) to active drugs, resulting in either a sub-therapeutic effect or non-responsiveness (underdosing), which eventually require alternate treatment or increased dosage. Poor metabolizers also may have a reduced capacity to eliminate active drugs, e.g. omeprazole, diazepam (Valium®, Roche, Basel, Switzerland), leading to accumulation and toxicity, resulting in life-threatening side effects (overdosing).

Such adverse effects can be predicted by determining the genotype-phenotype associations (Figure 1). The homozygous wild type allele of CYP2C19 designated CYP2C19*1, is defined as an EM. Nine common variants of the *CYP2C19* gene associated with impaired drug metabolism include *2 to *10. While the wild-type form of the *CYP2C19* gene (*1) encodes an enzyme with normal activity, other genetic variants (*2 to *8) encode enzymes with loss of activity, and two other variants (*9 and *10) have reduced activity. The most common PM phenotypes are from CYP2C19 with homozygous *2 and *3 alleles. Homozygous *4 to *8 alleles are less common PM; *9 and *10 alleles have reduced activity. One of the variants (*17) confers augmented enzyme activity, leading to a UM phenotype. Inheritance of one normal allele and one loss of function allele can result in an IM phenotype.

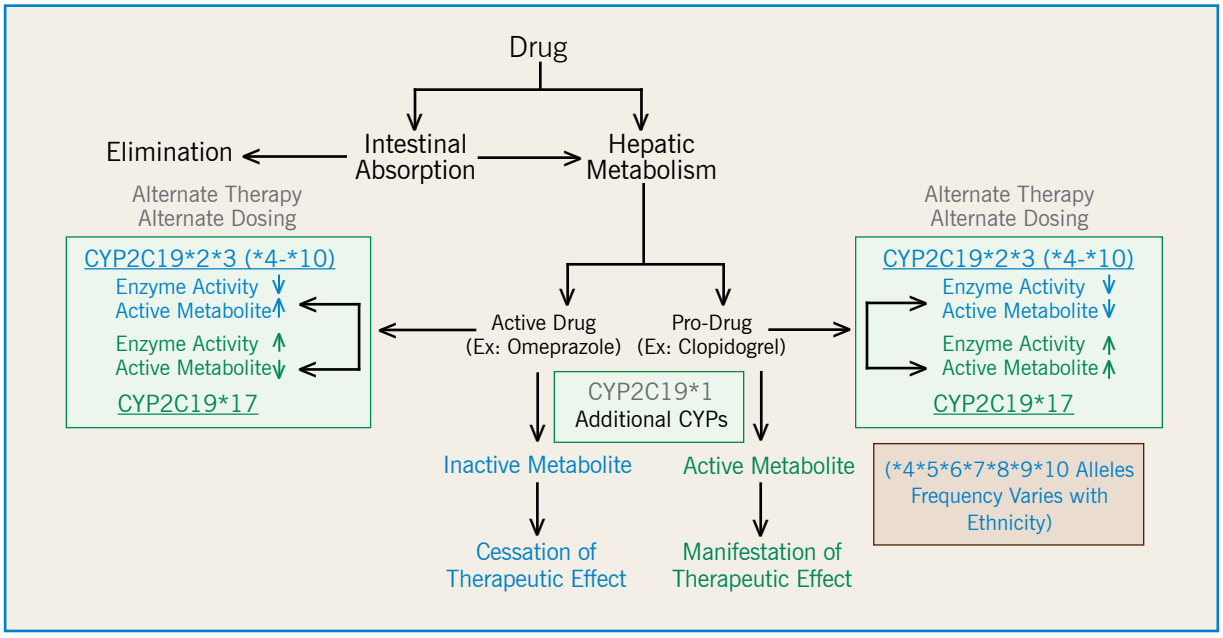

Figure 1. Pharmacogenetics and mechanism of action of clopidogrel and other drugs metabolized by cytochrome P450 2C19

Validation of the *CYP2C19* Genetic Test

We have analytically validated a microarray-based test from AutoGenomics Inc., Vista, Calif. for genotyping *CYP2C19* variants. The assay involves multiplex PCR amplification of genomic DNA followed by allele-specific primer extension using fluorescently labeled dCTP and hybridization on to a microarray coated with capturing oligonucleotides, which are specific for complementary oligonucleotides linked to the allele specific primer-extended products. A built-in confocal microscope is enabled to capture fluorescent signal from the pre-determined hybridization spots corresponding to specific products and genotypes deciphered from signal ratio.

Validation was performed following the guidelines of the Clinical Laboratory and Standards Institute (CLSI). Accuracy of the CYP2C19 test was evaluated by genotyping specimens (n=69) of known genotypes and comparing the results for a subset of specimens (n=26) genotyped in an external reference laboratory that uses the same assay system. Inter- and intra-assay precision and the limit of detection were determined using three specimens representing extensive, intermediate and poor metabolizers. Specificity of the assay was confirmed by specified criteria.

Interpretation of the *CYP2C19* Genetic Test

This genetic test is used to identify patients at risk for adverse events and to determine appropriate therapy by

- Detecting six variants of the *CYP2C19* gene associated with a non-functional enzyme
  *CYP2C9*2, *3, *4, *6, *7*, and **8*
- Detecting two variants of the *CYP2C19* gene associated with a decreased-function enzyme
  *CYP2C9*9* and **10*
- Detecting one variant of the *CYP2C19* gene associated with an increased-function enzyme
  *CYP2C9*17*

Interpretation of the assay is based on the presence of specific genetic variants of *CYP2C19* (Table 1).

1. Individuals with *CYP2C19* variant alleles designated *2, *3, *4, *6, *7 and *8 have no functional activity compared with *1 (wild-type) individuals. Administration of a pro-drug such as clopidogrel can result in markedly reduced or no conversion to the active metabolite and a subtherapeutic effect or non-responsiveness, which require an alternate treatment or increased dosage. Conversely, administration of active drugs, such as diazepam and omeprazole, may result in impaired elimination, potential risk of drug toxicity and life-threatening side effects, also requiring an alternate treatment or reduced dosage.
2. Individuals with *CYP2C19* variant alleles designated *9 and *10 have decreased functional activity. Such individuals may experience sub-therapeutic effects for pro-drugs. Conversely, administration of active drugs may result in impaired elimination and potential risk of drug toxicity.
3. Individuals with *CYP2C19* variant alleles designated *17 have augmented enzyme activity compared to *1 (wild-type) individuals, leading to increased conversion of pro-drugs to active metabolites with potential risk for toxicity and requiring reduced dosage. Conversely, it also may lead to rapid elimination of active drugs and sub-therapeutic effects.
4. Individuals with a *CYP2C19* genotype comprised of one of the non-functional alleles (*2, *3, *4, *6, *7 or *8) and *17 have an unknown metabolizer phenotype. Due to the low population frequency of these alleles, the level of CYP2C19 activity cannot be predicted based on genotype.
5. Individuals with a *CYP2C19* genotype comprised of one of the reduced-function alleles (*9 or *10) and *17 have an unknown metabolizer phenotype. Due to the low population frequency of these alleles, the level of CYP2C19 activity cannot be predicted based on genotype.
6. Prevalence of *CYP2C19* gene variants differs depending on racial and ethnic background. The frequency of allele *CYP2C19*2* has been reported as approximately 30-35% in Asians and 15-26% in Caucasians and African Americans. The frequency of allele *CYP2C19*3* has been reported as approximately 10% in Asians and less than 2% in Caucasians and African Americans.

Limitations of the *CYP2C19* Genetic Test

Analysis for specific genetic variants detected in this test does not rule out the possibility of the presence of other variant alleles that may influence drug effects and metabolism. Non-genetic factors such as concurrent medications, impaired hepatic function, obesity, insulin resistance and non-compliance can also affect CYP2C19 metabolism. These can lead to increases or decreases in function relative to the predicted genotype.

CYP2C19 mediates the metabolic activation and elimination, and hence the therapeutic effect, of a variety of drugs including anticonvulsants, antidepressants, antimalarial drugs, antithrombotics, antiulcer drugs, beta blockers, antineoplastic drugs and proton pump inhibitors. Co-administration of drugs metabolized by CYP2C19 may increase or decrease the CYP2C19 activity. Drugs metabolized by CYP2C19 are listed in Table 2.

CYP2C19 genotyping should not replace clinical monitoring of patients when required. Genotype-phenotype interpretation should be made in the context of a patient's clinical condition and concomitant medications, which may be substrates, inhibitors or inducers of CYP2C19. For clopidogrel therapy, this genotyping assay should be interpreted in conjunction with the aspirin/clopidogrel aggregation assay as a functional screen to determine platelet response to clopidogrel.

Table 1. Cytochrome P450 2C19 Genotypes and Phenotypes for Drug Metabolism

Allele	Polymorphism	Phenotype / Enzyme Activity
*CYP2C19*1*	None (wild-type)	EM / normal function
*CYP2C19*2*	681G>A	PM / non-functioning
*CYP2C19*3*	636G>A	PM / non-functioning
*CYP2C19*4*	1A>G	PM / non-functioning
*CYP2C19*6*	395G>A	PM / non-functioning
*CYP2C19*7*	IVS 5+2T>A	PM / non-functioning
*CYP2C19*8*	358T>C	PM / non-functioning
*CYP2C19*9*	431G>A	IM / decreased function
*CYP2C19*10*	680C>T	IM / decreased function
*CYP2C19*17*	-806C>T	UM / increased function

Abbreviations: EM = extensive metabolizer; IM = intermediate metabolizer; PM = poor metabolizer; UM = ultra-metabolizer

Table 2. Summary of Drugs Metabolized by Cytochrome P450 2C19

Drugs Metabolized by Cytochrome P450 2C19:

Anticoagulants:	clopidogrel
Anticonvulsants:	mephenytoin, phenytoin, primidone, mephenytoin
Antidepressants:	amitriptyline, citalopram, S-citalopram, clomipramine, imipramine, fluoxetine, escitalopram, moclobemide, trimipramine, sertraline
Antineoplastic drugs:	cyclophosphamide
Antiretroviral/antifungal drugs:	nelfinavir, voriconazole, vfend
Proton pump inhibitors:	lansoprazole, omeprazole, pantoprazole
Miscellaneous drugs:	diazepam, progesterone, propranolol, R-warfarin, proguanil, malarone, carisoprodol, flunitrzaepam, soma

Cytochrome P450 2C19 Inhibitors

Co-administration of these drugs decreases the rate of drug metabolism and increases the possibility of toxicity

chloramphenicol, cimetidine, delavirdine, efavirenz, felbamate, fluconazole, fluoxetine, fluvoxamine, indomethacin, isoniazid, ketoconazole, lansoprazole, modafinil, omeprazole, oral contraceptives, oxacarbazepine, probenicid, ticlopidine, topiramate, voriconazole

Cytochrome P450 2C19 Inducers

Co-administration of these drugs increases the rate of drug excretion and reduce the drug's effectiveness

carbamazepine, ginko biloba, prednisone, rifampin, secobarbital, St. John's wort

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Latest Advances in Treatment of Blood Disorders

PLMI Reports from ASH Annual Meeting

Cleveland Clinic was a dominant presence at the American Society for Hematology (ASH) annual meeting in San Diego in December, presenting 112 abstracts and posters. Department of Clinical Pathology Chairman Eric Hsi, MD, co-authored six of these abstracts with colleagues from Clinical Pathology, Cleveland Clinic Taussig Cancer Institute and the Department of Hematology and Oncology.

Eric Hsi, MD

Xiaoxian Zhao, PhD

ASH is the world's largest professional society concerned with the causes and treatments of blood disorders.

The following is a summary of Dr. Hsi's abstracts and their conclusions.

The t(14;18)(q32;q21) Characterizes a Subset of Patients with Diffuse Large-B Cell Lymphoma of Germinal Center Origin with Poor Outcome: Report From the International DLBCL Rituximab-CHOP Consortium Program Study

Patients with the GCB subtype and t(14;18) exhibit a significantly worse prognosis than patients without t(14;18) when treated with R-CHOP. The assessment of t(14;18) by FISH approach not only functions as a valuable prognosticator for individual risk estimation in GCB-DLBCL patients in addition to the established parameters, but also provides valuable result for therapeutic intervention.

A Proof of Principle Clinical Trial in Myelodysplastic Syndromes of Non-Cytotoxic Differentiation Therapy with Decitabine

This study provides clinical proof of principle that a decitabine regimen rationalized for non-cytotoxic epigenetic-differentiation effects is active in myeloid malignancy, correlates with molecular markers of terminal differentiation, and has potentially important safety and efficacy advantages over cytotoxic therapy that warrant further evaluation and optimization.

Phospho-SFKs (Y416) As a Potential Predictive Marker for Dasatinib Therapy in B-Cell Non-Hodgkin Lymphoma and Chronic Lymphocytic Leukemia

(Co-author Xiaoxian Zhao, PhD, PLMI Department of Clinical Pathology)

P-SFK (Y416) may be a useful predictive marker of response to dasatinib. Potential uses include pharmacodynamic monitoring or integral biomarker for selecting appropriate patients with B-cell malignancies for clinical trials.

Cell of Origin Determination in Diffuse Large B-Cell Lymphoma: Performance of Immunohistochemical (IHC) Algorithms and Ability to Predict Outcome

The Hans and Choi algorithms are reasonable methods for identifying PFS and OS differences based on CoO for de novo DLBCL treated with chemoimmunotherapy. The positive predictive value is universally high for all algorithms tested, but the sensitivity of IHC for identifying CoO was fair, particularly for the Tally method. IHC represents a valid biomarker to identify non-GCB cases. Clinical trials of DLBCL that stratify patients by IHC are feasible provided the performance characteristics of the algorithms are taken into consideration during study design.

Whole Genome and Exome Sequencing Reveals the Genetic Landscape of Burkitt Lymphoma

This study represents one of the first in-depth analyses of a BL genome and one of the largest applications of exome sequencing in cancer. Our data provide the most comprehensive genetic portrait of human BL to date, and provides a significant first step to identifying the genetic causes of the disease.

The Impact of Molecular Lesions in Post-Transplant Acute Myeloid Leukemia (AML) in Correlation with Cytogenetic Abnormalities

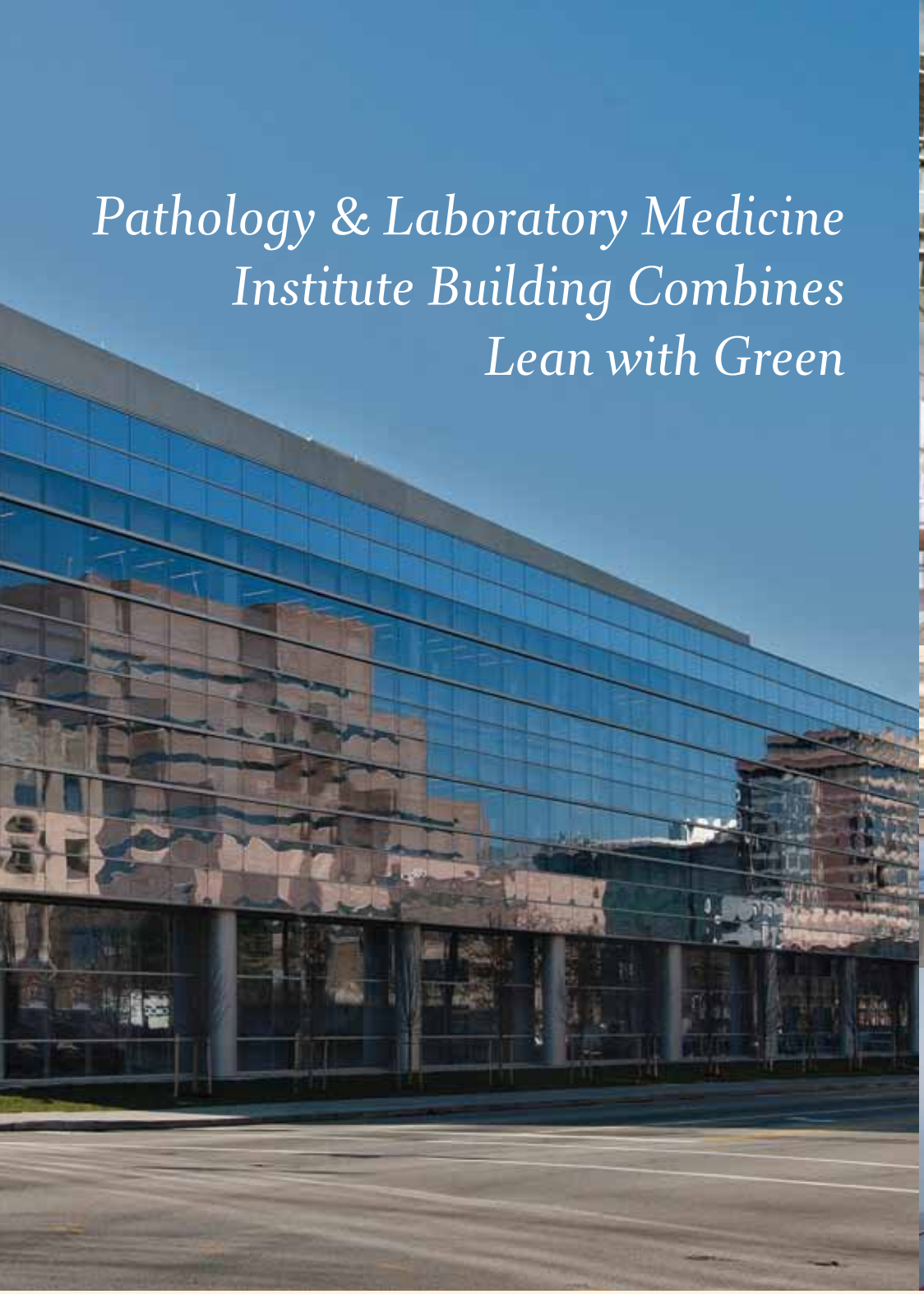
Preliminary results suggest that myeloablative AlloHCT could minimize the prognostic impact of adverse molecular markers in AML. These mutations potentially serve as important biomarkers in determining the management of AML. Further studies designed to determine their precise role are planned.

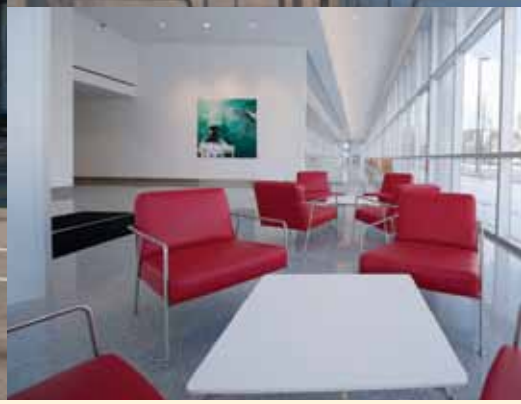
New Combination Therapy for Relapsed/Refractory Multiple Myeloma Enters Phase III Trials

Building on initial pre-clinical work done in part at Cleveland Clinic by Dr. Hsi with collaborators at Protein Design Labs (ASH 2006 and Hsi ed *et al*. *Clin Cancer Res.* 2008), clinical investigators reported Phase II results of elotuzomab with lenalidomide and low dose dexamethasone in patients with relapsed/refractory multiple myeloma (abstract 303). Patients demonstrated a 92% overall response rate, and this novel monoclonal antibody is now being evaluated in Phase III trials.

The Cleveland Clinic Pathology & Laboratory Medicine Institute (PLMI) has expanded its laboratory space on main campus with the January opening of a new, state-of-the-art, 138,000-square-foot facility. The building was designed by Vlad Novakovic of Perspectus Architecture and laboratory architect Roger Maynard.

Pathology & Laboratory Medicine Institute Building Combines Lean with Green





The new building, situated at the eastern end of the Cleveland Clinic campus on Carnegie Avenue, houses central processing, microbiology, molecular pathology, immunology and special chemistry laboratories, and Cleveland Clinic Laboratories' administration, marketing and logistics.

"The role of pathology and laboratory medicine in patient care is going to grow exponentially over the next decade. The Pathology & Laboratory Medicine Institute is now positioned to take advantage of the opportunities that growth will bring in testing, technology and training."

— Kandice Kottke-Marchant, MD, PhD, PLMI Chair

David Bosler, MD, Head, Cleveland Clinic Laboratories, adds, "The new building doubles our laboratory space. We have added staff as well as space, which will allow us to expand our test menu and our capacity and service our customers more efficiently and effectively."

The building incorporates Lean engineering and design principles, which focus on eliminating wasted effort, time and physical resources. By applying Lean principles, the goal was to create a lab where the techs can spend more time performing critical tasks and less time walking, searching and waiting, explains Joe Seestadt, PLMI Director of Continuous Improvement.

"Incorporating the continuous improvement elements of workbench optimization, specimen delivery and inventory management from the planning stage will ensure that the labs can function at optimal efficiency and turnaround time," he notes.

A total of 67 front-line laboratory technologists, coordinators and managers were involved in the design process through nine two-day Kaizen events. With their input, the overall result was a streamlined laboratory operation that:

- cut out 200 wasteful activities such as inefficient walking patterns and waiting time,
- increased the percentage of laboratory workstations in the most efficient location from 62 percent to 91 percent,
- increased the percentage of work steps performed within the optimal window from 38 percent to 91 percent,
- incorporated a more efficient inventory management system that decreased order replenishment time from 15 to three days and
- implemented a water spider delivery concept that uses offline associates to deliver specimens directly to techs.

In addition to the tangible benefits, "Involving front-line staff in the process increased employee engagement and made employees aware that their opinions count because their ideas were used," Seestadt adds.

The new building's first floor houses a new, expanded Microbiology lab and a spacious central processing area designed to optimize specimen handling and reduce turn-around times.

The second floor is home to Molecular Pathology and is fully equipped for the continued expansion in FISH, CISH, PCR and other molecular assays for DNA, RNA and protein analysis. The expanded Cytogenetics laboratory will allow for growth in in-house test volume and introduction of a cytogenetics technology training program.

The Special Chemistry lab on the third floor was designed to accommodate the migration to automated platforms. Immunopathology, also on floor three, now has the space, equipment and manpower to meet the expanding testing needs in this growing area.

For additional information about PLMI, please visit clevelandclinic.org/pathology. For more information about Cleveland Clinic Laboratories, visit clevelandcliniclabs.com.

Sustainability Features Complement Design

PLMI's new facility was constructed according to the U.S. Building Council's LEED (Leadership in Energy and Environmental Design) standards. Cleveland Clinic hopes to achieve LEED certification for the building, which would provide independent, third-party verification that it was designed and built using strategies aimed at achieving high performance in key areas of human and environmental health.

Highlights of the sustainability features incorporated in the PLMI building's design and construction include:

Sustainable site

- Constructed on a previously occupied site
- Close to public transportation

Water efficiency

- Designed to reduce potable water use by 83%
- Water-efficient irrigation systems
- Porous concrete, permeable pavers, green roof contribute to enhanced water quality and minimal runoff

Energy and atmosphere

- Extensive exterior and interior glazing to maximize daylighting and minimize need for sun control
- Sunshades to reduce solar heat gain in summer
- Daylight-responsive lighting controls, LED lighting, exhaust air energy recovery for 41.6% improvement in energy performance over standard building

Materials and resources

- Regionally manufactured materials with maximum recycled content
- 60% of construction waste recycled

Indoor environmental quality

- No interior finishes containing volatile organic compounds
- 90% of work spaces have view to outdoors

PCA3 Assay for Prostate Cancer

By Raymond Tubbs, DO

Since the introduction of the PSA (prostate specific antigen) test in 1989, it has gained almost universal acceptance as a screening tool for prostate cancer. PSA screening typically is used in conjunction with the digital rectal examination (DRE) and, in men with elevated PSA level, is followed by a biopsy. The PSA threshold for biopsy, originally 10 ng/mL, has been adjusted several times, and today the usual recommendation is to biopsy when serum PSA level >2.5ng/mL. The lowering of the PSA threshold has resulted in an increase in the number of biopsies. However, the positive predictive value of PSA and DRE are low, 25-30% and <20%, respectively (1), which means that many initial biopsies are negative. The Prostate Cancer Prevention Trial risk calculator demonstrates a significant risk of positive biopsy following one previous negative biopsy, independent of changes in PSA (2). This has resulted in a high rate of repeat biopsies in an effort to detect potentially harmful prostate cancer unrecognized in the initial biopsy.

While PSA is a useful marker for prostatic adenocarcinoma, its utility is limited because it is prostate-specific but not cancer-specific; consequently, there is no PSA lower limit of detection for which the risk of prostate cancer is zero. For predicting biologically significant disease, the predictive value of PSA is limited because higher grade tumors often express little PSA. Decisions regarding the need for re-biopsy in men with an elevated PSA are limited because PSA usually is more reflective of prostate volume rather than the risk of carcinoma.

The Role of the PCA3 Assay

The prostate cancer gene 3 (PCA3) assay is a genetic-based diagnostic test for prostate cancer that meets the need for a test with greater predictive value than the serum PSA. PCA3 is highly specific for prostate cancer and capable of distinguishing between clinically significant and insignificant prostate cancer and, therefore, can support more appropriate use of biopsy and reduces the risk of overtreatment.

PCA3 is expressed only in prostate tissue and is significantly overexpressed in malignant prostate tissue. The PCA3 gene's messenger RNA is highly overexpressed (median 66-fold) in more than 95% of prostate cancer tissue compared with expression in normal or benign prostate tissue from the same patients (3,4). PCA3 is specifically expressed by prostate carcinoma cells. The PCA3 assay addresses many of the issues related to serum PSA screening, improves the sensitivity and specificity of PSA interpretation and may add other information more reflective of tumor biology.

The PCA3 assay is a simple urine test that detects the presence of PCA3 mRNA. It is performed on 2.5 mL first-catch urine collected from the patient post-DRE. When transported under prescribed conditions, the specimen is stable at 4°C for 21 days and 5 days at 30°C.

In step 1 (top), target capture of the mRNA is performed, using magnetic bead (purple).

In step 2 (center), the captured gene is amplified using transcription-mediated amplification, a process that generates some 10 billion copies of PCA3 in one hour.

In step 3 (bottom), the hybridization protection assay is performed using DNA probes tagged with a chemiluminescent substance that is activated upon contact with detection reagents.

Image courtesy of Gen-Probe Incorporated, San Diego, Calif.
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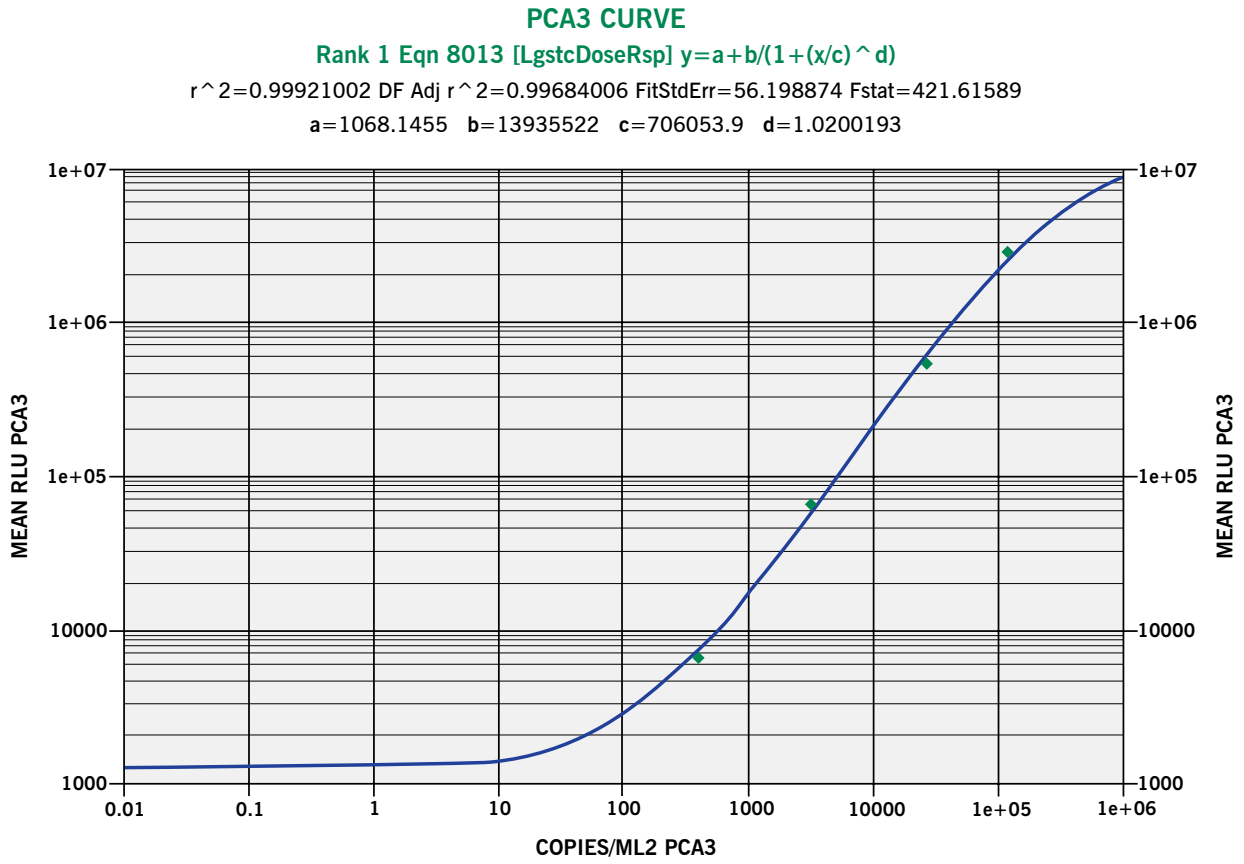

The PCA3 mRNA molecules are amplified using transcription-mediated technology, and the PCA3 score is calculated. Accuracy of the assay is independent of prostate volume or total PSA.

PCA3 and Biopsy

The current research focus is on the appropriate use and timing of the PCA3 assay in the prostate cancer diagnostic algorithm.

In a study of 260 American men with serum PSA of 8ng/mL, 111 men (43%) had a positive first biopsy. For men with a PCA3 score ≥ 35, the risk of positive biopsy was double that of men with a PCA3 score < 35. Men with a PCA3 score ≥ 35 had a 62% probability of a positive biopsy (5).

Several studies have proposed that a PCA3 assay should routinely follow an initial biopsy, regardless of biopsy results. Haese and colleagues (6) demonstrated a direct correlation between PCA3 score and positive repeat biopsy — the higher the PCA3 score, the higher the probability of a positive repeat biopsy. When the PCA3 score was 10, specificity for a positive repeat biopsy was 28%; when the PCA3 score increased to 35, specificity increased to 72%; when PCA3 score rose to 50, specificity was 81%. Likewise, the lower the PCA3 score, the lower the risk of a positive repeat biopsy.

In their study of 233 American men with an average PSA of 7.4 ng/mL and at least one negative biopsy, Marks and colleagues found that 60 men (27%) had a positive repeat biopsy (7). As PCA3 score increased, the percentage of men with a positive biopsy increased. Men with a PCA3 score ≥ 35 had a 2.5-fold increased risk of a positive biopsy compared to those with a PCA3 score < 35. In those men with a PCA3 score ≥ 35, the probability of a positive biopsy was 43%.

The correlation between PCA3 score and positive biopsy was demonstrated further in the REDUCE trial. In a population of 1140 men with a PSA of 2.5-10ng/mL and a negative initial biopsy, the second biopsy was positive in 18% of subjects.

Among those with a PCA3 score > 35, the risk of a positive repeat biopsy increased 3.5-fold. In subjects with a negative biopsy at two years, PCA3 score was predictive of a positive biopsy at four years. In subjects with a negative biopsy at year two and a PCA3 score > 35, the risk of a positive biopsy at year four was doubled (8).

Clinical Use of PCA3

Treatment for men with a positive biopsy and organ-contained disease may range from watchful waiting to radical prostatectomy, depending on tumor volume and grade. PCA3 offers a simple, noninvasive means to assist in treatment decisions derived from the assay's correlation with tumor volume.

Nakanishi *et al* (9) demonstrated a correlation between low PCA3 and low tumor volume, as measured in radical prostatectomy specimens. In 34 men with an average PCA3 score of 48.1, tumor volume was >2.0 mL; in 27 men with an average PCA3 score of 21.5, tumor volume was <0.5 mL. These investigators concluded that "PCA3 score is significantly lower (28.5) in low volume/low grade prostate cancer compared with PCA3 scores (58.5) in significant prostate cancer" and that "PCA3 was the best predictor of total tumor volume in prostatectomy." The authors suggested that a low PCA3 score signifies low volume, low grade disease, a conclusion that will require additional studies and confirmation by other investigators.

The medical and scientific community has adopted a PCA3 score of 35 as the cut-off for biopsy. This score appears to balance sensitivity and specificity to provide the greatest accuracy in predicting the results of an initial biopsy so that unnecessary biopsy can be avoided with minimal risk of missing clinically significant disease.

The PCA3 assay fills the need for a noninvasive diagnostic test for prostate cancer with higher specificity than serum PSA. The PCA3 assay has a role in cancer diagnosis and treatment planning by its ability to more accurately identify those men most likely to have a positive biopsy, thus reducing unnecessary biopsy and improving patient care by supporting better treatment decisions.

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PLMI Residents and Fellows Shine

Bryce Portier, MD, PhD

Zhen Wang, MD, PhD

Christi Wojewoda, MD

Shelley Redfern, MD

Continuing the Pathology & Laboratory Medicine Institute's (PLMI) legacy of leadership in the field, PLMI residents and fellows are making their mark in several of the leading professional societies.

Bryce Portier, MD, PhD
Zhen Wang, MD, PhD
Residents, Department of Molecular Pathology
Young Investigator Award
Association for Molecular Pathology (AMP) 2011 annual meeting

For their abstract, "A Quantitative Real-Time PCR Based Approach for Resolution of *HER2* Amplification"

AMP annually presents three Young Investigator Awards to recognize the best abstracts submitted by trainees who are AMP members and who present outstanding basic or applied research in poster format at the annual meeting. The mission of the award is to encourage junior investigators to ask important original questions, design sound, controlled experiments with a clear rationale, and present the results clearly in a poster format. Dr. Portier and Dr. Wang presented at the 2011 meeting in November in Grapevine, Texas.

Christi Wojewoda, MD
Clinical Microbiology Fellow
Trainee Travel Award 2011
Intersociety Council for Pathology Information (ICPI)

For her poster, "Performance Validation of Roche Cobas AmpliPrep/Cobas Taqman HIV-1 Test version 2.0 (CAP/CTM v2.0): Comparison to CAP/CTM v1.0 and Abbott Real Time HIV-1 Assay (m2000)"

The ICPI Trainee Travel Award supports trainee participation in scientific meetings by providing a stipend to offset the travel costs for attending one of ICPIs member societies meetings. Through her award, Dr. Wojewoda had the opportunity to participate in the AMP 2011 annual meeting with colleagues Dr. Portier and Dr. Wang, where she presented her poster and attended the scientific sessions.

Bryce Portier, MD, PhD
Resident, Department of Molecular Pathology
Scholar-in-Training Award 2011
Supported by Susan G. Komen for the Cure® Cancer Therapy & Research Center - American Association for Cancer Research San Antonio Breast Cancer Symposium

For his abstract, "HER2 Status Resolution in FISH and IHC "Double Equivocal" Breast Carcinomas by Quantitative Real-Time PCR"

The American Association for Cancer Research, with support from more than 40 cancer research organizations, corporations and individuals, annually awards grants to outstanding young investigators. Dr. Portier presented his abstract and received his award at the Cancer Therapy & Research Center - American Association for Cancer Research San Antonio Breast Cancer Symposium in December.

Shelley Redfern, MD
CP/AP Resident
Continuous Compliance Committee 2102
College of American Pathology (CAP)

The CAP Continuous Compliance Committee ensures that accredited laboratories maintain and improve continuous compliance with CAP and other regulatory requirements through oversight and education in the areas of proficiency testing (PT) and other mandated quality activities. The committee also monitors all CAP-accepted PT providers to ensure that they meet established criteria and develops guidelines for requiring PT assessment.

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Pathology Innovations Magazine offers information from the medical staff in the Cleveland Clinic Pathology & Laboratory Medicine Institute about its research, services and laboratory technology.

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A graduate of Kirksville College of Osteopathic Medicine, he served his residency in pathology at Cleveland Clinic and was named to the Cleveland Clinic professional staff in 1979. His former positions at Cleveland Clinic include Vice Chair of the Division of Pathology and Laboratory Medicine, Chair of the Department of Clinical Pathology and Section Head for Molecular Genetic Pathology, Department of Clinical Pathology.

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