LAYING the FOUNDATION for Next-Gen Cancer Diagnostics

Foundation Medicine prepares to apply NGS for individualized cancer care. BY KEVIN DAVIES

This summer, Foundation Medicine will launch what could be described as the next generation of cancer diagnostics.

The Cambridge, Mass.-based company, founded by a premier group of cancer researchers and funded by Third Rock Ventures, Kleiner Perkins Caufield & Byers and Google Ventures, will launch a comprehensive next-gen sequencing (NGS) profile screening some about 200 genes known to be clinically relevant and actionable in cancer using routine, formalin-fixed paraffin embedded patient cancer specimens.

"The care of oncology patients is on the verge of being individualized," says CEO Michael Pellini. "The molecular make-up of each tumor is going to drive personalized medicine. Our goal is to work with oncologists and pathologists and do nothing short of transform cancer care. We want to change the way cancer patients are managed, not just in academic medical centers but in communities across the U.S. and internationally."

"People in the field all say, 'If I or a friend was diagnosed with cancer, this is the test that I'd want run.' That's an almost universal »

Photos by Mark Gabrenya

Michael Pellini, Foundation Medicine CEO, intends to transform cancer care.

statement," says Pellini. "Our founders—Matthew Myerson, Levi Garraway, Todd Golub, and Eric Lander—understood the value of where cancer care and molecular diagnostics is heading and the role of next-gen sequencing."

Foundation offers an informative genomic profile in one broad-based test for all solid tumor cancer biopsies and DNA samples employing deep sequencing of about 200 genes that are clinically relevant to cancer. That information—mutation analysis, relevant targeted drugs, plus potentially appropriate clinical trials—will be communicated to physicians and oncologists in a user friendly report, helping guide their therapeutic decisions.

While the number of targeted therapeutics and molecular tests for cancer are growing in parallel, cancer biopsies are shrinking in size. "The oncologist or pathologist has to prioritize the molecular markers. That's a real issue," says Pellini. "Once you get beyond 3-5 markers, the costs to the system grow tremendously." The Foundation approach screens hundreds of genes simultaneously while preserving precious patient material.

Six months before full commercial launch, Pellini says everything is operational with specimens routinely running through billing. The company is already routinely processing specimens and producing reports for clinical cases on a by-request basis as it ramps up to full commercial launch. "We're doing things years ahead of anyone else right now. We're just at the tip of the iceberg," he says (see, "Pellini's Pitch").

Technology Review

Foundation's approach wasn't feasible a couple of years ago, let alone a decade ago. "Even if we had the technology, we didn't have the therapeutic options," says Pellini. But in addition to the breakthroughs that have brought us to the brink of the $1,000 genome, the recent FDA approval of two targeted cancer drugs—Pfizer's Crizotinib and Plexxikon's Venurafinib—has provided timely evidence that the oncologists' arsenal is strengthening. Hundreds more targeted anti-cancer compounds are currently in clinical trials.

Pellini also credits the launch of the KRAS genetic assay for colon cancer in 2007 (see, "Amgen's Personalized Medicine Story," *Bio•IT World*, April 2008) for triggering a wave of optimism that quickly spread to clinical practice. "Molecular markers were no longer confined to academic centers, they were being ordered by

Pellini's Pitch

Eight years ago, Pellini was running Genomics Collaborative, a leading biorepository start-up. The company was 5-10 years ahead of its time, Pellini says, gathering data on more than 100,000 clinical specimens (see, "Blood, Sweat and Tissue" *Bio•IT World*, March 2004). "We still need to access extremely well characterized clinical specimens. It's one of our great challenges," he says.

After the Genomics Collaborative assets were acquired by SeraCare (later transferred to BioServe), Pellini returned to Philadelphia. After a brief spell in the VC world, he founded and helped run a pathology company called Lakewood Pathology. Later he was invited to join the Board of Clarient in California. "I went out there in the summer of 2007 for six weeks, and never came home!"

Pellini signed on as president/COO and worked with their executive team to help Clarient build a robust diagnostics business with the notion of using a lab to help introduce new tests into the marketplace. Until Clarient, Pellini says diagnostics companies were either a service provider/standard lab, or a biotech-like company similar to Genomic Health, "betting the farm" on a single test or platform. There didn't seem to be much in the middle.

Clarient routinely tested tissue blocks from cancer patients for groups of standard molecular markers. "Roughly 75% of the time, an alteration would indicate another treatment option or a clinical trial option. Our chief medical officer would call the oncologist, and say, 'Here's a marker, there's a therapy that targets this alteration.' I can't say what happened to the patients in most cases, but we provided additional options. It was one of those 'Aha' moments."

Pellini was content to stay at GE Healthcare after he helped engineer Clarient's acquisition, but after a brief discussion with Alexis Borisy, he was convinced of Foundation's potential. "I believe we'll be the company to figure this out. This technology is going to penetrate the market, there's no question about it. It's just a matter of timing."

Pellini's biggest surprise since joining Foundation is big pharma's acceptance of the new rules of clinical trials and molecular diagnostics. "Ten years ago, they had blinders on; they felt threatened by the stratification of patients. I'm pleasantly surprised by pharma's appreciation that understanding each cancer at the molecular level is critical—on the science, medical, and marketing sides."

community doctors," he says.

The firm's core competencies extend beyond NGS, says Pellini. "The sequencing platform is not irrelevant, but it's simply a platform. The protocols are our protocols. The way we think about the application of the platform into clinical diagnostics is Foundation Medicine. This is the application of NGS into the cancer diagnostics world."

There are four critical pillars to Foundation's operation:

- Genome technology;
- Cancer biology;
- Information science; and
- Clinical oncology—the routine practice of cancer care.

Pellini isn't suggesting that NGS will replace other fundamental methods of cancer diagnostics, such as flow cytometry and immunohistochemistry. But he thinks NGS is destined to have a meaningful impact. "It's just a question of when. This is a complementary technology that will replace certain other molecular techniques."

While more and more academic centers are running small, tumor-type based cancer sequencing panels, Foundation aims to see its testing utilized in communities across the U.S. "We want to bring this technology to the community oncologist, that's where over 80% of cancer patients are treated," says Pellini. "We're investing tens of millions of dollars to get this

« right… Our goal is to gear up to run tens of thousands of profiles a year."

Taking the Tour

As the man in charge of Foundation's laboratory, Scotsman John Curran has to build a pipeline that satisfies not only in-house needs but also the various regulatory agencies that will grant the necessary certification. He graciously shows me around the facility even though he's preparing for an important state inspection.

"Next week is a very big week for us," he says. "Then we can start processing clinical samples." Sure enough, Foundation did indeed pass its Massachusetts state inspection and is now CLIA-certified. Until that point, Foundation was working only on samples from academic collaborators including Memorial Sloan Kettering Cancer Center and MD Anderson Cancer Center.

The labs consist of a series of spotless, interjoining rooms in which samples are received, DNA extracted and manipulated, and sequencing performed. The process is tied together by the Thermo Scientific Nautilus laboratory information management system (LIMS—see, "A Clinical LIMS").

Clinical labs typically employ a Laboratory Information System (LIS), but as Curran points out, in Foundation's environment, "You need a much more comprehensive informatics platform to handle information management, aggregate information across the enterprise, and a solution to handle a very dynamic business process" where new technologies can be plugged in with little notice.

Unlike a LIS, a typical LIMS isn't designed for patient data or HIPAA certification. So Thermo Fisher partnered with a clinical consulting firm to enhance the functionality of its Nautilus LIMS and provide the capabilities necessary for patient-centric data flow and physician-oriented interaction. "We offer the best of both worlds—a traditional LIMS with the patient-centric side of equation. We call it a clinical LIMS," says Dave Minicuci, Thermo Fisher's director of field marketing, informatics and laboratory automation.

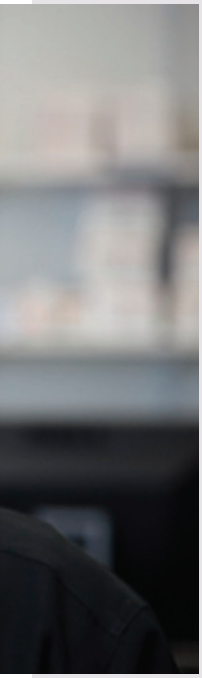
John Curran, Foundation lab manager

Similar systems have already been deployed at Children's Hospital (Philadelphia), Emory University, and the University of Miami.

The clinical LIMS not only tracks the receipt of samples, but a lab web portal provides an interface to exchange information with doctors, who can access the portal and order a test. "We send the shipping kit with barcodes, [they] apply the barcodes to the samples and forms, put it back in the box and send it here. We can track it, because it's already in the system," says Curran. "We envisage a turnaround time that starts when the oncologist orders a test. With

A Clinical LIMS

The choice of LIMS partner was a crucial decision for John Curran's team at Foundation Medicine. After narrowing selection to three vendors—Core LIMS, STARLIMS and Thermo Fisher—Curran's team judged that Core LIMS was better suited for biochemistry labs than molecular analysis, while the Thermo Scientific Nautilus LIMS edged STARLIMS based on references and demos.



Building a LIMS in house was never a viable option, not just because of the time constraints but also the need to ensure future flexibility. "We needed something that wouldn't constrain us to just DNA processing but could handle RNA processing or other pathology [applications]," says Curran.

Thermo Fisher's Dave Minicuci points to several features that made Nautilus a good fit, including reconciliation of arriving samples. "If there's a time delay—organizations don't make money when the samples aren't tested in the right sequence." Another advantage is the physician-friendly customization to the core Nautilus LIMS, which still supplies "the comprehensive information management layer, as well as a dynamic workflow capability," says Minicuci.

One area where the LIMS should shine is in assisting with a series of state and national inspections (including Massachusetts, CLIA, CAP, and New York state). "They want to make sure you're doing a good job processing clinical samples," says Curran. An inspector will typically pick a handful of patient samples at random, and ask to see the entire paper trail—requisition forms; training documentation forms (for everyone that touches the sample); maintenance documentation for all equipment (including pipettes) that touch the sample; reagent logs; control verification logs; and documentation for QC checks.

Curran typically pulls those forms and training records from a filing cabinet, but now he can enter an accession number, and have the information downloaded from the LIMS. "Even though the LIMS is not a reagent management system, we can capture all the information as we process it through, so I can get it all in one easy format at the end," he says.

the portal, we can track, call up, and ask if it's on the way."

After samples arrive, the barcode is scanned and the data entered into the LIMS. Tissues can arrive in many forms—in a block or on a slide, fresh or several years old—each requiring different processing steps. "Choosing a LIMS, we needed a system that could handle multiple sample input streams that would coalesce at extraction," says Curran.

A rigorous protocol maximizes the amount of nucleic acid extracted from tissue. "95% of the time, it should not be a limiting factor," says Pellini. "If you have to extract 50 nanograms (ng) for every single [sequential] gene test, you'd run out quite quickly," he says. The Foundation

The IT Guys

Like a car showroom, the data center at Foundation Medicine is proudly displayed in the center of the firm's corporate headquarters. Jared White leads Foundation's software engineering effort. He sees his role as to "take the big picture on how to wire together computation so it delivers the results of the pipeline in a robust way. We run over 50 analysis tasks/sample, and dozens of samples per plate, so how do you wire that together in a high performance, fault-tolerant way?"

White and colleagues integrate with the LIMS and the knowledgebase, while the computational biologists work closely on the individual analysis components. While some informatics tools came from the Broad, Sanger, and other sources, several key algorithms were developed in-house in order to obtain clinical-level performance in detection of low-frequency mutations, White explains.

Of the ten servers, two are master/slave, and the other eight are execution hosts. Because they are typically saturated, a hardware feature design called hyperthreading is leveraged that simultaneously supports two threads of execution per core, thereby effectively doubling throughput for a given number of rack units. "It's better than buying more physical cores—there's a greener energy footprint," White says. "Each box has 12 physical cores, but they're hyperthreaded so each box can support 24 concurrent CPU-intensive jobs."

Foundation currently uses 260 terabytes of Isilon storage, sufficient to store data on 10,000 patient samples through 2012. Each rack can hold up to 1.5 petabytes, so there is plenty of headroom. Virtualization minimizes the rack footprint and the power consumed.

"The pipeline is computationally intensive, but it also induces a lot of load on the Isilons," says White. The CPU load on the computational cluster as well as the I/O load on the storage cluster affects the performance of the analysis pipeline in different ways, and these need to be balanced.

"We have a very unique network," says Sam Tran, the head of IT Infrastructure. While one cluster supports the primary data center,

« lab is geared to working with as little as 50 ng DNA, although more sample can be requested if necessary. In the clinical marketplace, 15-20% of routine molecular tests fail. "Even though it is a complex NGS-based test, we still want to get it down less than 5% of the time we have a failure and have to make that phone call to the ordering physician."

Extra Cover

While research NGS applications typically settle for 30X genome coverage, a clinical grade test, particularly in cancer, requires much greater stringency. Due to tumor heterogeneity and the low purity of cancer samples, the frequency of important mutations in a given sample is often very low, and a clinical grade test must be able to detect them at a very high level of sensitivity and 100% specificity.

"100X does not work in routine cancer diagnostics, it only reveals a small percentage of the potentially important mutations [at a 1-5% frequency]," says Pellini. "The average lung cancer specimen is [only] 20% tumor. To find

Jared White leads Foundation's software engineering team, aided by a pristine data center.

another provides redundancy. The entire platform is tied together by a series of aggregated 10-Gigabit network connections that powers the servers and storage. The entire rack is cooled by 24 tons air conditioning—a cold air aisle in the front, fans sucking out the hot air in the back.

"One of the best features is there are no wires in the side or on top," says Tran. "We achieve that by coming from the ground." A strategically placed yellow Post-It note demonstrates "the enthusiasm of the fans."

an alteration that occurs at a frequency of 10% or less in a lung cancer specimen, you need to ratchet that coverage up north of 400X."

Foundation's median sequence coverage is 500-1000X on average, which Pellini believes will provide greater than 99% sensitivity and no false positives, which cannot be tolerated in a clinical setting.

Each sample is reviewed to ensure there is sufficient material to proceed, noting the tumor type, dimensions, and cellularity. In the DNA extraction lab, Foundation extracts DNA in as little as 30 minutes. Each DNA sample is graded on yield, purity, fragment size, and integrity. The information is plugged into the LIMS, which calculates whether the sample passes or not. "We've built an algorithm that enables us to predict which specimens we can't run through the sequencer to obtain the desired results," says Pellini.

The purified genomic DNA is next fragmented into 200-basepair fragments. A short (7-bp) molecular index barcode is integrated into each sample, which will eventually en-

able multiplexing. "We can track the samples with the molecular barcode, mix them all together, and at the end computationally separate them," says Curran. "My biggest fear is sample switching and sample contamination. That's why the LIMS is critical. If I see a barcode I wasn't expecting or misplaced, I know we have a problem."

Following PCR and library construction, the DNA is sized and normalized for hybrid capture, or what Curran calls "the meat of the assay." Foundation uses proprietary RNA baits for the optimal capture of the targeted genomic content. The captured material is then normalized for sequencing.

The LIMS creates a run sheet, detailing samples/lanes for one of the three (soon to be four) Illumina HiSeq 2000 instruments. HiSeq will be the workhorse, although Pellini expects Ion Torrent to play a role in the future. Curran also anticipates a role for Illumina's benchtop MiSeq or Ion Torrent's Personal Genome Machine, particularly in quality control. Leading Foundation's technology development group is Alex Parker, who joined from Amgen as employee #8 (see, "Ten Billion Genotypes," *Bio•IT World*, September 2008).

"We need to utilize technology that's ready for the clinical arena. We want to stay friends with Life Tech and with Illumina and whoever else enters the NGS field," Pellini says diplomatically. While the initial focus is on DNA, Pellini expects to add RNA analysis to "bridge the gap" between exome analysis and a full genome/transcriptome workup.

Mary Pat Lancelotta, director of strategic marketing

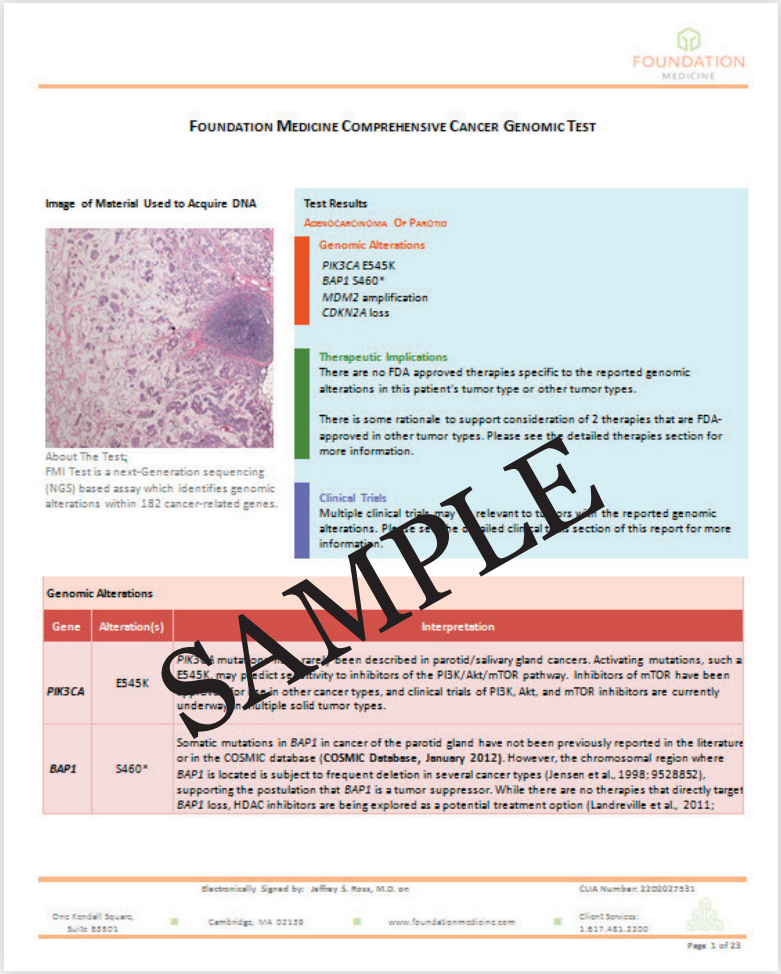
The informatics team led by Doron Lipson and Roman Yelensky (see, "The IT Guys"), formerly with Helicos and Novartis, respectively, processes the sequence data and performs variant analysis for detection of sequence mutations, copy-number alterations and genomic rearrangements. The detected alterations are fed back to the knowledge base, which in turn provides the necessary correlation to curated medical and scientific information to generate the result report. Coming full circle, that report

can be delivered through the lab web portal to the physician, ideally no more than 14 days from the test order date.

Final Answer

Mary Pat Lancelotta, Foundation's director of strategic marketing, shares with me a mock physician report (which will be provided in print or in a digital, interactive format through an online portal), which summarizes the key mutations, relevant drugs and clinical trials. As a diagnostics laboratory, Foundation doesn't have all of the information about the patient and can't recommend a particular action, but they can supply critical information that the physician might not know or have time to research.

"We can't recommend a particular action, but we can say, 'Here are the genomic alterations and what they mean for this patient, and based on an exhaustive search of the literature, here are the therapeutic agents and clinical trials that you and your patient could consider," she says. "We're giving the physician a fully informative genomic profile as a tool to help them make the next treatment decision with their patient."


FOUNDATION MEDICINE

FOUNDATION MEDICINE COMPREHENSIVE CANCER GENOMIC TEST

Image of Material Used to Acquire DNA

About The Test:
FMI Test is a next-Generation sequencing (NGS) based assay which identifies genomic alterations within 182 cancer-related genes.

Test Results

ADENOCARCINOMA OF PAROTID

Genomic Alterations
PIK3CA E545K
BAP1 S460*
MDM2 amplification
CDKN2A loss

Therapeutic Implications
There are no FDA approved therapies specific to the reported genomic alterations in this patient's tumor type or other tumor types.

There is some rationale to support consideration of 2 therapies that are FDA-approved in other tumor types. Please see the detailed therapies section for more information.

Clinical Trials
Multiple clinical trials may be relevant to tumors with the reported genomic alterations. Please see the detailed clinical trials section of this report for more information.

SAMPLE

Genomic Alterations

Gene	Alteration(s)	Interpretation
PIK3CA	E545K	PIK3CA mutations have been described in parotid/salivary gland cancers. Activating mutations, such as E545K, may predict sensitivity to inhibitors of the PI3K/Akt/mTOR pathway. Inhibitors of mTOR have been approved for use in other cancer types, and clinical trials of PI3K, Akt, and mTOR inhibitors are currently underway in multiple solid tumor types.
BAP1	S460*	Somatic mutations in *BAP1* in cancer of the parotid gland have not been previously reported in the literature or in the COSMIC database (**COSMIC Database, January 2012**). However, the chromosomal region where *BAP1* is located is subject to frequent deletion in several cancer types (Jensen et al., 1998; 9528852), supporting the postulation that *BAP1* is a tumor suppressor. While there are no therapies that directly target *BAP1* loss, HDAC inhibitors are being explored as a potential treatment option (Landreville et al., 2011;

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This mock-up of Foundation's physician summary highlights mutational profile, potential drugs and available clinical trials.

The final report will have a very strong human element, says Pellini. Every report is reviewed by a staff oncologist and pathologist with expertise in genomics before it is sent out. "Even if we jump ahead a year and populating the report is largely automated, our medical director will be responsible for the accuracy of the information. There will always be the involvement of an oncologist and pathologist in the final report." Pellini expects insurance companies to reimburse for the test "somewhere in the $4,000-5,000 range," but it will be a learning process, he admits.

But what will physicians do with that information? Foundation faces a massive challenge to improve physician education. "Many pathologists and oncologists today had no formal training in genetics/genomics because the field is so new," says Lancelotta. "We're trying to provide them with a tool to help them shift their thinking from an exclusively tumor-type way of thinking to a pathway model so that they can focus on their patients rather than molecular biology."

Commercial success hinges on more

Foundation's headquarters in Kendall Square has an element of Silicon Valley playfulness, from the bean-bag conference room furnishings to the wall of employee caricatures.

than just sophisticated technology and communication channels. Lessons can be learned from Genomic Health, the Bay Area company that commercialized the OncotypeDx gene expression test for breast cancer. Two former Genomic Health executives, Maureen Cronin and Gary Palmer, have joined Foundation, although Pellini doubts the Genomic Health model will be replicated. "It's very difficult for a diagnostics company to spend $60-90 million in marketing each year. They did a masterful job and paved the way for other diagnostics companies. We get to learn from what they did well."

As for Foundation's fortunes, "There's no magic here," says Pellini. It comes down to presentations at key medical and scientific conferences, peer review publications, and academic and pharma collaborations. "We have to arm the sales force with the publications, we'll have early adopters in academic medical centers. We have a clinical advisory board, and we'll have groups meeting around the country to [keep abreast of] the market… Over time, we see this moving more and more into the community where we can benefit the greatest number of patients."

Even as Foundation ramps up, the company has struck agreements with several pharma companies, including Novartis, Celgene, and Johnson & Johnson. "As compounds go through clinical trials, we're actively helping them understand who is responding, what they have in common, and how can we get drugs to market quicker," explains Lancelotta. "We're helping pharma get therapies to market faster. In turn, our test becomes more actionable because there are more therapeutic options for patients."

Pellini says pharma is looking for genomic data upfront, not only to characterize patient subpopulations that respond well, but also to rescue failed trials. "A few pharmas have told us that they plan to sequence every patient that enrolls in one of their cancer-based clinical trials going forward. They understand the value of these data," he says. "Doing this with a clinical-grade test has distinct advantages as the companies are under pressure to show how patients who are most likely to respond can be routinely identified."