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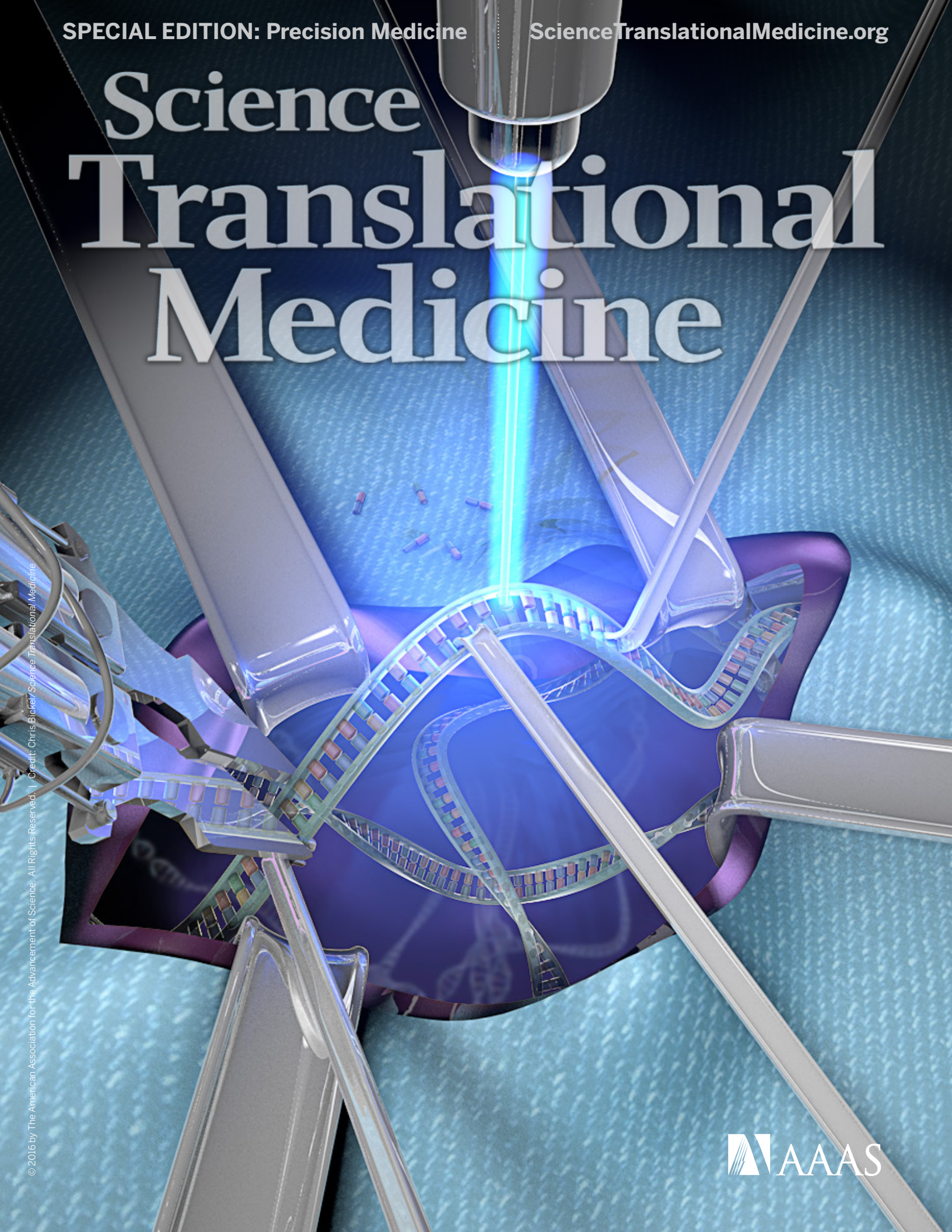

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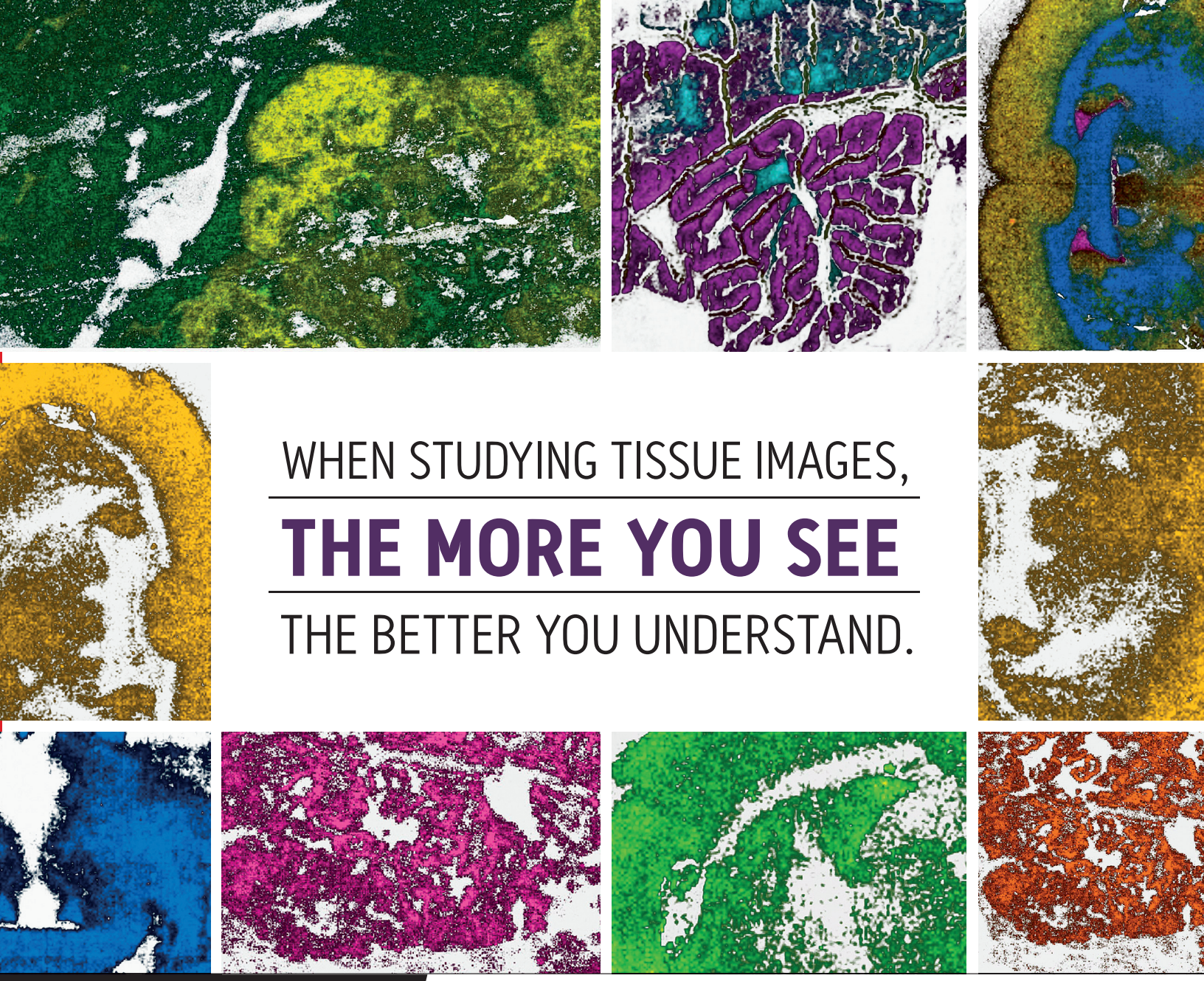

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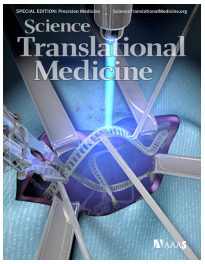

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INTEGRATING SCIENCE, ENGINEERING, AND MEDICINE

January 2016
Washington, DC

Dear Colleagues,

In this booklet on Precision Medicine, the Editors of *Science Translational Medicine* have assembled some of the best examples of precision medicine that have appeared in our pages. More than a traditional field, precision medicine represents a new way of thinking about human health and disease.

Precision medicine aims to factor in individual patients' genetics, epigenetics, phenotype, and environment in clinical decision-making and drug development. It promises to harness innovations in the biological, physical, engineering, computer, and health sciences to understand disease in more mechanistic detail than ever before. Sophisticated computational analysis of large patient cohorts and healthy individuals will facilitate understanding of normal and disease processes so we can develop better diagnostic and treatment approaches.

> *"I want the country that eliminated polio and mapped the human genome to lead a new era of medicine—one that delivers the right treatment at the right time."*
>
> –Barack Obama
> State of the Union Address, 2015

Here we include an array of policy-focused editorials and perspectives on the role of the patient, the confluence of new technologies, and how regulatory science and funding will need to be restructured to reach precision medicine objectives. We also present precision medicine research efforts in cancer, immunology, imaging, pharmacology, and infectious disease to illustrate the breadth of this new approach.

We thank our sponsors Affymetrix, Inc., Canon BioMedical, Inc., OriGene Technologies, and Waters Corporation for their support, and we hope you enjoy this collection.

Sincerely,

Katrina L. Kelner, Ph.D.
Editor

POLICY

From passengers to co-pilots: Patient roles expand

Margaret Anderson* and K. Kimberly McCleary*

The premier position of medical research on the U.S. national policy agenda offers an unprecedented opportunity to advance the science of patient input and marks a turning point in the evolution of patient engagement.

For most of history, patients have been the passive recipients of medical care with little or no role in research. Even as research subjects, patients were not required to give informed consent prior to adoption of the Nuremburg Code in 1947. Since then, patient participation has expanded dramatically, and today, opportunities abound to serve as active partners in defining and prioritizing research questions and solutions. As digital strategist Leonard Kish declared in 2012, "If patient engagement were a drug, it would be the blockbuster drug of the century and malpractice not to use it" (*1*).

Patient engagement offers the promise of advancing more personal and efficacious medical products faster than the typical ~15-year discovery-to-market timeline (*2*). Here, we explore the early foundations of patient engagement (table S1), where it occurs in the drug-development pipeline, the power of recent policy initiatives, and prospects for success in improving health outcomes.

FROM SIDELINES TO CENTER COURT

Early in the last century, patients began to mobilize to accelerate research for particular conditions. The March of Dimes, founded by President Franklin D. Roosevelt in 1938 to expand polio research, is one of the first examples of philanthropy directed at finding treatments and cures. Research supported by individuals through the March of Dimes led to development of the "iron lung" and a successful vaccine. Until recently, this case was an outlier, considering that until the 1973 Patient Bill of Rights was adopted by the American Hospital Association, patients did not necessarily expect to be told their diagnosis, much less have a voice in determining their care plan.

Addressing a patient's part in advancing biomedicine.

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Even in recent years, patients didn't always express their own preferences and expectations for care, deferring to choices the doctor deemed best.

The HIV/AIDS movement catapulted patient needs to the forefront of research and created the force for change that dramatically altered regulatory approval processes at the U.S. Food and Drug Administration (FDA), funding formulas and emphasis at the U.S. National Institutes of Health (NIH), and the path forward for disease organizations. People affected by HIV rallied together and created a movement that demanded change and got results (*3*): from the creation of Gay Men's Health Crisis in New York in 1982 and the AIDS Coalition to Unleash Power in 1987, to the National Institute of Allergy and Infectious Diseases' (NIAID's) formation of the largest HIV clinical trials network in the world, to protests at both NIH and FDA, to passage of the Ryan White Comprehensive AIDS Resources Emergency Act in 1990.

The HIV/AIDS model continues to provide a roadmap followed by other patient communities, demonstrating that it is not enough to question the status quo; you have to do the hard work of presenting well-founded alternatives. As Anthony Fauci, director of NIAID, noted at a *FasterCures* event in 2011, "If you really want to shake cages you have to be persistent. This is very different than coming to a meeting once a year. We knew the HIV/AIDS activists weren't going away."

Today, the role of patients as partners permeates the R&D landscape, extending far beyond the traditional model of funding basic science through donations. Spurred on by the increase of entrepreneurial philanthropy and the proliferation of technology that connects and empowers patient communities, patient influence on decision-making is increasing. In particular, the venture philanthropy drug-development model pioneered by the Cystic Fibrosis Foundation—which led to the codevelopment, with Vertex Pharmaceuticals, of Kalydeco, the first disease-modifying treatment aimed at the genetic cause of cystic fibrosis—is gaining steam and altering the landscape of disease research and cross-sector collaboration.

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The U.S. federal government recently initiated a series of efforts to more formally incorporate patient input into its decision-making processes. Efforts and entities have jumpstarted activities across the medical products industry to elicit and include patient perspectives along the full range of clinical development, such as the Patient-Centered Outcomes Research Institute (PCORI), established through the Affordable Care Act in 2010; the Patient-Focused Drug Development initiative at the FDA, mandated under the fifth reauthorization of the Prescription Drug User Fee Act (PDUFA) in 2012; and a Patient Preference Initiative launched by the FDA's Center for Devices and Radiologic Health (CDRH) in 2013.

POLICY PROSPECTS CONVERGE

The past year has ushered in a "perfect storm" of policy initiatives in biomedical research and opportunities for patient engagement. In April 2014, the chairman of the U.S. House of Representatives Energy and Commerce Committee, Fred Upton, partnered with Rep. Diana DeGette to launch the 21st Century Cures Initiative with a series of hearings and roundtable discussions around the country. These listening sessions solicited unprecedented public input about how Congress could help "accelerate the discovery, development, and delivery of promising new therapies and cures for patients and maintain our nation's standing as the biomedical-innovation capital of the world" (*4*). In recognition of the committee's patient-centered emphasis, Title 1 of the first draft of proposals—released on 27 January 2015—was titled "Putting patients first by incorporating their perspectives into the regulatory process and addressing unmet medical needs" (*5*). The proposals also include patient representatives in nearly every council, panel, advisory board, and body that would be created under the act.

Two days later, a companion effort was announced in the U.S. Senate under the Health, Education, Labor, and Pensions Committee entitled "Innovation for healthier Americans: Identifying opportunities for meaningful reform to our nation's medical product discovery and development" (*6*). It highlighted disease registries sponsored by nonprofit organizations as a "way for patients with a specific disease to signal their potential willingness to participate in research on that disease" and public-private partnerships as a means to "bring academia, government, patients, industry, and others together to solve complex scientific and process questions about medical product development."

The next day, the executive branch added its voice to the chorus when U.S. President Barack Obama announced the Precision Medicine Initiative, a "moon shot" type project that includes the building of a cohort of 1 million engaged participants to contribute data and insights over many years, enabling researchers to better understand how genomic variations and other health factors affect disease development. The president's invitation outlined a collaborative approach to identifying superior treatments and prevention strategies: "In order for us to realize [the Initiative's] potential, I'm asking more hospitals and researchers and privacy experts to join us in this effort. I'm asking entrepreneurs and nonprofits to help us create tools that give patients the chance to get involved as well. Because we want every American ultimately to be able to securely access and analyze their own health data, so that they can make the best decisions for themselves and for their families."

Negotiations for the sixth authorization of PDUFA will begin this fall among the FDA, Congress, and the biopharmaceutical industry. For the second time, patient representatives will have an active role in the process, although not quite full negotiating status, because user fees are paid by industry to FDA with oversight from Congress. Most recognize that patients' influence and the open dialogue among stakeholders under the 21st Century Cures initiative has served as a dress rehearsal—in particular, these new actors are given opportunities to contribute to the hashing out of ideas, alignment of goals, and vetting of approaches to meeting those goals.

For example, *FasterCures*, the Biotechnology Industry Organization, and Eli Lilly & Co. developed independent yet complementary proposals for Congress as part of 21st Century Cures to authorize a public-private partnership dedicated to developing tools and methods to support science-based approaches for collecting patient input. The bipartisan discussion draft includes such a body, the "Council for 21st Century Cures," whose mandate is to "accelerate the discovery, development, and delivery in the United States of innovative cures, treatments, and preventive measures for patients" (*7*).

THE SCIENCE OF PATIENT INPUT

Accompanying acceptance of the need to integrate patient perspectives is an increase in the demand for research-based methods and tools to measure the effectiveness of incorporating patient input into the system and, ultimately, its impact on patient health. What began as an extension of patient advocacy has evolved into an emerging scientific discipline aimed at understanding and incorporating patient needs into the processes of developing, regulating, and delivering new therapies.

A compelling "call to action" authored by thought leaders from international patient organizations and pharmaceutical companies describes the gap that must be closed: "Despite the increasing number and scope of patient-involvement initiatives, there is no accepted master framework for systematic patient involvement in industry-led medicines research and development, regulatory review, or market access decisions.… It is essential that all stakeholders participate to drive adoption and implementation of the framework and to ensure that patients and their needs are embedded at the heart of medicines development and lifecycle management" (*8*). Meetings convened in the first quarter of 2015 by the Clinical Trials Transformation Initiative, National Health Council, University of Maryland's Center of Excellence for Regulatory Science Innovation, and PCORI have provided opportunities to share emerging practices and lessons learned.

For medical devices and biologics, the call to action was answered by the FDA's CDRH and Center for Biologics Evaluation and Research on 13 May 2015, with a draft guidance entitled "Patient preference information—Submission, review in PMAs, HDE applications, and de novo requests, and inclusion in device labeling" (*9*). The guidance outlines "qualities" of patient-preference information acceptable for regulatory purposes and directions for submitting such data to the agency. On the same date, the Medical Device Innovation Consortium (MDIC), a public-private partnership, released its "Framework and catalog of methods for incorporating information on patient preferences regarding benefit and risk into the regulatory assessments of new medical technologies" (*10*). The catalog captures methods of assessing patient preference that are adapted from health economics, outcomes research, epidemiology, social sciences, and marketing sciences. Although compiled for medical technology development, the catalog is expected to be highly transferable to the development of pharmaceuticals and biologics as well.

For drugs, the groundwork has been laid by researchers who participated in early organized efforts to develop structured assessment of benefits and risks, including the Benefit-Risk Assessment Team convened by the Pharmaceutical Research and Manufacturers of America, the Centre for Innovation in Regulatory Sciences, and special interest groups within the International Society for Pharmacoeconomics and Outcomes Research. *FasterCures*'s Benefit-Risk Advisory Council comprises many of these experts along with patient leaders and provided the faculty for a one-day "benefit-risk boot camp" on this topic in September 2014.

On a parallel track, patient organizations have piloted new approaches to meet the demand for data that supplement personal testimony and participation of individual advocates as patient representatives in decision-making bodies. Parent Project Muscular Dystrophy (PPMD) demonstrated leadership in sponsoring a benefit-risk–preference study among parents of boys with the rare but fatal form of muscular dystrophy known as Duchenne. PPMD published the results, held a policy forum that attracted 17 FDA officials, and organized a community-based drafting of a regulatory guidance for drug development. The FDA opened a public docket to receive comments on PPMD's guidance document and is expected to issue its version in coming weeks. Other patient organizations are following PPMD's model—seeking academic partners, building patient registries, and educating their patient communities about new opportunities to reshape treatment pipelines and care delivery.

ACCOUNTABILITY ALL AROUND

To fulfill the prediction that patient engagement will be the blockbuster drug of the century, we offer five observations to guide the path forward:

- There is a need to expand the capacity of all participants—industry, academia, government, and patient organizations—to engage patients in biomedical research, medical product development, regulatory decision-making, and health care delivery. We must understand the full range of patient experiences and expectations across a representative cross section of individuals with a particular diagnosis or collection of conditions.
- Developing appropriate, scalable, sustainable methods and practices will require collaboration, experimentation, coordination, and transparency. Multiple types of expertise will be needed, and adoption will be highly iterative and require extreme focus on the goal: improved patient outcomes.
- It's too early to tout emerging practices as being "best," and standards are likely to change rapidly. This may challenge resources and introduce new sources of uncertainty, especially at first. We may all need to tolerate more turbulence in the ascent, with our seatbelts fastened, before we reach a comfortable cruising altitude.
- Different diseases, disease communities, stages of disease, and stages of life might warrant distinct approaches to patient engagement and integration of patient input. The role of the caregiver and family members is clear in pediatric disorders, disabilities, and conditions associated with aging such as Alzheimer's disease; individuals who surround the patients also should be factored into our understanding of unmet medical needs in mental-health conditions such as addiction and schizophrenia.
- Patients are found not only in conventional settings, such as disease-specific foundations and clinics, but also living their lives as members of social media networks and local community organizations. We need to rethink and expand the settings in which we recruit and equip individuals to be informed participants in research and care activities. This will take time to implement.

Medical products and interventions that begin with a solid understanding of patient needs and expectations promise better outcomes for the individual, families, communities, our nation, and global health. More than 75 years ago, patient engagement contributed to arresting the polio epidemic. The HIV/AIDS activists charted a path forward for the way patients can engage in all aspects of research and delivery of care under stunningly difficult circumstances. With advances in the tools we have for conducting science and communication, think of the potential we have to capitalize on the blockbuster that is patient engagement. The possibilities are endless.

SUPPLEMENTARY MATERIALS

www.sciencetranslationalmedicine.org/cgi/content/full/7/291/291fs25/DC1

Table S1. Patient engagement timeline.

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Citation: M. Anderson, K. K. McCleary, From passengers to co-pilots: Patient roles expand. *Sci. Transl. Med.* **7**, 291fs25 (2015).

HUMAN HEALTH

Precision medicine: Beyond the inflection point

Sam Hawgood,[1] India G. Hook-Barnard,[1] Theresa C. O'Brien,[1] Keith R. Yamamoto[1,2]*

A confluence of biological, physical, engineering, computer, and health sciences is setting the stage for a transformative leap toward data-driven, mechanism-based health and health care for each individual.

Despite staggering and persistent inequalities in health care access and clinical outcomes, there is no doubt that the past century's growth in our understanding of mechanisms that underlie biological processes and the application of such knowledge to medicine have steadily advanced human health and longevity. Now, early in the 21st century, convergence of the technological and health sciences has created the opportunity for a transformational leap forward in the way health care decisions are made for all individuals.

Biomedicine now sits at an inflection point, poised between what futurist Ian Morrison calls the first, or incumbent, curve marking steady progress and a second, or nascent, curve that would transform and dramatically accelerate progress (*1*). The first curve depicts biomedical scientists' incremental progress through iterative reductionist approaches loosely coupled with the advances of clinicians in diagnosis and treatment through the use of periodic patient histories, physical examinations, signs and symptoms, personal expertise and experience, and risk factors assigned to statistically defined groups.

We suggest that the second curve will be defined by precision medicine (*2*), in which scientists, clinicians, social and behavioral investigators, and patients collaborate to generate and use massive data networks that access, aggregate, integrate, and analyze information from huge patient cohorts, healthy populations, and experimental organisms in order to determine mechanisms of normal and disease processes and provide precise health advice, diagnoses, and treatments for each individual.

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POISED FOR PRECISION

Humans are not hardwired by their genomes. Rather, we sense and respond to internal and external signals, and the combinatorial output of likely hundreds of complex contributing factors and interactions defines one's overall health status as well as the onset and course of a disease. While capturing the excitement and promise inherent in the $1000 human genome, a defining assertion of precision medicine is that genomics—no matter how powerful or economical—is far from sufficient to understand human physiology and pathophysiology. Myriad other components—molecular, developmental, physiological, social, and environmental—also must be monitored, aligned, and integrated in order to arrive at a meaningfully precise and actionable understanding of disease mechanisms and of an individual's state of health and disease.

The 2011 U.S. National Academy of Sciences (NAS) report entitled "Toward precision medicine: Building a knowledge network for biomedical research" (*2*) used the analogy of Google Maps to illustrate the value and necessity of aligning and integrating diverse, often unstructured, data sets into a comprehensive knowledge network if we are to understand the complexities of human health and disease (Fig. 1).

Precision medicine, as named and detailed in the NAS report, is not a new field of study or a subspecialty but, rather, an

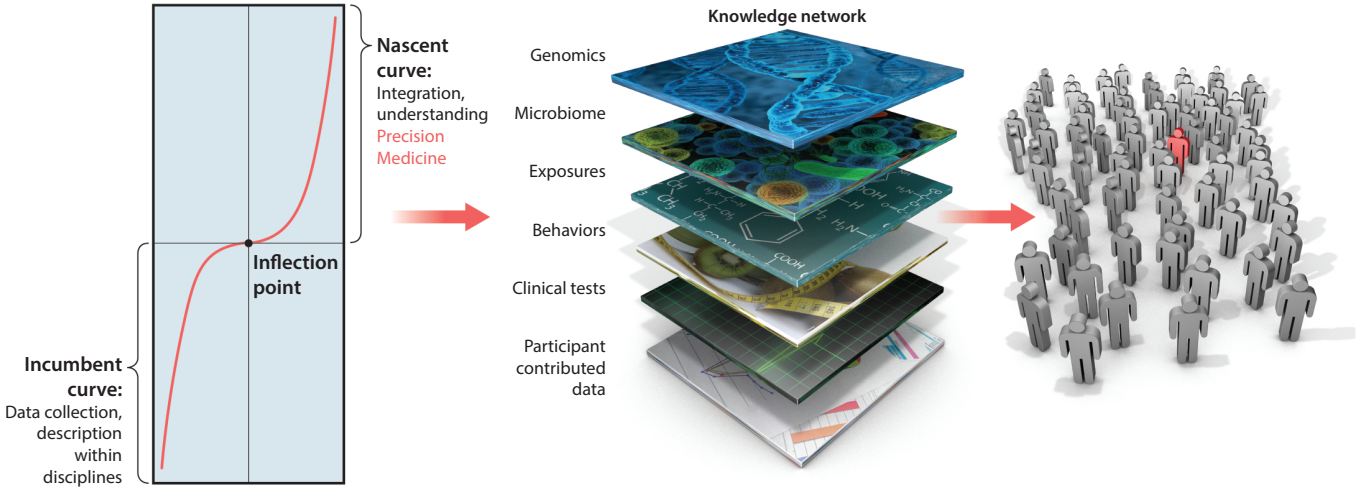

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Fig. 1. Surpassing single-layer health care. An inflection point marks an opportunity or moment of dramatic change between the first, or incumbent, curve, marking steady progress, and a second, or nascent, curve, indicating transformation and accelerated progress. In biomedical research, health, and health care, we are at an inflection point, poised for precision medicine. Whereas Google Maps links layers of transportation, land use, and other data, precision medicine aims to integrate and apply data from biomedical research, clinical practice, social/behavioral studies, and participant-contributed observations toward better diagnosis, treatment, and preventative strategies.

Table 1. Precision medicine approaches and pilot studies.

Study type	Description	Reference
Basic discovery	An experimental strategy has been devised that combines genetic, proteomic, structural, and computational approaches to proceed from patient-based systems data, such as genome-wide association studies, to functional complexes, to pathways, and ultimately to predictive networks. The approach reveals disease mechanisms and has implications for therapeutic decisions and drug development.	(*7*)
Clinical discovery	BRCA pathway mutations, known to be causative in certain breast cancers, have also been implicated in ovarian and pancreatic cancers thought previously to be unrelated. This common mechanism predicted correctly that therapies for BRCA pathway–defective breast cancers, such as poly (adenosine-diphosphate–ribose) polymerase (PARP) inhibitors, could be efficacious for other cancers with related defects.	(*8*, *9*)
Social and behavioral discovery	Analysis of genomic data linked to clinical records of a diverse cohort of >100,000 Californians has revealed genomic variants linked to prostate cancer, diabetes, and other diseases; uncovered molecular features related to aging; and provided insight into the relation between genetic ancestry and social categories of racial and ethnic identity.	(*10*)
Disease prevention	Health eHeart is a large study that seeks to harness data from smart phones, biosensors, and other wearable devices in order to collect longitudinal blood pressure, activity, sleep, diet, and other data on 1 million subjects to define patterns that will be informative and predictive and motivate behavioral changes so as to prevent cardiovascular disease.	www.health-eheartstudy.org

approach to knowledge acquisition that integrates across the spectrum of biomedical research and clinical practice; it is a computation-enabled platform for organizing, synthesizing, and rationalizing information in ways that fundamentally change how we conduct biomedical research and patient care. The success of this approach will depend on the engagement of wide stakeholder communities, notably including both patients and healthy people who become convinced that their contributions will benefit their own health and well-being as well as that of their children and grandchildren. U.S. president Barack Obama's precision medicine initiative, announced in January 2015 (*3*), gives voice to this complex task with his call to create a million-citizen cohort, assembled largely from existing cohorts, to contribute and share their health data while maintaining privacy and security.

BUILDING THE KNOWLEDGE NETWORK

In the 4 years since the release of the NAS report, work has progressed at different velocities within distinct data layers ranging from cancer-gene atlases to exchanges of clinical data in electronic health records to a growing appreciation, through metagenomic analyses, of the diversity and complexity of microbial communities resident in and on our bodies. Of course, progress has been slower in discerning correlations, patterns, and relationships between data layers and over time as well as in aligning health data collected in the ordinary course of care or in the course of daily life. The defining of such linkages will be the hard-fought product of insightful research designed to achieve a measure of understanding that moves us through the inflection point, extending beyond the collection of data to the creation of new knowledge. This is the work of decades, demanding sustained effort, effective partnerships, and a broad base of support.

For example, in April 2015, California governor Jerry Brown announced the California Initiative to Advance Precision Medicine, which provides funding to motivate diverse stakeholders to participate and contribute resources (http://gov.ca.gov/news.php?id=18921). Collaborative teams are currently designing and launching demonstration projects that take advantage of the state's diverse demographics, deep intellectual resources, and energetic entrepreneurial culture to illustrate the power of precision medicine and build tools with which to drive its application.

When contemplating the daunting challenge of such a massive endeavor as precision medicine, we can perhaps take heart in the assertion of Microsoft cofounder and philanthropist Bill Gates, who remarked that "most people overestimate what they can do in one year and underestimate what they can do in ten years" (*4*). However accurate or flawed the trajectory projections of Morrison's nascent curve for precision medicine may be, this approach has a crucial redeeming characteristic: Creation of the knowledge network need not be complete to demonstrate contributions to our understanding of the diverse natural histories and mechanisms of disease and to the impact of new knowledge on human health. Individual pixels of success derived from adding a single new data layer to those traditionally used to interrogate a disease mechanism or inform a therapeutic decision can have substantial impact. Indeed, the progressive merging of these pixels will begin to reveal the full image. At the University of California, San Francisco, where precision medicine is central to our overarching institutional vision, numerous pilot projects are under way across basic, clinical, and social and behavioral discovery research as well as in disease prevention studies (Table 1), and our knowledge network, initially rooted in oncogenesis/cancer and neuroscience/neurological disease, is expanding across a range of disciplines and disease areas. Our efforts, although still at an early stage, are already providing valuable insights and reinforcing the perception that we have entered a transformative period in life-science research, health, and health care.

PERSPECTIVES

Precision medicine is a bold approach that broadly integrates the endeavors and advances of biomedical science, physical science, and engineering research with health outcomes and health care. Although grand in overall scope, precision medicine can succeed iteratively and likely can move forward only through pilot studies—some that will establish standards and best practices and some that will be scalable, illuminating routes toward larger and broader efforts. Individual biomedical communities can and should undertake different pilot projects that are tailored to their strengths, resources, cultures, and environments.

The NAS "Toward precision medicine" report (*2*) envisioned a national or international enterprise, surely an audacious aspiration. However, success, even in much smaller increments, would demonstrate how insights gained from integrating many data elements—some drawn from engaged citizens seizing a new social contract (*5*)—will bring advances through data manipulation, modeling, and testing of predictions, toward a more intricate mechanistic understanding of fundamental physiological principles and processes. This knowledge—evidence-based and predictive in nature—will, in turn, promote new strategies for prevention, early diagnosis, treatment, and cure of diseases. Moreover, if the nascent curve of precision medicine yields a healthier, more productive workforce; better control of chronic disease; smaller, faster, and more successful clinical trials; and avoidance of unnecessary tests and ineffective therapies, the slope of the health care–cost curve could decline—a welcome consequence for the United States, in which health care costs account for 17.4% (and growing) of the gross national product (*6*). Thus, precision medicine holds promise for improvements in health, reduction of disease, and broad impacts—scientific, societal, and economic.

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CANCER

Personalized genomic analyses for cancer mutation discovery and interpretation

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Massively parallel sequencing approaches are beginning to be used clinically to characterize individual patient tumors and to select therapies based on the identified mutations. A major question in these analyses is the extent to which these methods identify clinically actionable alterations and whether the examination of the tumor tissue alone is sufficient or whether matched normal DNA should also be analyzed to accurately identify tumor-specific (somatic) alterations. To address these issues, we comprehensively evaluated 815 tumor-normal paired samples from patients of 15 tumor types. We identified genomic alterations using next-generation sequencing of whole exomes or 111 targeted genes that were validated with sensitivities >95% and >99%, respectively, and specificities >99.99%. These analyses revealed an average of 140 and 4.3 somatic mutations per exome and targeted analysis, respectively. More than 75% of cases had somatic alterations in genes associated with known therapies or current clinical trials. Analyses of matched normal DNA identified germline alterations in cancer-predisposing genes in 3% of patients with apparently sporadic cancers. In contrast, a tumor-only sequencing approach could not definitively identify germline changes in cancer-predisposing genes and led to additional false-positive findings comprising 31% and 65% of alterations identified in targeted and exome analyses, respectively, including in potentially actionable genes. These data suggest that matched tumor-normal sequencing analyses are essential for precise identification and interpretation of somatic and germline alterations and have important implications for the diagnostic and therapeutic management of cancer patients.

INTRODUCTION

High-complexity genomic analyses are changing the diagnostic landscape of oncology (*1*–*7*). Therapies targeting specific genetic alterations can be safer and more effective than traditional chemotherapies when used in an appropriate patient population (*8*). This has been successfully demonstrated for a number of therapeutics targeting the protein products of specific genes that are altered in human cancer, including the use of imatinib in chronic myeloid leukemias carrying the *BCR-ABL* fusion, trastuzumab in *ERBB2* (*HER-2/neu*) amplified breast cancer, and vemurafenib in *BRAF*-mutated melanoma. Molecular alterations have also been shown to have a predictive or prognostic effect. For example, mutations at codons 12 and 13 of *KRAS* predict a poor response to anti–EGFR (epidermal growth factor receptor) monoclonal antibodies such as cetuximab and panitumumab, so the use of these drugs is contraindicated in colorectal cancer patients with such mutations (*9*). Glioblastoma patients with *IDH1*-mutated tumors have an increased overall survival compared to those without such changes (*10*). In addition to established therapies, off-label indications and drugs in clinical trials can be used with knowledge of alterations in specific genes. Because the mutations driving each tumor are unique, identifying the specific mutations in each patient's cancer is critical for the development of a personalized treatment plan that takes advantage of the growing number of targeted therapies.

Each tumor contains inherited (germline) and tumor-specific (somatic) variants. Somatic alterations in oncogenes and tumor suppressors drive the development and growth of the tumor and are typically the targets of personalized therapies. Sequencing and comparison of matched normal DNA to tumor DNA from an affected individual would theoretically allow for accurate identification and subtraction of germline alterations from somatic changes. However, this method is not routinely used in cancer diagnostic assays, including next-generation sequencing approaches, where only tumor DNA is assessed, likely as a result of logistical difficulties in obtaining a blood or saliva sample, increased cost, and an underappreciation of the potential value of the matched normal (*2*, *11*–*13*). Additionally, a major question in the development and progression of human cancer has been the contribution of germline alterations to cancer predisposition. Although estimates have been proposed in specific tumor types (*14*, *15*), the comprehensive examination of cancer-predisposing alterations in apparently sporadic cancer patients has not been investigated.

To evaluate the clinical use of large-scale cancer genome analyses that incorporate these aspects, we performed whole-exome and targeted next-generation sequencing analyses in tumor and normal samples from cancer patients. We analyzed matched tumor and normal data together as well as separately for somatic mutation detection, potential clinical actionability, and identification of predisposing alterations.

RESULTS

Overview of the approach

To systematically assess somatic alterations in tumor samples, we designed capture probes for the targeted analysis of a set of 111 clinically relevant genes (table S1) and sequenced these regions or the complete

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set of coding genes (20,766 genes) using next-generation sequencing approaches (Fig. 1). These data were aligned to the human reference sequence and annotated using the Consensus Coding DNA Sequences (CCDS), RefSeq, and Ensembl databases. Tumor and normal data were compared to identify somatic and germline alterations using the VariantDx software pipeline, focusing on single-base substitutions as well as small insertions and deletions. Stringent criteria were used to ensure sufficient coverage at analyzed bases and to exclude mapping and sequencing errors (table S2). All candidate somatic alterations were visually inspected to remove remaining artifactual changes. Analysis of samples using both whole-exome Sanger and next-generation sequencing was used to demonstrate that the next-generation sequencing and bioinformatic approaches were able to detect somatic mutations in frozen and formalin-fixed paraffin-embedded (FFPE) tumor tissues with high sensitivity and specificity and to accurately distinguish between somatic and germline alterations (table S3).

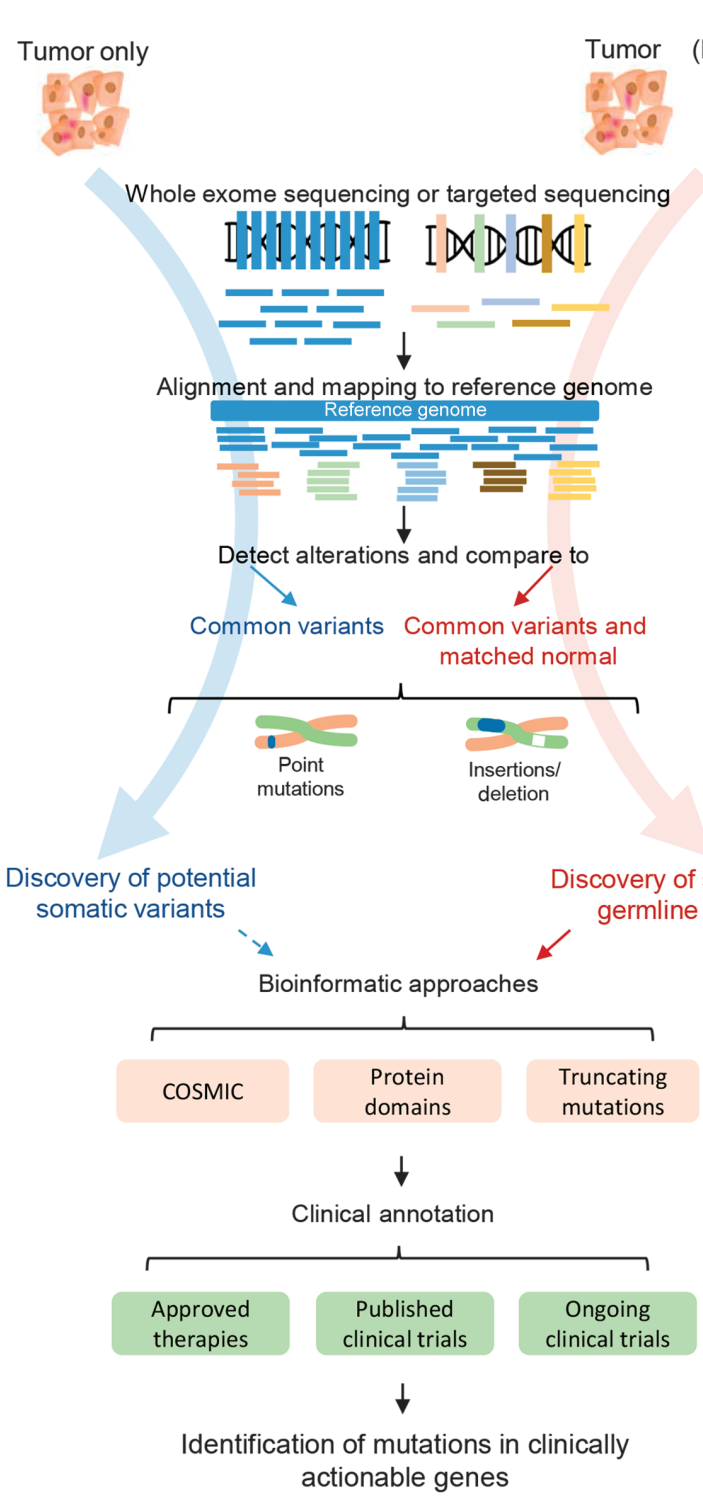


Fig. 1. Schematic description of whole-exome or targeted next-generation sequencing analyses. The approaches used tumor-only (blue arrow) or matched tumor and normal DNA (red arrow) to identify sequence alterations. Bioinformatic methods to separate germline and somatic changes included comparison to dbSNP, COSMIC, and kinase domain databases. Identified gene alterations were compared to databases of established and experimental therapies to identify potential clinical actionability and predisposing alterations.

Clinical actionability of targeted and exome analyses

Using the above approach, we analyzed matched tumor and normal specimens from 815 patients, with the tumor types indicated in table S4. A total of 105,672 somatic alterations were identified, with an average of 4.34 somatic mutations (range, 0 to 29) in the targeted analyses and an average of 140 somatic alterations (range, 1 to 6219) in the exome analyses. The number of somatic alterations in various tumor types was largely consistent with previous analyses of cancer exomes (*10*, *16*–*30*). To explore whether genetic alterations may be useful clinically, we investigated whether mutant genes observed in individual cases may be clinically actionable using existing or investigational therapies. We examined altered genes that were associated with (i) U.S. Food and Drug Administration (FDA)–approved therapies for oncologic indications, (ii) therapies in published prospective clinical studies, and (iii) ongoing clinical trials for patients with tumor types analyzed. Through these analyses, we identified somatic alterations in genes with potentially actionable consequences in 580 of the 753 patients analyzed (77%) (Fig. 2 and tables S5 and S6). Some tumor types, such as colorectal cancer and melanoma, had a much higher fraction of actionable changes than others. More than 90% of genes with potentially actionable alterations were mutated in <5% of cases, suggesting that actionable changes are predominantly different among cancer patients (table S5). Although the fraction of patients who had at least one actionable alteration was high, most of the actionable changes were associated with current clinical trials (67%) rather than established or investigational therapies (33%).

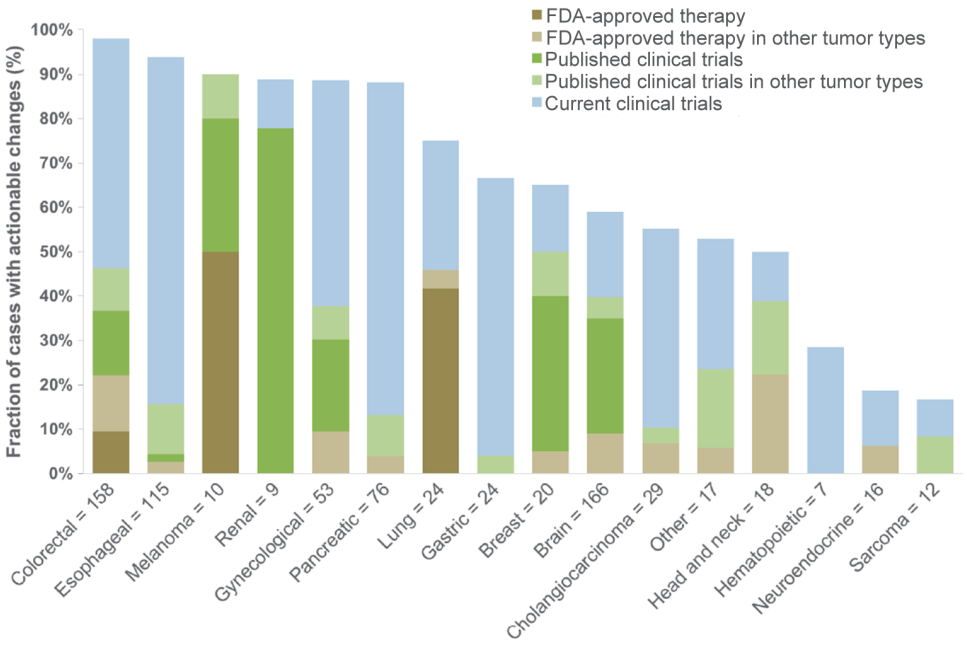


Fig. 2. Clinically actionable somatic genomic alterations in various tumor types. Each bar represents the fraction of cases with mutations in clinically actionable genes as determined by the comparison of alterations to genes that were associated with established FDA-approved therapies (brown), previously published clinical trials (green), or current clinical trials in the same tumor type (blue). For approved therapies and previously published clinical trials, potential actionability was also considered in tumor types that were different from those where the clinical use has been described (light brown and light green, respectively). Some of the colorectal tumors analyzed were from patients with tumors known to be *KRAS* wild type, resulting in a lower fraction of cases with actionable changes related to FDA-approved therapies.

Identification of patients with putative germline cancer predisposition mutations

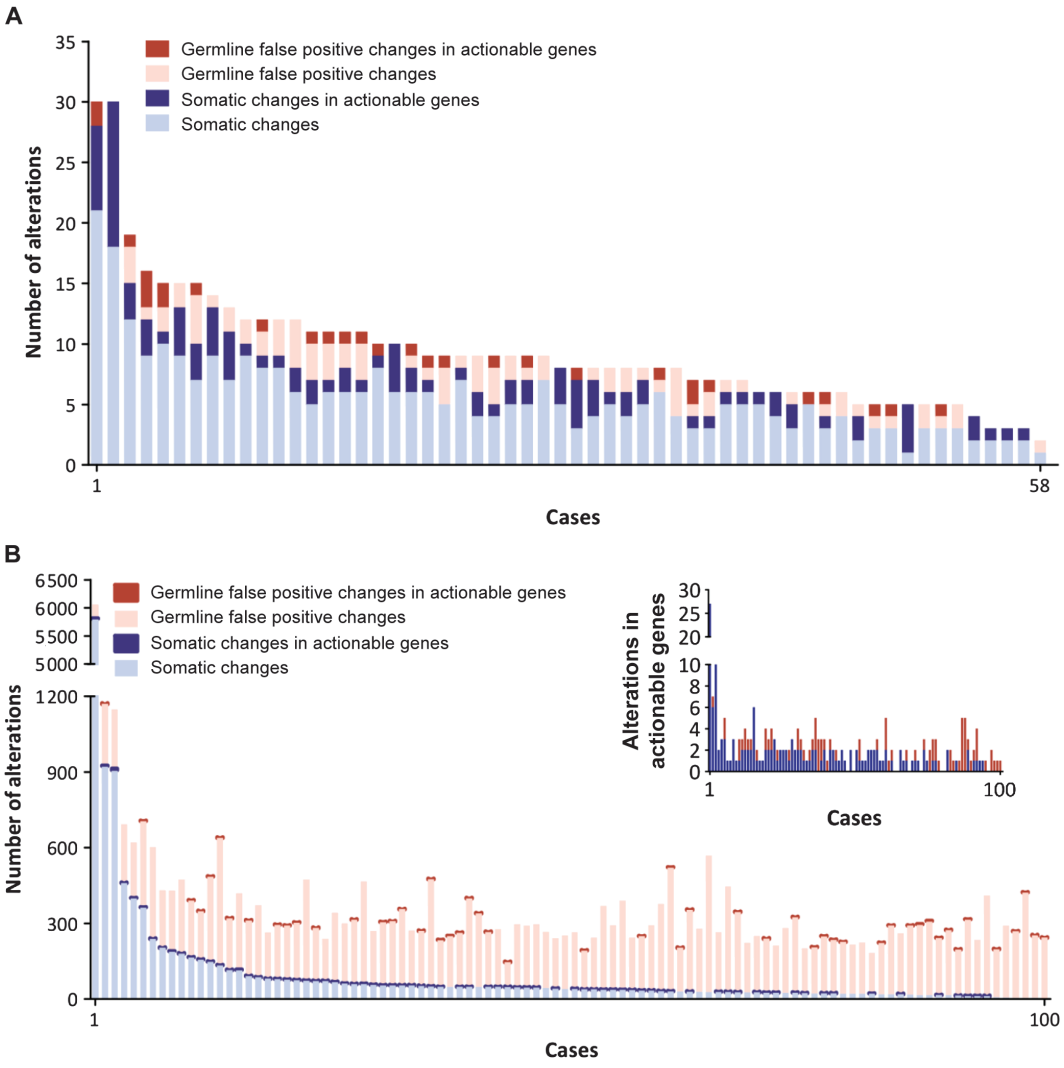
In addition to the detection of somatic alterations, we assessed whether our analyses identified cancer-predisposing changes in the genomes of apparently sporadic cancer patients. To perform this analysis, we examined a set of 85 genes associated with known cancer predisposition syndromes (table S7) in DNA from blood, saliva, or unaffected tissue samples from the 815 cancer patients. To conservatively identify protein-altering changes in these genes, we focused on truncating alterations, including insertions or deletions resulting in a frameshift, splice site changes, and nonsense alterations. Through these analyses, we identified 27 of the 815 patients (~3%) with truncating alterations in these genes (table S8). All but one of these cases were not previously known to have a cancer-predisposing alteration in their germ line. Fifteen mutations were predicted to be pathogenic or likely pathogenic based on previous publications. Examples of germline alterations included changes in genes in expected tumor types, such as *BRCA1* alterations in breast and ovarian cancer patients and a nonsense mutation (50Q>X) in *CDKN2A* in a melanoma case. However, less well-described examples were also detected, including *BRCA2* alterations in patients with other solid tumor types such as colorectal cancer and cholangiocarcinoma, *ATM* changes in an esophageal cancer case, *FANC* alterations in patients with a variety of tumor types, and alterations in the *BRIP1* (BRCA1 interacting protein C-terminal helicase 1) gene in a cholangiocarcinoma (800Y>X) and in an anal cancer case (624S>X).

Bioinformatic approaches for distinguishing germline and somatic mutations

Because many newly developed tests for alterations in cancer genes only examine the tumor tissue (*2*, *11*, *13*, *31*), we evaluated how effective bioinformatic approaches could be in separation of somatic from germline mutations without the use of a matched normal (Fig. 1). First, we reanalyzed only the tumor data from all 58 targeted cases and 100 whole-exome cases composed of about half frozen and half FFPE samples from a representative range of tumor types. We compared these to an unmatched normal sample that had been sequenced using the same methods as for the matched normal samples. We used these data to remove common germline variants, as well as sequencing and alignment errors. All candidate alterations were visually inspected to remove any remaining artifacts. An average of 11.53 mutations (range, 3 to 34) and 1401 mutations (range, 919 to 2651) were observed in the targeted and exome cases, respectively (Fig. 3).

To identify additional germline variants in the tumors that were not present in the unmatched normals, we compared the observed tumor alterations to those in single-nucleotide polymorphism (SNP) databases (dbSNP, version 38) and filtered variants identified through the 1000 Genomes Project or other sources (*32*) (including 42,886,118 total candidate variants). This approach removed between 0 and 9 alterations (average, 5.25) in the targeted analyses, including all germline alterations in 10 of 58 cases. However, an average of 1.95 germline variants remained per case through the tumor-only approach, resulting in a total of 113 remaining germline changes in the 58 cases analyzed (Fig. 3). A total of 1019 mutations were removed using dbSNP filters in each of the exome cases (range, 623 to 1911), but an average of 382 mutations remained per case. A considerable proportion of the remaining germline variants affecting 48% of patients analyzed included alterations that could have been classified as potentially actionable changes (Fig. 3 and table S9). For example, a *JAK2* (Janus kinase 2) mutation in the catalytic domain (1021Y>F), multiple missense alterations in *ERBB2*, an in-frame deletion (1508PF>P) in *TSC2* (tuberous sclerosis complex 2), and an *ALK* (anaplastic lymphoma kinase) change in the catalytic domain (1200A>V) would have been incorrectly identified through a tumor-only approach. Approved or investigational therapies targeting the altered protein products are available for these genes, including ruxolitinib for JAK2, neratinib for ERBB2, everolimus for TSC2, and crizotinib for ALK among others, that could have been inappropriately administered to patients on the basis of a tumor-only analysis. Overall, most of the cases filtered using germline databases had remaining germline alterations, with about half in potentially actionable genes.

The filtering of tumor-only data with variants present in germline databases has the potential to inadvertently remove somatic variants



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	Matched tumor/normal sample analyses			Tumor-only analyses			
	Total sequence coverage	Number of samples analyzed	Somatic mutations per tumor		Candidate mutations per tumor	Candidate mutations per tumor after removal of common germline variants (dbSNP)	Alterations in actionable genes after dbSNP filter
Whole exome	199×	100	135	Total alterations	1401	382	2.41
				True positives (somatic)	135 (10%)	133 (35%)	1.61 (67%)
				False positives (germline)	1266 (90%)	249 (65%)	0.80 (33%)
Targeted	1052×	58	4.34	Total alterations	11.53	6.28	2.31
				True positives (somatic)	4.34 (38%)	4.33 (69%)	1.79 (77%)
				False positives (germline)	7.19 (62%)	1.95 (31%)	0.52 (23%)

Fig. 3. Detection of tumor-specific and germline alterations using tumor-only and matched tumor and normal analyses. (**A** and **B**) Bar graphs show the number of true somatic alterations (blue) and germline false-positive changes (red) in each case for tumor-only targeted (A) and exome (B) analyses. The fraction of changes in actionable genes is indicated for both somatic (dark blue) and germline changes (dark red). For exome analyses, actionable alterations for somatic and germline changes are also indicated in the inset graph. (**C**) Summary of overall characteristics and the number of somatic and germline variants detected for each type of analysis. Total sequence coverage, the number of samples analyzed, and the number of somatic mutations per tumor in the matched tumor/normal analyses are included for reference.

that may be identical to germline variants. In our targeted analyses, two somatic mutations in *PDGFRA* (478S>P) and *ATRX* (929Q>E) matched identical mutations at the nucleotide level in dbSNP and were erroneously removed by this method. The analysis of all coding genes revealed 155 somatic mutations that were removed using this approach, including the 114R>C change in the catalytic domain of the mitogen-activated protein kinase *MAPK4* and 320P>R in the transcription factor *ESX1*, which have been previously reported to be somatically mutated in skin, and thyroid and liver cancers, respectively.

To further examine detection of somatic alterations using a tumor-only approach, we attempted to separate out the somatic mutations from the remaining germline alterations after dbSNP filtering using data from the COSMIC (Catalogue of Somatic Mutations in Cancer) database (Fig. 4). Mutations in our data set were considered more likely to be somatic if tumor-specific alterations had previously been reported within the same codon of the gene. In total, 108 mutations in 47 of the cases analyzed for the targeted set of genes and 1806 mutations in the exome cases were classified into this category. This approach was useful in identifying well-characterized mutations at hotspots in oncogenes such as *KRAS*, *TP53*, and *PIK3CA* but did not identify less frequent nonsynonymous somatic mutations. Nine of the potential somatic mutations in the targeted genes that overlapped with COSMIC were present in the matched normal samples and were, in fact, germline. In the exome data, 778 germline mutations occurred at codons in which somatic mutations had been previously described. Because somatic mutations can be clustered within certain regions

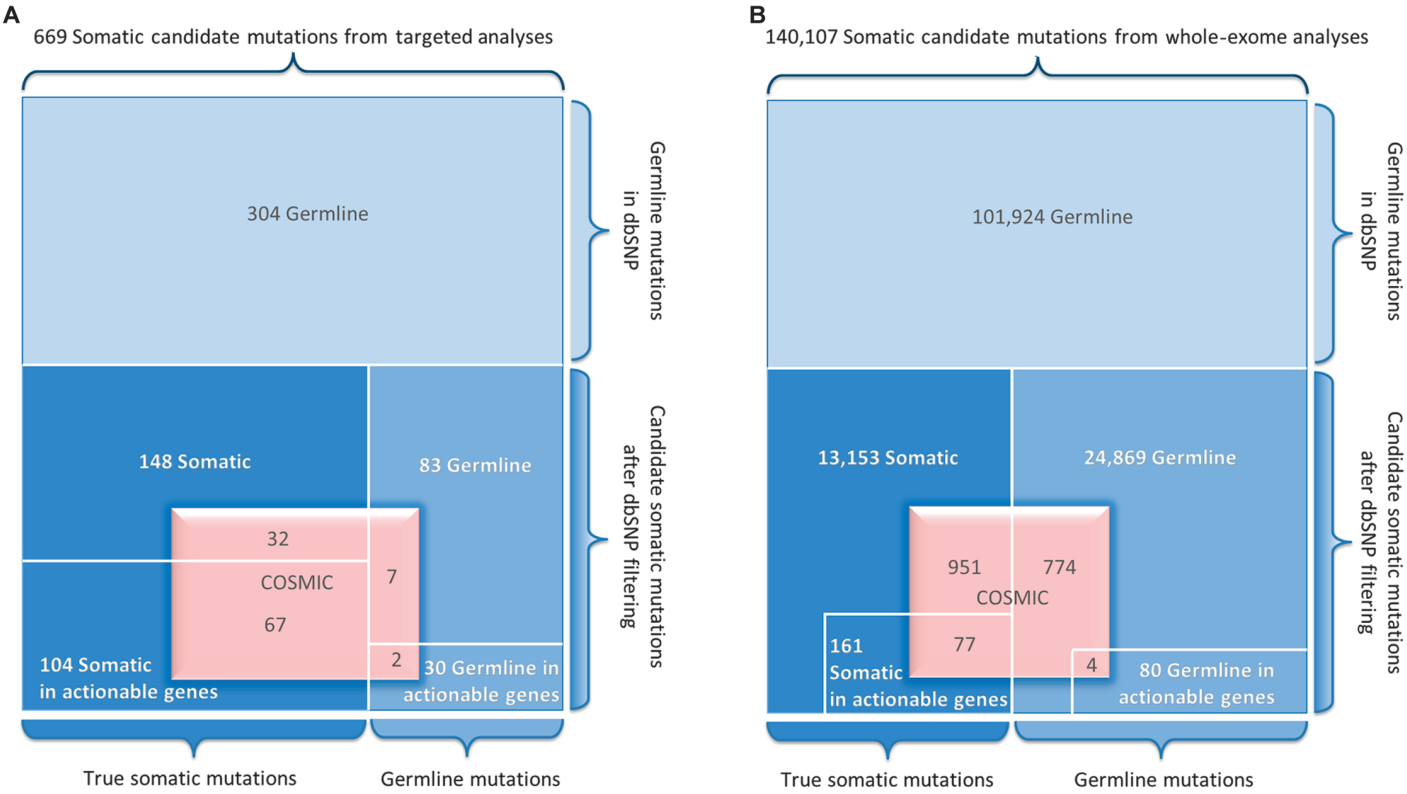

Fig. 4. Bioinformatic filtering approaches for detection of somatic and germline changes. (**A** and **B**) Somatic candidate mutations identified through targeted (A) and whole-exome (B) analyses. A total of 669 and 140,107 candidate mutations were found before any filtering in targeted and exome analyses, respectively. After filtering using dbSNP, 304 germline variants could be distinguished from 365 candidate somatic mutations in the targeted analyses; 101,924 germline changes were similarly filtered from 38,183 candidate somatic mutations in the exome analyses. Comparison to matched normal samples in each case allowed for distinction between true somatic mutations and germline variants. Filtered variants were compared to COSMIC data to determine the number of somatic mutations that could be distinguished from germline changes using this approach. In parallel, candidate somatic mutations were compared to genes described in FDA approval trials, published clinical trials, and active clinical trials to identify alterations present in clinically actionable genes. The overlaps between the COSMIC data and the categories indicated above are indicated with the designated areas in both targeted and exome analyses.

of a gene, we expanded our COSMIC criteria to include any mutations within five codons of the observed alteration. This increased the number of potential somatic mutations in the targeted genes by 152 to give a total of 270 (4.48 per patient) and increased the number by almost 15,000 in the exome cases to give a total of 16,731 (168 per patient). However, the specificity of the approach was substantially reduced, with 48 and 8929 of these mutations actually occurring in the matched normal in the targeted and exome genes, respectively. To determine the overall number of identical changes in the genome that had been reported as both germline variants and somatic changes through other studies, we examined the overall overlap between common dbSNP variants and the COSMIC databases. After excluding variants of known medical impact or annotated as somatic in dbSNP, we found 8606 nonsynonymous mutations that were present in both databases, of which 63 mutations were observed more than five times in COSMIC. These analyses suggest that a considerable number of variants in the germ line may be identical to those in somatic disease such as cancer, and the number of identical variants will increase as additional somatic and germline genomes are analyzed.

Because somatic mutations in tumor suppressor genes are often truncating, we also examined this mutation type as a means to positively select for alterations in the tumor-only data after filtering of common germline variants (fig. S1). Seventy-five mutations affecting genes such as *CDH1* (splice site), *PIK3R1* (frameshift), and *ARID1B* (nonsense) in 43 cases of the targeted analyses fell into this category. However, similar to the COSMIC approach, 13 of the alterations identified as candidate somatic changes using this method were germline. In the exome cases, there were 7424 truncating mutations, but 5108 of these were germline, not somatic. Finally, we looked to see whether any of the mutations were present in the kinase domains of the proteins, because activating somatic mutations often occur in these regions. Forty-two mutations, including the *EGFR* exon 19 deletion 745KELREA>T, 542E>K in *PIK3CA,* 1021Y>F in *JAK2,* and 867E>K in *RET*, were identified in the targeted analyses, and 786 mutations, including 309P>L in *MAPK12* and 201P>S in *CDK10,* were present in the exome cases. However, four mutations in the targeted set (including the alteration in *JAK2*) and 295 alterations in the exome set were, in fact, germline (fig. S2).

Using a combination of the COSMIC, truncating alteration, and kinase domain approaches, we correctly identified 216 of 252 somatic mutations in the targeted analyses. Of the 36 somatic mutations that were missed, several occurred in genes such as *ERBB2*, *ERBB3,* and

TSC2 that are under active clinical investigation and may have been clinically actionable. These approaches also identified 71 mutations (1.22 per case) that were germline from the analyses of the matched normal samples. These included changes in actionable genes such as *ERBB2* (1128V>I), *MSH6* (726F>L), and *RET* (977S>R). Furthermore, there were 78 mutations that were not removed by the SNP filters or positively selected by the additional criteria and could not be classified by these methods. When the entire coding region was analyzed, only 8941 of the 13,314 true somatic mutations were identified, 14,734 germline variants were incorrectly categorized as likely to be tumor-specific, and the remaining 14,508 mutations including 10,135 germline alterations could not be classified.

Use of tumor cellularity in distinguishing germline from somatic mutations

As an independent measure of the somatic or germline status of a variant, we examined the fraction of mutant alleles in an analyzed tumor sample. Germline mutations would be expected to have variant allele frequency close to 50% for heterozygous and 100% for homozygous changes, whereas the proportion of variant tags for somatic mutations would depend on the extent of normal tissue contamination in the tumor sample and would presumably be lower. Of the 43 targeted cases where tumor cellularity was available, only 5 had a pathological purity of less than 50%. In these cases, all of the alterations were correctly called as somatic or germline using this method. However, in most cases, the tumor cellularity exceeded 50%. In these cases, this approach could not reliably distinguish between somatic and germline alterations, correctly identifying on average only 48% of somatic mutations. Likewise, of the assessable 16 cancer-predisposing germline variants in these cases, only 2 could be distinguished from somatic alterations through an analysis of allele fractions.

DISCUSSION

Overall, these data provide a comprehensive analysis of the detection and interpretation of somatic and germline alterations in human cancer. These observations suggest that a high fraction of human tumors have alterations that may be clinically actionable and that a small but notable fraction of apparently sporadic cancer patients have pathogenic germline changes in cancer-predisposing genes. Additionally, these data support the notion that accurate identification and clinical interpretation of alterations benefit from analysis of both tumor and normal DNA from cancer patients.

As with all large-scale studies, our analyses have limitations. Although a variety of bioinformatic approaches were used in our analyses, additional computational methods could improve tumor-only analyses in the future. These include the use of additional germline databases, including the Exome Sequencing Project as well as other ongoing large-scale germline analyses such as the Genomics England 100,000 Genomes Project (*33*) and the Human Longevity sequencing initiative (*34*), that may not be well represented in the current dbSNP or the 1000 Genomes data sets. However, even if such approaches improve the filtering of germline changes, they are likely to increase the fraction of somatic variants that are inadvertently removed. Tools such as CHASM (cancer-specific high-throughput annotation of somatic mutations) (*35*), SIFT (*36*), PolyPhen (*37*), and others could potentially be used to predict whether a somatic mutation is likely a driver or passenger even in the absence of normal DNA. However, using CHASM as a final filter in our tumor-only data set approach did not identify any additional somatic mutations. Increasing the number of protein domains examined may also be helpful, although the mutations identified using this approach may be expected to be identified by the COSMIC clustering filter. Additionally, it is conceivable that some of the somatic changes identified represent genetic mosaicism affecting precursor cells from which the tumor originated. Although such changes would likely not affect actionable genes, careful genomic analyses of normal cells adjacent to neoplastic cells could be performed to resolve this issue.

From a clinical perspective, the use of matched tumor and normal DNA for genomic analyses as we have described is the most direct approach for accurate identification of actionable somatic and germline changes in cancer specimens. Although hotspot mutations in a few oncogenes can be readily detected with high sensitivity and specificity by analyses of tumor tissue alone, we expect that as many as a third of actionable changes in tumor-only analyses may be incorrectly classified as somatic changes when these actually represent constitutional alterations. Use of additional bioinformatic filtering approaches can improve the specificity but will miss a sizable fraction of somatic changes in actionable genes. Additionally, as we have shown, without analysis of germline DNA, cancer patients cannot be accurately screened for hereditary mutations in cancer predisposition genes that could inform the clinical management of the patient and indicate additional family members that could benefit from regular cancer screening. Larger-scale studies in patients with a family history of the tumor types identified in this study could be used to determine the contribution of mutations such as those indicated in table S8 to different cancers. Conversely, the identification of alterations in cancer-predisposing genes such as *BRCA1* and *BRCA2* in tumor-only analyses may lead to unnecessary referrals for genetic counseling and additional germline-specific testing in cases where these alterations are truly somatic. For example, we identified somatic alterations in *BRCA1* or *BRCA2* in 53 patients without any evidence of additional germline changes in these genes that may have led to unnecessary additional clinical follow-up had these been identified through a tumor-only analysis.

The current use of tumor-only sequencing analyses in many diagnostic laboratories may be a result of previous implementation of mutation hotspot assays but also reflects practical challenges in performing matched tumor and normal analyses, including obtaining normal DNA and the potential need to consent patients for such studies. However, germline DNA can now be routinely obtained from saliva samples and unaffected resected tissue in addition to blood, potentially simplifying logistical challenges. Additionally, patient consents may not be needed when constitutional changes are analyzed only for the purposes of filtering somatic alterations and germline changes are not directly reported. Some institutions are moving to blanket consents that permit comprehensive genomic analyses, including those identifying somatic as well as predisposing alterations in cancer and other diseases.

Given the anticipated widespread adoption of genomic analyses for cancer patients, these studies suggest that such genetic tests need to be carefully designed and implemented in the clinical setting. These results highlight that the sensitivity and specificity of clinical genetic tests can be compromised when analytical methods are used in an attempt to identify somatic mutations in the place of sequencing a matched normal sample. Our studies suggest that the use of tumor-only analyses may lead to inappropriate administration of cancer therapies with substantial effects on patient safety and health care costs. The consequences of

such analyses will become even more important through discovery of additional actionable genes and as new targeted therapies continue to be developed. The design of diagnostic assays must be carefully considered to ensure that patients receive the full benefit of these advances.

MATERIALS AND METHODS

Study design

The study was a retrospective analysis of targeted and whole-exome sequencing data from cancer patients with a range of different tumor types. We evaluated the clinical actionability of the mutations identified, determined the fraction of cases with a hereditary mutation in a known cancer predisposition gene and the effectiveness of different bioinformatic approaches in distinguishing between germline and somatic variants in the absence of matched normal samples.

Samples

Eight hundred fifteen tumor samples and matched normal tissues were obtained and analyzed with Western Institutional Review Board approval. A large range of cancer types including brain, breast, colorectal, cholangiocarcinoma, head and neck, neuroendocrine, renal, gastric, gynecological, esophageal, lung, melanoma, and pancreatic cancers, hematopoietic malignancies, and sarcomas were studied. Sample types included FFPE and frozen tissue, cell lines, DNA, and early-passage patient-derived xenografts. Patient-derived xenografts were included because we have previously shown a high concordance between such samples and matching primary tumors (*38*). Samples provided as FFPE blocks or frozen tissue underwent pathological review to determine tumor cellularity. Tumors were macrodissected to remove contaminating normal tissue. Matched normal samples were provided as blood, saliva, unaffected tissue, or normal cell lines (table S4).

Sample preparation and next-generation sequencing

Sample preparation, library construction, exome and targeted capture, next-generation sequencing, and bioinformatic analyses of tumor and normal samples were performed as previously described (*19*). In brief, DNA was extracted from frozen or FFPE tissue, along with matched blood or saliva samples using the Qiagen DNA FFPE Tissue Kit or Qiagen DNA Blood Mini Kit (Qiagen). Genomic DNA from tumor and normal samples was fragmented and used for Illumina TruSeq library construction (Illumina) according to the manufacturer's instructions or as previously described (*19*). Briefly, 50 ng to 3 μg of genomic DNA in 100 μl of TE (tris-EDTA) was fragmented in a Covaris sonicator to a size of 150 to 450 base pairs (bp). To remove fragments smaller than 150 bp, DNA was purified using Agencourt AMPure XP beads (Beckman Coulter) in a ratio of 1.0:0.9 of polymerase chain reaction (PCR) product to beads twice and washed using 70% ethanol per the manufacturer's instructions. Purified, fragmented DNA was mixed with 36 μl of H_2O, 10 μl of End Repair Reaction Buffer, 5 μl of End Repair Enzyme Mix [cat# E6050, New England BioLabs (NEB)]. The 100-μl end-repair mixture was incubated at 20°C for 30 min and purified using Agencourt AMPure XP beads (Beckman Coulter) in a ratio of 1.0:1.25 of PCR product to beads and washed using 70% ethanol per the manufacturer's instructions. To A-tail, 42 μl of end-repaired DNA was mixed with 5 μl of 10× dA-Tailing Reaction Buffer and 3 μl of Klenow (exo-) (cat# E6053, NEB). The 50-μl mixture was incubated at 37°C for 30 min and purified using Agencourt AMPure XP beads (Beckman Coulter) in a ratio of 1.0:1.0 of PCR product to beads and washed using 70% ethanol per the manufacturer's instructions. For adapter ligation, 25 μl of A-tailed DNA was mixed with 6.7 μl of H_2O, 3.3 μl of paired-end (PE) adapter (Illumina), 10 μl of 5× ligation buffer and 5 μl of Quick T4 DNA ligase (cat# E6056, NEB). The ligation mixture was incubated at 20°C for 15 min and purified using Agencourt AMPure XP beads (Beckman Coulter) in a ratio of 1.0:0.95 and 1.0 of PCR product to beads twice and washed using 70% ethanol per the manufacturer's instructions. To obtain an amplified library, 12 PCRs of 25 μl each were set up, each including 15.5 μl of H_2O, 5 μl of 5× Phusion HF buffer, 0.5 μl of a deoxynucleotide triphosphate (dNTP) mix containing 10 mM of each dNTP, 1.25 μl of dimethyl sulfoxide (DMSO), 0.25 μl of Illumina PE primer #1, 0.25 μl of Illumina PE primer #2, 0.25 μl of Hot Start Phusion polymerase, and 2 μl of the DNA. The PCR program used was 98°C for 2 min; 12 cycles of 98°C for 15 s, 65°C for 30 s, and 72°C for 30 s; and 72°C for 5 min. DNA was purified using Agencourt AMPure XP beads (Beckman Coulter) in a ratio of 1.0:1.0 of PCR product to beads and washed using 70% ethanol per the manufacturer's instructions. Exonic or targeted regions were captured in solution using the Agilent SureSelect version 4 kit or a custom-targeted panel for the 111 genes of interest according to the manufacturer's instructions (Agilent). The captured library was then purified with a Qiagen MinElute column purification kit and eluted in 17 μl of 70°C elution buffer to obtain 15 μl of captured DNA library. The captured DNA library was amplified in the following way: eight 30-μl PCR reactions each containing 19 μl of H_2O, 6 μl of 5× Phusion HF buffer, 0.6 μl of 10 mM dNTP, 1.5 μl of DMSO, 0.30 μl of Illumina PE primer #1, 0.30 μl of Illumina PE primer #2, 0.30 μl of Hot Start Phusion polymerase, and 2 μl of captured exome library were set up. The PCR program used was 98°C for 30 s; 14 cycles (exome) or 16 cycles (targeted) of 98°C for 10 s, 65°C for 30 s, and 72°C for 30 s; and 72°C for 5 min. To purify PCR products, a NucleoSpin Extract II purification kit (Macherey-Nagel) was used following the manufacturer's instructions. PE sequencing, resulting in 100 bases from each end of the fragments for exome libraries and 150 bases from each end of the fragment for targeted libraries, was performed using Illumina HiSeq 2000/2500 and Illumina MiSeq instrumentation (Illumina).

Primary processing of next-generation sequencing data and identification of putative somatic mutations

Somatic mutations were identified using VariantDx custom software for identifying mutations in matched tumor and normal samples. Before mutation calling, primary processing of sequence data for both tumor and normal samples was performed using Illumina CASAVA (Consensus Assessment of Sequence and Variation) software (version 1.8), including masking of adapter sequences. Sequence reads were aligned against the human reference genome (version hg18) using ELAND (Efficient Large-Scale Alignment of Nucleotide Databases) with additional realignment of select regions using the Needleman-Wunsch method (*39*). Candidate somatic mutations, consisting of point mutations, insertions, and deletions, were then identified using VariantDx across either the whole exome or regions of interest. VariantDx examines sequence alignments of tumor samples against a matched normal while applying filters to exclude alignment and sequencing artifacts. In brief, an alignment filter was applied to exclude quality-failed reads, unpaired reads, and poorly mapped reads in the tumor. A base quality

filter was applied to limit inclusion of bases with reported Phred quality scores >30 for the tumor and >20 for the normal (www.phrap.com/phred/). A mutation in the tumor was identified as a candidate somatic mutation only when (i) distinct paired reads contained the mutation in the tumor; (ii) the number of distinct paired reads containing a particular mutation in the tumor was at least 2% of the total distinct read pairs for targeted analyses and 10% of read pairs for exome; (iii) the mismatched base was not present in >1% of the reads in the matched normal sample as well as not present in a custom database of common germline variants derived from dbSNP; and (iv) the position was covered in both the tumor and normal. Mutations arising from misplaced genome alignments, including paralogous sequences, were identified and excluded by searching the reference genome.

Candidate somatic mutations were further filtered on the basis of gene annotation to identify those occurring in protein-coding regions. Functional consequences were predicted using snpEff and a custom database of CCDS, RefSeq, and Ensembl annotations using the latest transcript versions available on hg18 from University of California, Santa Cruz (https://genome.ucsc.edu/). Predictions were ordered to prefer transcripts with canonical start and stop codons and CCDS or RefSeq transcripts over Ensembl when available. Finally, mutations were filtered to exclude intronic and silent changes and retain mutations resulting in missense mutations, nonsense mutations, frameshifts, or splice site alterations. A manual visual inspection step was used to further remove artifactual changes.

Identification of putative somatic mutations without matched normal sample

One hundred cases with exome sequencing data and 58 targeted cases were selected for analyses both with and without their matched normal sample. For the identification of putative somatic mutations without a matched normal, additional filters were applied. First, mutations present in an unmatched normal sample, sequenced to a similar coverage and on the same platform as the matched normal, were removed. Second, alterations reported in the 1000 Genomes Project, present in >1% of the population, or listed as Common in dbSNP138 were filtered. In an attempt to positively select for somatic changes in the resulting data set, mutations occurring within the same amino acid or within five codons of previously reported somatic alterations were identified by comparison to the COSMIC database (version 68, http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/). In addition, frameshift, nonsense, and splice site changes predicted to truncate the protein as well as nonsynonymous mutations within the catalytic domain of protein kinases (*40*) were selected.

Comparison between dbSNP and COSMIC

Common germline mutations were obtained from the dbSNP human variation sets in VCF (variant call format) version 138 labeled "common" with a germline minor allele frequency of ≥0.01 and indicated with "no known medical impact." Mutations were filtered on dbSNP fields to exclude synonymous changes and those annotated with somatic origin. Mutations were compared to COSMIC version 68 to identify mutations that matched dbSNP both on genomic position and genomic change.

Clinical actionability analyses

We identified 196 well-characterized genes with potential clinical relevance and assessed the level of evidence for clinical actionability in three ways. First, we determined which of the genes were associated with FDA-approved therapies (www.fda.gov/Drugs/). Second, we carried out a literature search to identify published prospective clinical studies pertaining to genomic alterations of each gene and their association with outcome for cancer patients. Genes that served as targets for specific agents or were predictors of response or resistance to cancer therapies when mutated were considered actionable. Third, we identified clinical trials (http://clinicaltrials.gov/) that specified altered genes within the inclusion criteria and were actively recruiting patients in August 2014. In all cases, the tumor type relevant to the FDA approval or studied in the clinical trials was determined to allow the clinical information to be matched to the mutational data by both gene and cancer type.

Identification of germline mutations in cancer predisposition genes

We evaluated the coding regions of 85 previously well-characterized cancer predisposition genes for alterations in normal DNA from blood, saliva, or normal tissue of 815 cases using the VariantDx pipeline adapted to run on germline samples. Mutations resulting in frameshifts, nonsense, or splice site alterations were considered most likely to be causative and selected for further analysis. Each mutation was compared to published alterations using the ClinVar database (*41*) and locus-specific databases including the Breast Cancer Information Core (BIC) database for *BRCA1* and *BRCA2* (*42*), the Leiden Open Variation Databases (LOVD) for *ATM*, *BRIP1*, FA genes, and *PALB2* (*43*), the International Agency for Research on Cancer (IARC) *TP53* database (*44*), and the International Society for Gastrointestinal Hereditary Tumours Incorporated (InSiGHT) database for the mismatch repair genes *MLH1*, *MSH2*, *MSH6,* and *PMS2* (*45*). Any alteration designated in these databases as benign or likely benign was excluded.

SUPPLEMENTARY MATERIALS

www.sciencetranslationalmedicine.org/cgi/content/full/7/283/283ra53/DC1

Fig. S1. Bioinformatic approach to classify somatic and germline mutations on the basis of the consequence of the alteration.

Fig. S2. Bioinformatic approach to classify somatic and germline mutations based on the affected protein domain.

Table S1. Genes analyzed in the targeted approach (provided in a separate Excel file).

Table S2. Summary of sequencing statistics (provided in a separate Excel file).

Table S3. Summary of performance characteristics of whole-exome and targeted analyses (provided in a separate Excel file).

Table S4. Characteristics of the tumor and normal samples (provided in a separate Excel file).

Table S5. Fraction of cases with somatic mutations in actionable genes (provided in a separate Excel file).

Table S6. Fraction of cases with evidence for clinical actionability in different tumor types (provided in a separate Excel file).

Table S7. Hereditary cancer predisposition genes (provided in a separate Excel file).

Table S8. Putative germline predisposing mutations (provided in a separate Excel file).

Table S9. Germline false-positive mutations in actionable genes (provided in a separate Excel file).

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Science Translational Medicine **publishes timely Editorial, Focus, and Perspective articles on research and policy issues of interest to the broad translational research community. Here is a selection of these articles in precision medicine, with brief summaries.**

PRECISION MEDICINE

Immunotherapy: Making the case for precision medicine

Jeffrey Bluestone and Qizhi Tang

Citation
Sci. Transl. Med. 25 Mar 2015:
Vol. 7, Issue 280, pp. 280ed3

10.1126/scitranslmed.aaa9846

Comprehensive assessments of progress in the development of immunotherapy and other therapeutics in the areas of oncology, autoimmune and allergic diseases, and transplantation provide a glimpse into the transformative power of precision medicine.

POLICY

Evolution in translational science: Whither the CTSAs?

Garret FitzGerald

Citation
Sci. Transl. Med. 22 Apr 2015:
Vol. 7, Issue 284, pp. 284fs15

10.1126/scitranslmed.aab1596

Clinical and Translational Science Awards–funded institutions are naturally equipped to drive research on human phenotyping and, in turn, shape the practice of precision medicine in the clinic of the future.

HEALTH CARE

Engineering precision

Giovanni Traverso and Robert Langer

Citation
Sci. Transl. Med. 27 May 2015:
Vol. 7, Issue 289, pp. 289ed6

10.1126/scitranslmed.aab1943

New technologies could help facilitate the translation of precision medicine to patients.

REGULATORY SCIENCE

FDA as a catalyst for translation

Robert Califf and Stephen Ostroff

Citation
Sci. Transl. Med. 15 Jul 2015:
Vol. 7, Issue 296, pp. 296ed9

10.1126/scitranslmed.aab2404

To strengthen and speed translation, we require a new focus on key areas of an emerging discipline now called regulatory science—the development and application of new tools, standards, and approaches for the assessment of medical product safety, efficacy, and quality—not only at FDA but also among many other stakeholders—academia, the nonprofit community, policy-makers, and industry.

EDUCATION

How to know when physicians are ready for genomic medicine

Citation
Sci. Transl. Med. 13 May 2015: Vol. 7, Issue 287, pp. 287fs19

10.1126/scitranslmed.aaa2401

Jason L. Vassy, Bruce R. Korf, and Robert C. Green

Despite perceptions to the contrary, physicians are as prepared for genomic medicine as they are for other medical innovations; educational initiatives and support from genetics specialists can enhance clinical practice.

REGULATORY SCIENCE

Unmet needs: Research helps regulators do their jobs

Citation
Sci. Transl. Med. 25 Nov 2015: Vol. 7, Issue 315, pp. 315ps22

10.1126/scitranslmed.aac4369

Russ Altman *et al.* (Kathleen Giacomini)

New medical products and the need to apply modern tools for their evaluation has spurred opportunities in regulatory science.

REGULATION

Hearing voice: FDA seeks advice from patients

Citation
Sci. Transl. Med. 11 Nov 2015: Vol. 7, Issue 313, pp. 313ed12

10.1126/scitranslmed.aad5866

Sharon Terry and Bray Patrick-Lake

A new patient-engagement committee will advise the U.S. Food and Drug Administration on the regulation and use of medical devices.

A selection of graphical abstracts that provide a visual summary of the key points of a research article.

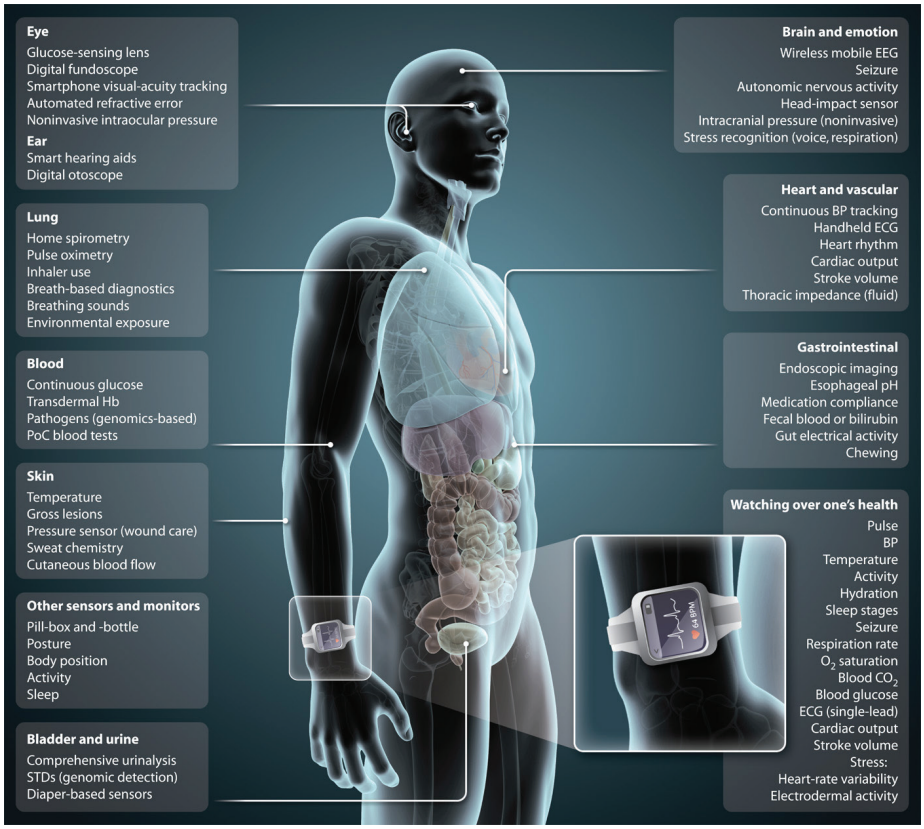
HEALTH CARE

The emerging field of mobile health

Steven R. Steinhubl, Evan D. Muse, and Eric J. Topol

Citation
Sci. Transl. Med. 15 Apr 2015:
Vol. 7, Issue 283, pp. 283rv3

10.1126/scitranslmed.aaa3487



CREDIT : H. MCDONALD/*SCIENCE TRANSLATIONAL MEDICINE*, ERAXION/ISTOCKPHOTO

Sensing a shift in health care. The surge in computing power and mobile connectivity have fashioned a foundation for mobile health (mHealth) technologies that can transform the mode and quality of clinical research and health care on a global scale. Several body-wide measurements by mobile health technologies are available to health care providers and patients to aid in the tracking, diagnosis, or management of various physiological processes and disease conditions. (Inset) Multiple developers have reported that certain physiological parameters—ranging from pulse to respiration rate to blood glucose—are measurable with sensors in wrist-worn devices. BP, blood pressure; Hb, hemoglobin; STDs, sexually transmitted diseases.

BIOENGINEERING

Citation
Sci. Transl. Med. 22 Apr 2015:
Vol. 7, Issue 284, pp. 284ra57

10.1126/scitranslmed.3010564

A technology platform to assess multiple cancer agents simultaneously within a patient's tumor

Richard Klinghoffer *et al.* (James Olson)

BIOENGINEERING

An implantable microdevice to perform high-throughput in vivo drug sensitivity testing in tumors

Oliver Jonas *et al.* (Robert Langer)

Citation
Sci. Transl. Med. 22 Apr 2015:
Vol. 7, Issue 284, pp. 284ra58

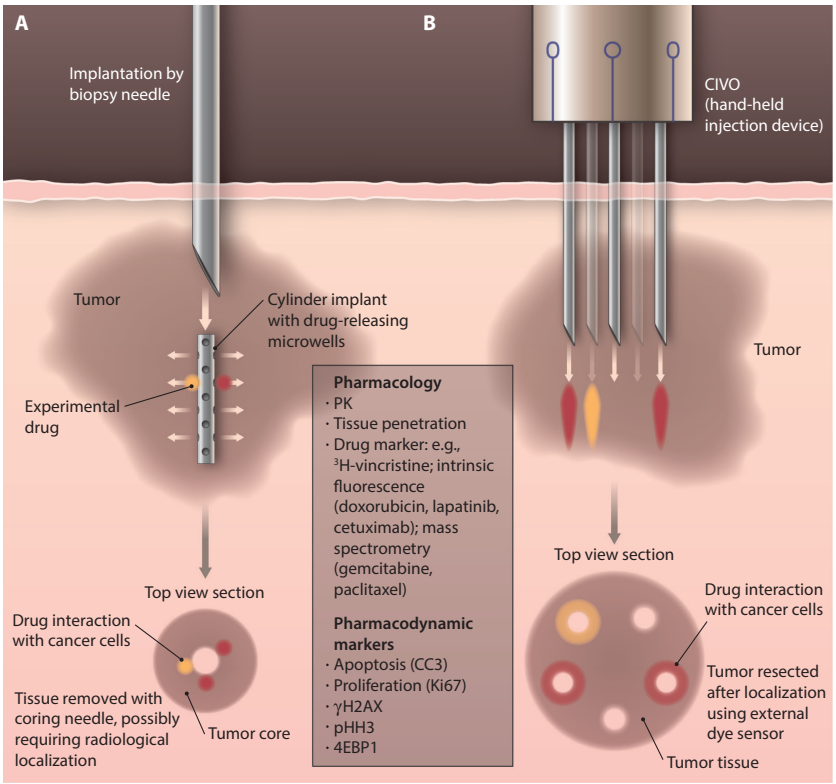
10.1126/scitranslmed.aaa7489

- In two related bioengineering studies, devices were engineered to deliver several cancer drugs to the tumor.
- Local tumor response to each drug could be evaluated upon biopsy or tumor resection.
- Optimal dosing and therapy can be determined for an individual patient.

See related Perspective
Coombes, "Drug testing in the patient: Toward personalized cancer treatment"

Citation
Sci. Transl. Med. 22 Apr 2015:
Vol. 7, Issue 284, pp. 284ps10

10.1126/scitranslmed.aab1214



CREDIT: CHRIS BICKEL/*SCIENCE TRANSLATIONAL MEDICINE*

Two different devices allow for in vivo drug sensitivity testing and biomarkers analysis in patient tumors. (**A**) The device created by Jonas and colleagues is implantable and can deliver up to 16 different drugs simultaneously, for evaluation after removal by biopsy coring needle. The device was tested in several mouse models of human tumors. (**B**) The handheld device engineered by Klinghoffer *et al.*, called CIVO, micro-injects up to eight different drugs prior to tumor resection, and has been tested in rodent and canine models and in human patients. Various pharmacologic and pharmacodynamic markers were evaluated in both studies to demonstrate that device outputs reflected the systemic response to therapy.

GENETIC MEDICINE

Integrated allelic, transcriptional, and phenomic dissection of the cardiac effects of titin truncations in health and disease

Ware *et al.* (Christine Seidman, Stuart Cook)

Citation
Sci. Transl. Med. 14 Jan 2015: Vol. 7, Issue 270, pp. 270ra6

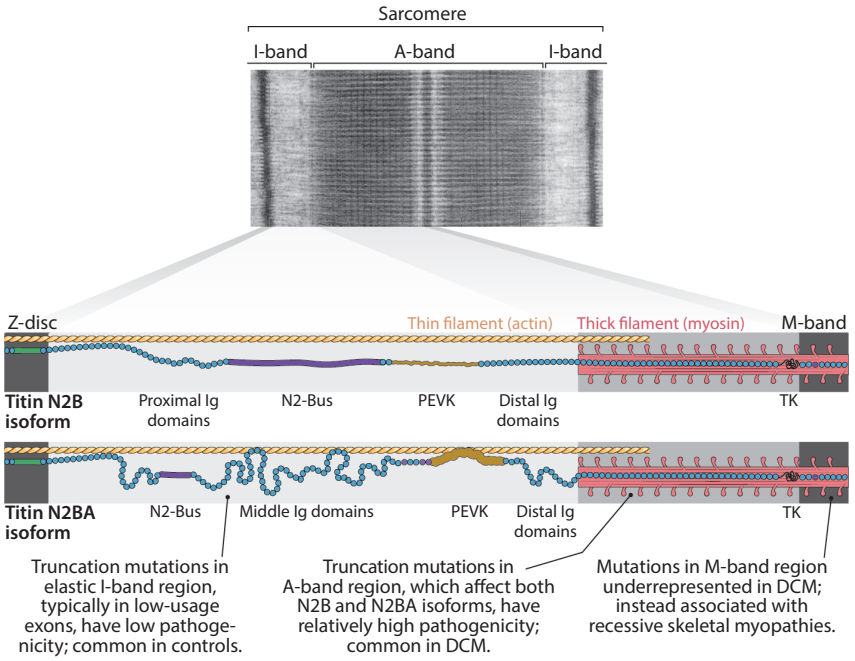
10.1126/scitranslmed.3010134

See related Focus
Watkins, "Tackling the Achilles' Heel of Genetic Testing"

Citation
Sci. Transl. Med. 14 Jan 2015: Vol. 7, Issue 270, pp. 270fs1

10.1126/scitranslmed.aaa4276

- Titin, a large heart protein, was sequenced in 5267 individuals, some with cardiomyopathy.
- Most of the disease-causing mutations in titin resulted in shortened RNA.
- The mutations caused cardiomyopathy primarily when located at the protein's carboxyl end or in highly transcribed exons.
- These results explain, in part, the variable penetrance of this disease.



ELECTRON MICROGRAPH [CREDIT: P. LUTHER/WWW.SARCOMERE.ORG]

Anatomy of a giant. The giant myofilament protein titin spans half the sarcomere of striated muscle, from the Z-disc that anchors the actin thin filament and many associated proteins to the M-band signaling complex of the myosin-containing thick filament. Two isoforms predominate in the heart, N2B (short and stiff) and N2BA (long and compliant), each with complex alternative splicing. Roberts and Leducq Network consortium colleagues showed that the impact of truncation alleles is determined by exon usage (low in the I-band, where most exons are symmetrical and can be spliced out without frameshift), occurrence in the different isoforms, and localization in terms of the sarcomeric region affected. This will aid, though not completely resolve, discrimination between benign (1% of the general population) and pathogenic truncation variants. Putative pathogenic variants are enriched in the A-band region but even here can be of low penetrance, suggesting other factors are also important. N2-Bus, N2B unique sequence (a site of phosphorylation); PEVK, titin region rich in proline, glutamate, valine, and lysine (contributes to spring function); TK, titin kinase domain (important signaling roles). Titin schematic is adapted from *Circulation Research.* (2014), doi: 10.1161/CIRCRESAHA.

INFLUENZA

Antibodies to influenza nucleoprotein cross-react with human hypocretin receptor 2

Syed Sohail Ahmed *et al.* (Lawrence Steinman)

Citation
Sci. Transl. Med. 01 Jul 2015: Vol. 7, Issue 294, pp. 294ra105

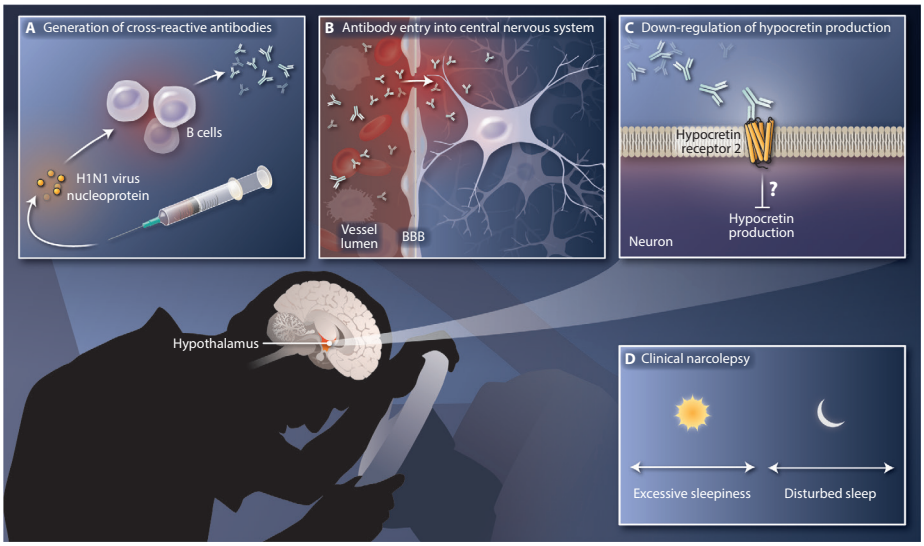
10.1126/scitranslmed.aab2354

See related Focus
Wekerle, "Vaccination and narcolepsy: Immune link found?"

Sci. Transl. Med. 01 Jul 2015: Vol. 7, Issue 294, pp. 294fs27

10.1126/scitranslmed.aac7091

- Narcolepsy, caused by a deficit in the brain hypocretin system, surged after a flu vaccination campaign.
- The particular vaccine used contained more flu virus nucleopeptide A than other flu vaccines.
- A peptide within nucleoprotein A mimics a fragment of the hypocretin receptor.
- Antibodies that cross-react with flu nucleoprotein and the hypocretin receptor were found in vaccinated narcoleptic patients.



CREDIT: H. MCDONALD/*SCIENCE TRANSLATIONAL MEDICINE*

Untimely siesta. Narcolepsy is linked to the HLA-DQB1*0602 haplotype and dysregulation of the hypocretin ligand–receptor pathway. In 2009, narcolepsy was associated with Pandemrix vaccination (an adjuvanted influenza pandemic vaccine) and also with infection by influenza virus during the 2009 A(H1N1) influenza pandemic. Differences in vaccine nucleoprotein (NP) content and respective immune response may explain these associations. The autoimmune concept of H1N1-related narcolepsy could involves four stages, according to Ahmed et al. (**A**) Stage I: Preferentially in HLA-DQB1*06:02+ individuals, anti-H1N1 vaccination triggers formation of antibodies that bind to the viral NP and hypocretin receptor 2. (**B**) Stage II: After perforation of the blood-brain barrier, the antibodies leak into the brain tissue. (**C**) Stage III: Antibodies bind to the hypocretin receptor 2 on the surface of neurons and disrupt signaling either by direct blockade or by secondary depletion of hypocretin formation. (**D**) Stage IV: Disrupted hypocretin signaling results in clinical narcolepsy.

Science Translational Medicine **publishes original research articles and reports that represent significant movements toward improving human health, including new biology and advances in the diagnosis and treatment of disease.**

PRECISION MEDICINE

Identification of type 2 diabetes subgroups through topological analysis of patient similarity

Li Li *et al.* (Joel Dudley)

Citation
Sci. Transl. Med. 28 Oct 2015: Vol. 7, Issue 311, pp. 311ra174

10.1126/scitranslmed.aaa9364

Type 2 diabetes (T2D) is a heterogeneous complex disease affecting more than 29 million Americans alone with a rising prevalence trending toward steady increases in the coming decades. Thus, there is a pressing clinical need to improve early prevention and clinical management of T2D and its complications. Clinicians have understood that patients who carry the T2D diagnosis have a variety of phenotypes and susceptibilities to diabetes-related complications. We used a precision medicine approach to characterize the complexity of T2D patient populations based on high-dimensional electronic medical records (EMRs) and genotype data from 11,210 individuals. We successfully identified three distinct subgroups of T2D from topology-based patient-patient networks. Subtype 1 was characterized by T2D complications diabetic nephropathy and diabetic retinopathy; subtype 2 was enriched for cancer malignancy and cardiovascular diseases; and subtype 3 was associated most strongly with cardiovascular diseases, neurological diseases, allergies, and HIV infections. We performed a genetic association analysis of the emergent T2D subtypes to identify subtype-specific genetic markers and identified 1279, 1227, and 1338 single-nucleotide polymorphisms (SNPs) that mapped to 425, 322, and 437 unique genes specific to subtypes 1, 2, and 3, respectively. By assessing the human disease–SNP association for each subtype, the enriched phenotypes and biological functions at the gene level for each subtype matched with the disease comorbidities and clinical differences that we identified through EMRs. Our approach demonstrates the utility of applying the precision medicine paradigm in T2D and the promise of extending the approach to the study of other complex, multifactorial diseases.

GENOMICS

Effectiveness of exome and genome sequencing guided by acuity of illness for diagnosis of neurodevelopmental disorders

Sarah Soden *et al.* (Stephen Kingsmore)

Citation
Sci. Transl. Med. 03 Dec 2014: Vol. 6, Issue 265, pp. 265ra168

10.1126/scitranslmed.3010076

Neurodevelopmental disorders (NDDs) affect more than 3% of children and are attributable to single-gene mutations at more than 1000 loci. Traditional methods yield molecular diagnoses in less than one-half of children with NDD. Whole-genome sequencing (WGS) and whole-exome sequencing (WES) can enable diagnosis of NDD, but their clinical and cost-effectiveness are unknown. One hundred families with 119 children affected by NDD received diagnostic WGS and/or WES of parent-child trios, wherein the sequencing approach was guided by acuity of illness. Forty-five percent received molecular diagnoses. An accelerated sequencing modality, rapid WGS, yielded diagnoses in 73% of families with acutely ill children (11 of 15). Forty percent of families with children with nonacute NDD, followed in ambulatory care clinics (34 of 85), received diagnoses: 33 by WES and 1 by staged WES then WGS. The cost of prior negative tests in the nonacute patients was $19,100 per family, suggesting sequencing to be cost-effective at up to $7640 per family. A change in clinical care or impression of the pathophysiology was reported in 49% of newly diagnosed families. If WES or WGS had been performed at symptom onset, genomic diagnoses may have been made 77 months earlier than occurred in this study. It is suggested that initial diagnostic evaluation of children with NDD should include trio WGS or WES, with extension of accelerated sequencing modalities to high-acuity patients.

MULTIPLE SCLEROSIS

Genetic variants associated with autoimmunity drive NFkB signaling and responses to inflammatory stimuli

William Housley *et al.* (David Hafler)

Citation
Sci. Transl. Med. 10 Jun 2015: Vol. 7, Issue 291, pp. 291ra93

10.1126/scitranslmed.aaa9223

The transcription factor nuclear factor κB (NFκB) is a central regulator of inflammation, and genome-wide association studies in subjects with autoimmune disease have identified a number of variants within the NFκB signaling cascade. In addition, causal variant fine-mapping has demonstrated that autoimmune disease susceptibility variants for multiple sclerosis (MS) and ulcerative colitis are strongly enriched within binding sites for NFκB. We report that MS-associated variants proximal to *NFκB1* and in an intron of *TNFRSF1A (TNFR1)* are associated with increased NFκB signaling after tumor necrosis factor–α (TNFα) stimulation. Both variants result in increased degradation of inhibitor of NFκB α (IκBα), a negative regulator of NFκB, and nuclear translocation of p65 NFκB. The variant proximal to NFκB1 controls signaling responses by altering the expression of NFκB itself, with the GG risk genotype expressing 20-fold more p50 NFκB and diminished expression of the negative regulators of the NFκB pathway: TNFα-induced protein 3 (TNFAIP3), B cell leukemia 3 (BCL3), and cellular inhibitor of apoptosis 1 (CIAP1). Finally, naïve CD4 T cells from patients with MS express enhanced activation of p65 NFκB. These results demonstrate that genetic variants associated with risk of developing MS alter NFκB signaling pathways, resulting in enhanced NFκB activation and greater responsiveness to inflammatory stimuli. As such, this suggests that rapid genetic screening for variants associated with NFκB signaling may identify individuals amenable to NFκB or cytokine blockade.

BIOENGINEERING

Predicting therapeutic nanomedicine efficacy using a companion magnetic resonance imaging nanoparticle

Miles Miller *et al.* (Ralph Weissleder)

Citation
Sci. Transl. Med. 18 Nov 2015: Vol. 7, Issue 314, pp. 314ra183

10.1126/scitranslmed.aac6522

Therapeutic nanoparticles (TNPs) have shown heterogeneous responses in human clinical trials, raising questions of whether imaging should be used to identify patients with a higher likelihood of NP accumulation and thus therapeutic response. Despite extensive debate about the enhanced permeability and retention (EPR) effect in tumors, it is increasingly clear that EPR is extremely variable; yet, little experimental data exist to predict the clinical utility of EPR and its influence on TNP efficacy. We hypothesized that a 30-nm magnetic NP (MNP) in clinical use could predict colocalization of TNPs by magnetic resonance imaging (MRI). To this end, we performed single-cell resolution imaging of fluorescently labeled MNPs and TNPs and studied their intratumoral distribution in mice. MNPs circulated in the tumor microvasculature and demonstrated sustained uptake into cells of the tumor microenvironment within minutes. MNPs could predictably demonstrate areas of colocalization for a model TNP, poly(D,L-lactic-co-glycolic acid)-b-polyethylene glycol (PLGA-PEG), within the tumor microenvironment with >85% accuracy and circulating within the microvasculature with >95% accuracy, despite their markedly different sizes and compositions. Computational analysis of NP transport enabled predictive modeling of TNP distribution based on imaging data and identified key parameters governing intratumoral NP accumulation and macrophage uptake. Finally, MRI accurately predicted initial treatment response and drug accumulation in a preclinical efficacy study using a paclitaxel-encapsulated NP in tumor-bearing mice. These approaches yield valuable insight into the in vivo kinetics of NP distribution and suggest that clinically relevant imaging modalities and agents can be used to select patients with high EPR for treatment with TNPs.

CANCER

Epigenetic therapy overcomes treatment resistance in T cell prolymphocytic leukemia

Zainul S. Hasanali *et al.* (Elliot Epner)

Citation
Sci. Transl. Med. 24 Jun 2015:
Vol. 7, Issue 293, pp. 293ra102

10.1126/scitranslmed.aaa5079

T cell prolymphocytic leukemia (T-PLL) is a rare, mature T cell neoplasm with distinct features and an aggressive clinical course. Early relapse and short overall survival are commonplace. Use of the monoclonal anti-CD52 antibody alemtuzumab has improved the rate of complete remission and duration of response to more than 50% and between 6 and 12 months, respectively. Despite this advance, without an allogeneic transplant, resistant relapse is inevitable. We report seven complete and one partial remission in eight patients receiving alemtuzumab and cladribine with or without a histone deacetylase inhibitor. These data show that administration of epigenetic agents can overcome alemtuzumab resistance. We also report epigenetically induced expression of the surface receptor protein CD30 in T-PLL. Subsequent treatment with the anti-CD30 antibody–drug conjugate brentuximab vedotin overcame organ-specific (skin) resistance to alemtuzumab. Our findings demonstrate activity of combination epigenetic and immunotherapy in the incurable illness T-PLL, particularly in the setting of previous alemtuzumab therapy.

ORGAN TRANSPLANTATION

Disseminated *Ureaplasma* infection as a cause of fatal hyperammonemia in humans

Ankit Bharat *et al.* (Robin Patel)

Citation
Sci. Transl. Med. 22 Apr 2015:
Vol. 7, Issue 284, pp. 284re3

10.1126/scitranslmed.aaa8419

Hyperammonemia syndrome is a fatal complication affecting immunosuppressed patients. Frequently refractory to treatment, it is characterized by progressive elevations in serum ammonia of unknown etiology, ultimately leading to cerebral edema and death. In mammals, ammonia produced during amino acid metabolism is primarily cleared through the hepatic production of urea, which is eliminated in the kidney. *Ureaplasma*species, commensals of the urogenital tract, are Mollicutes dependent on urea hydrolysis to ammonia and carbon dioxide for energy production. We hypothesized that systemic infection with *Ureaplasma* species might pose a unique challenge to human ammonia metabolism by liberating free ammonia resulting in the hyperammonemia syndrome. We used polymerase chain reaction, specialized culture, and molecular resistance profiling to identify systemic *Ureaplasma* infection in lung transplant recipients with hyperammonemia syndrome, but did not detect it in any lung transplant recipients with normal ammonia concentrations. Administration of *Ureaplasma*-directed antimicrobials to patients with hyperammonemia syndrome resulted in biochemical and clinical resolution of the disorder. Relapse in one patient was accompanied by recurrent*Ureaplasma* bacteremia with antimicrobial resistance. Our results provide evidence supporting a causal relationship between *Ureaplasma* infection and hyperammonemia, suggesting a need to test for this organism and provide empiric antimicrobial treatment while awaiting microbiological confirmation.

CANCER

STING agonist formulated cancer vaccines can cure established tumors resistant to PD-1 blockade

Juan Fu *et al.* (Young Kim)

Citation
Sci. Transl. Med. 15 Apr 2015: Vol. 7, Issue 283, pp. 283ra52

10.1126/scitranslmed.aaa4306

Stimulator of interferon genes (STING) is a cytosolic receptor that senses both exogenous and endogenous cytosolic cyclic dinucleotides (CDNs), activating TBK1/IRF3 (interferon regulatory factor 3), NF-κB (nuclear factor κB), and STAT6 (signal transducer and activator of transcription 6) signaling pathways to induce robust type I interferon and proinflammatory cytokine responses. CDN ligands were formulated with granulocyte-macrophage colony-stimulating factor (GM-CSF)–producing cellular cancer vaccines—termed STINGVAX—that demonstrated potent in vivo antitumor efficacy in multiple therapeutic models of established cancer. We found that rationally designed synthetic CDN derivative molecules, including one with an Rp,Rp dithio diastereomer and noncanonical c[A(2′,5′)pA(3′,5′)p] phosphate bridge structure, enhanced antitumor efficacy of STINGVAX in multiple aggressive therapeutic models of established cancer in mice. Antitumor activity was STING-dependent and correlated with increased activation of dendritic cells and tumor antigen–specific CD8+ T cells. Tumors from STINGVAX-treated mice demonstrated marked PD-L1 (programmed death ligand 1) up-regulation, which was associated with tumor-infiltrating CD8+IFNγ+ T cells. When combined with PD-1 (programmed death 1) blockade, STINGVAX induced regression of palpable, poorly immunogenic tumors that did not respond to PD-1 blockade alone.

CANCER

Clonal status of actionable driver events and the timing of mutational processes in cancer evolution

Nicholas McGranahan *et al.* (Charles Swanton)

Citation
Sci. Transl. Med. 15 Apr 2015: Vol. 7, Issue 283, pp. 283ra54

10.1126/scitranslmed.aaa1408

Deciphering whether actionable driver mutations are found in all or a subset of tumor cells will likely be required to improve drug development and precision medicine strategies. We analyzed nine cancer types to determine the subclonal frequencies of driver events, to time mutational processes during cancer evolution, and to identify drivers of subclonal expansions. Although mutations in known driver genes typically occurred early in cancer evolution, we also identified later subclonal "actionable" mutations, including BRAF(V600E), IDH1 (R132H), PIK3CA (E545K), EGFR (L858R), and KRAS (G12D), which may compromise the efficacy of targeted therapy approaches. More than 20% of IDH1mutations in glioblastomas, and 15% of mutations in genes in the PI3K (phosphatidylinositol 3-kinase)–AKT–mTOR (mammalian target of rapamycin) signaling axis across all tumor types were subclonal. Mutations in the RAS–MEK (mitogen-activated protein kinase kinase) signaling axis were less likely to be subclonal than mutations in genes associated with PI3K-AKT-mTOR signaling. Analysis of late mutations revealed a link between APOBEC-mediated mutagenesis and the acquisition of subclonal driver mutations and uncovered putative cancer genes involved in subclonal expansions, including CTNNA2 and ATXN1. Our results provide a pan-cancer census of driver events within the context of intratumor heterogeneity and reveal patterns of tumor evolution across cancers. The frequent presence of subclonal driver mutations suggests the need to stratify targeted therapy response according to the proportion of tumor cells in which the driver is identified.

CANCER

Clinical impact of the NKp30/B7-H6 axis in high-risk neuroblastoma patients

Michaela Semeraro *et al.* (Laurence Zitvogel)

Citation
Sci. Transl. Med. 15 Apr 2015:
Vol. 7, Issue 283, pp. 283ra55

10.1126/scitranslmed.aaa2327

The immunosurveillance mechanisms governing high-risk neuroblastoma (HR-NB), a major pediatric malignancy, have been elusive. We identify a potential role for natural killer (NK) cells, in particular the interaction between the NK receptor NKp30 and its ligand, B7-H6, in the metastatic progression and survival of HR-NB after myeloablative multimodal chemotherapy and stem cell transplantation. NB cells expressing the NKp30 ligand B7-H6 stimulated NK cells in an NKp30-dependent manner. Serum concentration of soluble B7-H6 correlated with the down-regulation of NKp30, bone marrow metastases, and chemoresistance, and soluble B7-H6 contained in the serum of HR-NB patients inhibited NK cell functions in vitro. The expression of distinct NKp30 isoforms affecting the polarization of NK cell functions correlated with 10-year event-free survival in three independent cohorts of HR-NB in remission from metastases after induction chemotherapy ($n = 196$, $P < 0.001$), adding prognostic value to known risk factors such as N-Myc amplification and age >18 months. We conclude that the interaction between NKp30 and B7-H6 may contribute to the fate of NB patients and that both the expression of NKp30 isoforms on circulating NK cells and the concentration of soluble B7-H6 in the serum may be clinically useful as biomarkers for risk stratification.

CANCER

Detection of somatic mutations and HPV in the saliva and plasma of patients with head and neck SCC

Yuxuan Wang *et al.* (Ken Kinzler, Bert Vogelstein, Nishant Agrawal)

Citation
Sci. Transl. Med. 24 Jun 2015:
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To explore the potential of tumor-specific DNA as a biomarker for head and neck squamous cell carcinomas (HNSCC), we queried DNA from saliva or plasma of 93 HNSCC patients. We searched for somatic mutations or human papillomavirus genes, collectively referred to as tumor DNA. When both plasma and saliva were tested, tumor DNA was detected in 96% of 47 patients. The fractions of patients with detectable tumor DNA in early- and late-stage disease were 100% ($n = 10$) and 95% ($n = 37$), respectively. When segregated by site, tumor DNA was detected in 100% ($n = 15$), 91% ($n = 22$), 100% ($n = 7$), and 100% ($n = 3$) of patients with tumors of the oral cavity, oropharynx, larynx, and hypopharynx, respectively. In saliva, tumor DNA was found in 100% of patients with oral cavity cancers and in 47 to 70% of patients with cancers of the other sites. In plasma, tumor DNA was found in 80% of patients with oral cavity cancers, and in 86 to 100% of patients with cancers of the other sites. Thus, saliva is preferentially enriched for tumor DNA from the oral cavity, whereas plasma is preferentially enriched for tumor DNA from the other sites. Tumor DNA in saliva was found postsurgically in three patients before clinical diagnosis of recurrence, but in none of the five patients without recurrence. Tumor DNA in the saliva and plasma appears to be a potentially valuable biomarker for detection of HNSCC.

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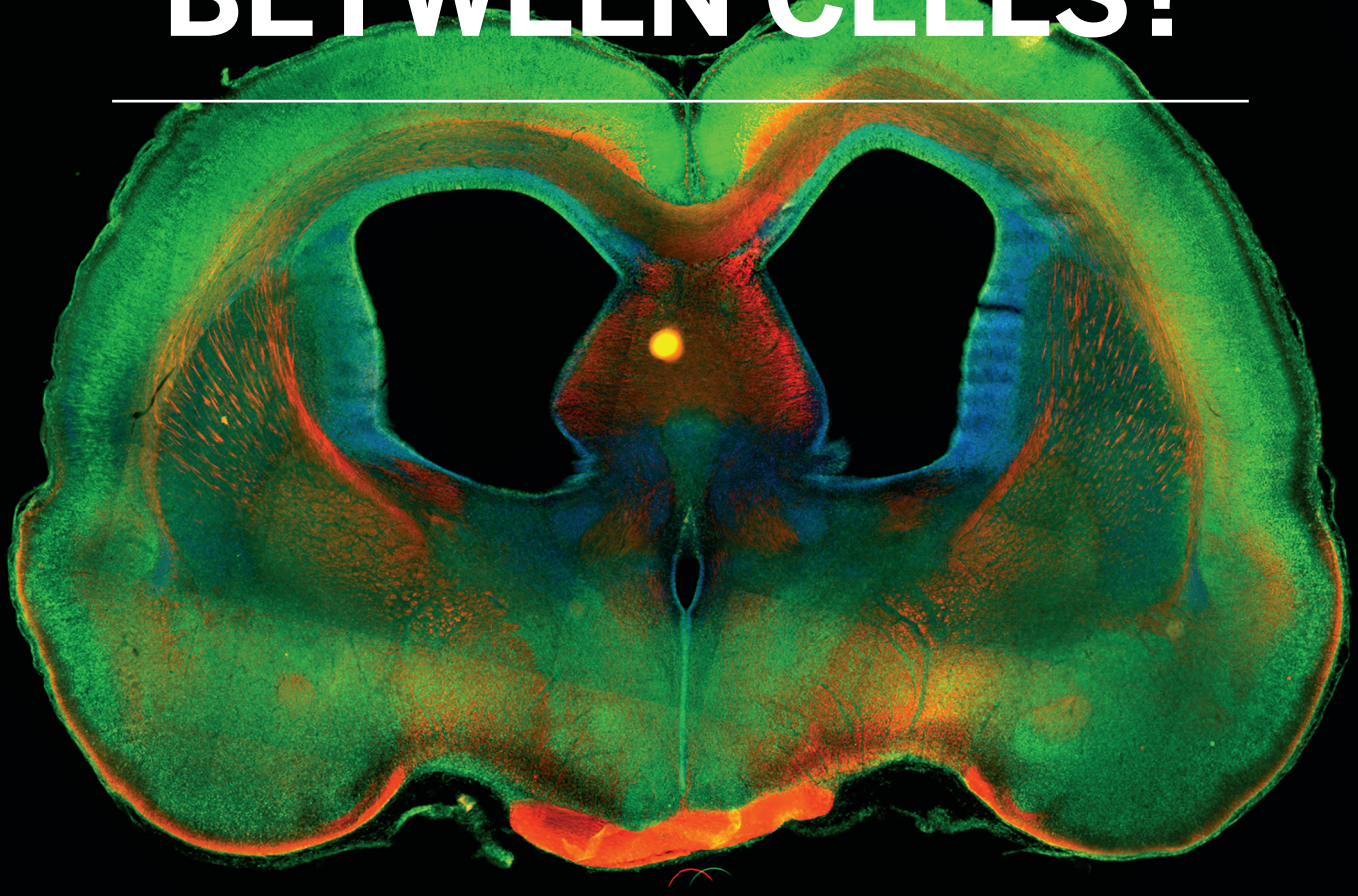

Kong-Yan Wu *et al.* (Zhen-Ge Luo), "Semaphorin 3A activates the guanosine triphosphatase Rab5 to promote growth cone collapse and organize callosal axon projections", *Sci. Signal.* 7, ra81 (2014). Rat Brain Slice. Image: Kong-Yan Wu and Zhen-Ge Luo, Chinese Academy of Sciences.

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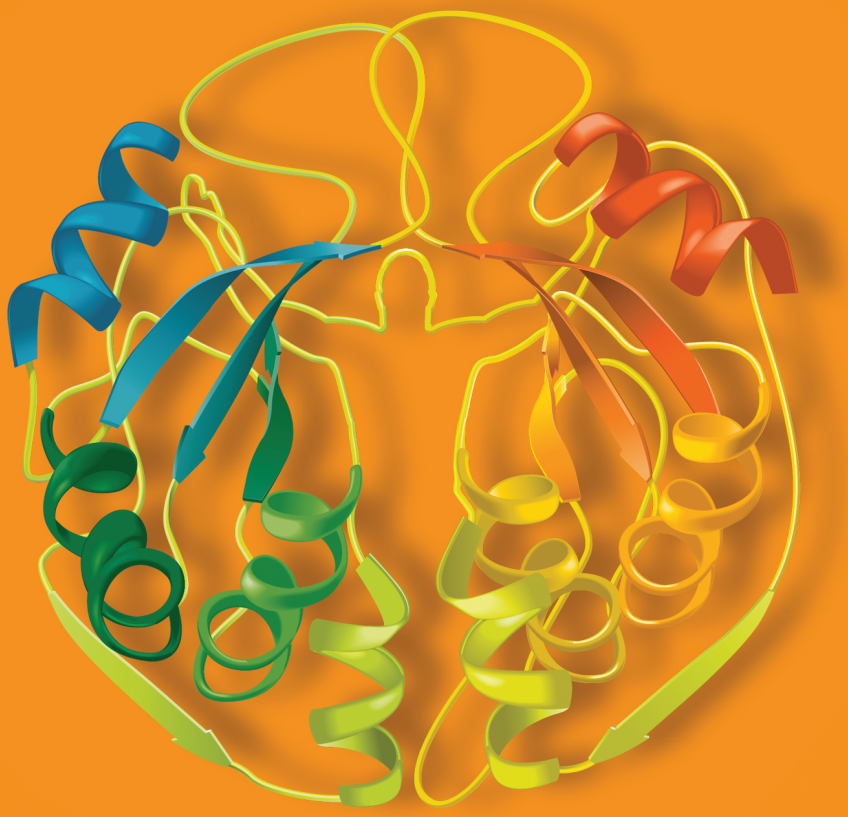

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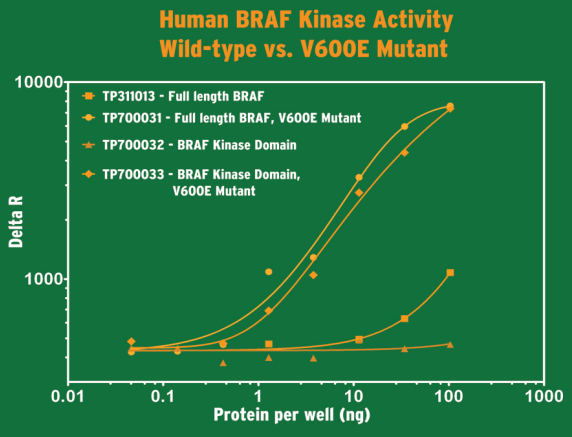

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