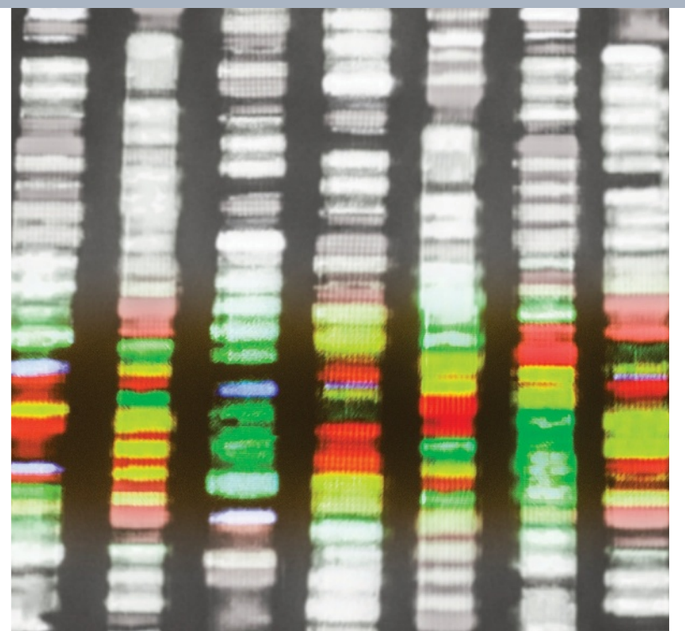
GEN eBook

Next-Gen Therapeutics Begin with Targeted Sequencing



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Welcome

A major step towards personalized medicine was achieved in early 2014, when the first report of sequencing a human genome for $1,000 was announced—hailed by some as one of the greatest scientific and medical achievements of the last decade.

Within the genome itself, approximately 85% of all genetic diseases can be traced to 1% of the genome that codes for genes, termed the exome. Target enrichment enables the sequencing of specific regions of interest in the human genome—such as the exome—which allows large volumes of exomes to be captured and sequenced simultaneously. This significantly lowers sequencing costs and reduces the barriers to access for personalized medical and high-throughput genetic research.

Twist Bioscience was founded in 2013, offering high throughput oligonucleotide synthesis on their innovative silicon-based DNA Synthesis Platform. Leveraging this state-of-the-art technology to elevate the next generation sequencing market, **Twist Bioscience** now offers a full range of Exome and Custom Next-Generation Sequencing (NGS) Target Enrichment Solutions.

Twist Bioscience's NGS Target Enrichment Solutions provide exceptional performance, greater flexibility, and maximum capture efficiency that allows researchers to increase the sequencing depth and increase sample throughput with each run. High-fidelity double-stranded DNA probes, a proprietary amplification method, optimized probe boosting and NGS QC of each oligo ensures very uniform capture efficiencies of probe pools. This eliminates exon dropout and minimizes false negatives that would arise without a built in QC methodology. Researchers have the flexibility to purchase the complete kit—including all modules needed for library preparation and target enrichment—or simply the high-quality capture probes compatible with multiple workflows. Rapid design iterations for custom panel designs as well as customized exomes allow the user's research to move at the pace of innovation.

Target capture technologies have already started to make personalized next-gen methods that involve genome screening more accessible to researchers and clinicians. **Twist Bioscience**, in partnership with GEN, present this eBook focused on how targeted sequencing is changing the development of modern clinical research.

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Next-Gen Therapeutics Begin with Targeted Sequencing

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Commonplace Sequencing Makes Disease Less Rare

Researchers and Clinicians Play a Balancing Act Between the Cost and Clinical Utility of WGS versus WES When Diagnosing Rare Diseases

Jeffrey S. Buguliskis, Ph.D.

It's not out of the ordinary upon hearing the word "rare" one conjures images of precious metals, dazzling jewels, or artifacts from a bygone era. It would be a unique person who would think of minute variations in the human genome as synonymous with rarity, but that is exactly how disease-hunting scientists tend to think. While the practical approach of empirical trial and error has produced strong therapeutic results for many maladies, rare diseases represent a particular challenge for investigators that has been seemingly insurmountable—until the recent dawn of the genomic era.

Rare diseases or as many investigators often call them, undiagnosed diseases, are in many ways a mathematical problem. The first part of the equation is the classification of prevalence. Where in the world an individual hails delineates how the prevalence of rare disorders are defined. In the United States, the Rare Diseases Act of 2002 states that "any disease or condition that affects fewer

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If the cost of whole-genome sequencing can be reduced, then we may begin to see a tipping point in molecular diagnostic use within the clinic. Firstsignal / Getty Images

than 200,000 people," or about one person in 1,500, is classified as rare. In Japan, however, rarity is defined as diseases that affect less than 50,000 people (about 1 in 2,500), with similar numbers for Europe (approximately 1 in 2,000).

The second part of the equation lies in the actual number of people with rare, undiagnosed disorders, which is actually quite a large number and seemingly antithetical to the idea of being "rare." For example, many estimates suggest that 5% to 10% of the U.S. population is afflicted, and more than 300 million people worldwide are living with, at least one of the 7,000 genes currently defined as rare.

The final mathematical challenge lies within the diagnostic and therapeutic realms. Currently, it takes an average of seven years for a diagnosis of a rare disease, which constitutes an average of eight different clinical visits and three misdiagnoses. This is incredibly frustrating for patients and their families as a significant bulk of undiagnosed disorders affect children. Moreover, 95% of rare disorders do not have a single FDA-approved treatment. Yet, clinicians and researchers are only as good as the diagnostic tools at their disposal that are validated for prognostic duty.

Advanced sequencing techniques and molecular diagnostic tests are facilitating rapid detection of rare genes, but

investigators still face a catalog of genetic variants that require disease confirmation status. "How do we deliver care using genomic medicine?" is the question that drives Howard Jacob, Ph.D., executive vice president and chief medical genomics officer for HudsonAlpha Institute for Biotechnology, to continually speak about the indispensable value of next-generation sequencing (NGS) technology for identifying and diagnosing rare disease.

Choose Wisely

With prices for NGS continuing to plummet, genomics is moving out of the laboratory as a tool for pure research and beginning to cross the threshold into the clinical space. But not all genomic tests are created equal and, with a variety of options to choose from, how do physicians decide which test to use, which is best, and which will be reimbursed?

"There's a big debate about this, with a roughly 50-50 split between whole exome sequencing (WES) and whole genome sequencing (WGS)," noted Shawn Baker, Ph.D., co-founder of AllSeq Consulting. "The arguments tend to center on the greater affordability of WES versus the greater diagnostic yield of WGS. When looking to maximize the number of diagnoses, WGS wins out. When trying to maximize the number of diagnoses per dollar spent, it's less clear. However, when we talk with actual clinicians, most are already struggling with targeted and exome approaches and simply aren't equipped to handle the analysis of whole genomes."

As with all emerging technologies, the various sequencing modalities come with their fair share of pros and cons. Many clinics use targeted exome sequencing for well-defined disorders that often have validated biomarkers. These tests require manufacturers to synthesize a small number of genes and gene variants, which keep test costs down and results rapid. However, since the overwhelming preponderance of rare disease cases are caused by *de novo* mutations (approximately 65%) occurring at some functionally important region, it becomes difficult for researchers to identify the particular genetic markers, let alone place them into a targeted genetic panel for clinical diagnostic use. This wrinkle causes researchers to swing the genetic pendulum in the other direction in an attempt to maximize the amount of genomic coverage per test.

Most commercially available exome capture kits cover approximately 99% of the reference sequence (RefSeq) databases' exome information, and over 95% of the targeted bases are covered at least eight times with a typical WES run—suggesting that there is a large of amount exome coverage being achieved. Yet the positive diagnostic rate of clinical

Validating sequence biomarkers lies at the heart of adopting clinical sequencing in the diagnosis of rare disorders. catalinr / Getty Images

WES for rare phenotypes settles in around only about 30%—signifying that a substantial portion of the remaining phenotypes might be caused by variants located outside of exons or are not detectable by WES. Although the majority of functionally critical and disease-causing mutations occur in protein-coding regions, most of the genome is noncoding and may contain variants with functional significance that have been overlooked.

> Building up the database of new variants, coupled with medical phenotypic data will really push clinical sequencing forward.

"The ideal technology for identifying disease-causing gene variants depends on the context of the question being asked," explained *Clinical OMICs* advisory board member Jason Park, M.D., Ph.D., who is also the medical director in the Advanced Diagnostics Laboratory at Children's Medical Center, Dallas. "If the context is a specific patient and a clinical test result is required, then the only approach is exome sequencing or syndrome focused gene panels. From a research context, the best approach is a combination of WGS and RNAseq."

WGS does have some clear advantages over WES, the obvious being that WGS covers the regions that are missed or not included in WES, which can be critically important in uncovering mutations that lead to rare disorders. Furthermore, WGS typically generates more uniform sequence coverage, and it can take advantage of longer reads, which provide much more useful information on copy number variations and other DNA structural alterations.

But WGS is not without its drawbacks. Cost and speed are intertwined aspects of modern healthcare and are factors when deciding which diagnostic test is to be utilized. Since there is far less genomic information to read, WES is undoubtedly faster than its counterpart, and currently, WGS projects range between $1,500 to $2,000, which gives WES the advantage. But that advantage is not as clear cut as it once was, as exome sequencing endeavors have slipped slightly below $1,000.

As strong an advocate as Dr. Jacob has been for the widespread clinical use of WGS, especially in children, he is still a pragmatic scientist who understands that a variety of sequencing methods exist, all with varying degrees of clinical usefulness. "I would never say that a single test does everything," Dr. Jacob told *Clinical OMICs*. "What we know in medicine is there are very few things that are absolute and what I can say about whole-genome sequencing is that there are still holes."

Where Do We Go from Here?

As stated previously, the problem of rare disease genomics is largely a mathematical one. To identify a significant proportion of rare disease variants, we need to accrue quite a large number of genomes from the population—most likely into the millions—to provide enough coverage and accuracy. "Each new genome sequenced contains millions of variants, most of unknown significance," Dr. Baker added.

"Building up the database of new variants, coupled with medical phenotypic data, will really push

It takes an average of seven years for a diagnosis of a rare disease, which constitutes an average of eight different clinical visits and three misdiagnoses.

Image Source / Getty Images

clinical sequencing forward. As NGS continues to improve and become more readily accessible, we should reach the critical mass necessary to have a real impact on undiagnosed disorders."

"Research has a very finite budget. So at some point, you have to say research has done its job, now it needs to be a commercial application," Dr. Jacob noted. "Science has done a really good job getting us to that launch point, but we still have a lot to learn about disease. Some of the really hard challenges of common disease are going to require tens of thousands or millions of people to have their genome sequenced, and the big question is how are you going to pay for that?"

Dr. Jacob continued stating that "we haven't hit the inflection point of adoption [for NGS], so we're still in that justification and validation phase before we hit that inflection point, where the price value proposition then makes it worthwhile to do it more—I think that's one of our biggest limitations."

Technology often has a way of leveling the playfield for the disenfranchised and remains healthcare's best hope for developing new therapeutic avenues for disease treatment. Newer NGS techniques like RNAseq, nanopore sequencing, and long-read sequencing have emerged from the research space and are being rapidly adopted into the clinic, filling in the gaps left by WGS and

WES, and in some instances, even surpassing the methodologies that paved the way.

"The key short-term challenges are improving quality, quality assurance systems, cost, and speed," Dr. Park stated. "The key long-term technologies are analytical (long-read sequencing) and informatic (expanded population and disease databases)."

Beyond the technological advances, investigators are beginning to explore new biological pathways that could have a significant impact on rare disease outcomes. A quick search of the current literature will turn up a small percentage of scientists who are looking at the influence of epigenetics on undiagnosed disorders. Though the number of scientists performing research from this angle is "rare," the evidence for epigenetic involvement is undeniable and could provide potentially novel markers for rare disorders.

"Researchers have had a vast arsenal of tools for examining epigenetic etiologies for over a decade," stated Dr. Park. "Initially the tools were targeted to specific genes or genetic loci, but now these same tools can be applied globally to a research subject's genomes. The tools include not only the examination of changes in DNA methylation but also examine the sites of DNA which are open to active transcription."

In the end, the best tests and methods in the world are still subject to the human decision-making process. Is this the right test for my patient? Will this approach provide physicians enough information to make accurate therapeutic decisions? Can the patient afford this? These are all valid questions that remain at the forefront of clinical NGS use—whether for rare or common diseases.

"If we can reach a point where insurers and physicians agree that this [NGS] is a standard of care, we'll see an explosion, because as you establish a standard of care, all of a sudden you move this out from the experimental to deploying it much earlier—so to me that's the tipping point," Dr. Jacob concluded.

Study Shows Universal Sequencing Detects More Cancer Mutations

Cancer Gene Mutations in People Were Not Detected Using Traditional Methods

Alex Philippidis

Simultaneous sequencing of tumor DNA and normal tissue for a broad panel of cancer-related genes may detect more potentially clinically significant heritable mutations than a targeted approach based on current clinical guidelines, according to a study published by researchers at Memorial Sloan Kettering Cancer Center (MSK).

The study by Kenneth Offit, M.D., chief of the Clinical Genetics Service and Robert and Kate Niehaus Chair in Inherited Cancer Genomics at MSK, found that more than half of inherited cancer gene mutations in people with advanced cancer were not detected using traditional methods based on family history.

Those results suggest that current guidelines for genetic testing based on family history may not detect all clinically actionable genetic mutations, the MSK researchers concluded.

But while knowledge of the additional mutations

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creates opportunities for precision prevention for patients' families, and can help guide therapy as well as preventive interventions among family members, the researchers added that they continue to investigate whether such interventions would improve outcomes.

The study, "Mutation Detection in Patients with Advanced Cancer by Universal Sequencing of Cancer-Related Genes in Tumor and Normal DNA vs Guideline-Based Germline Testing," appears in a recent issue of the *Journal of the American Medical Association*.

"What was surprising about this study was the large number of individuals with inherited mutations who would not have been aware of the risk to their families had we not provided them with tumor-normal sequencing at time of their treatment evaluation," Dr. Offit said in a statement. "At the time of a diagnosis of advanced cancer, we have a vital opportunity, through comprehensive genetic testing, to set the stage for precision prevention for patients' families. The major message for patients is that out of the challenges of a cancer diagnosis can come the opportunity for prevention in the family."

410-Gene Panel

From January 2014 until May 2016, 10,336 patients at MSK consented to tumor DNA sequencing through MSK-IMPACT™ (Integrated Mutation Profiling of Actionable Cancer Targets), a 410-gene panel designed to detect gene mutations and other critical genetic aberrations in rare and common cancers, as well as test both inherited DNA and tumor DNA. Since May 2015, 1,040 of these patients with advanced cancer additionally consented to germline analysis of 76 cancer predisposition genes.

> Tumor-normal sequencing facilitated personalized therapies and prevention by simultaneously detecting inherited markers of cancer risk and identifying tumor-specific genetic targets for treatments.

The researchers analyzed DNA samples from 1,040 patients, finding that 182 (17.5%) had mutations indicating cancer susceptibility. Of these 182 patients, 101 (55%) would not have had these mutations detected using traditional guidelines based on family history, age, and tumor type.

Clinical actionability of pathogenic variants was defined by evidence of their utility in cancer prevention or their potential utility as therapeutic targets. The frequency of inherited mutations was related to case mix, stage, and founder mutations.

MSK said the study was one of the first large-scale efforts to return germline findings in the context of tumor-normal sequencing to patients.

Germline analysis included the 76 genes on the MSK-IMPACT panel that are associated with hereditary cancer predisposition, including all of the cancer-predisposing genes identified in the American College of Medical Genetics and Genomics guidelines.

According to the study, germline findings led to discussion or initiation of change to targeted therapy in 38 patients, and to predictive testing in the families of 13—including six for whom genetic evaluation would not have been initiated by guideline-based testing.

Facilitating Personalized Therapies

"We found that tumor-normal sequencing facilitated personalized therapies and prevention by simultaneously detecting inherited markers of cancer risk and identifying tumor-specific genetic targets for treatments," added pathologist Diana Mandelker, M.D., Ph.D., a co-primary author of the study along with Dr. Offit, molecular geneticist Liying Zhang, M.D., Ph.D., and genetics counselor Yelena Kemel, M.S.

Researchers also found a significantly greater overall prevalence of germline mutations observed in patients with metastatic disease—a result they said may be explained through further molecular profiling and prospective studies of treatment response.

"Unlike most other studies, we reported results of inherited mutations directly to families who wished to know," stated Mark Robson, M.D., a medical oncologist and clinic director of the Clinical Genetics Service at MSK and the study's co-senior author. "Working with a team of experienced genetic counselors, we were able to provide predictive testing and counseling in a supportive and educational environment to families who would not have received counseling based on published decision rules."

Among study limitations acknowledged by researchers were physician discretion for referrals to tumor sequencing, and unique demographic characteristics of patients. Of 1,040 patients, the median age was 58; 65.3% were male, and 81.3% had stage IV disease at the time of genomic analysis, with prostate, renal, pancreatic, breast, and colon cancer as the most common diagnoses.

These and other factors limit the generalizability of study findings to a community practice environment, according to MSK.

The study was supported through MSK's Robert and Kate Niehaus Center for Inherited Cancer Genomics.

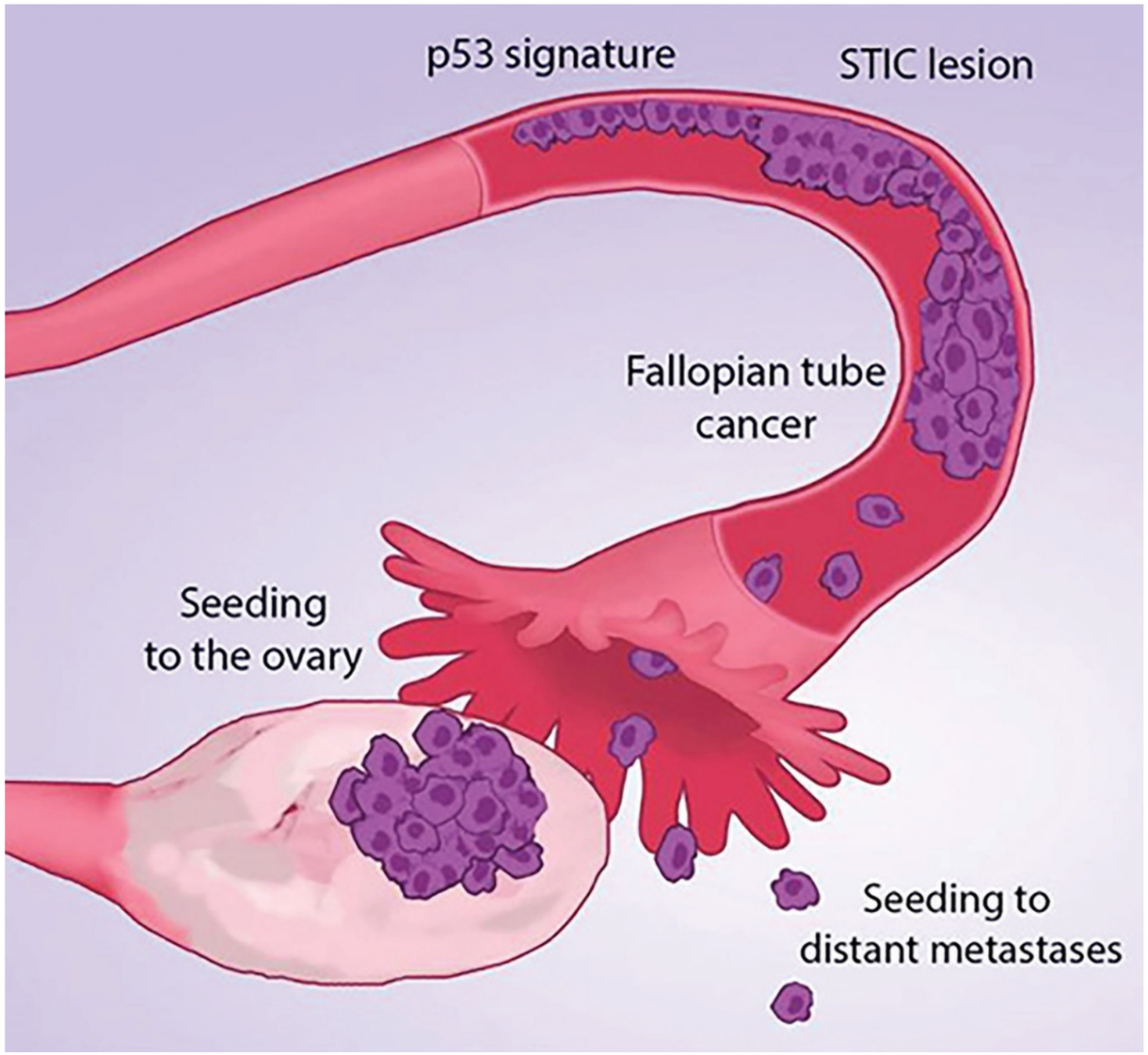
Discovery that Ovarian Cancer Originates in Fallopian Tubes May Aid Earlier Diagnosis

Fang Xia Nuo/Getty Images

Genetic studies by scientists in the U.S. suggest that the most common form of ovarian cancer actually starts in the fallopian tubes and takes about 6.5 years to progress to high-grade serous ovarian carcinoma (HGSOC). Research leader Victor Velculescu, M.D., Ph.D., a professor of oncology at the Johns Hopkins Kimmel Cancer Center, says that data from their small-scale genetic analysis study could lead to new approaches to preventing, diagnosing, and potentially treating the disease.

"Ovarian cancer treatments have not changed much in many decades, and this may be, in part, because we have been studying the wrong tissue of origin for these cancers," he claims. "If studies in larger groups of women confirm our finding that the fallopian tubes are the site of origin of most ovarian cancer, then this could result in a major change in the way we manage this disease for patients at risk."

The researchers report their findings in *Nature Communications*, in a paper entitled,



A genomic study suggests that most ovarian cancers originate in the fallopian tube. Carolyn Hruban

"High Grade Serous Ovarian Carcinomas Originate in the Fallopian Tube."

Ovarian cancer is the leading cause of death from gynecologic cancers, and the 10-year survival rate, at less than 30%, has not improved significantly during the last 30 years, the team notes. Ovarian cancer isn't just one disease, however, but a highly heterogeneous group of diseases that includes different histological subtypes with specific clinical and molecular genetic features that are classified more broadly as type I and type II. HGSOC is the most frequent, type II form of ovarian cancer, which accounts for about 75% of all cases. Unfortunately, about 70% of HGSOC isn't diagnosed until it has already reached an advanced stage.

> Ovarian cancer treatments have not changed much in many decades, and this may be, in part, because we have been studying the wrong tissue of origin for these cancers.

It has previously been suggested that fallopian tube cancers may be precursors of HGSOC, but there is little evolutionary evidence to support this idea, the researchers acknowledge. To try and provide new genetic insights, the Johns Hopkins Kimmel Cancer Center researchers, working with a team at the Dana Farber Cancer Institute, carried out whole-exome sequencing analyses of normal cells and ovarian cancers, metastases, and small fallopian tube cancers, including single-cell-layer p53 signatures and serous tubal intraepithelial carcinoma (STIC), from five women with HGSOC. The researchers also analyzed STIC lesions and normal cells from four additional women who had undergone prophylactic removal of ovaries and fallopian tubes because they either carried BRCA mutations or had a pelvic mass.

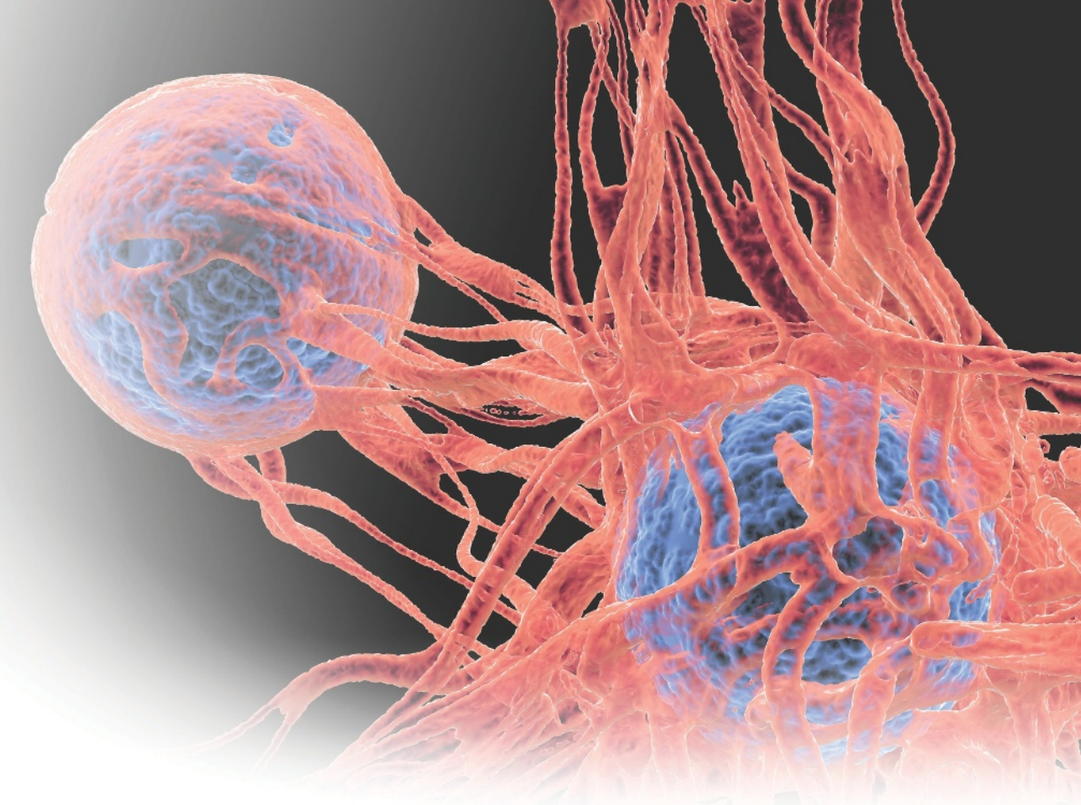
The results of genetic analysis showed that all nine patients had lost the same p53-harboring region of chromosome 17 in each cancer sample, including the early-stage STIC lesions. This indicated that abnormalities in p53 might be involved in the early stages of ovarian cancer development. "Our study highlights the role of p53 signatures as early lesions in this evolutionary paradigm," the researchers write. Each of the nine patients had also lost parts of the chromosomes harboring BRCA1 and/or BRCA2, while four patients exhibited deletions in chromosome 10, which encompasses PTEN.

With their genomic data to hand, and reasoning that early cancer cells will exhibit fewer mutations than later-stage cancer cells, the team created an evolutionary tree of ovarian cancer among the five HGSOC patients. They concluded that the disease starts with genetic changes in STIC or earlier lesions in the fallopian tubes, which already contain sequence and structural changes in key driver genes. Statistical modeling indicated that while it probably takes an average of 6.5 years for ovarian cancer to develop from the early STIC lesions, once the cancer has reached the ovary it progresses to metastatic diseases within just 2 years. "This aligns with what we see in the clinic, that newly diagnosed ovarian cancer patients most often already have widespread disease," Dr. Velculescu commented.

The authors admit that more work will be needed in much larger groups of women to validate their findings, but they suggest that identifying precursor lesions in the fallopian tubes could help earlier diagnosis of HGSOC. "Currently, the typical histopathologic evaluation of FTs [fallopian tubes] typically involves a cursory evaluation of one or two representative sections," they write. "Our study suggests that systematic sectioning and extensive examination of total FTs should become common practice in pathology…."

A confirmation of the results could mean that fewer women would need to have their ovaries removed. "The window of time that exists between the development of a STIC lesion and metastatic disease highlights the importance of new screening approaches, such as liquid biopsy methods, for detection of ovarian cancer," Dr. Velculescu concluded.

Melanoma Neoantigen Vaccine Shows Strong Antitumor Response



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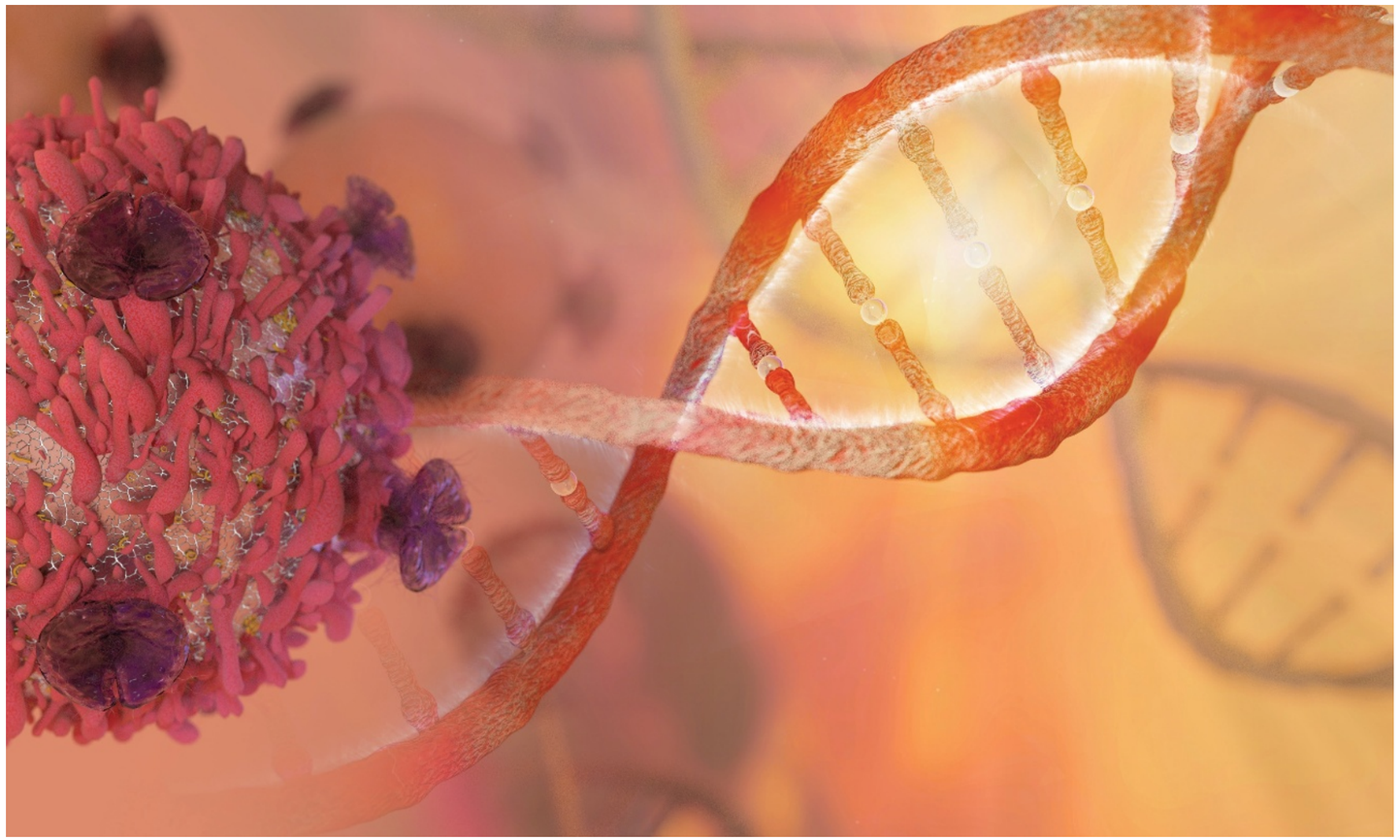
While possibly not the most pleasant topic to discuss while on holiday, cancer and especially melanoma are becoming omnipresent topics among worshipers of the sun and shade alike. Now, a new study from investigators at the Dana-Farber Cancer Institute and the Broad Institute of MIT and Harvard shows that a personal cancer treatment vaccine that targets distinctive neoantigens on tumor cells can stimulate a potent, safe, and highly specific immune antitumor response in melanoma patients.

Findings from the new study "provides proof-of-principle that a personal vaccine tailored to a patient's tumor can be produced and generates highly specific responses to that patient's tumor after vaccination," explained senior study investigator Catherine Wu, M.D., associate professor at the Dana-Farber Cancer Institute. The study results were published recently in *Nature* in an article entitled "An Immunogenic Personal Neoantigen Vaccine for Patients with Melanoma."



A personal cancer treatment vaccine that targets distinctive neoantigens on tumor cells has been shown to stimulate a potent, safe, and highly specific immune antitumor response in melanoma patients. CIPhotos/Getty Images

Antigens are molecules that are displayed on the surface of cells and stimulate the immune system. Neoantigens are molecules on cell surfaces that are produced by DNA mutations present in cancer cells but not in normal cells, making neoantigens ideal targets for immune therapy against cancer, say the scientists. The vaccines used in the Phase I trial reportedly contained up to 20 neoantigens derived from an individual patient's tumor. The vaccines were administered to patients to train their immune system to recognize these neoantigens, with the goal of stimulating the immune system to destroy the cancer cells that display them.

"We've long recognized in cancer that every patient's tumor is different," the authors noted. "With recent advances in technology, it's now becoming possible to create a therapy that's suited to target an individual's tumor. Although neoantigens were long envisioned as optimal targets for an antitumor immune response, their systematic discovery and evaluation only became feasible with the recent availability of massively parallel sequencing for detection of all coding mutations within tumors, and of machine-learning approaches to reliably predict those mutated peptides with high-affinity binding of autologous human leukocyte antigen (HLA) molecules."

While other immunotherapies, such as checkpoint inhibitor drugs, also trigger immune responses against cancer neoantigens, they are not designed to be specific. They can also induce responses against normal tissue antigens, leading the immune system to attack normal tissues and cause toxicity in a subset of patients. The researchers found that the personal vaccine induced a focused T-cell response against several tumor neoantigens, beyond what is normally seen in response to existing immunotherapies.

> With recent advances in technology, it's now becoming possible to create a therapy that's suited to target an individual's tumor.

In the current study, the vaccine—known as NeoVax—was administered to six patients with melanoma whose tumors had been removed by surgery and who were considered at high risk for recurrence. The vaccinations were started at a median of 18 weeks after surgery. At a median of 25 months after vaccination, four of the six patients showed no evidence of cancer recurrence. In the other two patients, whose cancer had spread to their lungs, the disease recurred after vaccination. At that point, they began treatment with the drug pembrolizumab, which inhibits the programmed cell death protein 1 (PD-1) immune checkpoint. Both patients had complete resolution of their tumors and remain free of disease according to imaging scans.

"We hypothesized that vaccination with neoantigens can both expand pre-existing neoantigen-specific T-cell populations and induce a broader repertoire of new T-cell specificities in cancer patients, tipping the intra-tumoral balance in favor of enhanced tumor control," the authors wrote. "Here we demonstrate the feasibility, safety, and immunogenicity of a vaccine that targets up to 20 predicted personal tumor neoantigens. Vaccine-induced polyfunctional $CD4^+$ and $CD8^+$ T cells targeted 58 (60%) and 15 (16%) of the 97 unique neoantigens used across patients, respectively. These T cells discriminated mutated from wild-type antigens, and in some cases directly recognized autologous tumor."

To create the vaccine, samples from a patient's tumor and normal DNA from the patient's blood underwent whole-exome sequencing to reveal mutations present only in the tumor's genetic program. Because some mutations are present in the DNA, but the gene is not made into RNA and protein, the researchers used RNA sequencing to identify mutations that caused the production of a mutated RNA, which is then normally translated into a protein.

T cells can only recognize neoantigens that are presented to them by HLA molecules of the immune system, thus an integral step in making

the vaccine is using computer algorithms to predict which neoantigen peptides will bind strongly to the HLA molecules for recognition by T cells. Utilizing these algorithms yielded dozens of unique neoantigens for each patient's personal vaccine.

Consequently, the selected neoantigen peptides were synthesized and mixed with an adjuvant—a mixture that helps to jump-start the immune response. The vaccine was aimed at generating responses to the neoantigens from T cells of two kinds: $CD8^+$ killer cells and $CD4^+$ helper cells. When the team monitored the vaccine's effects on the immune system in each patient, they found that both T-cell types had indeed been activated by the vaccine and could recognize the neoantigens bound to HLA molecules. Most importantly, many of the T cells were able to recognize the tumor cells directly, demonstrating that the vaccine had triggered a tumor-specific immune response that could target the patient's tumor.

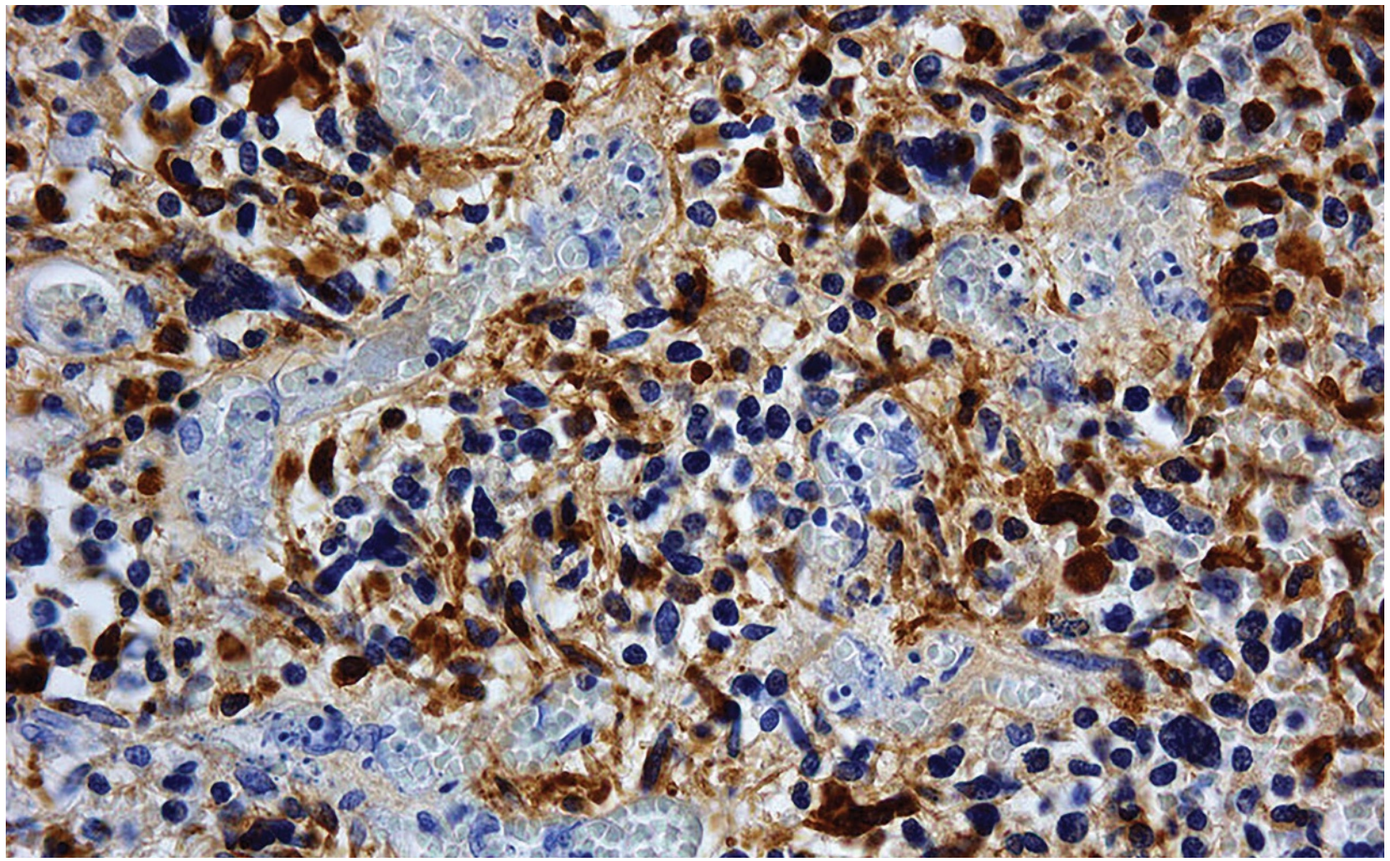
"Future neoantigen vaccine trials will recruit more patients with advanced disease to test the efficacy of the vaccine, take advantage of improved methods for predicting antigen presentation to boost the number of effective neoantigens, and test for synergy with checkpoint blockade and other immunotherapeutics," the researchers remarked. "If successful in subsequent trials, a personal vaccine has the potential to be applied to any cancer that harbors sufficient numbers of neoantigens for vaccination."

Genome-Wide Molecular Profiling Informs Therapy for Recurrent Glioblastoma

Lajos Repasi/Getty Images

Scientists report that several patients with recurring glioblastoma survived for more than a year in a clinical trial the researchers believe was the first to use DNA and RNA sequencing of a patient's tumor to provide treatment for these patients in real time. The study ("Prospective Feasibility Trial for Genomics-Informed Treatment in Recurrent and Progressive Glioblastoma"), published in *Clinical Cancer Research*, was led by the Translational Genomics Research Institute (TGen), University of California, San Francisco (UCSF), and the Ivy Foundation Early Phase Clinical Trials Consortium.

"We conducted a prospective genomics-informed feasibility trial in adults with recurrent and progressive glioblastoma. Following surgical resection, genome-wide tumor/normal exome-sequencing and tumor RNA-sequencing was performed to identify molecular targets for potential matched therapy. A multidisciplinary molecular tumor board issued treatment



Glioblastoma (pictured above) is an aggressive disease, with a median overall survival of only 15 months for newly diagnosed patients. Marvin 101/Wikimedia

recommendations based on the genomic results, blood–brain barrier penetration of the indicated therapies, drug-drug interactions, and drug safety profiles. Feasibility of generating genomics-informed treatment recommendations within 35 days of surgery was assessed," write the investigators.

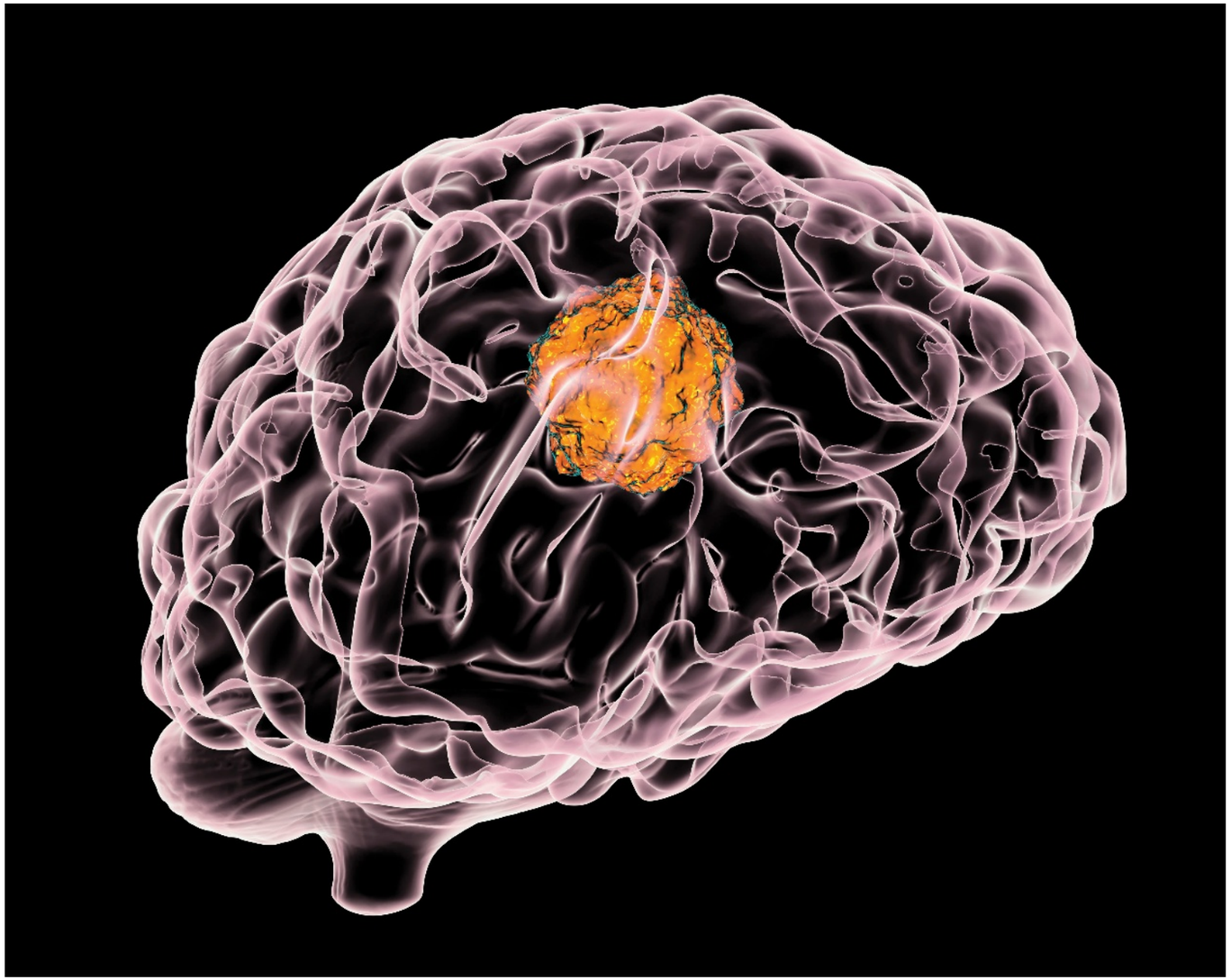
"Of the 20 patients enrolled in the study, 16 patients had sufficient tumor tissue for analysis. Exome-sequencing was completed for all patients and RNA-sequencing was completed for 14 patients. Treatment recommendations were provided within the study's feasibility time frame for 15 of 16 (94%) patients. Seven patients received treatment based on the tumor board recommendations. Two patients reached 12-month progression-free survival, both adhering to treatments based on the molecular profiling results. One patient remained on treatment and progression-free 21 months after surgery, three-times longer than the patient's previous time to progression. Analysis of matched non-enhancing tissue from 12 patients revealed overlapping as well as novel putatively actionable genomic alterations."

"Use of genome-wide molecular profiling is feasible and can be informative for guiding real-time, central nervous system (CNS)-penetrant, genomics-informed treatment recommendations for patients with recurrent glioblastoma."

"To our knowledge, this is the first report of a prospective profiling study in recurrent glioblastoma to show patients with extended time to progression following treatment with genomics-informed therapy," said Sara Byron, Ph.D., research assistant professor in TGen's Integrated Cancer Genomics Division and the study's lead author. "This is a primary example of the benefits of genomics-driven precision medicine being applied for patients with aggressive and refractory tumors."

Key to this study was the fact that all genomic sequencing, genetic analysis, and recommendations for treatment were completed in less than 35 days after surgery, ensuring that suggested therapies could begin within "a clinically acceptable time frame."

Glioblastoma is an aggressive disease, with a median overall survival of only 15 months for newly diagnosed patients. One of the major difficulties in treating glioblastoma is its intrusive penetration into adjoining tissues, which prevents the complete surgical removal of the tumors from the brain, even with follow-up radiation and chemotherapy. As a result, nearly all glioblastomas recur. Patients whose brain cancer returns are often encouraged to enter experimental clinical trials. However, even in clinical trials, further progression of the disease is seen, on average, within four months.



Knowledge of the underlying genomics surrounding aggressive cancers such as glioblastoma could be the lynchpin toward developing effective therapeutics.

Dr_Microbe / Getty Images

"Notably, two of the patients experienced progression-free survival—meaning their tumor did not return or increase in size—for more than a year, with one of these patients progression-free at 21 months, three times longer than the time to progression on their previous therapy," said Michael D. Prados, M.D., the Charles B. Wilson Endowed Chair in Neurological Surgery at UCSF, and the study's senior author.

Another major challenge in treating brain tumors is finding drugs that can penetrate the blood–brain barrier, which buffers the brain from the rest of the body's blood-circulatory system. Located along small capillaries, the blood–brain barrier protects the brain from rapid changes in the body's metabolic conditions and minimizes exposure to large molecules that are toxic to neurons in the brain. The only FDA-approved standard-of-care drugs to treat glioblastoma are temozolomide, nitrosoureas, and bevacizumab.

In this study, more than 180 FDA-approved agents were reviewed, including all FDA-approved oncology drugs and a selection of repositioned agents that are approved by the FDA for other indications but show promising activity against cancer pathways. The tumor board considered the drugs supported by the genomic data for each patient, and discussed each drug's ability to penetrate the blood–brain barrier, potential opportunities to combine treatments, drug-to-drug interactions, and drug-safety profiles.

> A precision-medicine study provides one of the first prospective demonstrations of using genome-wide molecular profiling to guide treatment recommendations for patients with recurrent glioblastoma.

One of the patients was a 58-year-old woman with recurrent glioblastoma. Genomic sequencing showed several alterations with potential therapeutic relevance. Based on mutations in her NF1 and PALB2 genes, the UCSF Molecular Tumor Board recommended treatment with a combination of trametinib, olaparib, and carboplatin. "This patient continued on treatment without disease progression (for more than) 665 days after surgery," according to the new study which adds, "Additional preclinical and clinical studies will be needed to determine the role of genomic context and combination therapy in the response observed for this patient."

Another patient was a 35-year old man with recurrent glioblastoma. The study's tumor board, focusing on the tumor's mutations in the IDH1 and ATRX genes, recommended treatment with a combination of CCNU (lomustine), carboplatin, and metformin. The patient and treating oncologist decided to pursue treatment with CCNU and metformin. "This patient remained on treatment and progression-free for just over one year," the study reported.

"This precision-medicine study provides one of the first prospective demonstrations of using genome-wide molecular profiling to guide treatment recommendations for patients with recurrent glioblastoma within a clinically actionable time frame," said Michael Berens, Ph.D., TGen deputy director for research resources, and professor and director of TGen's Cancer and Cell Biology Division. "These findings provide a rationale and framework for larger prospective studies to further assess the efficacy of employing genomics-guided treatment for patients with recurrent glioblastoma."