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10TH ANNIVERSARY

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The cover image shows a collection of *Science Translational Medicine* covers, one for each year of the journal's publication. To celebrate *Science Translational Medicine*'s 10th anniversary in 2019 and a decade of exciting translational research, the journal published a special Focus series "*Science Transforming Medicine*". Focus articles in this series highlight key translational research advances in different fields achieved since the journal began publishing in October 2009. Authored by leadin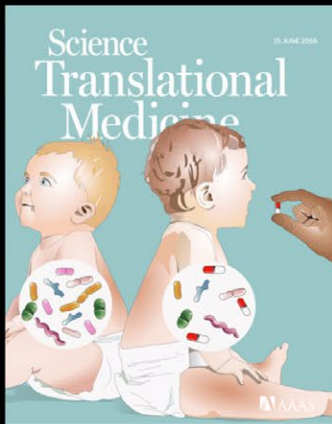g researchers, the Focus articles discuss progress over the past decade and the barriers still to be surmounted to translate these exciting translational advances into improved treatments for patients.

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IN THIS BOOKLET
FOCUS ARTICLES

10TH ANNIVERSARY SERIES

Transforming medicine with the microbiome

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Advances in microbiome research are spurring the development of new therapeutics for a variety of diseases, but translational challenges remain.

The study of microorganisms has been revolutionized by complementing the centuries-old art of microbiology with next-generation sequencing of complex bacterial communities (collectively termed "the microbiome") within and around the eukaryotic host. Microbiome research initially focused on associations between certain microbial compositional features and human medical conditions. The field has quickly evolved, unraveling causative links between distinct microbial consortia, their collective functions, and impacts on host pathophysiology. In addition to the microbiome's emerging role as an orchestrator of biological processes, it also has plasticity in its composition and function, thereby constituting an attractive target for therapeutic intervention. In this Focus, the first in a special series to celebrate the 10th anniversary of *Science Translational Medicine*, we introduce a paper published in the journal a decade ago and discuss progress in developing translational approaches involving the host-microbiome interface (Fig. 1).

HOST-MICROBIOME MODULATION BY DIET

For decades, nutritional research focused on seeking direct links between dietary constituents and human health, aiming to establish universal guidelines to combat disease. However, a large body of research has not resulted in conclusive findings, contributing to various unsubstantiated nutritional trends and unsupported practices. Gut microbiome studies have added an important facet to nutritional research by incorporating the microbiome as a major contributor to host metabolic phenotypes, thus clarifying some of the unresolved questions in the field. In their pioneering work published a decade ago, Turnbaugh *et al.* (*1*) showed that host adiposity could be modulated by the gut microbiome's ability to harvest energy from food; transplantation of microbiome consortia obtained from lean or genetically obese mice into germ-free mice transferred the donor's phenotype to the recipient animal. In subsequent work published in *Science Translational Medicine* (*2*), these investigators demonstrated in germ-free mice transplanted with fecal microbiomes from human volunteers that microbiome composition and function could be rapidly and reproducibly altered by diet. These discoveries have led to potential approaches to treat cardiometabolic disease, and attempts have been made to find prebiotic dietary components to shape the microbiome and confer health benefits on the host. An example of such prebiotic intervention was described by Zhao *et al.* (*3*); they showed that dietary fiber intake improved glycemic control in patients with type 2 diabetes mellitus to a greater extent than standard care through modulation of the microbiome. With these examples of "one size fits all" nutritional interventions notwithstanding, heterogeneity among individuals in gut microbiome composition and function is increasingly appreciated to hamper universal food-based interventions. Accordingly, Zeevi *et al.* (*4*) showed that glycemic responses to food were person specific and dictated by a combination of clinical, laboratory, and microbiome characteristics. Individual postprandial glycemic responses became predictable with a machine-learning algorithm, enabling personalized diets that maintained normoglycemia.

In the next decade, microbiome-based dietary and prebiotic interventions may emerge as essential tools for health care and dietary planning, enabling precision therapies, for example, as a complementary preventive treatment of uncontrolled inflammation in inflammatory bowel disease (IBD). Fecal microbiome profiling could become a component of medical evaluation, leading to tailor-made diets or ad hoc medications. However, conclusive evidence of prebiotic and personalized diets as inducers of sustained metabolic improvements in humans still remains to be determined. Future studies should concentrate on long-term impacts and safety of such therapies and on their potential extension to health conditions beyond obesity and its metabolic complications, such as malnutrition, dietary constituent deficiencies, inflammatory states, and neoplastic diseases.

HOST-MICROBIOME MODULATION BY PROBIOTICS

Bacterial supplements, termed probiotics, have been used to promote health for more than a century, yet their efficacy remains inconclusive. Gut microbiome research offers an opportunity to study live microbial interventions in terms of colonization, interactions with the indigenous microbiome, and impact on the host. Recent work (*5*) suggests that some inconsistencies regarding live microbial effects on the human host might stem from interindividual differences in probiotic gut colonization patterns and their impact on the indigenous microbiome. As "resistance" and "permissiveness" to probiotic gut mucosal colonization could be predicted by baseline host and microbiome features, an opportunity emerges for context-specific tailoring of distinct probiotic strains to optimize gut colonization and downstream activity.

There are still major obstacles to implementing live microbial therapy in clinical practice. These challenges include the need to develop noninvasive approaches for direct sampling of the gut mucosa and technologies to enable reliable characterization of the microbiome in different regions of the gut. In addition, we need to determine mechanisms of activities of probiotic strains in vivo, thereby enabling the prediction of alterations in the microbiome after treatment. Last, we need to generate high-quality and conclusive clinical data in the form of large multicenter randomized, placebo-controlled trials in different clinical scenarios and human subpopulations.

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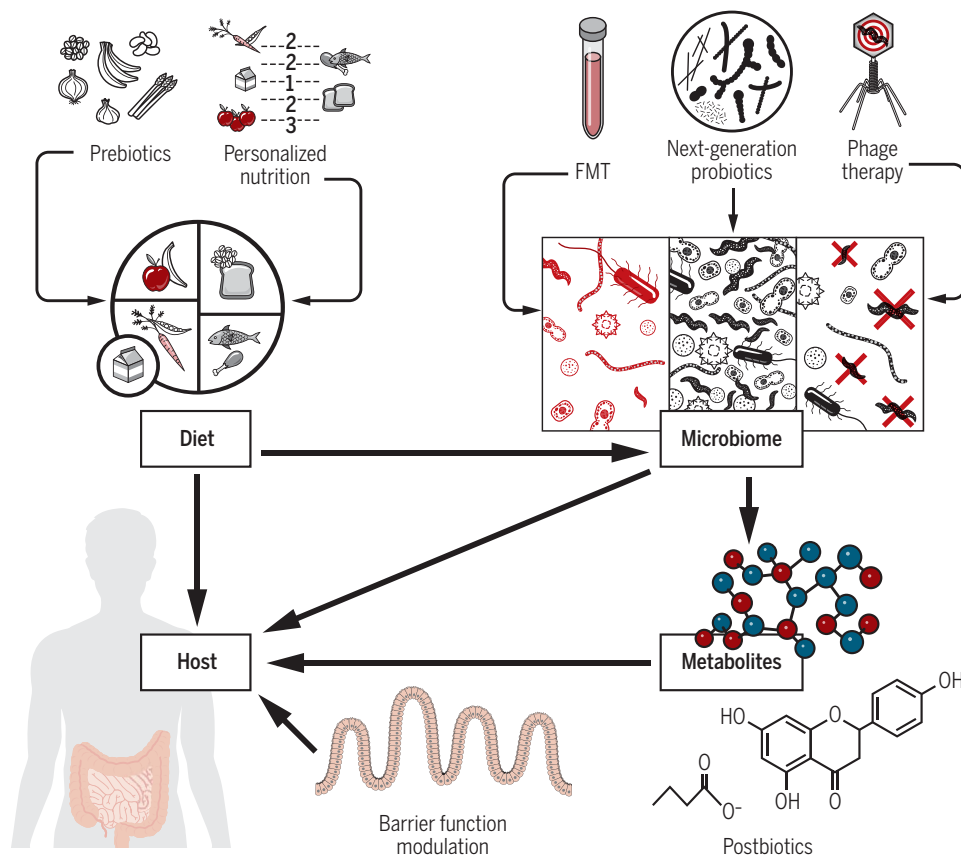
Fig. 1. Gut microbiome–based therapeutic approaches. Recent research has elucidated gut microbiome interventions for promoting human health and for combating disease. These approaches include microbiome modulation or direct impact on the host through nutritional intervention, either by prebiotics or by individualized diets (top left). Strategies to affect the gut microbiome or directly impact the host through live bacteria supplementation or exclusion include fecal microbiome transplantation (FMT), treatment with custom-made probiotics, or targeted elimination of bacterial members of the microbiome (top right). The host and potentially its microbiome can also be modulated by administration, reduction, or activity blocking of bacteria-derived metabolites through treatment with or inhibition of postbiotics (bottom right) or by manipulation of host gut barrier function (bottom left). Collectively, these modalities, when used alone or in combinations, will affect the host-microbiome interface.

fourth century, is fecal microbiome transplantation (FMT). In a landmark study, van Nood *et al.* (*9*) found that intraduodenal infusion of a healthy fecal microbiome administered to patients suffering from recurrent *Clostridium difficile* infection decreased the rate of infection recurrence within 10 weeks of follow-up compared to treatment with the antibiotic vancomycin. Since then, FMT has been studied in other disease contexts, such as cardiometabolic disease and IBD. One emerging limitation of FMT is that efficacy varies between fecal donors because of unknown factors. Another concern involves the risk of transmission of communicable diseases or other microbiome-mediated traits from donor to recipient.

An alternative, more personalized approach involves an autologous FMT using fecal samples from the individual that were banked before disease onset. Such an approach would necessitate large-scale fecal banking facilities. However, it still carries underlying risks, as microbiomes from individuals who may appear healthy could harbor causal factors of the condition to be treated, resulting in unforeseen resurgence of the disease. Future studies should investigate the factors that render some FMT donors superior to others, decipher the interactions between the transplanted and host microbiomes, and elucidate gut colonization. New mechanistic insights could enable development of "designer" therapies of custom-made microbiome signatures conferring distinct functions.

HOST-MICROBIOME MODULATION BY BACTERIAL METABOLITES

Another strategy for microbiome-based therapies is to use supplements of bacteria-derived metabolites or to block their generation, rather than attempting to enrich or deplete the bacteria that produce them. One example of these so-called "postbiotics" was described by Maslowski *et al.* (*6*). They showed that short-chain fatty acids produced by fermentation of dietary fiber by the gut microbiome or those administered exogenously could attenuate gut inflammation in mouse models of colitis. In animal models of recurrent obesity, diminished flavonoids from an altered microbiome drove exaggerated weight regain after successful dieting (*7*). Postbiotic replenishment of the depleted metabolites mitigated the accelerated weight regain by affecting adipocyte energy expenditure. Similarly, Koeth *et al.*

(*8*) revealed that the gut microbiome metabolizes L-carnitine, a compound abundant in red meat, into the proatherogenic molecule trimethylamine N-oxide. Follow-up studies have tested inhibitors targeting a gut microbial enzyme in this pathway to combat platelet hyperreactivity and to decrease the risk of atherothrombotic events, such as myocardial infarction and stroke. Together, these findings highlight the potential of postbiotic therapy with microbiome-derived molecules in animal models. Additional studies are warranted to shed light on the intended and off-target effects of such compounds and to examine their long-term safety in humans.

HOST-MICROBIOME MODULATION BY FMT

One of the oldest microbiome-based interventions in humans, which dates back to the

TARGETED ELIMINATION AND GUT BARRIER REGULATION

One unmet need is an intervention that specifically eliminates harmful members of the microbiome (pathobionts) from the ecosystem. Although antibiotics are commonly used against pathogens, they are nonspecific, inflict collateral damage both to commensal bacteria and to the host, and are associated with the emergence of antibiotic-resistant bacterial strains. Bacteriophages are now attracting renewed attention because they can target specific bacteria and result in fewer side effects than antibiotics due to their lack of tropism for eukaryotic cells. Norman *et al.* (*10*) showed that patients with IBD exhibited abnormal enteric viromes with an increased richness of bacteriophages. Exogenous administration of lytic bacteriophage combinations or designer nanomolecular structures that

use bacterial recognition sites and phage-associated membrane penetration machinery could serve as a strain-specific pathobiont-targeting modality. Although having great potential, bacteriophage therapy faces major challenges, including an inability to recapitulate in vitro antibacterial action in vivo. This could be attributable to dosing issues, phage mutagenesis, interaction with the microbiome, neutralization by host antibodies, or the emergence of phage-resistant bacterial strains. Combinations of phages targeting distinct receptors on pathobionts of interest may offer a solution to some of these issues.

Another underexplored methodology to regulate host-microbiome interactions and microbial immunomodulatory products lies in direct targeting of the host intestinal barrier. Emerging regulators of gut barrier function include biophysical factors such as osmotic pressure, microbiome-generated molecules, and host-related modulators. Comprehensive understanding of the repertoire and mechanisms of these barrier-modulating factors is an exciting avenue of future research.

CHALLENGES AND PROSPECTS

The last decade has witnessed a remarkable leap in microbiome research. In its infancy, such research focused on important but inherently limited descriptive studies, providing a detailed characterization of microbiome alterations during health and disease and in response to distinct dietary regimens. These studies are now being followed by more mechanistic approaches to establish causal links between microbiome assemblages and various phenotypes. A new and exciting aspect of microbiome research focuses on personalization of interventions, as well as harnessing the inherent individualized variability in microbiomes and other physiological features to explain and even predict human health and disease states.

In addition to the specific challenges presented so far, there are some general limitations to be considered when attempting to draw clinical conclusions from gut microbiome research. Conceptual pitfalls include distinguishing between associative and causative relationships, which should be validated by appropriate experimentation. This could be accomplished by ablation of the disease phenotype after antibiotic treatment or by mimicking the phenotype with the administration of a postbiotic compound. The ideal validation would reproduce the phenotype by transplan-

tation of different microbiome configurations into germ-free mice. It is crucial to account for differences between preclinical models and humans in terms of anatomy, physiology, and microbiome composition. Humans tend to have more heterogeneous microbiomes than do animal models because of variations in geographic, ethnic, and nutritional backgrounds and thus manifest a wider spectrum of phenotypes. In addition, nonbacterial members of the microbiome, such as the virome, mycome, and parasitome, are currently understudied but are increasingly recognized to mediate important regulatory functions in the host gut. Furthermore, with the development of techniques to handle low-biomass samples, other organs such as the skin, genitourinary tract, and respiratory tract are being explored as treatment targets. Last, several technical challenges need to be addressed, such as establishing standardized protocols for sample collection, storage, processing, sequencing and analysis, and harmonization of interpretation.

Clinical translation necessitates stringent testing, preferably in the form of randomized placebo-controlled clinical trials. In these trials, feasibility, efficacy, adverse events, and long-term safety issues need to be assessed in large cohorts to ensure that the tested interventions are used responsibly, avoiding unsubstantiated claims and contemporary hype. As part of the process, regulatory authorities, such as the U.S. Food and Drug Administration and the European Food Safety Authority, will have to adapt their procedures to accommodate new data mining techniques such as machine learning and artificial intelligence, while enabling the testing of microbial and metabolite consortia, rather than individual components of consortia. Uniform, rigorous, and unbiased experimental and regulatory approaches, similar to the careful and stringent testing and approval processes practiced in other human interventions, will allow the safe and efficacious long-term integration of microbiome-based therapies into the treatment of a variety of different diseases.

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Driving CAR T cell translation forward

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Successes in CAR T cell translation have propelled their commercial launch, but expanding the impact of cancer immunotherapies remains challenging.

Adoptive cell therapy for cancer has been revolutionized by the development of chimeric antigen receptor (CAR) T cells. CARs are synthetic T cell receptors that fuse tumor-specific binding domains to T cell activation signals to confer tumor-specific cytotoxicity. Patient-derived T cells can be collected and transduced ex vivo to express CARs and then returned to the patient to mediate tumor eradication. Following decades of preclinical development, striking clinical responses have been achieved using CAR T cells targeting CD19, a B cell antigen with conserved expression by the majority of B cell malignancies. The success of CD19-CAR T cells has propelled commercial launch of this class of therapeutics, but realizing the full potential of CAR T cells will require parallel scientific progress to overcome primary and secondary resistance and addressing practical challenges relating to affordability and scalability (Fig. 1).

DISCOVERING CAR T CELL POTENCY

In addition to a successful trial in indolent lymphoma, an early report of CAR T cell efficacy in patients with chronic lymphocytic leukemia (CLL) by Kalos et al. (1) was published in Science Translational Medicine. These studies demonstrated that CD19-CAR T cells could induce lasting antitumor responses and establish CAR+ memory T cells. Subsequent results in pediatric and adult acute lymphoblastic leukemia (ALL) revealed exquisite sensitivity to CD19-CAR T cell therapy, driving global collaborative efforts to centralize manufacturing and confirm results at scale. A phase 2 multi-institutional trial testing CD19-specific CAR T cells in pediatric patients and young adults reported a remission rate of 81% within 3 months of infusion in the subset of patients for whom ex vivo T cell engineering was successful and whose clinical status was such that they could wait for manufacture of the T cell product (2). This led the U.S. Food and Drug Administration (FDA) in 2017 to approve tisagenlecleucel, a CD19-specific 4-1BB-ζ CAR construct for treating relapsed or refractory CD19+ B cell ALL in children and young adults. Shortly thereafter came FDA approval of axicabtagene ciloleucel, a CD19-specific CD28-ζ CAR construct, for treating refractory large B cell lymphoma after a phase 2 multi-institutional study in adults, which demonstrated complete responses in 54% of patients (3). Thus, in the 8 years since the Kalos et al. (1) study was published, the field has progressed rapidly, resulting in FDA-approved CAR T cell therapies for treating hematological malignancies. Commercialization of individualized and engineered cell products was a major hurdle but now provides post-marketing clinical data to enhance understanding of the correlates of success or failure for these therapeutics.

CAR T CELL EXPANSION AND PERSISTENCE

Dissecting drivers of durable clinical responses remains a challenge. Studies have variably reported on the relationship between CAR T cell expansion, persistence, and clinical responses. The experience in CLL demonstrated that in vivo expansion correlated with CAR T cell persistence and clinical response, and studies in large B cell lymphoma also showed that expansion correlated with clinical response (3). The phase 2 study of tisagenlecleucel in pediatric ALL did not reveal differential CAR T cell expansion between responders and nonresponders, but persistence did correlate with sustained clinical responses (2).

Lymphodepletion of the recipient is vital for permitting homeostatic CAR T cell expansion after infusion. Comparison of CAR T cell expansion and clinical outcomes in patients with non-Hodgkin's lymphoma who received cyclophosphamide-based lymphodepletion demonstrated superior survival outcomes with the addition of fludarabine (8% clinical response compared to 50% clinical response) (4). Although inter-institutional conditioning regimens were quite variable during early stages of CAR T cell translation, cyclophosphamide- and fludarabine-based regimens are now standard for lymphodepletion.

CAR design and intrinsic properties of resident T cells influence CAR T cell expansion and persistence. Second-generation CARs, incorporating a CD3-ζ signal domain and a costimulatory domain (typically CD28 or 4-1BB), remain the most common constructs in clinical use but demonstrate distinct kinetics. CD28 achieves a more rapid expansion of CAR T cells and potentially faster tumor elimination, as demonstrated using a preclinical model where CAR T cells are titrated down to identify differences in efficacy (5). However, costimulation also impacts CAR T cell persistence and exhaustion. A model of chronic CAR signaling demonstrated that T cell exhaustion could be ameliorated by 4-1BB costimulation (6), providing a biologic explanation to why 4-1BB–bearing CAR T cells are more persistent than CD28-bearing CAR T cells. Third-generation CARs, including those with two costimulatory domains, have not demonstrated clinical superiority over second-generation CARs.

Regarding CAR T cell persistence, the type of disease influences clinical outcomes. Persistent 4-1BB–containing CAR T cells are associated with sustained clinical remission in B-ALL. CD28-bearing CAR T cells unable to achieve long-term persistence are effective in lymphoma but ALL. We anticipate that decreased persistence of CAR T cells may be effective and even desirable for treating acute myeloid leukemia (AML). Many AML targets of CAR T cells are primed for translation but have conserved expression on hematopoietic stem cells and progenitor cells, making long-term persistence

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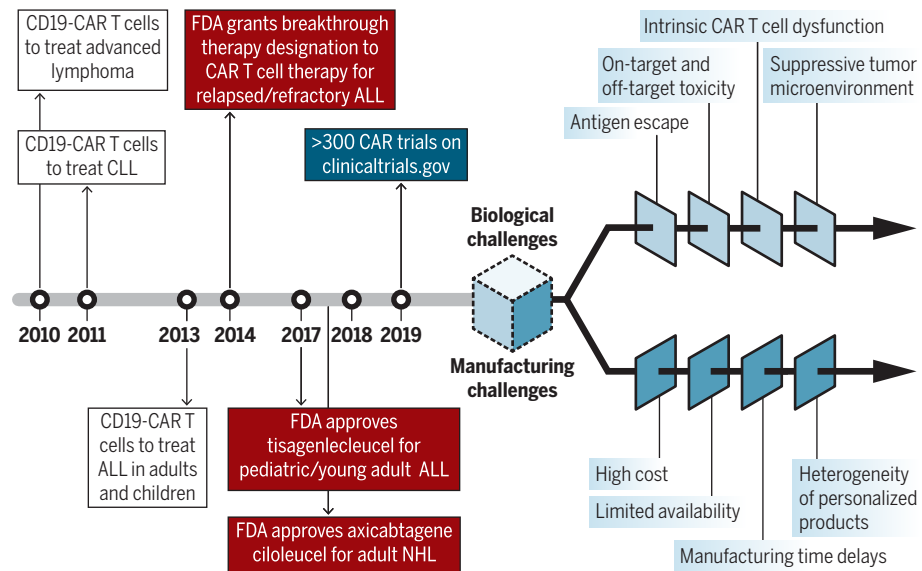
Fig. 1. Progress in CAR T cell therapy. Success in early CAR T cell clinical trials propelled rapid evolution of the field from small early-phase trials to FDA approval of commercialized cell products within a decade. Continued progress will require solutions to biological challenges, including barriers that have limited the efficacy of CAR T cells in non–B cell malignancies, as well as addressing the manufacturing challenges currently limiting accessibility of these therapies for patients.

possibly undesirable. It seems that desirable CAR T cell properties vary among diseases, and thus it is unlikely that one configuration will emerge as the optimal therapeutic for all malignancies.

NAVIGATING CAR T CELL TOXICITY

Cumulative experience has facilitated standardized clinical guidelines to enhance the safety of CAR T cells. Cytokine release syndrome and immune effector cell–associated neurotoxicity syndrome (ICANS), which can range from mild to life-threatening, have emerged as dominant CAR T cell–mediated toxicities, with the toxicity risk paralleling disease burden (7). Cytokine release syndrome is now treated with the IL-6 receptor–targeting antibody tocilizumab, although other cytokines are involved as well. Early in clinical translation, it was unclear if aborting cytokine release syndrome would in parallel disrupt CAR T cell efficacy, and so this intervention was often delayed until later stages of toxicity. Studies have since shown that tocilizumab or steroids are not independent covariates influencing clinical response rates (3) and the use of these agents to manage CAR T cell–related toxicities has been liberalized. Clinical management algorithms for both types of toxicity are now established, allowing more centers to safely offer this treatment (7).

CD19 ANTIGEN LOSS IS A MAJOR DRIVER OF RELAPSE

Although the initial clinical remission rates after CAR T cell therapy in ALL patients are as high as 90%, survival rates with extended follow-up are substantially lower. In the previously mentioned phase 2 pediatric ALL study, despite an overall remission rate of 81% at 3 months postinfusion, event-free survival at 12 months decreased to 50%. In 15 of 16 evaluable patients who relapsed in this study, the cause was the emergence of leukemia that lacked CD19 and thus escaped recognition by the CD19–CAR T cells (2). Mechanistic studies of relapsed tumors negative for CD19 describe alternatively spliced isoforms lacking exons critical for CAR binding, including loss of epitopes recognized by CAR or proteins involved in surface expression. Patients have additionally experienced relapse associated with myeloid transformation and CD19 loss. Future work focused on more precise immune profiling of disease to quantify antigen density and identify minor subclones with subthreshold CD19 expression, or variant exon mutations could identify predictive biomarkers that confer increased risk of immune escape.

WHAT IS BEYOND CD19?
Hematological malignancies
The phenomenon of CD19-negative B cell leukemia relapse has prompted targeting of

alternative B cell antigens. A major advance has been the successful treatment of patients with B-ALL who had, in some cases, been previously treated with CD19-CAR T cell therapy using CD22-specific CAR T cells. Patients achieved clinical remission rates of 73% using CD22-CAR T cells at biologically active dosing (8). Clinical remission rates were comparable to rates seen with CD19-CAR T cells, but antigen remodeling and CD22 downregulation were also observed. Preclinical studies developing CAR T cells with dual targeting of CD19/CD22 or CD19/CD20 have demonstrated promise, and trials studying bispecific targeting to circumvent antigen down-regulation are ongoing. Efforts targeting alternative antigens, including CD30 in refractory Hodgkin's lymphoma; CD33, CD123, and FLT3 in AML; and BCMA in multiple myeloma are under way. These promising agents are still in the early stages of clinical translation.

Solid tumors
Studies are ongoing to extend CAR T cell applications to solid tumors, yet effects comparable to CD19-CAR T cell therapy for hematological cancers have not yet been achieved. Constrained clinical responses seen with solid tumors can be explained by limited CAR T cell trafficking, intrinsic T cell dysfunction in the recipient due to T cell exhaustion, extrinsic T cell suppression mediated by a hostile tumor microenvironment, and antigenic heterogeneity. Identification of appropriate antigens with high on-tumor expression and absent or subthreshold expression on normal tissues has been challenging. Enhancing CAR T cell efficacy using next-generation CAR design and intratumoral injections for solid tumors is under way. Notably, IL-13Rα2-specific CAR T cells delivered intracranially achieved a 7.5-month regression in patients with glioblastoma (9). This experience emphasizes the possible need for alternative dosing and delivery strategies in solid tumors and invigorates promise for CAR T cells for treating solid tumors.

DESIGNING NEXT-GENERATION CAR T CELL PRODUCTS
The basic CAR structure is modular, allowing targeted modification of single chain variable fragments (scFvs), flexible linkers within scFvs, activation domains, spacers, and transmembrane domains to improve therapeutic effects. Further engineering strategies are also under way to permit CAR T cell–mediated

cytokine delivery, secretion of checkpoint-blocking moieties, modulation of T cell exhaustion, and regulatable "on/off" switches. Precise gene editing techniques are being leveraged to develop off-the-shelf CAR T cell products, including CRISPR-mediated elimination of endogenous T cell receptors on donor CAR T cells to prevent graft-versus-host disease and to generate CAR T cells that are less likely to be rejected by host allogeneic immune responses. The majority of clinical studies to date use mixed populations of T cells within the CAR T cell product, which vary across individuals. The consequences of variable T cell subset composition are currently being investigated to determine whether defined and potent subsets can be identified (4).

ONGOING CHALLENGES

Although scientific challenges relating to CAR T cell therapy optimization are manyfold, the need to render these therapies more affordable and available is equally pressing. The field, which sprung from individual academic centers, has evolved into a centralized model of commercial distribution. The prices for these products are high, at $373,000 per product for axicabtagene ciloleucel and $475,000 for tisagenlecleucel. Although CAR T cells in ALL can at times replace stem cell transplantation, CAR T cell therapy is often used as a bridge to transplant, incurring the costs of both therapies (10). It is anticipated that improvements in the manufacturing process and reductions in the cost of goods may ultimately result in lower prices and support scalability. The generation of off-the-shelf CAR T cells, currently in early-phase clinical trials, is an alternative strategy to address the complexities of manufacturing and high costs of individualized CAR T cell products.

The rapid translation of CAR T cell therapies from studies in academic centers to commercialized global manufacturing within the span of a decade is a remarkable success story. Future scientific and clinical progress will extend the reach of these therapeutics to even more patients. Unleashing the full curative potential of this potent therapy hinges on advancing our understanding of the basis for primary and secondary resistance in hematological cancers and solid tumors, development of next-generation products that leverage improvements in CAR engineering, as well as overcoming major barriers related to cost and scalability.

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10ᵀᴴ ANNIVERSARY SERIES

Advances in artificial pancreas systems

Charlotte K. Boughton and Roman Hovorka*

The artificial pancreas for managing type 1 diabetes has progressed from research into clinical practice, revealing areas for future advancements.

In type 1 diabetes, immune-mediated destruction of the pancreatic β cells causes insufficient insulin production and life-long dependency on exogenous insulin administration. In health, insulin is released in response to elevated blood glucose and facilitates glucose transport from the blood into cells throughout the body. To mimic the healthy pancreas, basal exogenous insulin administration replicates the background insulin produced by the pancreas, and additional insulin boluses are required at mealtimes, when glucose concentrations rise in response to carbohydrate consumption. Maintaining blood glucose concentrations as close as possible to the nondiabetic range is essential for people with type 1 diabetes to avoid the long-term complications of high blood glucose concentrations (hyperglycemia). Although intensive insulin therapy, with multiple daily insulin injections or insulin pump therapy and frequent blood glucose measurements, is routinely applied to manage type 1 diabetes, many people fail to achieve adequate glycemic control due to limiting low glucose concentrations (hypoglycemia). As the burden of diabetes self-management remains high, there is a growing need for devices that continuously monitor glucose concentrations and automatically adjust insulin delivery rates—the so-called "artificial pancreas"—to help maintain blood glucose in a healthy range. In this Focus article, the third in a special series to celebrate the 10th anniversary of *Science Translational Medicine*, we discuss advances in artificial pancreas systems achieved over the past decade and considerations for continued progress toward widespread clinical adoption. There has been much progress since *Science Translational Medicine* published a study by El-Khatib *et al.* (*1*) in 2010, showing that a bi-hormonal artificial pancreas, automatically delivering insulin and glucagon, was a feasible approach to achieving near-normal glucose concentrations in people with type 1 diabetes.

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THE ARTIFICIAL PANCREAS

Automated insulin delivery has long been an enticing goal to accommodate variable hour-to-hour and day-to-day insulin requirements while also reducing the burden of self-care. Improvements in the accuracy and reliability of continuous glucose monitors (small sensors worn subcutaneously that continuously measure interstitial glucose concentrations) have enabled progressive development and recent adoption of automated insulin delivery systems in clinical practice. The simplest automated insulin delivery system suspends insulin delivery when sensor-detected glucose concentrations cross a prespecified threshold (low glucose suspend) or are predicted to cross the prespecified threshold within a certain time period (predictive low glucose suspend). Closed-loop insulin delivery systems, which increase and decrease insulin delivery to achieve target glucose concentrations, are more sophisticated. An adaptive control algorithm (often personalized initially using body weight and/or total daily insulin dose and then based on individual sensor glucose data) automatically and continuously adjusts insulin delivery in response to sensor-detected glucose concentrations. An insulin pump delivers insulin via a subcutaneous cannula to the user; the insulin infusion rate is directed by the algorithm hosted on a hand-held device such as a smartphone or on the insulin pump itself, which receives and processes continuous glucose monitoring data (Fig. 1).

Both single-hormone systems (delivering insulin only) and dual-hormone systems (delivering insulin and glucagon or another hormone) are being pursued clinically. The addition of glucagon has the potential to further alleviate the risk of hypoglycemia but increases the system's complexity with separate drug reservoirs and infusion sets. From a patient perspective, the ideal closed-loop system requires minimal user interaction, device burden, and inconvenience while achiev-

ing optimal glucose control. Fully closed-loop systems that detect and automatically dose insulin for meals have been attempted, but glucose control is compromised because of delays in the absorption of subcutaneous rapid-acting insulin analogs. Therefore, most closed-loop systems adopt a hybrid approach, requiring manual administration of insulin boluses for meals. Simplified meal announcements using qualitative estimates of meal size rather than accurate carbohydrate counting have been used to attain reasonable post-prandial glucose control.

In 2010, a study by El-Khatib *et al.* (*1*) published in *Science and Translational Medicine* sparked interest in the development of dual-hormone closed-loop systems. Algorithm-directed subcutaneous delivery of insulin lispro (rapid acting insulin analog) and glucagon in 11 adults for a period of 27 hours under supervised conditions in a clinical research facility achieved near-normal mean blood glucose concentrations with minimal hypoglycemia. The algorithm did not receive meal announcements but, due to the delay in insulin lispro absorption, led to delivery of more insulin than required, using glucagon delivery to mitigate insulin overdelivery. An adjustment to account for variability in insulin lispro kinetics led to improved glucose control. Concurrently, several other groups demonstrated feasibility and safety of automated insulin delivery using single-hormone closed-loop prototypes in supervised settings. A series of randomized crossover clinical studies undertaken in Cambridge, UK, demonstrated that closed-loop insulin delivery could increase the duration the glucose concentration was within target glucose range while reducing time spent in hypoglycemia.

SINGLE-HORMONE CLOSED-LOOP STUDIES

The first study exploring the feasibility of prolonged use of a single-hormone artificial pancreas system overnight in the home setting compared closed-loop insulin delivery with sensor-augmented pump therapy in 24 subjects over 6 weeks with remote monitoring (availability of glucose data to health care
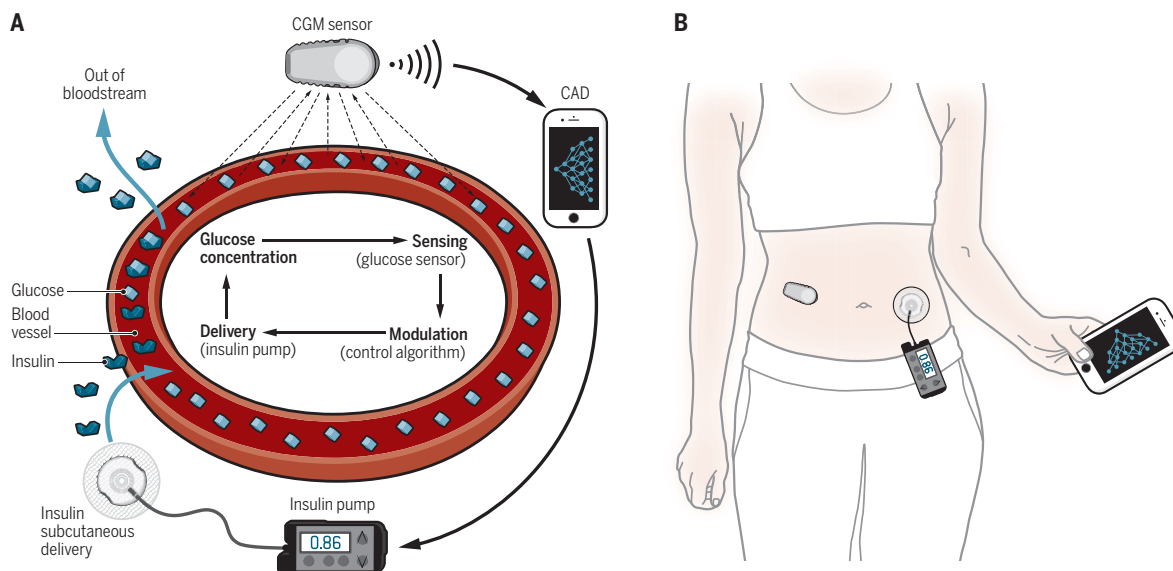
Fig. 1. Closed-loop artificial pancreas systems. (**A**) Diagram depicting closed-loop insulin delivery. A continuous glucose monitor (CGM) worn subcutaneously transmits information about interstitial glucose concentrations to a smartphone or other control algorithm device (CAD), which hosts a control algorithm that translates information from the CGM and computes the amount of insulin to deliver. An insulin pump delivers rapid-acting insulin analog subcutaneously. Insulin delivery is modulated in real time by the control algorithm. Communication between system components is wireless. (**B**) Schematic depicting where the components of a closed-loop insulin delivery system may be worn and held by a human subject with type 1 diabetes.

professionals not in the immediate vicinity of the user) (*2*). Closed-loop control overnight reduced time in hypoglycemia and increased time in the target glucose range compared with control nights. This demonstrated the feasibility of using closed-loop insulin delivery in real-world settings.

The efficacy and safety of closed-loop glucose control in the outpatient setting has been demonstrated in multiple studies using different closed-loop prototypes and in meta-analysis. The proportion of time spent in target glucose range (between 3.9 and 10 mM) was higher with closed-loop compared with control therapy, both overnight and over a 24-hour period (*3*). Closed-loop insulin delivery reduced the proportion of time with sensor glucose above 10 mM and the time below 3.9 mM over 24 hours; closed-loop systems also decreased glycated hemoglobin by 0.3%.

Key to demonstrating robustness of closed-loop technology and generalizability of therapeutic benefits is the inclusion of a wide range of people with type 1 diabetes (different ages, baseline glycemic control, and backgrounds) in studies. In a recent multinational randomized controlled trial, 86 children and adults with suboptimal glycemic control (glycated hemoglobin, 7.5 to 10.0%) using insulin pump therapy received either hybrid closed-loop or sensor-augmented pump therapy over 12 weeks under free-living conditions (*4*). The proportion of time that glucose was within target was higher with closed-loop compared

with pump therapy (65% versus 54%, respectively), and time in hypoglycemia was also reduced. Glycated hemoglobin was reduced by 0.4% with closed-loop use compared with 0.1% in the control group (*4*).

The first commercially available artificial pancreas, a hybrid single-hormone closed-loop system (MiniMed 670G Insulin Pump System, Medtronic), is approved for use in people aged 7 years and older and reportedly has more than 100,000 users. The pivotal study designed to demonstrate safety of this first-generation closed-loop system, a single-arm study without a control group, involved 124 people aged 14 to 75 years who used the closed-loop system initially during a 6-day hotel stay and then unsupervised in free-living conditions for 3 months (*5*). Over 12,389 patient days, there were no episodes of severe hypoglycemia or ketoacidosis. Limitations to the system include frequent exits from closed-loop (auto) mode due to prolonged hyperglycemia, loss of sensor glucose data, or insulin delivery above or below calculated safety levels. Usage of auto mode has also been reported to decline over time in users, and reasons for this need to be explored. Safety of this technology in real-world settings supports clinical adoption of hybrid closed-loop systems for people with type 1 diabetes, and future generations of this system with modified features are under development. Several other companies are also developing commercial single-hormone closed-loop systems, includ-

ing Insulet, Bigfoot Biomedical, Beta Bionics, Tandem Diabetes Care, Roche, and Diabeloop. These systems use different combinations of technologies and algorithms and are currently in clinical trials. Regulatory support for interoperability has been orchestrated by the FDA (U.S. Food and Drug Administration), defining a new type of devices such as iCGM (interoperable CGM) and ACE (alternate controller enabled) pumps.

The #WeAreNotWaiting diabetes community has developed alternative noncommercial artificial pancreas systems. The OpenAPS (Open Artificial Pancreas System) movement includes individuals building their own do-it-yourself (DIY) closed-loop systems from commercially available insulin pumps (although sometimes out of warranty), continuous glucose monitoring devices, and an open source algorithm. DIY systems benefit from a fast innovation cycle and customization alongside transparency of algorithm decision-making; these systems appeal to an increasing population of people with type 1 diabetes, with more than 1000 users to date worldwide (*6*). The responsibility of health care professionals in supporting users of these non-regulatory approved systems remains controversial.

DUAL-HORMONE CLOSED-LOOP SYSTEMS
Dual-hormone closed-loop systems have progressed since the key initial study in 2010 (*1*). In the first free-living remotely monitored

crossover study, dual-hormone hybrid closed-loop control was compared to standard insulin pump therapy in 52 adolescents and adults for 5 days with close supervision (*7*). Among adults, mean glucose was lower with closed-loop, time in target glucose range was greater (80% versus 59%), and time in hypoglycemia was reduced (4.1% versus 7.3%) compared to standard pump therapy. Similar glycemic benefits were observed in an outpatient study of 19 pre-adolescent children aged 6 to 11 years in a diabetes camp setting (residential camp where specialist staff provide supervision to facilitate a medically safe environment) (*8*). The dual-hormone closed-loop system was associated with lower mean glucose, increased time in target range, and less time in hypoglycemia with fewer rescue carbohydrates than standard pump therapy. A study of dual hormone closed-loop control in the home setting with remote monitoring in 43 adults over 11 days using optional meal announcements without carbohydrate counting showed increased time in target glucose range and reduced hypoglycemia compared to insulin pump therapy (*9*). Meta-analyses report that addition of glucagon is associated with a greater increase in time in target glucose range and a greater decrease in time in hypoglycemia versus comparator, compared with single-hormone systems (*3*).

The appeal of dual-hormone systems to reduce the risk of hypoglycemia and achieve more physiologic glucose control is self-evident; however, progress has been limited because of lack of commercially available room-temperature-stable glucagon, device complexity, and the short duration and small size of clinical studies. Currently, there are no commercially available dual-hormone closed-loop systems. Companies including Beta Bionics in collaboration with Xeris Pharmaceuticals, Zealand Pharma, and Lilly are developing such systems.

PSYCHOSOCIAL IMPACT OF CLOSED-LOOP SYSTEMS

Understanding expectations and experiences of users of closed-loop systems is important for effective and sustainable system usage. Individuals may perceive the same technology differently due to previous experiences and general psychological attitudes. The impact of closed-loop technology on quality-of-life measures has been explored in several studies (*10*).

Interviews of closed-loop users generally report positive user experience. Benefits include improved glucose control leading to reassurance and reduced anxiety, improved sleep and confidence due to improved overnight glucose control, relaxed eating habits, and "time off" from the demands of diabetes. Challenges include technical difficulties, alarm intrusiveness, and size of equipment in addition to variable trust and difficulties incorporating closed-loop systems into activities of daily life (exercise and bathing). Low-frequency users, with regard to time spent using the closed-loop system, reported little benefit during the day and more interruptions to their daily lives from alarms. Most participants in closed-loop studies reported that they would personally continue using or would recommend closed-loop therapy to a friend or relative if available because the clinical benefits outweigh system imperfections. Exploring the psychosocial aspects of closed-loop technology and related training among health care professionals will be instrumental in ensuring that clinical benefits are realized in routine care.

LOOKING TO THE FUTURE

Improvements in closed-loop system components are likely to enhance performance and user experience. Noncalibrating continuous glucose monitoring devices with increased accuracy and longer wear time, and algorithms incorporated into insulin pumps or as an application on a smartphone, will reduce device burden. Flexibility to choose different combinations of "open protocol" devices for automated insulin delivery, which allow seamless and secure connectivity with other devices, will improve user choice and experience and has been supported by a JDRF initiative to expedite regulatory approval of interoperable devices.

Ultrafast-acting insulin analogs or adjuncts that reduce postprandial glucose responses may facilitate progress from hybrid closed-loop systems requiring prandial boluses to fully automated systems. Algorithms integrating multiple signals, including measures of activity, may more accurately reflect rapidly changing insulin requirements than sensor glucose input alone.

Remote monitoring systems (for example, Dexcom Share) allow glucose data sharing among selected individuals and will likely increase appeal and acceptability of closed-loop control. Data management platforms such as Diasend/Glooko will be important in making data from closed-loop systems readily accessible to both users and health care professionals and for supporting optimal usage of this technology. Clinical studies applying closed-loop to particular cohorts of individuals with type 1 diabetes will be im-portant in determining those who can benefit most from closed-loop technology and will provide key evidence to support reimbursement by health care providers.

The artificial pancreas for people with type 1 diabetes has been successfully translated from research into clinical practice. Future closed-loop systems will likely improve performance and acceptability. Widespread adoption of closed-loop systems as the standard of care will require a clear understanding of the training needs of both users and health care professionals to ensure successful implementation. Cost-effective analyses are required for health care systems to support reimbursement of this technology.

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10ᵀᴴ ANNIVERSARY SERIES

Exacting Edward Jenner's revenge: The quest for a new tuberculosis vaccine

Steven A. Porcelli* and William R. Jacobs Jr.*

Recent experimental and clinical work has reinvigorated the pursuit of a better tuberculosis vaccine.

The power of vaccines was first realized with the eradication of smallpox. Ironically, Edward Jenner, the inventor of the first smallpox vaccine, lost his wife and son to tuberculosis (TB), a major global infection that continues to thwart efforts to create a highly effective vaccine. In this fourth installment of the 10th-anniversary Focus series, we examine progress in the decade since Bertholet *et al.* reported in *Science Translational Medicine* their strategy to boost immunity in animal models of TB (*1*). Although progress on developing new and more effective TB vaccines has been slow and uncertain, recent advances have generated renewed optimism.

Disease caused by infection with the bacterium *Mycobacterium tuberculosis* remains a substantial global infectious disease problem. This bacterium primarily infects macrophages and other phagocytic cells in the lungs as a result of inhalation of aerosolized infectious microdroplets, and quickly spreads to adjacent lymph nodes and other tissues. Current estimates suggest that at least 1 billion people harbor latent *M. tuberculosis*. *M. tuberculosis* bacilli can persist in tissues as viable organisms without causing apparent disease for the lifetime of the host and can eventually progress or become reactivated in a fraction of individuals to cause active TB and transmission to new hosts. Although a well-developed public health infrastructure combined with effective chemotherapy regimens has effectively controlled and largely eliminated TB from most developed nations, the disease continues to rage unchecked in underdeveloped and resource-poor countries. In these settings, the availability of effective vaccines for prevention of *M. tuberculosis* infection and active disease is widely viewed to be a key strategy for breaking the cycle of transmission and finally controlling the ongoing epidemic (Fig. 1).

An attenuated strain of *Mycobacterium bovis* known as Bacille Calmette-Guérin (BCG) has been available as a TB vaccine since 1921, and it is the only TB vaccine currently licensed for use in humans. Vaccination with BCG is widely used in many areas of the world and is routinely administered to newborns within a few days after birth in most countries with high rates of *M. tuberculosis* infection. Despite rates of newborn vaccination with BCG exceeding 90% in many of the countries most seriously affected by TB, rates of infection have not been consistently declining. This is in line with the generally accepted view that infant BCG vaccination reduces the risk of severe disseminated TB in infants and young children but does not provide consistent or durable protection against pulmonary TB in adolescents and adults. In addition, given that infants and young children rarely transmit TB, the protection afforded them by BCG contributes relatively little to halting the cycle of transmission that perpetuates the global TB epidemic. Over the last several decades, major effort has been directed toward generating new candidate TB vaccines and to improving the potential impact of the existing BCG vaccine. Very recently, these efforts have begun to yield tangible evidence of success.

CAN BCG BE IMPROVED OR SHOULD IT BE REPLACED?

It is surprising that a vaccine developed 100 years ago that shows at most partial efficacy has not been replaced by now with something more effective, particularly given the extraordinary advances achieved in the fields of immunology and vaccinology. Much effort has been expended on reengineering BCG to improve its immunogenicity, and to developing live attenuated vaccine strains of *M. tuberculosis* to replace BCG entirely. Very few, if any, of these vaccine strains have shown more than marginal evidence of improved protective immunity compared to that induced by BCG in standard animal models of *M. tuberculosis* infection (*2*). Relatively few of these new vaccine strains have advanced to clinical trials in humans, and even those candidates have not yet been shown to be clearly better than the standard BCG vaccine.

Because of the standard practice of BCG vaccination in many TB-endemic areas, diagnostic methods have been developed to sensitively and specifically detect *M. tuberculosis* infection even in BCG-vaccinated subjects, for which the standard tuberculin skin test can often yield false-positive results. Most notable has been the increasing use of the whole blood interferon-γ release assay (IGRA), which detects latent or active *M. tuberculosis* infection based on T cell responses to a small number of antigens that are present in all *M. tuberculosis* isolates but absent from BCG (*3*). The IGRA is now well established as a valuable test for diagnosing *M. tuberculosis* infection and for guiding treatment decisions, particularly in cases of suspected latent infection. However, one consequence of the increasing use of this test to guide treatment decisions is that it will become increasingly difficult to test or implement new vaccine strains that generate false-positive results for TB infection. Indeed, in ongoing clinical trials, the live attenuated *M. tuberculosis* vaccine strain MTBVAC (*2*) has been reported to cause IGRA conversions from negative to positive that cannot be distinguished from actual TB infection, confounding decisions on when to apply prophylactic antibiotic treatment.

MOVING FORWARD WITH IMPROVED BCG-BASED STRATEGIES

Given the enormous experience with BCG, the substantial investment into implementing its widespread use, and the validation of diagnostic approaches designed for use in the context of previous BCG vaccination, it is likely that BCG vaccination of newborns will remain as a component of any TB vaccination regimens introduced in the foreseeable future. With these realities in

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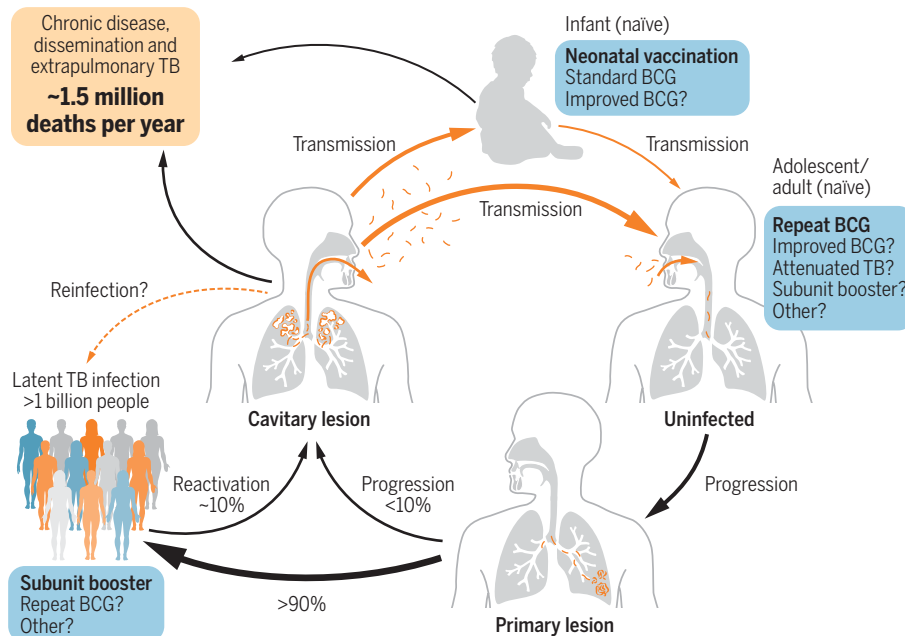
Fig. 1. Breaking the cycle of TB transmission. *M. tuberculosis*, the pathogen causing TB, is transmitted by inhalation of aerosolized microdroplets that are released by the coughing of infected individuals, usually those with cavitary lung lesions that have advanced into the bronchi. Infants and young children exposed to such infectious aerosols are highly susceptible to infection and have a high risk of developing severe, progressive disease with dissemination beyond the lungs to other organs. Cumulative deaths from TB currently total about 1.5 million globally per year. The blue boxes indicate key points in the infectious cycle where vaccination could have the greatest impact. Neonatal BCG vaccination is already well established in most countries with a high prevalence of TB. Improvements to this vaccine will likely involve introduction of modified BCG strains with better safety and immunogenicity profiles. Vaccination of uninfected or latently infected adults or older children may involve a more diverse array of new vaccine candidates such as subunit or live attenuated vaccines. Recent reports suggest benefit from revaccinating adults with BCG, and one trial has shown efficacy of the M72 subunit vaccine in prevention of disease in adults with latent TB infection.

mind, greater attention has turned toward rationally engineering BCG to improve its safety and immunogenicity. Although BCG has generally been accepted as safe, it retains its replicative potential and can lead to disseminated disease in substantially immunodeficient neonates or adults. This risk can be eliminated by genetic manipulation of the parent BCG strain to generate auxotrophs that propagate normally in culture but cannot grow at all in the body of an animal host because of an inability to synthesize required nutrients. Many such auxotrophies have been introduced into both *M. tuberculosis* and BCG (*4*), although their effects on immunogenicity and vaccine efficacy are incompletely studied. It remains unclear whether the marginal advantage in safety with such strongly attenuated BCG strains will provide sufficient justification to advance these into further studies in nonhuman primate models or in humans.

Various strategies to improve the immunogenicity, and hence the vaccine efficacy, of BCG

are also being considered. As a mutated version of a pathogenic mycobacterium, BCG retains many immune evasion mechanisms that prevent the development of optimal host immune responses. By disabling some of these mechanisms, it may be possible to create BCG strains that stimulate greater protective immunity. One such modified BCG strain that has progressed into clinical trials is the VPM 1002 strain (*2*). This is a variant of standard BCG with genetic changes that increase phagosomal acidification and release of antigens into the cytosol of infected macrophages and dendritic cells, thus enhancing antigen presentation and T cell responses. Initial phase 2 clinical trials to determine whether VPM 1002 should be adopted as a replacement for standard BCG in vaccination of newborns are currently ongoing.

BOOSTING BCG-INDUCED IMMUNITY
Various approaches to boost preexisting BCG-induced immunity have emerged as perhaps the most promising area for delivering practical

solutions in the near term for improving immunity to prevent TB. An important study published in 2018 reexamined the impact of homologous prime-boost regimens for BCG, in which human subjects who had received neonatal BCG priming underwent a second BCG immunization as adolescents to boost the waning protective effects of the initial vaccination (*5*). Evaluation during 2 years of follow-up after the second vaccination in a high transmission setting revealed evidence for differences in the acquisition of latent TB infection. Although initial conversions to IGRA positivity (indicating new TB infections) were similar in BCG-boosted versus placebo-boosted groups, a greater proportion of these reverted to negative in the BCG-boosted group. One interpretation of these results is that reversion of the positive IGRA test indicates an immunologically mediated clearance of recent infection, consistent with benefits from homologous boosting. This study raises many questions about the potential for repeated administration of BCG to reduce rates of actual disease (particularly in light of previous large BCG revaccination trials that failed to show effects) but is likely to encourage further studies to evaluate the impact of BCG revaccination.

A second approach to boosting protective immunity in BCG-vaccinated hosts involves the use of specific protein antigens of *M. tuberculosis*, delivered either with viral vectors or as purified recombinant proteins formulated with adjuvants. This approach provides many options, and the questions of which adjuvants to use and which specific antigens to select from the ~4000 proteins encoded by the *M. tuberculosis* genome are currently major areas of study. Initial efforts to create subunit booster vaccines have focused mainly on the relatively small group of immunodominant secreted protein antigens, such as the members of the Antigen 85 family (e.g., Ag85A and Ag85B) and secreted substrates of type VII secretion systems (e.g., ESAT-6 and CFP-10) (*2*). However, a major phase 2b clinical trial using an attenuated vaccinia virus producing Ag85A to boost immunity in BCG-vaccinated infants revealed no impact on subsequent acquisition of clinical TB infection (*6*). This finding raises serious doubts about using immunodominant secreted antigens to stimulate protective immune responses against *M. tuberculosis*. Indeed, given that strong immune responses are generally seen against these antigens in animals or humans with active TB infection, they may be unlikely to serve as points of vulnerability for *M. tuberculosis*.

A second approach that has developed in parallel over the last decade is that of targeting less immunodominant antigens for the design of BCG-boosting subunit vaccines, often with two or more antigens linked as recombinant fusion proteins. In general, this approach has emphasized protein antigens associated with immune responses in subjects with latent TB who have successfully controlled their infections. One important early demonstration of this approach involved the creation of a single recombinant fusion protein called ID93, composed of four *M. tuberculosis* antigens associated with bacterial virulence or latency (*1*). Studies of ID93 in multiple animal models demonstrated the ability of this antigen combined with a suitable adjuvant to stimulate polyfunctional CD4 T cell responses against *M. tuberculosis*, with effective control of bacterial growth in the lungs of infected mice. Perhaps most striking was the ability of ID93 to strongly enhance control of *M. tuberculosis* infection when administered to previously BCG-vaccinated guinea pigs (*1*). This finding established the concept of using properly selected *M. tuberculosis* protein antigens to boost BCG to increase immune control of infection in a highly susceptible host and represents an important landmark achievement in the TB vaccine field.

The evaluation of ID93 in humans for its ability to increase protective immunity against primary TB or reactivation is currently at an early stage with at least one phase 2 study being planned (*2*), but a major success was recently reported using another recombinant fusion protein antigen. This fusion protein, known as M72, was produced by combining two *M. tuberculosis* antigens: one a secreted protease and the other a putative immune evasion or virulence mediator. M72 has been tested in formulation with the adjuvant AS01$_E$ in a major phase 2b clinical trial for boosting immunity to prevent active TB in adults with latent TB infection documented by a positive IGRA test (*7*). The vast majority of subjects in this study had received infant BCG vaccination, so the protocol could be viewed as one for boosting immunity induced by subclinical TB infection in the context of previous childhood BCG vaccination for prevention of subsequent clinical disease. After 2 years of follow-up, a statistically significant reduction in the proportion of subjects free of TB disease was observed in the M72/AS01$_E$ group compared to the placebo group, with an estimated vaccine efficacy of 54%. Further follow-up on these study populations is planned to determine the durability of the observed protective effect.

FUTURE PERSPECTIVES

The recent progress in preclinical studies relevant to principles for design of TB vaccines will undoubtedly maintain a steady flow of new candidates for testing in the current validation pipeline. Now, with a notable glimmer of positive results on improving vaccination against *M. tuberculosis* from recent clinical trials, there is sure to be renewed interest in accelerating the testing of additional new products and protocols in this space. Although vaccine regimens that are compatible with continued neonatal BCG vaccination will likely be emphasized, we also will see continued experimentation with radically different platforms that could further enhance or ultimately replace BCG. One such approach that has already achieved remarkable early success in nonhuman primate studies is the cytomegalovirus-based polyepitope vaccine, which achieves high numbers of sustained circulating effector T cells through persistent antigen delivery (*8*). Another area that is likely to continue to drive preclinical experimentation is the delivery of BCG or other live vaccines through alternate routes of administration, particularly by inhalation or possibly intravenously, which may lead to greater immunogenicity (*9*). In addition, improvements in tractable animal models such as mice for higher throughput of early-stage vaccine testing (*10*), as well as development of more sophisticated in silico models, should accelerate the design and testing of new TB vaccines. Although we are still at a relatively early phase in the discovery of a vaccine that will truly make a major contribution to eliminating TB as a leading global health problem, the stage may now be set for finally achieving Edward Jenner's long-awaited revenge.

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10ᵀᴴ ANNIVERSARY SERIES

Depicting brighter possibilities for treating blindness

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Advances in preclinical research are now being translated into innovative clinical solutions for blindness.

The World Health Organization (WHO) estimates that approximately 1.3 billion people live with some form of vision impairment. Degenerative diseases of the retina, such as age-related macular degeneration (AMD) and inherited retinal dystrophies (IRDs), are major causes of untreatable blindness, with glaucoma, pathological myopia, and corneal blindness also having a major health impact (*1*). Recent clinical trials have led to approval in 2017 of the first treatment, gene therapy, for treating blinding retinal degeneration. This follows decades of research on visual processing, genetics, animal models, mechanisms of vision loss, vector design, imaging, and microsurgery that have provided invaluable preclinical data and clinical proof-of-concept results. Substantial investment by charities, public agencies, and businesses has moved innovative therapeutic strategies, such as pharmacotherapy, gene therapy, stem cell therapy, and optogenetics, into clinical development. In this Focus article, celebrating *Science Translational Medicine*'s 10th anniversary, we examine some of the exciting advances—from gene and cell therapy to prosthetics and neuroprotection—made in treating retinal degeneration over the past decade.

Progress in ophthalmology is intrinsically linked to increased understanding of the morphology and function of the visual system. Vision is a complex process that begins in the retina, the specialized neurosensory organ that is established in the eye during embryonic development (*2*). Retinal photoreceptor cells (rods and cones) convert light into neuronal signals that are processed by other retinal cell types including retinal ganglion cells. The resulting visual information travels via the optic nerve to higher centers in the brain, where

it is processed and decoded into visual perception. Specific characteristics make the eye particularly suited for diagnostic and therapeutic exploration: easy access, small volume, high internal compartmentalization, and stable cell populations. The optical transparency of the eye allows direct visualization with high-resolution imaging and precise evaluation of disease stage and response to therapy. Moreover, the relative immune privilege of the eye, especially the subretinal space, reduces adverse responses to injected vectors and gene products. However, the impact of this immune privilege on cell-based therapies is less clear.

AMD is a leading cause of irreversible blindness and central vision loss in the elderly (Fig. 1). It is a multifactorial disease in which cumulative damage over a lifetime leads to progressive deterioration of key retinal structures, including the retinal pigment epithelium (RPE), Bruch's membrane, the choroid, and photoreceptor cells. Although there has been substantial progress in treating the neovascular form of AMD (characterized by growth of abnormal, leaky blood vessels), there is no effective or approved treatment for the atrophic "non-neovascular" form (associated with gradual loss of photoreceptors and RPE cells). Oxidative stress, inflammation, heredity, environmental factors, and demographic factors are implicated in AMD etiology but not fully understood and are potential therapeutic targets. Despite continued gains in understanding AMD pathophysiology, relevant animal models and prospective controlled clinical studies are lacking. Furthermore, early markers of the disease that could be targets of future preventive treatment have yet to be identified or validated.

IRDs may be inherited as Mendelian traits or through mitochondrial DNA, involve the entire retina or just the macula, affect either rod or cone photoreceptor cells predominantly, and may occur as single, syndromic, or systemic forms, with peripheral and central loss of vision (Fig. 1). The numerous challenges associated with the development of therapies should be considered alongside the extensive genetic and phenotypic heterogeneity of IRDs. In addition, genotype-phenotype correlations are difficult: Different mutations in the same gene can cause different diseases, or the same mutation can result in different phenotypes.

THERAPEUTIC STRATEGIES FOR TREATING BLINDING DISEASES

There have been some notable successes over the past decade using gene replacement strategies for treating blinding retinal diseases. Leber congenital amaurosis (LCA) is the most severe IRD that usually develops in early childhood. In the autosomal-recessive form of LCA caused by *RPE65* mutations, a biochemical deficit impairs the ability of photoreceptor cells to respond to light. Delivery of wild-type *RPE65* complementary DNA (cDNA) to RPE target cells in animal models and in humans led to substantial improvements in light sensitivity, visual fields, and functional vision as demonstrated on multiluminance mobility testing. These studies led to regulatory approval in 2017 for gene therapy to treat LCA in children and adult patients with confirmed biallelic *RPE65* mutation–associated retinal dystrophy (*3*). The successes with *RPE65* gene therapy have paved the way for more than 30 gene replacement trials worldwide. Phase 3 trials of gene replacement therapy for choroideremia, achromatopsia, and Leber hereditary optic neuropathy are ongoing. A recent promising strategy delivered an antisense oligonucleotide to restore correct splicing of a common LCA-causing variant, *CEP290*, that results in a splicing defect (*4*).

Gene-independent strategies could overcome the complexities of the mechanisms underlying photoreceptor cell degeneration in AMD and the genetic heterogeneity of

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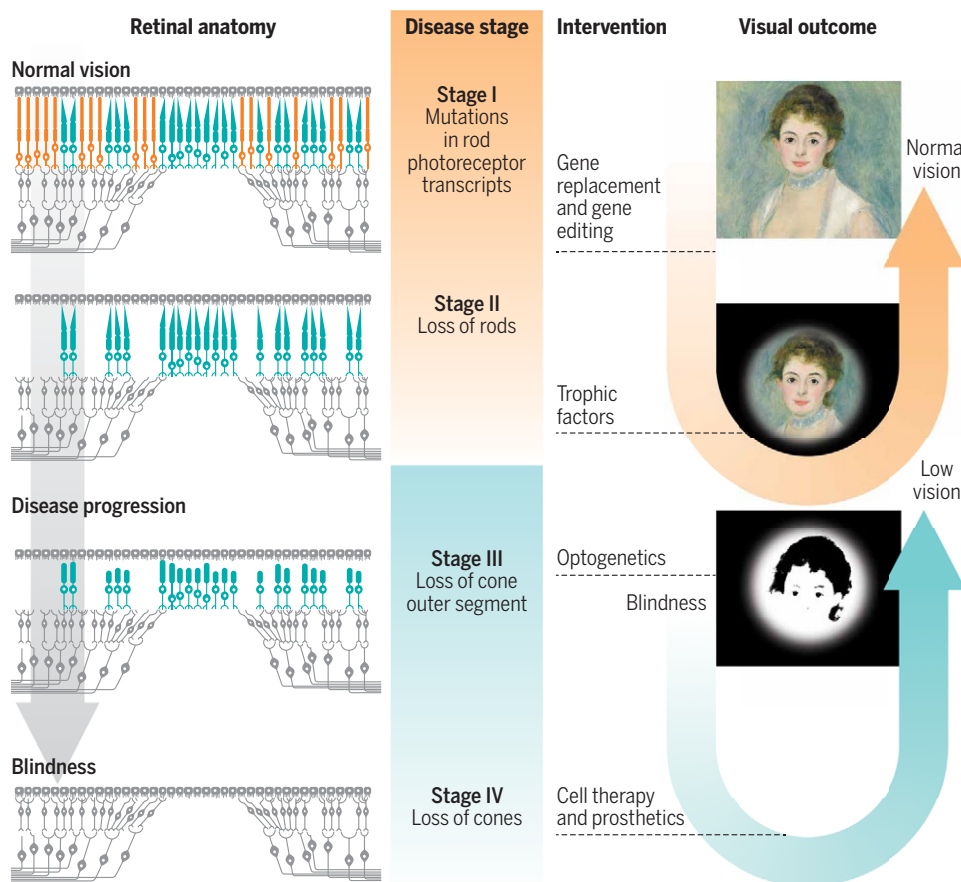
Fig. 1. Relevance of therapies for treating retinal degeneration and stage of disease. Gene replacement therapy is appropriate during the early stages of retinal degeneration when the photoreceptor cells (rods and cones) are still intact (stage I). Early intervention with gene replacement or gene editing could potentially reverse vision loss and lead to close to normal visual outcomes (stage II). Neuroprotective strategies, particularly those for preserving cones, are the best approach for treating disease where there is ongoing photoreceptor cell degeneration (stage III). Neuroprotection of cones can stave off loss of high acuity vision by protecting the fovea (stage III). Stem cell therapy, optogenetic therapy, and retinal prostheses are used to restore vision during the later stages of retinal degeneration, after the loss of cone outer segments (stage III). These approaches can be applied independently of the causal mutation and are expected to restore a low degree of vision in blind patients (stage IV). Pierre-Auguste Renoir, "*Madame Henriot*," National Gallery of Art, Washington, DC.

IRDs and their often dominant transmission. Of these strategies, neuroprotection aims to prevent or slow the progressive degeneration of photoreceptor cells. The retina-specific neurotrophic factor rod-derived cone viability factor rescued cone structure and function independently of genetic mutations and the mechanisms and extent of rod degeneration. This is a potential therapeutic strategy for a broad spectrum of retinal dystrophies (*5*).

Stem cells are at the center of another mutation-independent approach for vision restoration that replaces degenerated cells. The proposed cell therapies are based on human pluripotent stem cells (PSCs), which expand indefinitely in culture and are a potentially unlimited source of retinal cells (RPE cells, photoreceptor cells, and retinal ganglion cells) for cell replacement. Methods to differentiate human PSCs toward retinal lineages have improved in the past decade, particularly the development of three-dimensional culture systems for generating human retinal organoids. Based on preclinical data, several stem cell–based therapies for RPE replacement for AMD and IRDs are currently under development or clinical evaluation (*6*). Stem cell therapy could be used to restore vision in a wide range of retinal degenerative conditions, provided that functional integration into the host tissue occurs and that immune responses to transplanted cells can be avoided or limited.

TECHNOLOGICAL STRATEGIES FOR TREATING BLINDING DISEASES

Retinal prostheses are able to reactivate remaining retinal circuits at the level of bipolar or ganglion cells after photoreceptor cell loss. Both epiretinal and subretinal implants are able to stimulate a light-insensitive degenerated retina and to restore partial vision in blind people (*7*, *8*). One implant has received market approval in Europe and in the United States; a photovoltaic wireless subretinal prosthesis is currently under clinical evaluation. The wireless device is characterized by photovoltaic electrodes with their own local return circuit and independent function (potentially giving higher resolution) and a simpler surgical procedure than for wired implants.

Another technology called optogenetics makes cells light sensitive through expression of an optogene encoding a light-activated channel or pump in the remaining inner retinal cells. Optogenes can be targeted to specific cell types using adenovirus-associated viral vectors equipped with cell type–specific promoters (*9*). Optogenetic therapy could be used to resensitize a degenerated retina to visible light independent of the mutation causing photoreceptor cell loss. Optogenes targeted to cones, bipolar cells, or ganglion cells in animal models of retinitis pigmentosa have been shown to restore visual function and behavioral responses to visual cues. Furthermore, efficient expression of optogenes in cones and ganglion cells has been demonstrated in the retina of nonhuman primates in vivo and in postmortem human retinas in vitro. The choice of the target cell type depends on the state of retinal degeneration. Cone targeting is expected to produce optimal results, followed by targeting of bipolar cells and, lastly, ganglion cells. All of these approaches require the patients to wear goggles that stimulate the optogenes with appropriate light intensity. Ganglion cell–based optogenetic stimulation is in phase 1 clinical trials for treating advanced retinitis pigmentosa (ClinicalTrials.gov: NCT03326336).

ONGOING CHALLENGES AND FUTURE POTENTIAL

Recent therapeutic strategies for treating blindness are very encouraging, and the field is poised to address the next set of challenges. Efficiently timed delivery of genes or small molecules to the appropriate cells is critical for success. For developmental IRDs, maximum efficacy would likely require intervention

during gestation, which is fraught with safety and ethical issues. The rescue of photoreceptor cell function also depends on whether there are any viable cells left in the retina; there may be nothing left to treat if the intervention is too late. Remodeling of the retina late in disease may also limit the ability of therapies to restore retinal function. Retinal remodeling may also affect the feasibility of gene transfer through subretinal injection. Subretinal gene transfer requires general anesthesia and carries risks, making delivery through the routine office procedure of intravitreal injection appealing. However, there are very few gene delivery agents that can reach the appropriate cellular targets in an organ the size of the human eye after intravitreal delivery. Furthermore, some test compounds are unstable (antisense oligonucleotides), necessitating repeated injections. Delivery of antisense oligonucleotides via a slow-release compound may sustain the therapeutic effect and avoid the risk of repeated injections.

Several common IRD genes have relatively large cDNAs that are incompatible with the packaging constraints of the current set of viral vectors. Effective delivery will require, for example, developing vectors with a larger cargo hold, trimming the transgene cassettes, halving the cargo and delivering multiple complementary drugs, and developing methods to safely and specifically edit the endogenous DNA or RNA. So far, clinical trials have not targeted autosomal-dominant mutations causing retinal degeneration. This may require delivery of two different reagents to inactivate the native faulty gene and deliver the normal gene. A gene-editing approach for autosomal-dominant IRDs, for example, would also require delivery of two different components, CRISPR and Cas9 (10). Thus, from dosage, safety, and regulatory perspectives, gene transfer of large genes or genes for autosomal-dominant retinal degenerative diseases is far more complicated than "simple" gene replacement.

The validation of a robust response to a therapeutic intervention can also be a challenge for diseases causing profoundly abnormal baseline vision. Traditionally, drugs for ophthalmic indications have been approved based on one criterion: the ability to read lines of letters on an eye chart. This standard reflects the function of the foveal cone cells that occupy 1/1000 of the area of the retina. Additional criteria are required to assess potential benefits for aspects of vision carried out by the extrafoveal retina. National regulatory agencies now request evaluation of the impact of disease and therapies on functional vision (patient-reported outcomes, performance-based tests, or daily activities).

IRDs and other diseases that affect ganglion cells can lead to atrophy of the optic nerve. Therapy cannot improve vision after optic nerve loss. However, brain-machine interface technologies using electrode arrays or optogenetics can stimulate the visual pathway downstream of the retina. Electrical stimulation of the primary visual cortex is one possible scenario that is currently in clinical trials. An early feasibility study is evaluating the safety of the visual cortical prosthesis and surgery, as well as the reliability of the system and the usefulness of any restored vision (ClinicalTrials.gov: NCT03344848).

The expanding armamentarium of gene therapy and gene-editing agents will allow testing of interventions for a variety of IRDs. As safety data accumulate on new vectors and routes of administration, regulatory bodies may relax the regulatory burden. This, in turn, will reduce the cost of clinical trials. Improved properties of therapeutics will allow many of them to be delivered safely during routine office procedures. As interventions specific to single genetic targets continue to develop, interventions that could treat IRDs regardless of genetic cause will also emerge. The latter include neuroprotective agents, those that enhance metabolic and nutritional pathways and those that can activate more distal neurons in the visual pathway. The next major challenges will be to understand the effects of such therapies on brain plasticity and to demonstrate the impact of vision preservation or restoration in real life.

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10ᵀᴴ ANNIVERSARY SERIES

A decade of digital medicine innovation

Eric J. Topol

The field of digital medicine has matured over the past decade, but validation will require careful randomized, controlled clinical trials.

Digital medicine is a new field that got its start around 2007, the time when smartphones were introduced. The connectivity of mobile devices with the internet ushered in technology platforms like telemedicine and wearable sensors, endowing hand-held devices with the ability to acquire images and perform lab assays. This introduced a new path for generating health and medical data—by the individual, in real time, in a real-world environment. Although these features are alluring, the benefits of digital medicine have to be proven through rigorous research, especially validation through randomized, controlled clinical trials. This Focus article, the sixth in a special series celebrating the 10th anniversary of *Science Translational Medicine*, discusses successes and challenges of digital medical devices over the past decade (*1*) and strategies for enabling this key technology to transform medicine.

WEARABLE SENSORS AND SMARTPHONES

Sensors available during the early days of digital health mainly tracked physical activity, namely, step counting. Since then, there has been a proliferation of biosensors, most of which are wearable, that track nearly every physiological system of the human body. The field has progressed rapidly with regulatory clearance or approval by the U.S. Food and Drug Administration (FDA) for continuous heart rate and rhythm detection (the Apple Series 4 Smartwatch), a six-lead electrocardiogram (AliveCor), continuous glucose tracking without fingerstick calibration and with factory calibration (Dexcom G6 and Abbott Libre sensors), oximetry to detect sleep apnea, and smartwatches that measure blood pressure (Omron HeartGuide smartwatch), to name a few. These approvals are an outgrowth of the FDA Digital Health Action Plan, which has the goal of "ensuring all Americans have timely access to high-quality, safe and effective digital health products." Sensors are also digitizing our environment, or human exposome, collecting such metrics as air quality or radiation (*2*).

Besides sensor development and increased interest in telemedicine, the ability to perform imaging using a smartphone is expanding. Smartphone-embedded cameras have remarkable resolution for capturing photos of skin lesions, which are one of the most common conditions for which a person visits a primary-care doctor. Smartphone ultrasound has afforded the ability to go well beyond the skin, imaging every part of the body (except the brain) with quality comparable to that of the expensive imaging machines used in hospitals (*3*). To illustrate this capability, I present a medical selfie (Fig. 1) that I performed using my smartphone connected to an ultrasound probe, one of five currently approved by the FDA. The frontier of smartphone lab assays, with or without wearable sensors [recently reviewed in (*4*)], is also moving forward with the ability to determine transcutaneous hemoglobin concentrations, electrolytes via sweat, nitrite via breath (for asthma), sperm counts for male infertility, quantification of nucleic acids, point-of-care pathogen detection, and sepsis management, among others.

VALIDATING DIGITAL MEDICINE IN RANDOMIZED CONTROLLED TRIALS

These new technologies have enabled site-less, digital clinical trials where suitable participants are identified, consented, and enrolled remotely; wearable sensors are sent by mail, and the data they collect are returned wirelessly without the trial participants and doctors ever having to physically meet (*5,6*). By avoiding the classical clinical trial model that requires clinic facilities and intensive human resource support, clinical trial digitization has the ability to revolutionize the way many clinical trials can be performed, with greater efficiency and speed and with less inconvenience for participants.

Several randomized clinical trials and one large population observational study using digital interventions have demonstrated favorable outcomes among diverse and common conditions, such as asthma, heart failure, diabetes, and cancer (*7, 8*). For example, in a study in Louisville, Kentucky, the use of inhalers that transmit data wirelessly led to a 48% reduction in asthma attacks and a 78% reduction in the need for rescue inhaler use (*7*). In the Telemedical Interventional Management in Heart Failure II (TIM-HF2) trial, there was a 20% reduction in the percentage of days lost due to unplanned cardiovascular hospital admissions or all-cause death (*8*). However, these trials were preceded by many small negative studies that left some disillusioned about the role of digital interventions in medicine. In the Medication Adherence Improvement Support App For Engagement—Blood Pressure (MedISAFE-BP) trial, Morawski *et al.* (*9*) found that the use of a smartphone app improved self-reported medication adherence among patients with poorly controlled hypertension; however, app use did not result in lower blood pressure. The expectation for immediate success using digital interventions did not take into account the warm-up phase required to amass experience, plan, execute, and publish clinical trials. There are multiple ways in which digital interventions may prove useful for addressing health conditions. For example, a mobile health system used to screen Kenyan schoolchildren for visual impairment improved follow-up attendance at hospital appointments (*10*), a finding of particular importance for public health. The use of large, randomized, controlled trials will remain the gold standard for validating the benefit of digital medical interventions.

GENOMICS AND MULTIMODAL DATA

Genomics is a useful tool to help understand the medical "essence" of human beings, and at some point, there will likely be general

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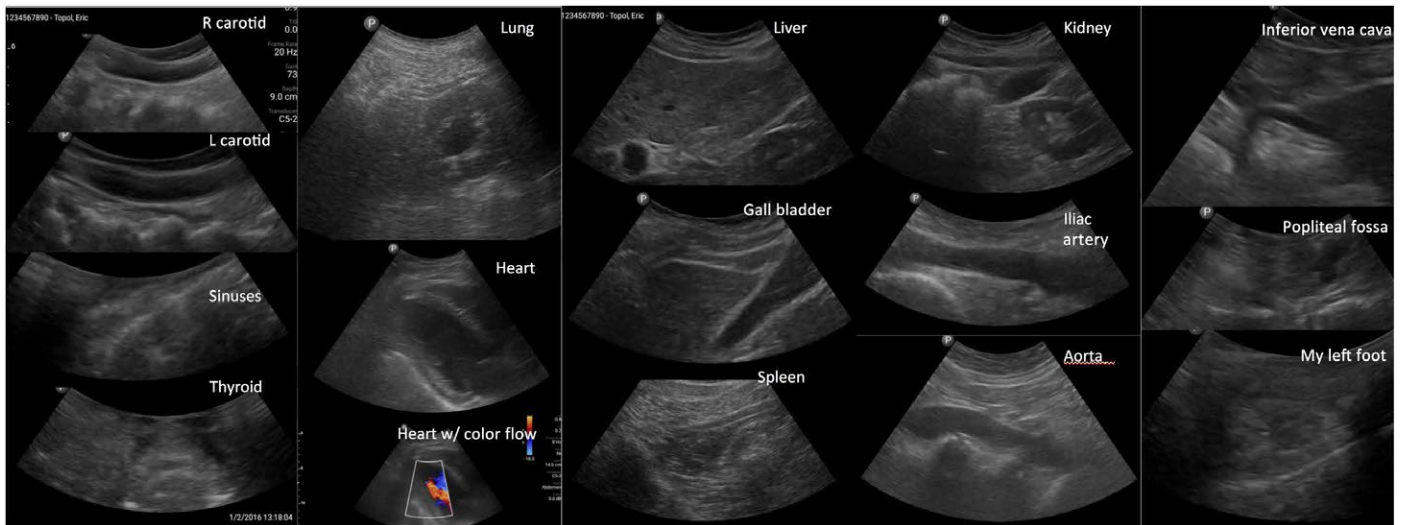
Fig. 1. Medical selfie. Shown is a medical selfie of the author's tissues taken using a smartphone connected to an ultrasound probe. The quality of the images is equivalent to those taken with a hospital ultrasound machine.

acceptance that genomics is integral to digital medicine. A, C, T, and G nucleotides can be considered an extension of the binary 0 and 1 digital code. With millions of people having had high-throughput genotyping, and exome or whole genome sequencing, we are now in a position to provide polygenic risk scores for many common conditions, including heart disease, breast cancer, prostate cancer, colon cancer, inflammatory bowel disease, type 2 diabetes, atrial fibrillation, and more. Individuals in the top decile for a polygenic risk score typically have a high risk that is comparable to that for rare monogenic diseases. Knowledge of heightened risk can be used to implement established preventative strategies, such as lifestyle changes, medications, or screening. With this increased accessibility to genome sequencing data comes the necessity to adequately educate and inform individuals of the implications of their personal risk scores.

To date, most efforts in digital medicine have been pursued in one dimension—one sensor, one type of image, or solely genomics—with little convergence. This approach captures a very narrow, constrained view of a person and, in general, is grossly insufficient. For example, in June 2000 when the human genome draft was first announced, it was naïve to expect that having a map of the genome alone would transform the future of medicine. Rather, multimodal data are needed to understand the uniqueness of human beings and to personalize medicine. This requires an aggregation of anatomical, physiological, biological, environmental, and demographic data, where the biological layers include DNA, RNA, protein, and the microbolome, metabolome, immunome, and epigenome.

Just as the genomics field embraced bioinformatics strategies to address the large datasets generated by sequencing, digital medicine will need to incorporate advanced computational analytics, specifically the use of machine and deep learning artificial intelligence tools, to parse the overwhelming amount of multimodal data that will be generated. For example, continuous glucose sensors for individuals with diabetes can alert a person if their glucose is trending up or down. But, we need a smart algorithm that integrates the person's physical activity, stress, sleep, food and beverage intake, gut microbiome, and possibly other relevant layers of data. For an individual with diabetes, deep learning algorithms can enable greater understanding of the drivers of their glucose regulation and management of their condition. A smart algorithm has potential to be useful for prevention in people with a high risk of developing type 2 diabetes. As voice assistants have become popular recently, it would not be surprising to witness the emergence of the voice medical coach, delivering health-related feedback to the person via the voice assistant platform. Although such coaches will likely be initiated for specific conditions, over time a virtual medical coach, with deep learning of all of a person's relevant data, has the potential to promote the general health of individuals.

WHERE ARE WE HEADED?

The next phase of digital medicine will greatly impact clinicians across disciplines. Much has been written about anticipated impact for radiologists and pathologists, whose primary role is interpreting images that can be improved—both in accuracy and in speed—by deep learning algorithms. However, every discipline in medicine stands to benefit. Digital medicine can incorporate natural language processing of voice during clinic visits to eliminate tedious keyboard use, or use machine vision in the hospital to improve patient safety by monitoring patients to prevent falls or to avoid removal of endotracheal tubes by patients in the intensive care unit. Many of the back-office functions of health systems, such as coding charts, billing, and administrative functions, will likely be supplanted by machines. Overall, there is potential for a marked enhancement of efficiency and productivity. Digital medicine will continue to increase accessibility to personalized medicine, placing tools to monitor personal health in the hands of the individual.

The next decade of digital medicine will need to confront the challenges of processing massive, multimodal datasets. It will also need to fully address potential liabilities in order for the best interests of patients to be advanced. Many serious concerns loom, including algorithmic bias, black box issues defying explainability and transparency, worsening of health inequities, and compromise of privacy and security. Just like any new drug or device, the implementation of digital medical technologies will require rigorous validation with randomized, controlled clinical trials. Digital medicine has considerable promise for improving the accuracy and efficiency of medical practice

and for fostering a greater degree of empowerment for patients.

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10ᵀᴴ ANNIVERSARY SERIES

Conformable bioelectronic interfaces: Mapping the road ahead

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Translating conformable bioelectronic interface research into clinical reality foretells a promising future for an aging society.

Today's technologies hold enormous promise for improving health and well-being. Chronic conditions such as cardiac arrhythmia, deafness, Parkinson's disease, diabetes, and chronic pain are increasingly monitored and treated with wearable or implantable electronic medical devices. Medical technology is evolving at a rapid pace in response to clinical needs, progress in manufacturing, and research and development conducted within medical device companies. In parallel, bioelectronics research in academic laboratories is fueling innovation in materials, device integration, and therapeutic applications to answer the increasing demand for medical devices that can meet the expectations of a growing and aging population, support more personalized health care, and harness large-scale health data. In this Focus article, the seventh in a special series celebrating the 10th anniversary of *Science Translational Medicine*, we discuss recent progress and ongoing challenges posed by the translation of conformable bioelectronic interfaces.

IMPROVING BIOINTEGRATION

Within the bioelectronics field, miniaturized devices with high conformability to complex anatomical structures and wireless data transfer capabilities are highly desirable. Although smart (wireless network-connected) medical devices, fueled by high-tech companies, have become accepted both by patients and practitioners, their form factor (shape, size, and physical specifications) remains a challenge.

Wearable and implantable devices with better biointegration, bidirectional modalities, and higher spatiotemporal resolution compared to current clinically approved technology are anticipated to emerge as technology advances. Groundbreaking proof-of-concept work in conformable bioelectronic devices a decade ago triggered exciting opportunities. For example, in 2010, Viventi *et al.* (*1*) demonstrated a mechanically flexible 288-channel active silicon-based array adhered to the three-dimensional moving surface of the porcine heart. This flexible array enabled in vivo mapping of cardiac electrophysiology with unmatched spatiotemporal precision. A few months later, Kim *et al.* (*2*) reported an ultrathin system that conformally laminates onto the surface of human skin to enable intimate human-machine interfaces with high-performance electrophysiological monitoring functionalities.

Since 2010, different materials and technologies have been explored (*3–6*), yet the general consensus suggests that the next technological breakthroughs will be enabled by the efficient and reliable transfer of microelectronic capabilities onto conformable substrates. Microtechnology offers several advantages. The miniaturization capabilities introduced by lithographic patterning enable fabrication of devices with higher functional density in smaller form factors, therefore reducing the invasiveness of implantation. The batch fabrication techniques of the microelectronics industry permit manufacturing at markedly lower cost compared to today's state-of-the-art electronic medical devices, which are assembled manually by highly skilled personnel. In addition, microelectronic fabrication frameworks offer well-established quality control procedures that can accelerate clinical translation.

In wearable applications, the emerging format is a skin-like patch that hosts thin and/or thinned powering, transmission, and transducing devices for imperceptible and ubiquitous physiological monitoring (*6*). Wearable bioelectronics enable continuous tracking of predefined biomarkers in people of any age in clinical and nonclinical environments, such as in the home. One major line of ongoing investigation for wearable interfaces is adhesive solutions to bond electronic components together, ensure that the bioelectronic devices stay in place for extended periods of time, and offer reversibility (removal), if desired.

In implantable applications, many designs are driven by the optimization of the mechanical properties of the bioelectronic system. Because mechanics have been shown to play a key role in the onset of foreign body reactions, research groups are developing strategies to make bioelectronics less detectable by host biology. Of the many potential ways to mitigate the rejection of implanted devices, the most common approach is to engineer mechanical compliance in the materials and/or device architecture. This can be achieved by using substrate materials of low Young's modulus (*4*) or bioresorbable matrices (*7*); designing reduced stiffness (*3*, *6*), small footprint (*5*), and radically different form factors such as meshes (*8*); and advancing wireless, untethered interfaces (*6*). Other exploratory and complementary solutions include locally administering bioactive molecules to reduce inflammation and promote neural growth and carefully selecting materials based on their surface chemistry to coat the implanted interfaces (*9*).

TRANSLATING FORM AND FUNCTION

Conformability in a bioelectronic interface indicates its ability to envelop a surface and maintain functionality under dynamic and multiaxial deformation. This is an essential property for the man-made interface to comply with the convoluted, moving structures that are typical of biology. For example, the skin on the forehead and around the eyes stretches and compresses extensively. The heart beats 60 to 100 times per minute, sustains a total volume variation of about 8% throughout

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the cardiac cycle (*10*), and operates at this capacity for a lifetime. When the heart beats, periodic variations in arterial blood pressure result in recurrent, localized motion and deformation of the brain. Such mechanical specifications are currently unmet by rigid bioelectronic interfaces, and considerable efforts are deployed toward implementing bioelectronic functions within conformable carrier substrates.

In today's research landscape, there is a trade-off between functionality and conformability: Complex, multimodal, high channel count systems are typically built on rigid to flexible or bendable substrates, whereas only much simpler devices use soft materials such as elastomers or gels. Advancing conformable bioelectronic interfaces requires the successful combination of these two fronts, with technologies enabling complex functionality on ultraconformable materials. As new materials and engineering strategies are proposed, research efforts are needed to assess their translational potential, recalibrate expectations, and define a sound way forward to clinical use. Table 1 identifies and summarizes critical challenges associated with the development of bioelectronic interfaces, which once tackled will help to convert technological hype into medical hope. Challenges include scaling, hermetic encapsulation, system-level integration, and compliance to handling in a typical health care use scenario. Wireless telemetry and rechargeable batteries are additional components for which there are currently no proscribed paths forward.

Prototypes of implantable bioelectronic interfaces are often tested in small animal models, and dimensional scaling requires more than simply a linear transformation of the interface geometry. Translation necessitates not only adjusting the overall dimensions to fit larger anatomical structures but also reevaluating the layout and performance of the scaled devices. For example, considering a bioelectronic interface designed to deliver functional electrical stimulation to tissue, the charge injection capacity of a given electrode coating scales down with increasing electrode geometrical surface area. This implies that higher current (voltage) may be required to deliver equivalent charges per phase to the tissue and an improved electrode coating may need to be introduced. Compatibility with medical implantable pulse generators, especially in terms of voltage compliance and leads resistive load, should also be anticipated. Iterative design cycles are therefore needed to scale the electrical and electrochemical performance of the bioelectronic interface.

SURMOUNTING FAILURES

A common failure mechanism of current bioelectronics is the ingress of conductive fluids (blood, interstitial fluid, cerebrospinal fluid, sweat, and water) over time, resulting in loss of insulation between separate conductors. This is particularly challenging for implantable bioelectronics because the water permeability of common substrate materials used in conformable bioelectronics (silicones or plastic foils) does not guarantee sufficient insulation over years when implanted in the body. Brittle inorganic layers such as oxides or nitrides offer excellent barrier properties but at the expense of conformability. A prominent solution that is currently being investigated is the integration of thin multilayer stacks of polymer and inorganic insulators, which promise a combination of conformability and barrier properties. Future embodiments will have to demonstrate reliable material interfaces and integration on soft carrier substrates.

Another current bottleneck for the successful translation of bioelectronic interfaces is the need for reliable integration in stand-alone, fully implantable systems. Today, most bioelectronic interfaces of any kind must be physically connected to the corresponding driving electronics that relay electrical signals into and out of the body via transdermal connectors or wireless transmission. Wires and cables prevent truly wearable applications. Although electronic boards can be packaged in hermetic implantable capsules, the challenge lies in the interconnection of high channel count devices to such packages with reliable feedthroughs. Current technology enables implanted systems with this type of connection scheme for pacemakers and neuromodulation therapy devices, which use only a small number of channels ($\leq$16). Although the approach originally shown by Viventi *et al.* (*1*) reduced 288 channels to a mere 36 multiplexed channels, new feedthrough solutions are required for devices with higher channel counts to be implanted chronically with minimal invasiveness.

Last, the ability of devices to perform "as expected" and "on demand" by the user (surgeons and clinicians) in the intended setting (inside and outside of the operating theater or medical unit) is often overlooked in research prototypes. Rigid devices are relatively easy to implant, position, manipulate during procedures, and remove but do not provide conformal contact with tissue. Conversely, conformable materials require ad hoc surgical tools that enable the surgeon to easily place the device where needed and remove it when required. With time, researchers may become accustomed to handling conformable materials; however, it is important

Table 1. Innovative strategies address challenges in developing next-generation conformable and implantable bioelectronic interfaces.

Conformable microfabrication	Hermetic encapsulation	System-level integration	Regulatory adaptation
Manufacturing standards for thin-film devices on conformable carrier substrates	Multilayered stacks of polymer/inorganic barriers	Compact wireless transmission modules	Policy changes for approval of tailor-made medical devices
Hybrid integration of rigid complementary metal-oxide-semiconductor (CMOS) components and polymer-based transducers	Deposition processes, interface, and barrier properties of inorganic films such as silicon carbide (SiC) and hafnium oxide (HfO_2)	Power management (new battery technology, battery life/heating)	New mechanical norms for conformable devices
Fabrication of soft active electronic components (diodes, transistors, light-emitting diodes, and combinations thereof)	Implantable plug-and-play connectors/feedthroughs	Complete kits including tools for clinical use	

to consider and include during the initial design, development, and modification stages all of the tools that will be required for ultimate clinical deployment and use of a device or system by health care professionals.

REGULATION AND THE ROAD AHEAD
From a technical point of view, the inherent design freedom and rapid manufacturability offered by conformable microtechnology pose no obstacles to current methods of surgical planning and device use. However, developments in regulatory compliance for medical devices may tend to favor the status quo over innovation, imposing ever-stricter validation protocols on medical device producers and clinicians willing to introduce bold changes to medical practice. Such divergence between research directions and the norms regulating innovation in the clinic is another aspect of bioelectronic interface development that warrants careful consideration and ongoing dialogue.

The different ethical facets of current and future research in bioelectronics also require further discussion. Academic work seldom crosses laboratory boundaries to venture into the clinic, and leveraging innovative technology to produce new medical devices for health care use is a long and costly challenge. From the academic perspective, considerable effort is required to bring new technology to the clinic, with important validation milestones that must be demonstrated before applying for a first clinical trial. Extensive tests must be conducted in compliance with relevant standards, or in the absence thereof, convincing proof must be provided regarding the fitness and robustness of new candidate devices. In vivo testing using translational animal models plays an important role in demonstrating long-term functionality of new devices when coupled with existing clinical systems and practices. This process entails heavy investment into development work that, per se, is not sufficiently acknowledged and valued as scientific innovation

and is therefore often difficult to publish in the scientific literature.

From an industrial perspective, regulatory compliance and a widespread conservative approach in medical practice often mean that medical technology companies tend to remain anchored to well-established frameworks. As extensive deviation from standard practice entails higher approval barriers, the general trend in the field is to carefully weigh innovation against regulatory requirements. This scenario is in net opposition to the ideological trend of personalized health care, which advocates that both technology and therapy must be tailored to the individual needs of each patient, with the aim of improving the therapeutic outcomes. Adaptation of policies should be the subject of discussions among all stakeholders, including clinicians and technologists.

Over the past decade, innovation in conformable bioelectronics has advanced rapidly to the point that it is implausible that conformable interfaces will not eventually convert to a standard in health care. Although the translational road is arduous and costly, investigators should be encouraged to push their laboratory research toward clinical adoption. Multidisciplinary collaboration and training programs across the life sciences, engineering, and medicine should be fostered, and long-term funding support through public and private partnerships intensified to maximize the impact of technological research and productively bring new conformable bioelectronic technologies to patient care.

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10TH ANNIVERSARY SERIES

Applications of liquid biopsies for cancer

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Liquid biopsies have the potential to detect, characterize, and monitor cancers earlier than is possible with conventional approaches.

INTRODUCTION

Finding minimally invasive methods to assess cancers has long been a central goal of oncology research. In the past decade, there have been major advances in our ability to examine tumor-derived material in the circulation and other biofluids, including urine, saliva, and cerebrospinal fluid. This has been possible due to the development of sensitive assays capable of detecting rare cancer-specific analytes immersed in a vast excess of analytes derived from normal cells. The analytes used for liquid biopsy include circulating tumor cells (CTCs), cell-free tumor DNA (ctDNA), proteins, metabolites, exosomes, mRNA, and miRNAs. Each analyte has its own advantages and disadvantages that must be considered when choosing a marker to answer specific clinical questions. For example, whereas CTCs are relatively rare in early cancers, they provide a particularly powerful approach to detect a variety of cancer-specific abnormalities in advanced cancers, such as androgen receptor splice variants. Moreover, studies of CTCs have led to remarkable insights about cancer biology that could not have been achieved using other analytes, such as the importance of cell aggregates in seeding metastases (*1*, *2*). In this eighth installment of *Science Translational Medicine*'s 10th anniversary Focus series, we discuss the current status of liquid biopsies and their applications for cancer detection.

TYPES OF LIQUID BIOPSIES

Many recent liquid biopsy studies use DNA as the analyte, partially due to the ease of DNA isolation and the availability of massively parallel sequencing technologies to assess tumor-specific alterations. Although DNA mutations occur in normal cells at a rate of five mutations per genome per cell division, only clonal proliferations of cells, containing tens of millions of cells with the identical mutation, can contribute enough mutant DNA templates to be detected at frequencies above 0.01% in plasma. This is in part because the half-life of circulating cell-free DNA is less than 1 hour, mandating a continuous outpouring of mutant DNA templates to be detectable in a randomly collected plasma sample. In addition to point mutations and small insertions and deletions, other genetic aberrations such as DNA fragment sizes, copy number changes, translocations, and epigenetic changes can be detected. When cancers release sufficient DNA into the circulation (or other bodily fluids), they can be detected with sensitive digital technologies such as massively parallel sequencing, wherein each individual DNA molecule is assessed independently.

Most cell-free DNA in plasma is derived from dying cells, namely, leukocytes (*3*). In general, there are between 3 and 9 ng of cell-free DNA per milliliter of plasma from normal individuals and patients with early-stage cancer. However, in patients with advanced cancer, there can be more than a 10-fold increase in the amount of cell-free DNA per milliliter of plasma, but the fraction of mutant DNA templates is still less than 10% of the total templates. The origin of the vast excess of nonmutant cell-free DNA in patients with advanced cancer remains unexplained. Empirically, we know that there is generally more mutant cell-free DNA in the plasma of patients with larger and more advanced cancers than in patients with smaller and less advanced tumors. However, the amount of mutant DNA found in plasma varies widely even in patients with the same tumor type, size, and stage, excluding the simplest explanations.

Protein biomarkers, such as carcinoembryonic antigen and carbohydrate antigen 19-9, were among the first analytes used to assess cancers through blood tests. Such biomarkers have been approved for assessing tumor burden in patients already diagnosed with cancer, particularly during therapy in patients with advanced cancer. Recent studies have indicated that protein biomarkers, when carefully applied, may also prove valuable for the detection of early cancers (*4*). With advances in mass spectrometry, we expect that a new generation of protein biomarkers for cancer will soon be available. Mass spectrometry should also enable the discovery of metabolites that can provide clues about the presence of cancer not possible with either proteins or DNA (*5*). Multi-analyte tests that include evaluations of DNA, proteins, metabolites, and RNA could theoretically be used to detect early cancers in a highly sensitive manner. Practically, however, there are challenges associated with using several platforms within a single test while maintaining sufficient specificity, throughput, and cost-effectiveness.

APPLICATIONS OF LIQUID BIOPSIES

To date, there are four clinical scenarios in which liquid biopsies are being evaluated.

At initial diagnosis

Because precision medicine for patients with cancer is now largely based on the mutations harbored by tumors, it is essential to identify these mutations at initial diagnosis for individuals being considered for adjuvant therapies, such as chemo- or radiotherapy, to prevent the cancer from recurring. Liquid biopsies are not optimal for this purpose; they are always less preferable than the evaluation of DNA from the primary tumor. The fraction of neoplastic cell DNA in liquid biopsies, particularly from patients with early-stage cancer, is usually low (often <0.1%) and often undetectable. In contrast, the fraction of neoplastic DNA in conventional solid tumor biopsies is usually >10 times higher, particularly after macrodissection of selected areas of the tumor. It can be difficult to confidently detect low mutant fractions and the mutations identified in the plasma of such

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cases can be artifactual and not present in the actual primary tumor (*6*).

One postulated advantage of liquid biopsies is that they reflect a random sampling of all the alterations in a tumor, whereas single biopsies from primary tumors are inadequate to reveal heterogeneity within the tumors. We do not think this advantage is compelling for two reasons. First, the heterogeneity within primary tumors is generally confined to passenger mutations. Targeted therapies are always tied to driver gene mutations, and these mutations are usually homogeneous throughout the primary tumor (*7*). Second, if the heterogeneity in primary tumors was reflected in independent metastases, then therapies directed against such a driver gene would not be helpful. Unless a targeted mutation is present in all metastatic lesions, the therapy will be of limited use.

On the other hand, liquid biopsies at initial diagnosis may be useful for prognostication. Another potential advantage of liquid biopsies over solid tumor biopsies is that, in unresectable cancers, the only solid tumor biopsy available may be a fine-needle aspirate. There may be insufficient tissue available from this aspirate for DNA sequencing, and a liquid biopsy may be the only noninvasive source of DNA available. Similarly, it can be problematic to obtain formalin-fixed paraffin-embedded sections of primary tumors, causing potential delays in the initiation of therapy. Regardless, it would be imprudent to use mutations detected in liquid biopsies for choosing first-line therapy unless the frequency of the mutant allele is high enough to warrant complete confidence that the mutation is likely to be derived from the tumor itself, rather than from leukocytes or technical errors. Otherwise, mistakes are bound to occur (*6*).

After surgery

In many patients without evident metastatic disease, the value of chemotherapy following complete surgical excision is not clear. Patients will nearly always suffer side effects from such therapies, but patients without residual disease or occult metastases cannot possibly benefit. The standard of care is to treat virtually all patients with a certain stage of cancer [e.g., stage III colorectal cancer (CRC)] with adjuvant therapy, even though ~70% of these patients with stage III CRC will not live longer as a result of the treatment. Similarly, standard of care dictates that patients with a lower stage of cancer (e.g., certain stage II CRC patients) do not receive adjuvant therapy,

even though 20% of these stage II CRC patients have micrometastatic lesions that will eventually cause them to relapse. Suppose there was a way to more reliably know which of these patients should be treated with adjuvant therapy regardless of their stage? This would not only reduce suffering from the toxic effects of therapy but also save time, effort, and money by focusing care on the patients that most need it. Moreover, it could considerably simplify future clinical trials for new adjuvant agents; only patients who have micrometastatic disease need be enrolled.

Liquid biopsies taken after surgery are promising in this context. There are already studies showing that patients who have circulating tumor DNA or CTCs following surgery are very likely to relapse, whereas patients without circulating tumor DNA relapse less frequently (*8*). It would seem reasonable to consider treating most patients with a positive test for circulating tumor DNA with adjuvant therapy as long as the identified mutation was present in the primary tumor. On the other hand, it would not be prudent to assume that patients with a negative test for circulating tumor DNA will not relapse. At the present state of the art, the sensitivity for detecting occult disease is far from 100%. A negative test should therefore be considered as another feature guiding the need for therapy rather than the sole feature used for decision-making.

After additional therapies

Liquid biopsies are able to detect early recurrences prior to the tumors becoming radiographically or clinically apparent, potentially giving clinicians a larger window of opportunity during which treatment regimens could be altered (*8*). Once a patient relapses, liquid biopsies may reveal new mutations not present in the primary tumor that could guide choices for second-line therapy. In lung cancers with anaplastic lymphoma kinase (ALK) mutations, for example, an identical new mutation occurs in many of the residual lung lesions and could dictate the best next-generation ALK inhibitor to use (*9*). In many cancers treated with other agents, however, a liquid biopsy will reveal heterogeneous new mutations, one or two in each of several different metastases. No single drug will be able to target all these mutations. The only mutations found in all metastatic lesions will be those identified in the primary tumor through evaluation of the original solid tumor biopsy. Targeting another of these clonal mutations from the primary

tumor is thus the best choice for second-line targeted therapy.

Screening for cancer

Using liquid biopsies before cancer is clinically detected is discussed last because it is by far the most difficult application, but it also has the greatest potential to reduce morbidity and mortality from cancer. The three applications noted above are designed for patients already known to have cancer, i.e., they are diagnostic tests. Cost and specificity are less of an issue for such patients; sensitivity is most important, as patients untreated on the basis of a false negative test are likely to die. The converse is true for screening tests; the test has to be cost-effective if it is to be applied to millions of patients at relatively low risk for cancer. Unless the test is exquisitely specific, then the number of false positives will greatly outnumber the number of true positives, engendering anxiety in patients and needless additional, sometimes invasive, procedures. Much of the controversy around current screening tests such as mammography and prostate-specific antigen is based on the relatively low positive predictive value (high ratio of false positives to true positives). A related problem is overdiagnosis—the detection of cancers that are indolent and never would cause morbidity or mortality if they had remained undetected.

Another issue that is especially important for screening applications of liquid biopsies concerns communication. How should information about the test be relayed to the patient? No liquid biopsy on the horizon is going to be 100% specific. On the basis of current publications, we envision that many average-risk patients testing positively with a liquid biopsy will not actually have cancer. How can patients be informed of a positive test in a way that causes the least degree of anxiety? What will the follow-up of such tests be, and what are the least invasive ways of following up a positive test in situations where the source of the tumor cannot be identified with certainty? Conversely, no liquid biopsy in the near term is going to be 100% sensitive, especially for early cancers. How can we make sure that patients do not think that they are at zero risk for cancer if their tests are negative, thereby unintentionally stimulating them to discount other primary or secondary prevention measures?

THE NEXT DECADE OF LIQUID BIOPSIES

Although many challenges abound for liquid biopsies in screening applications, the

opportunities are equally bountiful. One often misunderstood but critical point is that screening tests do not have to detect cancers that are very early and amenable to surgical resection to save lives and reduce morbidity. All they have to do is to detect cancers earlier than they would be detected otherwise. Studies using conventional chemotherapeutics, oncogene-targeted agents, immune checkpoint inhibitors, or T cells have all shown that therapies are much more effective when the tumor burden is low rather than high. As just one example, conventional chemotherapeutic agents can cure 47% of patients with micrometastatic CRC but nearly zero patients with bulky disease. The development of early detection tests is therefore intertwined with the development of new therapeutics: The earlier cancers are detected, the better any drug will work. As previously discussed, new therapeutics will require robust biomarkers to stratify responders from nonresponders, thereby treating patients most likely to benefit and preventing unnecessary harm to those patients least likely to be helped. Liquid biopsies are likely to provide such markers for many types of cancer.

Another often unappreciated point is that screening tests do not have to be 100% sensitive to make a difference. Suppose a liquid biopsy–based screening test was able to detect 20% of patients with solid tumors of diverse types and that half of these patients could be cured. Would such a test be useful? A common answer to such a question would be "No, this sensitivity is not high enough." But another answer to this question is provided by comparison to an analogous therapeutic. Suppose a new drug were developed that could cure 10% of all cancer patients—not just put them into remission but actually cure them, with minimal side effects. Would that be considered a major advance? This comparison illustrates the double standard that is often used to judge new diagnostic tests versus new therapeutic agents (*10*).

Overdiagnosis may be a problem faced by new early detection tests, but we currently face the opposite problem: underdiagnosis. Most of the 600,000 patients who die each year from cancer in the United States die only because their cancers were not detected early enough to be cured by surgery or other currently available treatments. How to balance the current, intolerable underdiagnosis with potential overdiagnosis is a challenge that further research will hopefully solve. However, a solution to this problem will only be possible if reliable early diagnostic tests for major cancers are developed and used. With technologies advancing rapidly, and with the accelerating interest in liquid biopsies from both academia and industry, we look forward to the day when liquid biopsies that detect cancers earlier become a routine part of preventive medicine.

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10TH ANNIVERSARY SERIES

Wrangling RNA: Antisense oligonucleotides for neurological disorders

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Effective treatment of spinal muscular atrophy with antisense oligonucleotide therapy opens the door to treating other neurological disorders with this approach.

Neurological diseases pose unique problems for medical therapy. Neurons are postmitotic, have limited capacity for regeneration, are vulnerable to age-related degeneration, and function as part of complex and precisely configured neuronal networks mostly laid down during development. Moreover, the nervous system is essentially sequestered from the systemic circulation behind the blood-brain barrier. A major challenge in advancing promising preclinical treatments is that the neurological pathways underpinning voluntary movement have intrinsic reserve capacity so that considerable undetected and potentially irreversible damage is likely to have occurred before a patient reports symptoms to a physician. Another reason why therapeutic progress in neurological disorders is slow compared to fields such as oncology is that cellular pathophysiology is less well defined. However, we are now entering an era where the first steps are being taken in precision medicine for inherited neuromuscular and other neurological diseases based on a detailed mechanistic understanding of the molecular basis of genetic mutations and how these can be manipulated.

Early treatment successes in animal models of the neuromuscular disease spinal muscular atrophy (SMA) using short single-stranded DNA–like nucleic acid compounds known as antisense oligonucleotides (ASOs) (1) have led to clinical trials and regulatory approval of ASOs for treating SMA. Altering the natural history of diseases using ASOs, which target genes through specific Watson-Crick base pairing resulting in modulation of gene splicing or expression, is now a clinical reality, with inherited neuromuscular diseases at the forefront of this new era of therapeutic in-

tervention. In this installment of the *Science Translational Medicine* 10th anniversary Focus series, we discuss the hurdles that need to be overcome to expand ASO therapy beyond SMA to other neuromuscular and neurodegenerative disorders.

PIONEERING ASO THERAPY IN SMA

SMA is a neuromuscular disease caused by loss of the *SMN1* gene and reduction in the widely expressed survival motor neuron (SMN) protein, resulting in selective loss of spinal cord motor neurons. Its commonest and most severe form (SMA type 1) results in lethal infantile paralysis, but all forms of SMA lead to severe disability. Because cells have an obligate requirement for SMN, a key factor in the essential cellular process of small nuclear riboprotein assembly, complete loss of *SMN1* is incompatible with cell viability. The copy gene *SMN2*, which is differentially spliced, thus excluding exon 7 in most of the transcripts, produces small amounts of full-length SMN protein, sufficient for normal function in most cells but below the threshold required for spinal motor neurons. The number of *SMN2* copies varies between individuals and correlates with disease severity in SMA. Crucially, the absolute difference in SMN protein concentrations between patients with severe or mild disease is small, suggesting that minor increases in SMN protein could have a profound clinical impact. Whereas neuroscientists are still vigorously debating which of the several functions of SMN explain selective motor neuron vulnerability, an effective treatment for SMA has arrived.

A systematic analysis of regulatory sequences in *SMN2* that modulate exon 7 splicing using ASOs showed that blocking the intronic splicing silencer (ISS-N1) of the 5′ splice site led to the greatest increase in exon 7 inclusion. Although the evolutionary *SMN1* duplication is not present in rodents, a series of mouse models (with human *SMN2* expressed on a null *Smn* background) replicated

the genomic architecture of the human disease and produced robust animal models of SMA, which have provided the essential confirmation that intrathecal injection of the anti–ISS-N1 ASO restored neuromuscular function (1). Subsequent work in nonhuman primates to establish dose and safety led to the first open-label and subsequent sham-controlled clinical trials of nusinersen (Spinraza) in infants with SMA, with encouraging evidence of benefit (2).

Coupled with a newborn screening program enabling ASO treatment to be started soon after birth, SMA, a previously fatal disorder, has become treatable. The development of Spinraza for SMA establishes the principle that modulating mRNA splicing can be effective therapeutically. In many ways, however, SMA is unique as a disease, and translation of ASO therapy to other neuromuscular and neurological disorders will require substantial refinements.

CHALLENGES OF ASO THERAPY FOR OTHER NEUROMUSCULAR DISEASES

In contrast to the rapid and impressive development of Spinraza for SMA, progress toward effective ASO therapy for other neuromuscular disorders such as the fatal X-linked disease Duchenne muscular dystrophy (DMD) has been much slower. The *DMD* gene was cloned in the 1980s, and its essential function encoding the structural protein dystrophin is well understood. A wide range of deletion/duplication and nonsense mutations cause DMD by disrupting the open reading frame, resulting in the absence of full-length functional dystrophin protein. The first evidence that the effects of such mutations could be abrogated through use of splice site–modifying ASOs to correct an aberrant reading frame emerged in the mid-1990s. In the early 2000s, in vivo efficacy of such ASOs was demonstrated in the dystrophic *mdx* mouse model of DMD (3), in which the therapeutic benefit of a morpholino phosphorodiamidate ASO

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that rescued muscle dystrophin expression was shown. This ultimately led to the 2016 approval by the U.S. Food and Drug Administration (FDA) of an ASO (eteplirsen) that resulted in skipping of exon 51 in the transcript encoding dystrophin and restoration of dystrophin protein (*4,5*). However, the approval of eteplirsen was not without controversy. Although the safety data were supportive, the efficacy of the intravenously administered ASO was in question, especially given the very low amount of dystrophin protein generated (<1% of normal dystrophin protein abundance).

Key differences between ASO development for DMD and SMA must be considered. Given that numerous mutations in *DMD* lead to disease, multiple exon-skipping ASOs targeting separate exons will be required to treat a majority of patients with DMD (e.g., only about 13% of patients would be candidates for eteplirsen treatment). Although the fold difference in SMN protein concentrations between SMA patients with severe or mild disease is relatively small, this is not true in the case of DMD, where increasing dystrophin protein to at least 10% of normal expression will likely be required for therapeutic efficacy. Further, although both diseases could be regarded as systemic in nature, the major cellular target in SMA is the spinal cord motor neuron, which can conveniently be targeted via the local intrathecal route. In the case of DMD, all skeletal muscle groups (especially those relevant to respiration) and cardiac muscle should be targeted, given that cardiorespiratory failure is the primary cause of premature death in patients with DMD. Achieving this challenging goal requires substantially higher ASO drug doses administered through a systemic route. Notably, an exon 51–skipping ASO (drisapersen) was rejected by the FDA, principally on the basis of safety concerns. There are encouraging clinical trial data regarding related exon-skipping ASOs, including Sarepta Therapeutics's golodirsen and NS Pharma's viltolarsen that both target exon 53, most likely reflecting improved ASO length, target sequence, and dose (compared to eteplirsen). However, accelerated approval for golodirsen was very recently declined by the FDA, highlighting the continued challenges in developing ASO therapy for DMD.

EMERGING ASO TREATMENTS FOR NEURODEGENERATIVE DISEASES

Most genetically determined neurodegenerative disorders are late-onset autosomal dominant conditions in which the gene mutation acts through altering the protein product in a way that leads to acquired toxicity. The task for therapy is therefore to antagonize the aberrant gene product, at the RNA or protein level, without driving toxicity through loss of function of the normal protein.

Amyotrophic lateral sclerosis (ALS) is an aggressive neurodegenerative disease of motor neurons in which the average survival is 2 to 3 years from onset and for which substantially disease-modifying treatments are currently lacking. About 12 to 15% of patients with ALS carry a disease-determining genetic mutation. There appear to be many biological triggers of ALS, given that mutations in more than 20 different genes have been implicated. The *SOD1* and *C9orf72* genes, however, account for about 60 to 70% of ALS mutations, and therefore, these two genes have become the focus for ASO therapy. Although highly expressed in the nervous system, rodent knockout experiments suggest that ablation of *SOD1* expression is tolerated. This has led to the development of an ASO targeting both mutant and normal *SOD1*, with the primary aim of reducing the accumulation of misfolded mutant SOD1 protein. This is required because of the impracticality of delivering personalized ASO therapy for the more than 100 separate missense mutations described in ALS cases. Rodent ALS models treated by intrathecal administration of tofersen (previously IONIS-SOD1Rx), a 2′MOE gapmer (which induces RNA degradation via the intracellular enzyme RNase H), resulted in mutant SOD1 protein reduction and an extension of survival. This ASO is now in phase 3 clinical trials in patients with ALS (clinicaltrials.gov: NCT02623699).

A hexanucleotide expansion mutation in the first intron of the gene *C9orf72* accounts for up to 10% of all ALS cases and also a substantial fraction of cases of the neurodegenerative disorder frontotemporal dementia. The mechanism of disease toxicity is still debated, but most evidence suggests that a rational therapeutic strategy is to block the production of the repeat RNA to mitigate direct RNA toxicity or the production of dipeptide repeat proteins, which arise when intronic repeat RNA is translated via a non–ATG-dependent mechanism. Because concern exists that haploinsufficiency may play a role in *C9orf72*-related neurodegeneration, ASOs have been designed to target the *C9orf72* pre-mRNA. These ASOs reduce the repeat-containing transcript without affecting total C9orf72 protein and have shown positive effects in reducing toxicity in cellular models. These cellular models include induced pluripotent stem cell–derived motor neurons, which have been crucial due to the lack of appropriate rodent models for *C9orf72* mutations (*6*). A clinical trial to assess the safety and toxicity of the ASO IONIS-C9Rx is now under way in patients with ALS who carry *C9orf72* mutations (clinicaltrials.gov: NCT03626012).

Similarly, IONIS-HTTRx, a 5-10-5 2′MOE gapmer targeting the *HTT* gene responsible for the neurodegenerative disorder Huntington's disease (HD), at a site distant from the CAG repeat mutation, has undergone initial clinical studies in patients with early manifest HD (*7*). The early signals from this clinical trial (clinicaltrials.gov: NCT02519036), with reduced mutant huntingtin protein in the cerebrospinal fluid, are encouraging. Initial analysis has not yet demonstrated any difference in clinical outcome related to reduced mutant huntingtin protein. A competing clinical approach in HD by Wave Life Sciences takes an allele-specific strategy in which the stereochemistry of the oligonucleotides is controlled with the aim of improved efficiency in mitigating toxicity and preserving function of the wild-type huntingtin protein.

THE FUTURE

Evidence from SMA in particular, and emerging data from other disorders, indicates that we are entering a new age of precision genetic medicine for neurological disorders, led by a maturing ASO technology (Fig. 1). Rapid progression beyond a few of the most obvious neuromuscular disease targets requires the development of next-generation ASO technologies. At present, the intrathecal route remains the most practical, as it allows local delivery to the central nervous system, limits the potential for systemic toxicity, and minimizes production costs. To achieve nervous system disease modification using a systemic route more practical for long-term use, these next-generation drugs will have to offer improved potency and safety. Advanced delivery technologies could drive ASO delivery across the blood-brain barrier, thus circumventing the need for repeated intrathecal drug administration. A potentially interesting technological advance is the advent of stereopure ASO chemistry (*8*), permitting chirally controlled ASO synthesis (current ASOs are typically chiral mixtures), with improvements in both potency and safety, and offering the potential of allele-specific targeting. A plethora of delivery technologies, including protein/peptide-based (*9*) and exosome-based nanotechnologies (*10*) are emerging for enhanced intracellular ASO
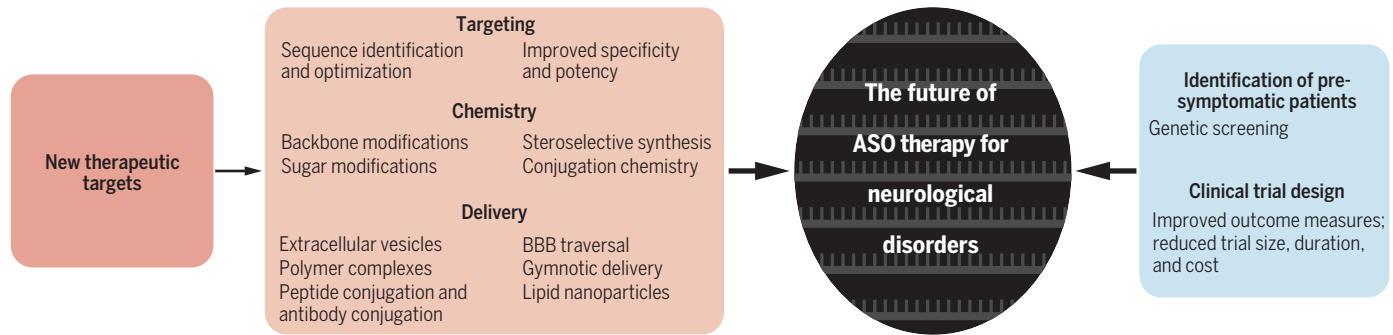
Fig. 1. Advancing ASO therapy for neurological disorders. Future advances in ASO therapy for neuromuscular and neurodegenerative disorders will depend on both technical advances (left) and improved screening and biomarker strategies for identifying patients during the earliest stages of disease (right). Development of next-generation ASO compounds will arise from advances in oligonucleotide chemistry, including stereoselective synthesis, coupled with technologies that solve the challenges of intracellular ASO delivery and delivery across the blood-brain barrier. Last, the ability to assess the true clinical effectiveness of ASO therapies will be driven not only by presymptomatic genetic screening of neonates or at-risk family members but also by development of more robust clinical trial outcome measures, including development of digital technologies and devices that will have implications for reductions in clinical trial size, duration, and ultimately cost.

delivery to overcome the very poor intracellular bioavailability of nucleic acid drugs. These, coupled with next-generation ASO chemistries, are likely to herald an age of much wider application of ASO medicines. Ultimately, transformation of the therapy of neuromuscular and neurodegenerative disorders necessitates presymptomatic treatment (as is now beginning for SMA), requiring appropriate early screening programs and biomarkers to guide effective treatment intervention. Although there are many technical challenges ahead, the first steps toward enabling the realization of disease-modifying therapies for currently untreatable neuromuscular and neurodegenerative diseases have been taken.

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10TH ANNIVERSARY SERIES

Multiple roles for HIV broadly neutralizing antibodies

Barton F. Haynes[1]*, Dennis R. Burton[2,3], John R. Mascola[4]

Potent broadly neutralizing antibodies may be used to treat or prevent HIV and to help guide HIV vaccine design.

Although great progress has been made in treating and preventing HIV infection with combination antiretroviral drug treatment globally, the epidemic continues with ~38 million people living with AIDS and 1.7 million new infections occurring each year (~4600 infections/day) (www.aidsdatahub.org/unaids-data-2018-unaids-2018). Thus, despite efficacious antiretroviral drug therapy (ART) and other prevention modalities that theoretically could stem the HIV epidemic, the development of new modes of treatment and prevention remains a high priority.

The HIV envelope (Env) trimer mediates virus entry through binding to the CD4 receptor on T cells and is the sole target for neutralizing antibodies. Although remarkably variable, the Env trimer has a number of relatively conserved regions to which broadly neutralizing antibodies (bnAbs) are elicited. These regions include the CD4 binding site, the V3- and V1/V2-glycan sites, the membrane-proximal external region (MPER), the Env silent face, the gp41-gp120 interface, and the fusion domain (1–3). Early on in the epidemic, bnAbs were discovered in individuals chronically infected with HIV (1), although these bnAbs inhibit a narrower range of viruses and are less potent than more recently isolated bnAbs. Studies mapping virus and bnAb coevolution in acutely infected individuals over time demonstrated that bnAbs only arise after years of infection and viral diversification (4). Over the past decade, new techniques have led to isolation of hundreds of bnAbs, some of which have been found to be extraordinarily potent (1–3). Thus, efforts now focus on developing antibodies for passive therapy for prevention or treatment of HIV. In addition, a major goal of HIV vaccine development is to develop immunogens that can induce bnAbs. In this 10th installment of *Science Translational Medicine*'s anniversary Focus series, we review the status of progress in both areas of research.

PASSIVE ADMINISTRATION OF BNABS FOR HIV TREATMENT OR PREVENTION

Some HIV bnAbs are now being tested in humans for their ability to promote immune control of HIV in infected individuals and potentially to eliminate HIV-infected cells. These include VRC01, VRC07-523, 3BNC117, and N6 (CD4 binding site–targeting antibodies); 10-1074 and PGT121 (V3-glycan–targeting antibodies); PDGM1400 and CAP256-VRC26 (V1/V2-glycan–targeting antibodies); and 10E8 (MPER-targeting antibody) (1–3, 5). In mice engrafted with human cells (humanized mice), in macaques chronically infected with simian human immunodeficiency virus (SHIV), or in HIV-infected humans, single bnAbs can lower the viral load by ~10- to 100-fold, but antibody-resistant viruses rapidly emerge. Combinations of bnAbs are more effective in preventing the emergence of antibody-resistant virus. Administration of bnAbs early after SHIV infection can lead to persistent viral clearance in some animals (5, 6). This viral clearance may occur because bnAb administration helps preserve helper CD4$^+$ T cells and because the formation of antibody-antigen immune complexes may help stimulate a more effective CD8$^+$ T cell response (6). Similarly, treatment of HIV-infected humans with the CD4 binding site–directed bnAb 3BNC117 was associated with a modest increase in host neutralizing antibodies to HIV (5). In SHIV-infected macaques, treatment with bnAbs prolonged viral control in a subset of animals after discontinuation of ART (5, 6). In humanized mice, bnAb treatment promoted enhanced clearance of HIV-infected cells (5). Future clinical studies will include modified forms of antibodies, such as bispecific molecules, which may improve the potency and duration of antiviral activity. It remains to be seen if antibodies can reach HIV virions or infected cells in protected sites such as the brain. Nonetheless, antibodies that target HIV-infected cells could be combined with latency reactivating agents (LRAs) to reduce or eliminate infected cells and contribute to curing individuals infected with HIV. An LRA could be used to activate latently infected CD4 T cells, followed by addition of bnAbs or multispecific antibodies, such as bispecific antibodies that can bind to both Env and CD8 T cells. Such strategies have been shown to result in reduction of replication-competent HIV in vitro and in a decrease in the virus reservoir in a humanized mouse model.

There is also strong evidence that bnAbs can prevent HIV acquisition in animal models. For example, passive administration can protect against SHIV infection of macaques, including repetitive mucosal challenges, with some antibodies protecting at in vivo serum inhibitory concentrations of less than 1 ug/ml (6). Modification of the Fc antibody region for increased half-life conveys the ability to prevent low-dose SHIV infection for more than 6 months (5). Although animal model data demonstrate protection, it remains to be proven that bnAbs can protect against HIV acquisition in humans. Thus, the NIAID HIV Vaccine Trials Network (HVTN) and HIV Prevention Trials Network (HPTN) are carrying out the Antibody Mediated Prevention (AMP) efficacy trials with intravenous administration of the CD4 binding site bnAb, VRC01 (NCT 02716675 and NCT02568215). With clinical trial sites in Africa, South America, Europe, and the United States, 4600 volunteers are already enrolled. The AMP trials are designed to assess if a single bnAb can prevent HIV acquisition in humans and to determine how much serum antibody is needed for protection. The results of the trials will likely be available in 2020. Based on the animal model studies, it is expected that bnAbs can prevent HIV acquisition in humans by sensitive strains, but, due to the antigenic diversity of HIV, a

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two- or three-bnAb combination may be required to protect against globally diverse strains.

INDUCTION OF BNABS BY ENV IMMUNIZATION

Generating bnAbs through Env vaccination continues to be challenging. BnAbs induced as a result of chronic viral replication during HIV-1 infection are the result of B cells undergoing extensive affinity maturation in germinal centers. HIV Env antibodies associated with protection from HIV acquisition in animal models include bnAbs and nonneutralizing Env antibodies (nnAbs) (*4, 7*). The former have been shown directly to mediate protection in nonhuman primates. In vitro, HIV Env Abs can act antivirally by direct neutralization and by Fc-mediated effector functions such as antibody-dependent cellular cytotoxicity (ADCC), although the relative contributions of these activities in vivo to protection is unclear and may differ between bnAbs. nnAbs rely solely on Fc-mediated activities for antiviral function, and evidence for protection is less direct than for bnAbs. HIV nnAb responses are readily induced by Env vaccination, but bnAb responses have not been robustly induced as yet in humans or nonhuman primate models. Many bnAbs have long heavy-chain third complementarity-determining regions that have low frequencies in the human naïve B cell repertoire and may subject such antibodies to deletion by immune tolerance. Some bnAbs share characteristics of autoantibodies such as auto- or poly-reactivity (*4, 7*) and thus may be excluded by immune tolerance mechanisms from productive immune responses. We now know that development of bnAbs in HIV infection is associated with high viral loads. Moreover, bnAbs are unusually mutated (affinity matured) by the enzyme activation–induced cytidine deaminase, indicating long periods of germinal center development induced by persistent antigen contact. Thus, there are multiple factors working against the elicitation of bnAbs, including host control roadblocks (*4, 7*).

The Env structure presents multiple issues as an antigen. Induction of bnAbs is hindered by instability of the native fusion-competent Env and by non-native forms that induce nnAbs. Moreover, HIV Env consists of ~50% glycans by mass, and thus, almost all bnAbs must bind to or accommodate these glycans to bind the native trimer and neutralize viral infection of host cells. Protein-glycan interactions are typically of relatively low binding affinity, further disfavoring bnAb development. In addition, HIV Env has five variable loops that may increase in length and vary in glycosylation with virus evolution over time, and bnAbs must evolve to accommodate such Env loops. Thus, in addition to host controls and repertoire restrictions, Env structural constraints conspire to make bnAb B cell lineages difficult to induce (*1–3*).

Although our ability to induce bnAbs in animal models and humans remains a challenge, there has been substantial progress in structural definition of neutralization epitopes on the native Env trimer, leading to new vaccine design approaches. Given that binding to the native Env trimer is required for virus neutralization, our ability to stabilize and express soluble mimics of Env as vaccine antigens is a major step forward (*8*). However, Env trimers alone do not induce bnAbs, in part because most of the glycoprotein is not highly immunogenic and because extensive antibody affinity maturation is required for neutralization. Thus, it is likely necessary to use informed immunogen design to stimulate the appropriate B cell precursors and then guide maturation pathways to yield bnAbs. In addition, use of strong adjuvants may be needed to overcome immune tolerance mechanisms and to support prolonged bnAb germinal center responses.

Most HIV infections are caused by one or a few transmitted/founder viruses. Antibody-virus co-evolution studies have led to investigation into how bnAb lineages arise and develop during the course of HIV infection, demonstrating an "arms race" between the evolving viral quasi-species and host neutralizing antibody responses. In some individuals, virus Env proteins evolve that eventually select B cell receptors encoding bnAb activity (*4, 7*). Reproducing the results of these pathways via immunization is one approach for vaccine development. The concept of using bnAbs as the scaffold for vaccine design has been termed reverse vaccinology or epitope-based vaccine design (*9, 10*). Here, vaccine immunogens are designed to mimic known bnAb epitopes. Additionally, using information about immunological pathways leading to development of antibody lineages with broad neutralizing activity has been termed "lineage-based" vaccine design (*7*). Knowledge of the unmutated common ancestor (UCA) of a bnAb lineage is paired with knowledge of the optimal Env immunogen to trigger the appropriate precursor B cell expressing the UCA antibody on its surface (*7*). Combining structural and immunological information for some antibodies, such as the CD4 binding site antibodies of the VRC01 class, has led to an approach termed "germline targeting" (*7, 8*). Germline targeting begins with the estimation that critical bnAb precursor features are sufficiently common within and among different individuals to make them targetable by appropriately templated immunogens. For the CD4 binding site, an immunogen (eOD-GT8) was templated from VRC01 class bnAb precursors from multiple HIV-infected individuals. Additional immunizations (boosts) must then be designed to drive affinity maturation toward mature bnAbs.

DEVELOPING IMMUNOGENS FOR INDUCTION OF BNABS IN CLINICAL STUDIES

Notably, many of the above immunization approaches have advanced to phase 1 clinical trials. This includes vaccines comprising soluble Env trimers that mimic native Env, lineage-based sequential immunogens for inducing CD4 binding site antibodies, or germline targeting for CD4 binding site VRC01 class or gp41 MPER antibodies. Over the next 2 years, several additional immunogens in each of these categories will enter clinical testing, including lineage-based and germline-targeting approaches for the V3-glycan and V1/V2-glycan region and epitope-based vaccines to target the fusion peptide region of Env. Immunization strategies likely will require sequential immunizations to prime and then boost bnAb lineages with the necessary affinity maturation to recognize Env and potently neutralize HIV. Once early-stage antibody lineages are stimulated and expanded, intermediate immunogens could foster further affinity maturation, and native trimer Env boosts could lead to final development of systemic bnAbs (Fig. 1). This level of immunogen design and sequential immunization has no precedent in vaccinology and will require iterative phase 1 experimental medicine clinical studies to assess, modify, and improve initial immunogens and immunization strategies.

MAKING A PROTOTYPE HIV BNAB VACCINE

The challenges posed by the antigenic diversity, glycosylation, and immune evasion
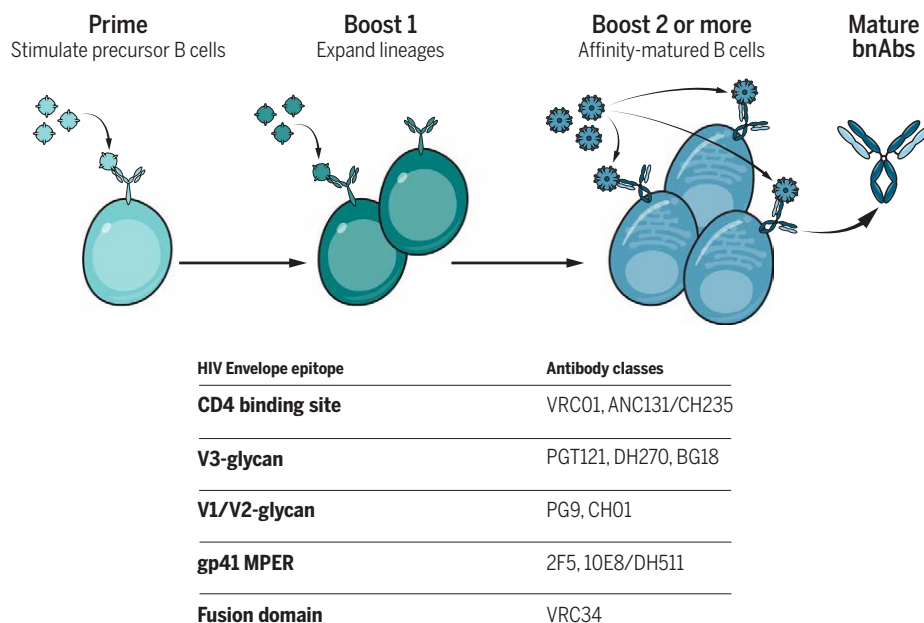
Fig. 1. Strategy for induction of broadly neutralizing antibodies. Shown here is the vaccine strategy of sequential prime and boost to induce mature broadly neutralizing antibodies (bnAbs) against HIV. The initial immunogen would engage the precursor naïve B cell of bnAb B cell lineages, and subsequent boosts would select for neutralizing members of the antibody lineage. The classes or types of bnAbs and representative bnAbs are shown in the bottom panel. A successful vaccine will likely need to induce a polyclonal antibody response against multiple bnAb epitopes to cover the full diversity of HIV virus quasi-species to which a vaccinee could be exposed and to avoid transmitted/founder virus escape.

of HIV Env, together with the unusual antibody characteristics required to achieve virus neutralization, call for a highly coordinated approach to phase 1 vaccine studies in order to achieve the key goal of eliciting bnAbs. As shown in Fig. 1, sequential Env immunogens for each bnAb B cell lineage, of necessity, must be coordinated in their design such that one can follow the other to select for desired bnAb B cell receptor mutations in intermediate and mature bnAbs. This will in effect keep bnAb lineages "on track" in germinal centers to acquire the required mutations necessary for neutralization. Thus, the biology of bnAbs with disfavored B cell lineages, resulting in the need to direct or shepherd the lineages to full maturation with sequential immunization regimens, coupled with the need for good manufacturing practice (GMP) production of a number of Env vaccine candidates, necessitates unprecedented cooperation and communication among HIV vaccine investigators. Such cooperation will allow optimization of clinical trial design by taking advantage of available immunogens with the aim of finding sequential immunization regimens that are able to induce

durable cross-reactive serum neutralizing antibody responses.

The NIH, NIAID Consortia for HIV/AIDS Vaccine Development (CHAVD) centered at Duke University and The Scripps Research Institute, the NIAID intramural Vaccine Research Center (VRC), and the HVTN have come together to form the Collaborative HIV Immunogen Project (CHIP) to coordinate logistics and priorities for preclinical testing, GMP manufacturing, and entry into human phase 1 clinical trials. The coordination provided by the CHIP will both optimize the utilization of resources and speed the development of a broadly protective HIV vaccine. The CHIP will both invite and welcome all groups of investigators engaged in HIV vaccine development with the goal of inducing bnAbs. Examples of the efforts of other groups that would be important to include are the two European Vaccine Consortia, other HIV vaccine development teams funded by the NIAID, the Bill & Melinda Gates Foundation, and the International AIDS Vaccine Initiative.

In summary, the isolation of bnAbs has provided a new category of biologic anti-

retroviral drugs for treatment and prevention and provided hope that bnAbs could be induced by vaccination. However, the discovery of the complex biology of bnAbs and their response to HIV Env conformations has necessitated a far more complicated vaccination strategy than has been used for any currently approved vaccine. Preclinical data suggest that we are on the verge of major progress, although ultimate success in humans will require closely tied scientific and clinical studies. We hope that with the complexity of the problem will come unprecedented collaboration and cooperation in the HIV vaccine field that will soon result in a safe and effective bnAb-based vaccine.

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10TH ANNIVERSARY SERIES

Promising disease-modifying therapies for Parkinson's disease

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To date, there is no disease-modifying therapy for Parkinson's disease; however, promising new agents have advanced into clinical trials.

Parkinson's disease (PD) is the second most common neurodegenerative disease. Individuals with PD have both motor and nonmotor symptoms. The motor symptoms include slowness of movement, rigidity, and tremor at rest. Nonmotor symptoms range from gastrointestinal and autonomic nervous system abnormalities to neuropsychiatric and cognitive dysfunction (1). Most of the neurodegenerative process of PD is driven by pathological α-synuclein, a presynaptic neuronal protein that aggregates and accumulates in Lewy bodies and Lewy neurites (2) in the nervous system. Although drugs that treat the motor symptoms are relatively effective and therapies addressing some of the nonmotor symptoms have shown modest efficacy, these treatments are less effective at treating advanced disease. To date, there are no disease-modifying therapies that slow, halt, or reverse the progression of PD. Development of disease-modifying agents remains a high priority. Advances in understanding the molecular pathogenesis of neurodegeneration in PD, development of new animal models, and human dopaminergic neuronal cell culture systems together with the identification of new therapeutic targets (1) over the past decade have led to the initiation of exploratory clinical trials. In this 11th installment of *Science Translational Medicine*'s anniversary Focus series, we highlight promising agents that have the potential for altering the progression of PD and the science behind them.

AGENTS TARGETING THE GENETIC CAUSES OF PD

Over the past two decades, there has been extensive characterization of the genetic architecture of PD, including identification of the genes encoding leucine-rich repeat kinase 2 (LRRK2) and glucocerebrosidase as risk factors for PD. From this foundation, LRKK2 kinase inhibitors and agents that modulate glucocerebrosidase function have recently advanced to clinical studies.

Disease-causing mutations in *LRRK2*, which play a major causal role in the inheritance of PD, lead to enhanced activity of this kinase and neurodegeneration (3). LRRK2 phosphorylates a conserved threonine or serine in the switch II domain of certain Rab GTPase family members. Rab GTPases, in part, regulate vesicle trafficking (4). More work needs to be done to clarify the roles of individual Rab GTPases in the pathogenesis of PD. The high abundance of Rab GTPases has enabled the development of high-affinity and specific antibodies, leading to the democratization of tools to monitor LRRK2 activity. The ribosomal protein s15 is a physiological substrate of LRRK2, and phosphorylation of s15 is involved in the loss of neurons in PD through altered protein translation (5). Targeting LRRK2 kinase substrates for therapeutic benefit is under active exploration.

Since the identification of the causal role of LRRK2 in PD, academic and industry investigations have led to the development of LRRK2 kinase inhibitors with improved potency, selectivity, and blood-brain barrier penetration (3). Denali's LRRK2 kinase inhibitors, DNL-151 and DNL-201, have completed phase 1 studies in healthy volunteers (Table 1). Individuals with PD or those who are at risk of developing PD due to an LRRK2 mutation potentially could benefit from treatment with an LRRK2 kinase inhibitor. There is some evidence suggesting that LRRK2 kinase activity may also be involved in the pathogenesis of idiopathic (sporadic) PD, indicating that a substantially larger patient population potentially could benefit from treatment with these inhibitors.

A major genetic risk factor for PD is mutation of the gene that encodes the enzyme glucocerebrosidase (6). These mutations lead to retention of glucocerebrosidase in the endoplasmic reticulum and decreased glucocerebrosidase activity. Mutant glucocerebrosidase can lead to pathological accumulation of α-synuclein. Clinical efforts have focused on translocating mutant glucocerebrosidase from the endoplasmic reticulum into lysosomes using chemical chaperones. A phase 2 clinical trial of Ambroxol and a phase 1 study of the Allergan compound LTI-291—agents that increase the activity of glucocerebrosidase—are currently testing this idea (Table 1). In addition, Genzyme is testing the hypothesis that PD in those patients with glucocerebrosidase mutations is due to the accumulation of the glucocerebrosidase substrate glucosylceramide. Individuals with PD are being treated in a phase 2 study with ibiglustat (GZ/SAR402671, venglustat L-malate), a glucosylceramide synthase inhibitor that blocks the formation of glucosylceramide (Table 1). Given that glucocerebrosidase activity is reduced in idiopathic PD and there is an inverse relationship between glucocerebrosidase activity and the accumulation of pathological α-synuclein (6), agents that enhance glucocerebrosidase activity are also being explored in idiopathic PD but have yet to enter the clinic.

AGENTS EXPLOITING THE CELL BIOLOGY OF PD

Research focused on how pathological α-synuclein leads to neurodegeneration in idiopathic PD has also resulted in agents that are now in clinical trials. Under physiological conditions, α-synuclein exists in both a soluble and membrane-bound state with the

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Table 1. Agents in clinical trials for PD.

Target	Action	Agent	Trial phase	Clinical trial identifier
LRRK2	Decrease LRRK2 kinase activity	Denali—DNL-151	Phase 1	NCT04056689
		Denali—DNL-201	Phase 1	NCT03710707
Glucocerebrosidase	Chemical chaperone to translocate mutant enzyme from the endoplasmic reticulum into lysosomes	Ambroxol	Phase 2	NCT02941822 and NCT02914366
		Allergan—LTI-291	Phase 1	Netherlands Trial NL7061
	Block accumulation of the glucocerebrosidase substrate, glucosylceramide	Genzyme/Sanofi—Ibiglustat (GZ/SAR40267)	Phase 2	NCT02906020
α-Synuclein	Anti–α-synuclein monoclonal antibodies to lower α-synuclein concentrations and block cell-to-cell transmission	Biogen—Cinpanemab	Phase 1/2	NCT03318523 and NCT03716570
		Hoffmann–La Roche/Prothena—Prasinezumab	Phase 2	NCT03100149
		Abbvie—ABBV 0805	Phase 1	NCT04127695
		Takeda/AstraZeneca—MEDI1341	Phase 1	NCT03272165
		Lundbeck—Lu-AF-82422	Phase 1	NCT03611569
	Inhibitors of pathological α-synuclein aggregation	Enterin—ENT-01	Phase 1	NCT03938922
		Alterity Therapeutic—PBT434	Phase 1	U1111–1211-0052
		Neuropore Therapies Inc./UCB S.A.—NPT200-11	Phase 1	NCT02606682
c-Abl kinase	c-Abl kinase inhibition	SPARC—K0706	Phase 2	NCT03655236
		Novartis—Nilotinib	Phase 2	NCT02954978 and NCT03205488
		1ST Biotherapeutics, Inc—FB-101	Phase 1	NCT04165837
GLP-1 receptor	GLP-1 receptor agonist, decreases inflammation	Amylin Pharmaceutical/ AstraZeneca—Exenatide	Phase 3	NCT03456687, NCT04154072, and ISRCTN14552789
		Novo Nordisk—Liraglutide	Phase 2	NCT02953665
		Sanofi—Lixisenatide	Phase 2	NCT03439943
		Neuraly—NLY01	Phase 2	NCT04154072

monomer in an inherently unfolded state. α-Synuclein can transition between nontoxic monomers and tetramers and toxic oligomers and fibrils. There is growing evidence that the central role of α-synuclein in α-synucleinopathies and PD is mediated by the aggregated α-synuclein fibrils. Pathological α-synuclein toxicity may be partly engendered through cell-to-cell transmission, in which pathological α-synuclein may act like a prion (2). Thus, monoclonal antibodies against α-synuclein are being used to reduce concentrations of α-synuclein and to prevent cell-to-cell transmission of pathological α-synuclein (Table 1). Biogen's Cinpanemab (BIIB 054) and Hoffmann–La Roche/Prothena Biosciences' Prasinezumab (PRX002) monoclonal antibodies are currently in phase 2 clinical trials. Meanwhile, Abbvie's ABBV 0805, AstraZeneca's and Takeda's MEDI1341 (TAK-341), and Lundbeck's Lu-AF-82422

monoclonal antibodies against α-synuclein are in phase 1 trials (Table 1). Preclinical studies using these different monoclonal antibodies and an immunotherapy approach are testing the hypothesis that lowering α-synuclein or interfering with cell-to-cell transmission may have a beneficial effect on disease progression and severity. There is a concern that the timing of immunotherapy could affect the efficacy of this approach. A distinct challenge for anti–α-synuclein monoclonal antibodies to lower α-synuclein in the brains of individuals with PD exists, because α-synuclein is predominantly an intracellular protein. Small-molecule inhibitors of α-synuclein, which in theory would prevent the formation of pathological aggregated α-synuclein, have entered phase 1 trials. These include Enterin's ENT-01, Alterity Therapeutic's PBT434, and Neuropore/UCB's NPT200-11 (UCB0599) (Table 1). Ideally, treatment

initiated during the early stages of the disease would be optimally efficacious in limiting seeding and misfolding of α-synuclein; however, extensive work still needs to be done to identify presymptomatic subjects. Phase 2 and 3 studies will ultimately determine the usefulness of these approaches in modifying the course of disease in individuals with early symptomatic PD.

After α-synuclein misfolds, it sets in motion both cell-autonomous (action within a cell) and non–cell-autonomous (action outside a cell) pathways, which contribute to neurodegeneration. The nonreceptor tyrosine kinase c-Abl, which is overactive in idiopathic PD, may play an important role in cell-autonomous death of neurons because loss or inhibition of c-Abl is protective in multiple animal models of pathological α-synuclein–induced neurodegeneration (7). c-Abl phosphorylates several substrates that

may contribute to the neurodegenerative process, including the ubiquitin E3 ligase, parkin, which leads to its inactivation. Inactivation of parkin induces accumulation of the parkin substrates PARIS (ZNF746) and aminoacyl tRNA synthetase complex interacting multifunctional protein 2 (AIMP2), which down-regulate peroxisome proliferator–activated receptor-gamma coactivator-1α and activate poly(ADP-ribose) polymerase-1, respectively. This kills neurons through formation of poly(ADP-ribose) and activation of parthanatos. c-Abl also phosphorylates α-synuclein on tyrosine 39, turning it into a pathological species. Other c-Abl substrates that may participate in the neurodegenerative process include cyclin-dependent kinase 5 and p-38 kinase (7). Enhanced c-Abl activity also impairs the autophagic degradation of pathological α-synuclein through unclear mechanisms (7). c-Abl inhibitors were developed for the treatment of cancer and are poorly brain penetrant. A small phase 1b study with Novartis's nilotinib suggested that individuals with PD who received nilotinib experienced a slight benefit (7). There are two phase 2 trials attempting to confirm this initial report (Table 1). Efforts to develop safer and brain-penetrant compounds have resulted in Sun Pharma Advanced Research's brain-penetrant c-Abl kinase inhibitor, K0706, which is currently in phase 2 clinical trials and 1ST Biotherapeutics' FB-101, which is in a phase 1 trial.

Non–cell-autonomous cell death is gaining traction as an important contributor to the pathogenesis of PD. Recent studies suggest that glucagon-like peptide-1 (GLP-1) receptor agonists, which lower blood glucose in diabetes, could be disease-modifying agents in PD. GLP-1 receptor agonists have diverse targets, but their primary mechanism of action may be through preventing pathological microglial activation and secretion of the proinflammatory cytokines tumor necrosis factor, interleukin-1α, and complement component 1q, which in turn prevents the conversion of resting astrocytes to the toxic and reactive A1 phenotype (8). Amylin Pharmaceuticals' and AstraZeneca's GLP-1 receptor agonist exenatide has been through a double-blinded and placebo-controlled phase 2 trial in patients with moderate PD. Individuals with PD who received exenatide experienced a slight improvement in motor function. However, it remains unclear whether the improvement was due to symptomatic relief or modification of the disease process (9). Exenatide has advanced to a phase 3 clinical trial. Other GLP-1 receptor agonists in phase 2 trials include Novo Nordisk's Liraglutide, Sanofi's Lixisenatide, and Neuraly's NLY-01. LRRK2 inhibitors and agents targeting glucocerebrosidase may also interfere with non–cell-autonomous cell death because both LRRK2 and glucocerebrosidase are enriched in glia and immune cells (3, 6).

POINTS TO CONSIDER

The presence of multiple potential disease-modifying therapies in early-phase clinical trials is exciting. One GLP-1 receptor agonist, exenatide, has already advanced to a definitive phase 3 clinical trial. Barring safety concerns or phase 2 trial futility, it is likely that many of the agents highlighted in Table 1 will advance to phase 3 clinical trials. However, as has been observed in prior clinical studies founded on sound science that failed to meet primary end points, major impediments to success remain. These include the heterogeneity of the clinical course of PD and the lack of biomarkers for diagnostic certainty and monitoring of disease progression. Development of tools for diagnosis and monitoring of disease progression beyond clinical assessments that are used by master clinicians will be critical to identify disease-modifying compounds that have small to modest but significant effects (10). An agent that halts disease progression may obviate the need to address these issues. However, because of the inherent challenges to achieve success, tools to aid in the diagnosis and monitoring of disease progression will ultimately be essential for identifying, proving, and accelerating the clinical use of agents that modify the course of PD. Finally, genetics and rigorous cell and animal studies have identified many additional disease targets not mentioned here. Agents directed at these targets are in the drug development pipeline. We remain optimistic that ultimately agents will be identified that can slow and alter the course of PD.

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10ᵀᴴ ANNIVERSARY SERIES

Advances in clinical MRI technology

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Advances in MRI technologies have the potential to detect, characterize, and monitor a wide variety of diseases.

INTRODUCTION

Nuclear magnetic resonance (NMR) is the emission of electromagnetic signals by atomic nuclei in response to magnetic fields. NMR has long been of interest to the scientific and translational medical research communities, given its broad potential in chemical characterization, biosensing, and imaging (*1*, *2*). Early work by Haun *et al.* (*1*) showed the utility of a handheld micro-NMR device for rapidly characterizing fine-needle aspirates to diagnose cancer. Magnetic resonance imaging (MRI) produces images by measuring the radiofrequency signals arising from the magnetic moments of hydrogen protons abundantly found in water and lipids. In 2010, Wu *et al.* (*2*) used MRI and magnetic resonance spectroscopy to map the metabolomic profiles of prostate cancer samples. MRI has progressed to a great extent over the past 10 years; indeed, it has revolutionized contemporary medicine, building on advances in image reconstruction (parallel imaging and spatiotemporal reconstruction) developed over the past 20 years. It is estimated that more than 36 million MRI exams are performed annually on nearly 12,000 installed systems in the United States alone. Compared to other imaging technologies, MRI offers superb soft tissue contrast, multiple different contrast mechanisms, absence of radiation, and the ability to colocalize anatomic and functional or molecular information. Accordingly, MRI is routinely used clinically to evaluate disease in all major organ systems. In translational research, MRI has enabled progress in entire fields, including neuroscience, psychiatry, and oncology, among others. In this final installment of *Science Translational Medicine*'s 10th anniversary Focus series, we broadly discuss key technological advances achieved over the past decade and the future of clinical and translational MRI for disease management.

KEY TECHNOLOGICAL ADVANCES

The technical capabilities of current MRI systems have been driven largely by advances in computers, material science, engineering, and physics (Fig. 1). Although some extraordinary feats, such as single-atom imaging and ultrahigh-field anatomical imaging, have received considerable media attention, there are a myriad of incremental advances that have improved clinical imaging.

Better image quality and higher spatial resolution of today's MRI are largely due to advances in MRI pulse sequences (structured sets of alternating magnetic gradients used to probe for tissue properties) and hardware, including higher field strengths, improved multichannel coils, stronger gradients, and more homogenous magnets. Whereas it was common to image at 0.5 and 1.5 T several years ago, many magnets currently operate at 3 T. Today, coils that collect the body's MRI signatures are typically phased array, flexible printed, or blanket coils rather than the bulky cage coils used a decade ago (*3*). Beyond improving image quality, these advances have enabled functional MRI (the combination of morphological data with biological information) of the brain, vascular mapping, and real-time cardiac imaging. Engineering advances have also led to development of wide-bore and open magnets, allowing interventional procedures and surgeries and accommodating claustrophobic patients.

Faster imaging has become a clinical reality, although much work remains to be done in this area. Increasing imaging speed improves patient comfort and throughput, decreases cost, reduces motion artifacts, and enables imaging of joints and muscle function, such as in the heart. Newer approaches to achieve faster imaging include undersampling of *K*-space (partial Fourier reconstruction and parallel imaging), compressed sensing, and non-Cartesian sampling with constantly improving reconstruction algorithms.

Computational analysis and artificial intelligence (AI) play increasingly important roles in all aspects of MRI acquisition and data processing. For example, AI-based image reconstruction greatly reduces the time required for image reconstruction, producing images of comparable quality while providing the ability to reconstruct large datasets in near real time (*4*). In addition, the increasing availability of AI has laid the foundation for automated image post-processing, including segmentation and volumetric analysis. Volumetric analysis is particularly useful in imaging Alzheimer's dementia and medial temporal lobe quantification, although predominantly used in a research setting at present. Last, AI can be used for decision support to create automated reports, flag imaging abnormalities, or classify organ structures as normal or abnormal.

New techniques have emerged over the past several years. Perhaps the most noteworthy are integrated MRI–positron emission tomography (PET) systems that can synchronously map molecular information (glucose uptake and receptor density) onto anatomic structures. Because it can coimage multiple molecular probes, MRI-PET has particularly important applications in oncologic and neurologic imaging (*5*). Beyond these exciting advances in MRI-PET fusion imaging, advances in MRI pulse sequences have enabled other new imaging approaches. For example, innovative distinct pulse sequences allow quantitation of fat, fibrosis (elastography), iron content (hemochromatosis), and water content and distribution. The latter forms the basis of diffusion-weighted imaging (DWI) and MR tractography, which can physiologically, directionally assess brain tissue or myocardial fibers. Deuterium metabolic imaging (DMI) is a new spectroscopic technique that generates three-dimensional metabolic maps of ^{2}H-glucose or other ^{2}H-labeled substrates given systemically or orally. In the cancer field, intravenously administered gadolinium chelates (contrast agents) enhance

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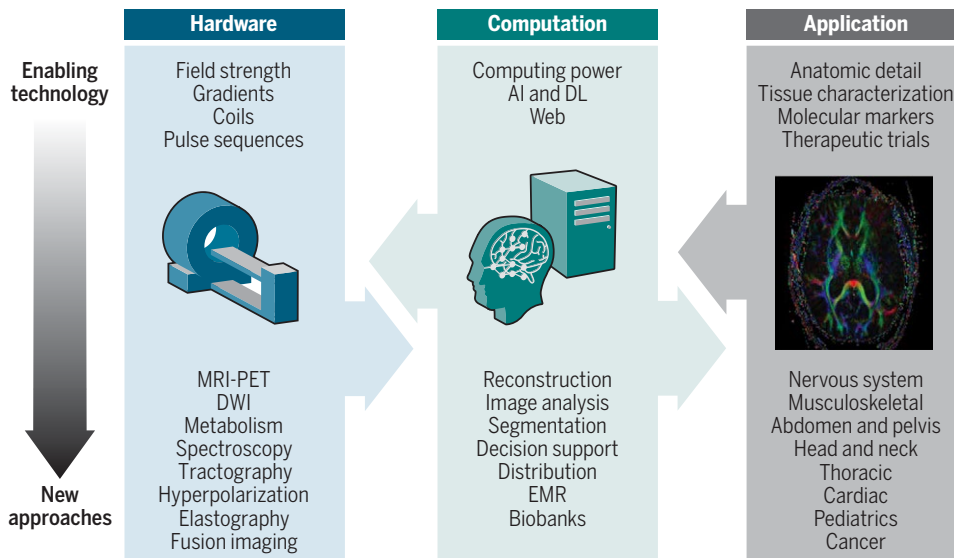*Corresponding author. Email: ralph_weissleder@hms.harvard.edu

Fig. 1. Advances in clinical MRI. Depicted are previous and projected advances in magnetic resonance imaging (MRI). Complementary developments in imaging hardware and computational analysis are facilitating improved image quality, which has enabled a broadening of MRI applicability across organ systems and will likely continue to do so in the future. DWI, diffusion-weighted imaging; EMR, electronic medical record.

tumor detection and tissue characterization; however, their use may be problematic in patients with renal failure who are at risk of developing nephrogenic systemic fibrosis. Consequently, gadolinium-free alternatives are under investigation. MRI can be used with systemically administered magnetic nanoparticles for nodal staging in cancer (6); MRI can also be used to quantify innate immune cells in tissue (inflammation imaging) and predict the efficacy of nanotherapeutics (7). MR spectroscopy can measure metabolites such as phosphocholine, lactate, citrate, and creatine within tissues. Last, hyperpolarized MRI using labeled metabolites, such as ^{13}C pyruvate, allows basic metabolic measurements (8) that can safely and effectively detect key metabolic conversions in patients with a variety of diseases. For example, in prostate cancer, increased conversion of pyruvate to lactate can be measured and is associated with increased tumor aggression.

Although many current MR techniques focus on qualitative assessment of tissue characteristics (hypointense versus hyperintense lesions), significant advances in quantitative MR techniques are now being realized. MR fingerprinting quantitatively characterizes tissues; the resultant numerical values permit data collation across differing time points for individual patients and between differing datasets of patients for enhanced precision. Handling these large volumes of numerical data will be facilitated by established and

emerging accomplishments in AI. Furthermore, image reconstruction for these datasets is complemented by concurrent advances in MR physics, including Bloch equation–based reconstruction techniques with dictionary mapping. These reconstruction techniques are necessary for accelerated data acquisition through undersampling while maintaining image quality. Similarly, advances in numeric T_1 mapping and extracellular volume analysis in cardiac MRI have improved our assessment of myocardial function. These permit, for example, the detection of diffuse cardiac fibrosis, which would otherwise go undetected on conventional late gadolinium-enhanced MRI, which is more sensitive to focal fibrosis.

THE NEXT DECADE OF CLINICAL MRI
Although clinical challenges abound for MRI, the opportunities are equally expansive. Looking forward, the most pressing clinical needs are shortening MRI acquisition times, optimizing image quality and content, automating analyses, perfecting fusion imaging, and enabling whole-body imaging. Approaches to achieve these goals will likely be similar to those described above. Deep learning (DL) algorithms are likely to play a central role in image acquisition (sub-Nyquist sampling strategies using DL), reconstruction (AUTOMAP), and automated image post-processing. More seamless integration of imaging results (including structured reporting and alerts of significant

findings) into electronic medical records will be essential. Currently, this remains cumbersome because the different imaging technologies and platforms are not optimized to interact with and learn from each other. Below we discuss established, emerging, and still-needed clinical MRI applications for cancer and other diseases.

Cancer imaging using MRI for initial cancer diagnoses, staging, serial imaging in therapeutic trials, and recurrence/progression monitoring is already established. In general, MRI has been shown to accurately stage cancer; triage patients to appropriate therapy; and support patient follow-up, particularly for colorectal, gynecologic, and prostate cancers. It is generally accepted that MRI is the most sensitive imaging method for identifying early metastatic disease in the liver and brain. MRI is also routinely used to establish the extent of bone marrow involvement and to identify skin or satellite lesions in bone malignancies. Furthermore, MRI is increasingly relied upon to phenotype cancers by extraction of quantitative imaging features (radiomics) (9). Several newer applications in cancer imaging include the following: screening for breast cancer in high-risk populations carrying *BRCA* mutations; planning radiation treatment, whereby superior soft tissue contrast permits accurate boundary delineation and dose painting; and predicting and monitoring patient response to chemotherapy. Another example is using whole-body MRI in oncology staging, particularly for lymphoma in younger patients for whom radiation exposure should be limited. Intraoperative MRI will likely play an increasing role in neurosurgical oncology by providing real-time information about the precise spatial relationship between tumors and adjacent areas in the brain to optimize surgical resection while limiting inadvertent damage to healthy cerebral parenchyma. The continued evolution of open-bore scanners will further enable MRI-guided procedures in interventional oncology.

Of the many potential clinical advances likely to result from expanding imaging technologies, several hold notable potential. A key interest in neurologic imaging is to translate emerging ultrahigh-field MRI (>3 T), which increases the signal-to-noise ratio, into clinical practice. This will allow better anatomic and ultrastructural imaging and will likely open new doors to disease characterization. Although a few such ultrahigh-field systems are operational in clinical research settings,

practical challenges will need to be overcome for the approach to be adopted more broadly. Improved signal-to-noise ratio and increased imaging sensitivity as a function of higher field strength have had profound effects on advanced techniques such as blood oxygen level–dependent (BOLD) imaging, which may help uncover the neurobiology of complex processes in addiction and the locoregional actions of drugs targeting the central nervous system. For musculoskeletal imaging, a central goal is the ability to perform MRI with stress loading on joints. This may seem simple but will likely require new magnet designs. In cardiology, fast data acquisition will allow routine real-time imaging in patients with arrhythmias. Similarly, advanced thoracic imaging will require the development of image acquisition methods during quiet breathing rather than the current approach of breath holding, which often is difficult for patients. Important aspirations in abdominopelvic imaging include establishing MRI as a surrogate end point for metabolic disorders such as hemochromatosis and validating MRI as a readout in drug development and trial assessment (for example, in drug trials for nonalcoholic steatohepatitis).

While acknowledging the advances made in decreasing scan time and optimizing image acquisition over the past decade, there remains considerable potential for improving the patient experience in the MRI suite. At present, advanced imaging techniques, such as functional and cardiovascular MR, require protracted scan times (sometimes up to or more than an hour). Consequently, patients often become uncomfortable during the examination, and motion artifacts remain a considerable problem. Although retrospective motion correction algorithms are useful, adaptive dynamic imaging, including prospective motion correction currently in development, is expected to expand the applications of cardiovascular and functional MRI. Within the field of cardiovascular imaging, efforts are being made to combine these prospective motion correction algorithms with free breathing techniques to further enhance the patient experience. Previously, complex sequence acquisition required electrocardiogram gating, breath-hold sequences, and respiratory gating to achieve artifact-free images. New efforts are emerging on MR "multitasking," which continuously collects geometric data and resolves for these artifacts. Undoubtedly, the secondary gains from this progress will include enhanced image quality, shorter scan times, and improved throughput.

MRI biobanking programs by global initiatives seek to acquire multiorgan imaging from large cohorts of patients. Such biobank efforts also include genomic, proteomic, and metabolic outcomes and other patient data often collected at multiple time points. These large repositories (U.K. Biobank and The Cancer Imaging Archive; OpenNeuro) will be invaluable to advance research, education, and training. Although many of these programs are just beginning, they present an exciting opportunity in population-based health care, where MRI will improve understanding of disease mechanisms. The breadth of information acquired by biobanks presents its own challenges and will very likely require automated techniques for data collection and storage.

The advances in MRI technologies described here have not been realized without growing pains, and a considerable amount of work must be done to improve them further. The aspirations of the field are ambitious and will require a community of basic and translational scientists, as well as physicians, to achieve them. Such efforts have been catalytic in the past, as evidenced by the expeditious development of MRI in the past decade. Many new applications will necessitate prospective clinical trials and cost-effectiveness analyses so that emerging techniques can become reimbursable. Further broadening our horizons in MRI may not simply extend to increasing field strengths or improving sequence technology but to providing ubiquitously available low-field MRI at the bedside. Commensurate advances in the allied fields of AI and MR physics will be necessary to facilitate all of these improvements. Collaborative efforts and public-private partnerships will be essential to driving these technologies and realizing the full potential of MRI in the years to come (*10*).
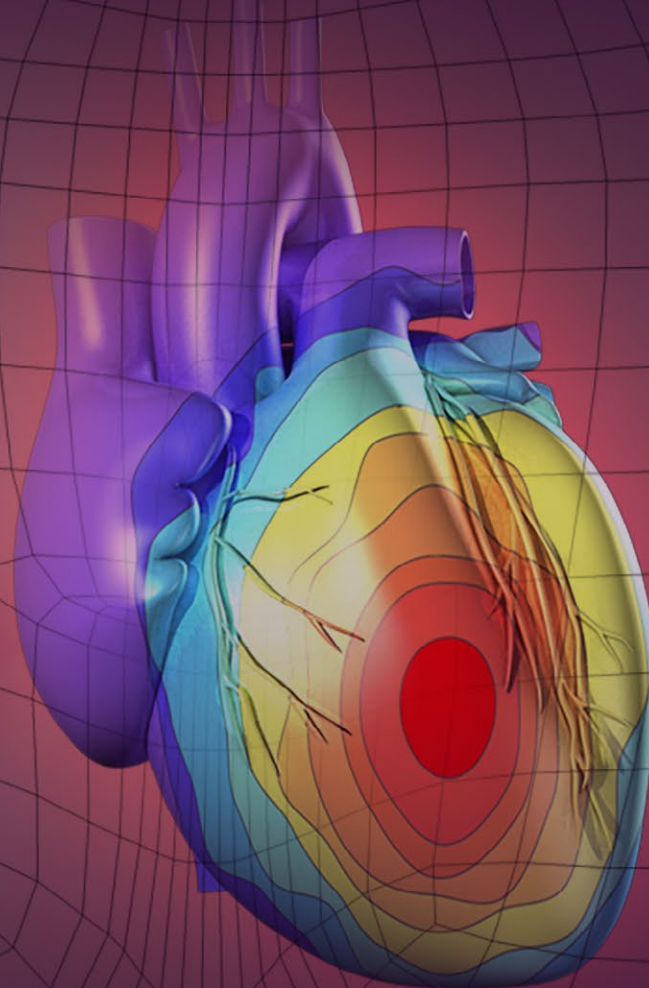
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