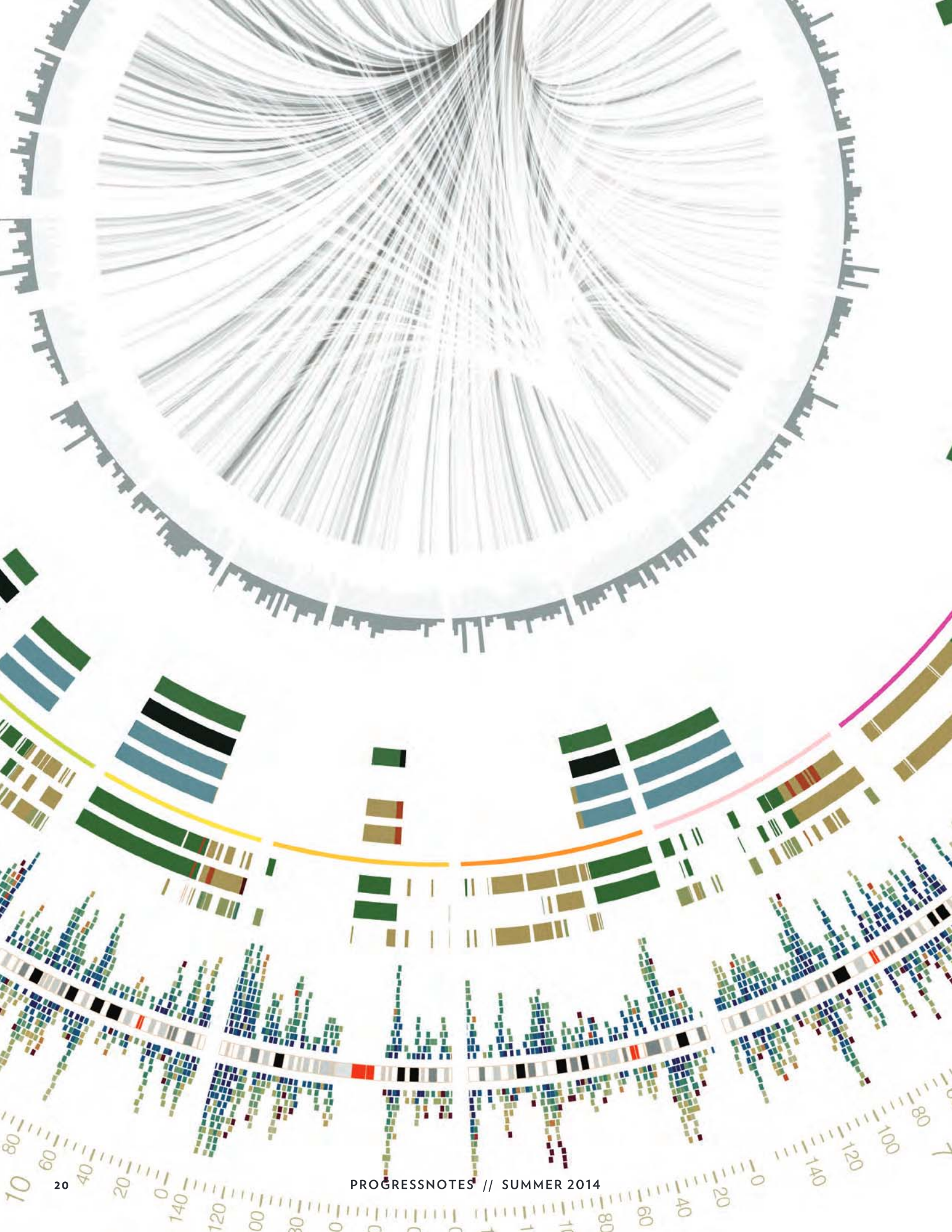
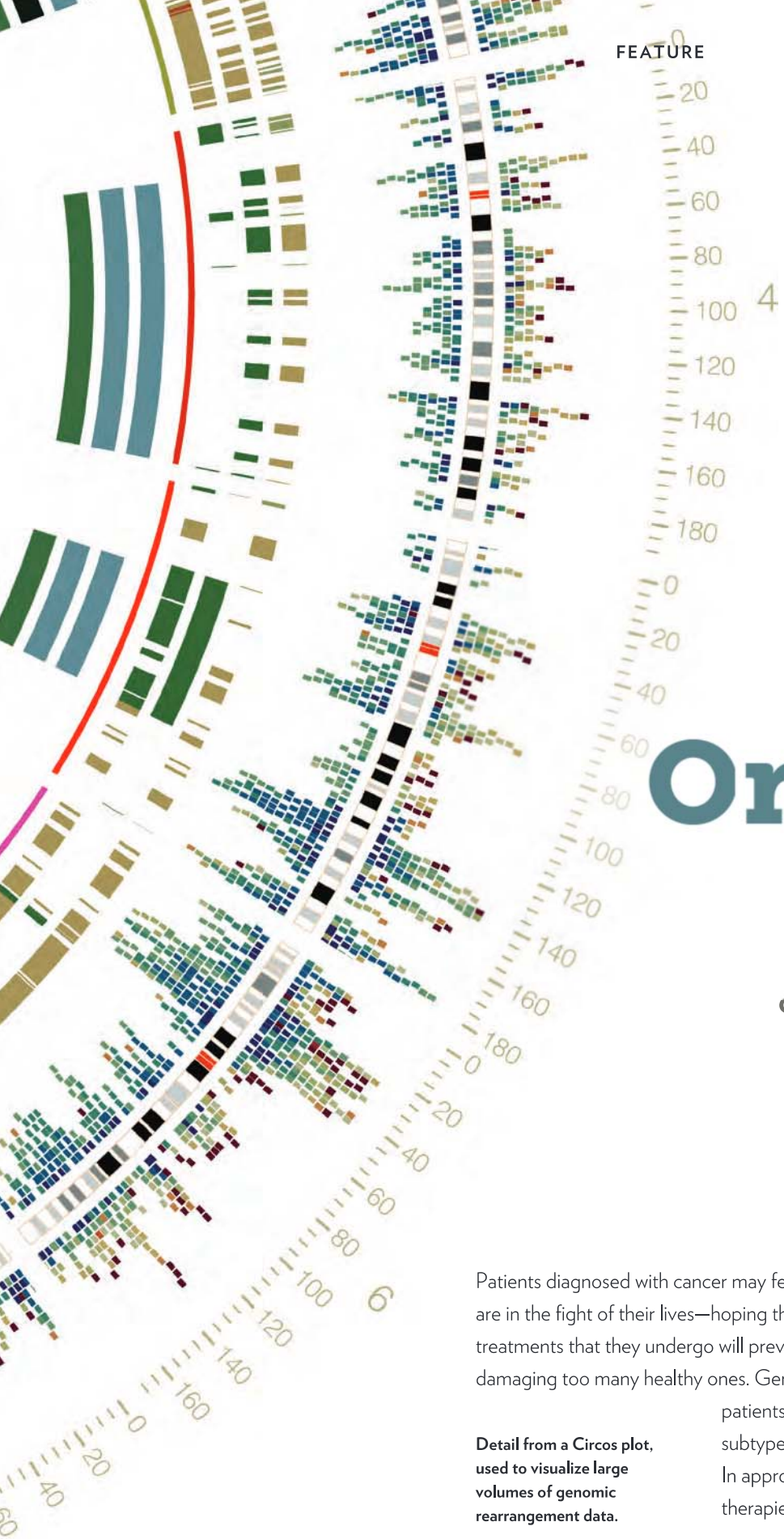




Right On Target

Clinical genomics identifies cancer patients likely to benefit from targeted therapies

BY KIMBERLY MCGHEE
ILLUSTRATION BY MARTIN KRZYWINSKI

Patients diagnosed with cancer may feel as if the odds have been stacked against them. They are in the fight of their lives—hoping that the sometimes harsh chemotherapeutic or radiation treatments that they undergo will prevent the rapid proliferation of cancerous cells without damaging too many healthy ones. Genomics screening can improve the odds for some cancer patients, potentially identifying mutations associated with cancer subtypes for which targeted therapies exist or are in development. In appropriately selected patients, response rates for targeted therapies are often higher than for conventional chemotherapy.

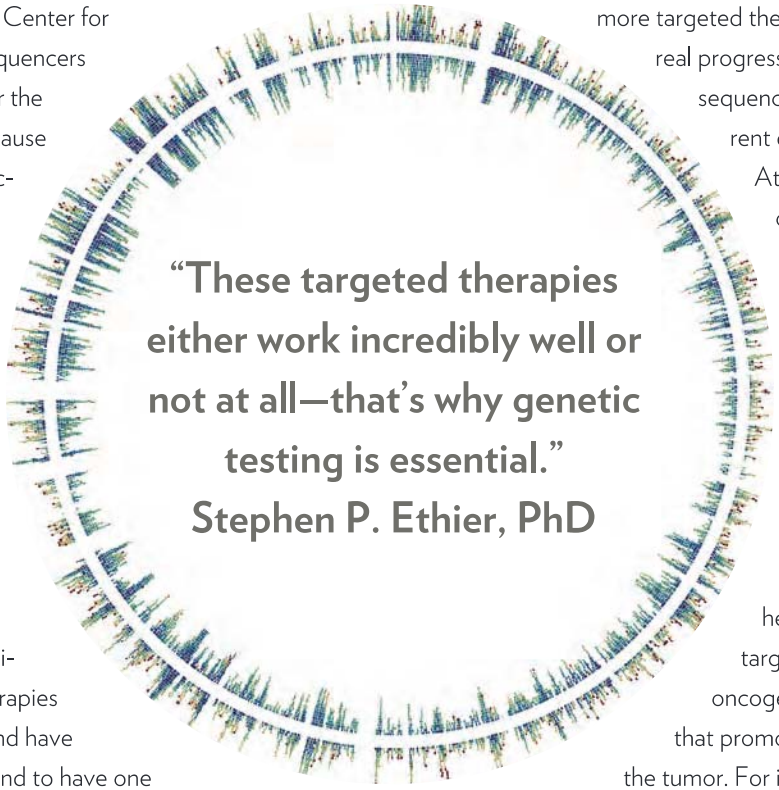
Detail from a Circos plot, used to visualize large volumes of genomic rearrangement data.

In January 2014, the CLIA-certified clinical genomics laboratory at MUSC expanded to include state-of-the-art next-generation sequencing (NGS). These efforts were led by **Daynna Wolff, PhD**, Director of Cytogenetics and Molecular Genetics, and **Julie Woolworth, PhD**, Associate Director of the Molecular Pathology Laboratory. The NGS technology allows biopsy samples from select cancer patients to be screened against a panel of 26 known oncogenes for which targeted therapies have been developed.

The MiSeq™ desktop sequencer (Illumina; San Diego, CA) used in the clinical genomics laboratory is the "kid brother" of HiSeq 2500™, Illumina's high-throughput sequencer, which was recently acquired by the MUSC Center for Genomic Medicine. Desktop sequencers such as the MiSeq™ are ideal for the clinical diagnostic laboratory because they offer the expanded sequencing ability of NGS together with practical advantages: they do not take up much space, provide user-friendly interfaces, automate many of the required steps for sequencing, and incorporate bioinformatics software that provides easy-to-interpret and actionable reports for clinicians.

Screening results can help clinicians to make treatment decisions. For example, targeted therapies would likely be more effective and have fewer side effects in patients found to have one of the mutations, whereas conventional chemotherapy would be the treatment of choice for patients without any of the mutations. Genomics screening can also identify resistance mutations that are predictive of poor response to targeted therapies, sparing patients unnecessary treatment exposure and expense.

Genomics screening is not indicated in all cancer patients; it makes most sense in cancers for which clear targets have been identified and for which effective targeted therapies exist. Initial plans at MUSC are to screen all patients with later-stage non–small cell lung cancer, colon cancer, or melanoma, all of which are cancers with identified targets and available targeted therapies. Patients whose cancer recurs after conventional chemotherapy and patients with metastatic disease would also be screened.



Targeted Therapies

Interest in targeted therapies has grown as researchers have realized that cancer is not a single disease entity, as once thought, but a plurality of subtypes, many of which are associated with characteristic mutations. To paraphrase Leo Tolstoy's famous opening line to *Anna Karenina*, healthy cells are all alike; every cancer subtype is unhealthy in its own way.

More than four decades after Richard Nixon declared a war on cancer with the National Cancer Act of 1971, mortality due to cancer in those aged forty and older remains high, leading many to believe that a shift from conventional chemotherapies to more targeted therapies will be necessary before real progress can be made. The successful sequencing of the human genome, current efforts by the Cancer Genome Atlas to sequence the genome of many cancer subtypes, and rapid advances in sequencing technology have further fueled interest in these targeted therapies. Conventional chemotherapy and radiotherapy take broad aim at cancer, trying to kill as many cancerous cells as possible but inevitably inflicting collateral damage on healthy ones as well. In contrast, a targeted therapy attempts to disrupt oncogene-activated signaling pathways that promote the growth and spread of the tumor. For instance, 10% of patients with non–small cell lung cancer have tumors with a mutation in the epidermal growth factor receptor (EGFR) that renders them susceptible to the small molecule drug erlotinib. These patients are very likely to benefit from targeted therapy with erlotinib, whereas those without the mutation would not.

According to **Stephen P. Ethier, PhD,** Interim Director of the Center for Genomic Medicine, "These targeted therapies either work incredibly well or they work not at all—that's why the genetic testing is essential."

Clinical Genomics Testing and Clinical Trials

Genomics-based drug development challenges some of the basic assumptions of traditional clinical trials, which were developed to test

GLOSSARY

Bioinformatics/Computational Genomics: Use of computational and statistical analysis to determine the biological relevance of genomic sequencing data.

ChIP Sequencing: A combination of chromatin immunoprecipitation and next-generation sequencing that can precisely identify binding sites for proteins.

Epigenetics: Heritable, functionally relevant changes in gene activity, possibly due to environmental pressures, that do not involve sequence changes. Examples are DNA methylation and histone modification.

Exome: The portion of the genome that codes for protein production. Sequencing of the exome can be a time- and cost-efficient alternative to sequencing of the whole genome when screening for mutations.

Genome: The totality of the genetic material of an organism, usually encoded as DNA.

Metagenomics: The genomic analysis of the viral and bacterial communities resident in a human and their potential links to diseases that involve disruptions of a microenvironment (e.g., chronic colitis).

Mutation: A change to the nucleotide sequence of a gene.

Nucleic Acid Sequence: A succession of letters (e.g., GACT) that indicate the order of nucleotides within a DNA molecule.

Oncogene: Mutated forms of normal cellular genes (proto-oncogenes) that, when activated, are capable of transforming normal cells into cancerous ones.

Next-Generation Sequencing: Also known as massively parallel sequencing, next-generation sequencing can analyze millions of loci simultaneously, providing far more comprehensive genomics data than previous technologies such as microarrays and Sanger sequencing and without the need for a probe that can bias results.

Pharmacogenomics: The use of genomics screening data to predict a drug's efficacy and side effect profile in an individual.

Single-Nucleotide Variant: A one-letter (one-nucleotide) variation in the nucleic acid sequence of a gene.

Targeted Therapy: A drug or immunotherapeutic therapy that has been developed to precisely target and disrupt cancer-promoting cellular signaling pathways in cancer subtypes associated with a particular gene mutation.

Whole-Genome Sequencing: A technique for obtaining the complete DNA sequence of an organism's genome.

more conventional, broad-based therapeutic regimens. Traditionally, the gold standard for proving efficacy was evidence of treatment response in phase 3 trials that recruited large numbers of patients with cancer of a single tissue (e.g., breast cancer). When tested in appropriately selected patients (i.e., those with the relevant mutation), targeted therapies have much higher response rates and can attain statistical power with far fewer trial participants. Pharmaceutical companies have now begun to develop targeted therapies and the diagnostics needed to identify appropriate patients simultaneously.

For many genomics experts, the current clinical trial system is ill-suited to bringing cutting-edge targeted therapies to the patients who need them. Oncogenes identified by genomic sequencing are often associated with more than one type of cancer; for example, the HER2 oncogene is associated with breast, ovarian, and lung cancers, among others. However, the efficacy of the therapy has only been proven in phase 3 clinical trials for breast cancer. Patients with HER2^{+} ovarian cancer could only be offered the treatment off-label, without hope of insurance reimbursement, making it prohibitively expensive for most.

Realizing the Promise of Personalized Medicine At Last

Fifteen years have passed since personalized medicine was first heralded as the future of health care, yet its promise has not been realized as quickly as many predicted. This is largely because the technology seemed out of reach for many health care institutions and the expense was prohibitive.

However, the cost of genomic sequencing has been decreasing even as its reach has been expanding. Next-generation sequencing, the development of which was spurred by efforts to sequence the human genome, can generate far more genetic data far more quickly and at a lesser cost than traditional sequencing technologies such as Sanger sequencing and DNA microarrays. The sequencing of

"The oncogene is everything when it comes to targeted drugs—it trumps the tissue of origin." —Stephen P. Ethier, PhD

Dr. Ethier believes that this needs to change if we are to realize the promise of genomic screening: "There is a mindset in the community that the tissue of origin trumps the oncogene. And people like me are trying to flip this around and say that the oncogene is everything when it comes to targeted drugs—it trumps the tissue of origin." In his view, the U.S. Food and Drug Administration must revamp the clinical trials process so that therapies that prove effective against oncogenes in one setting can be used in other patients with that oncogene, regardless of tissue of origin, though this does need to be done in a clinical trial setting.

Although the clinical genomics laboratory is currently using a commercial panel of mutations for its screens, Dr. Wolff plans to create customized panels in the future, in part to attract more clinical trials of targeted therapies to MUSC. She is collaborating with **Carolyn D. Britten, M.D.**, Director of the Phase 1 Clinical Trials Program at MUSC's Hollings Cancer Center, to develop diagnostics for targeted therapies that are just entering phase 1 trials. Once pharmaceutical companies realize that such diagnostic testing is available to identify patients with the targeted mutation, they will be more likely to open phase 1 trials at MUSC, making some of the newest and most cutting-edge treatments available to South Carolinians. MUSC could even become the central genomics testing site for some of these studies.

the 3 billion base pairs of the human genome required several years using the older technology at a cost of about $13 billion. In contrast, the latest iterations of NGS platforms such as the HiSeq 2500™ can sequence that number of base pairs in 25 hours for around $1000. NGS can sequence exponentially more base pairs than previous techniques because it can simultaneously analyze millions of DNA fragments and because, unlike those older technologies, it does not require decoupling of the sequencing and detection steps (i.e., they occur simultaneously, not sequentially) or the use of a probe (sequence segment) that could bias results. According to Dr. Wolff: "The nice thing about this is that when we have lots and lots of copies of the pieces of DNA, we can reassemble them and look at the entire genome. It gives us higher fidelity of results and it's a lot faster."

Gary T. Hardiman, PhD, the new Director of the Informatics Core for MUSC's Center for Genomic Medicine, not surprisingly reaches for an IT analogy when describing the potential of this new sequencing method: "NGS takes you away from technologies like microarrays and replaces this analog technology with digital technology. In the past, we were interrogating just a small region of each transcript

delineated by a 60- to 70-base pair probe and now we can sequence across the entire transcript."

Rapid advances in NGS technology will enable care to be tailored to the individual patient's needs, especially as whole-genome sequencing becomes more feasible in the clinic. Researchers with The Cancer Genome Atlas project are using NGS technology to sequence the genome of many subtypes of cancer, spurring research to develop new therapies against identified targets and speeding a much wider-scale implementation of targeted therapies in clinical practice. The rapid availability of robust genomics data will also be a boon to pharmacogenomics, enabling better prediction not only of whether a person will likely respond to a therapy but also whether they are likely to develop side effects. The pharmaceutical industry has been somewhat reluctant to embrace personalized medicine and targeted therapies. However, it is beginning to warm to these therapeutic paradigms as NGS identifies new uses for older drugs as well as subpopulations that might benefit from drugs that have been previously abandoned because of disappointing clinical trial results.

It is not surprising that early genomics efforts have focused on cancer. Characterized by mutations of a patient's own healthy cells into lethal ones, cancer is, in some ways, the most personal of diseases. Next-generation sequencing offers a better understanding of these mutations in a clinically relevant timeframe and offers to identify therapies for cancer patients that are at once more effective and less toxic because they are profoundly personal.

Physicians who would like to use the services of the clinical genomics laboratory for screening for lung cancer, colorectal cancer, and melanoma may contact MUSC Health Lab Client Services at 843-792-0707. The clinical genomics laboratory will also evaluate metastatic tumors of other origins for screening of variants that may help to identify a potential therapy or clinical trial. Any identified potential clinical trials will be reported back to the ordering physician.

Harnessing Big Data

Next-generation sequencing (NGS) creates exponentially more data than previous sequencing technologies, making data interpretation the next big challenge for the field. High-throughput systems such as the HiSeq 2500™ can produce up to 1 terabyte of data when running at maximal performance. Having access to this much genomic data is unprecedented, but it will not bring about the revolutionary changes it promises in our understanding and treatment of disease if we do not find ways to efficiently interpret it. This quantity and complexity of data requires analysis by bioinformatics experts specializing in computational genomics.

MUSC's Center for Genomic Medicine, led by **Stephen P. Ethier, PhD**, recently recruited **Gary T. Hardiman, PhD**, a specialist in computational genomics, to ensure that maximal research and clinical benefit is derived from recent investments in expanding its genomic sequencing capacity (e.g., the purchase of MiSeq™ and HiSeq™ 2500). Dr. Hardiman works closely with **Robert C. Wilson, PhD**, who heads up the NGS core and is responsible for providing HiSeq 2500™ sequencing data to researchers.

Dr. Hardiman joined MUSC in February 2014 from the University of California at San Diego, where he established and led the Biomedical Genomics Facility. At MUSC, he has been charged with building the IT infrastructure needed to store, transfer, and interpret genomics data for both clinical and research projects. He is currently working to put into place the pipelines needed for RNA sequencing analysis, variant calling DNA sequencing using exome capture, ChipSeq™, and metagenomics (16s ribosomoal RNA sequencing).

The expanded sequencing offered by NGS will reveal many more single-nucleotide variants relevant to disease that could become new targets for intensive research. Because it screens so many loci from across the genome, it can detect epigenetic alterations (e.g., changes in DNA methylation and modification of histone) that do not actually alter the genomic sequence. Such changes, some of which have been linked to cancer and all of which are heritable, are thought to result in part from exposure to environmental toxins such as smoke or pollutants.

Metagenomics explores the microenvironment inside of each human, recognizing that we are constituted not only of human DNA but also of the DNA of resident bacteria and viruses. Sequencing the DNA of these resident microorganisms could improve our understanding of diseases characterized by chronic inflammation due to disruptions of the body's microenvironments (e.g., chronic colitis), disruptions that some think may set the stage for cancer.

Dr. Gary T. Hardiman, Director of the Informatics Core at MUSC's Center for Genomic Medicine

To realize the potential of NGS for research, the data generated by the sequencer, typically accessible only to the bioinformatics expert, must be made available to other clinicians and researchers for analysis. **E. Starr Hazard, PhD**, Director of MUSC's Computational Biology Resource Center, who is working to optimize an open source graphical user interface called Galaxy (usegalaxy.org) for this purpose, describes the program as "an open source analysis and workflow management software that can provide scientists without command line skills access to state-of-the-art genomics software via a user-friendly interface." Rollout of Galaxy is expected in the coming months and will allow all MUSC researchers to interrogate the massive amounts of genomics data being produced by NGS.