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Guest Editorial

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Role of Biomolecules and Biologics in Precision Medicine, Personalized Medicine, and Emerging Therapies

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Abstract

In the 1990s, DNA sequencing technologies could only read bite-sized pieces of DNA. Then came the human genome project (HGP), a thirteen-year international effort, 1990-2003, with the primary goal of discovering the complete set of human genes, sequencing nucleotides, and making the information accessible worldwide for further biological studies. We have come a long way since that time in terms of sequencing the genes of the human genome. Now the researchers can sequence the DNA and analyze gene-expressed proteins in individual cells, allowing them to dissect the complexities of genetic diseases with exceptional details. Currently, technologies are available for single-cell or multi-omics platforms to analyze genotype and phenotype. The completion of this one-of-a-kind project created public expectations for immediate, better health care delivery and possible cures for 'so called' incurable diseases. The HGP was the single most influential investment made in modern basic science research. A monumental breakthrough in medicine has given us the ability to sequence the DNA in cancer cells to identify possible errors in mutations. The impact of the HGP's success was so significant that President Barack Obama initiated a very ambitious new 'precision medicine' research initiative and announced the launch of this project during his State of the Union Address in 2015. The benefits of precision and personalized medicine include predicting susceptibility to diseases, improving disease diagnostics, preempting disease progression, customizing disease prevention strategies, and developing personalized drugs and therapies. As examples of emerging therapies, we have discussed the role of biomolecules and biologics in precision medicine applications like 'The All of Us,' personalized medicine approaches for monogenic diseases like hemophilia, sickle cell disease, and other rare genetic disorders, and CRISPR gene-editing technologies. Biomolecules play an essential role in all life processes, a variety of signaling processes, which are vital for normal functioning of physiological responses, in the early diagnosis of risk factors for various diseases, in the development of diseases and their progress. Furthermore, biomolecules, RNAs, DNAs, molecular and cellular engineering, genetic engineering of biologics, cells, tissues, and organs, play an important role in emerging therapeutic applications. The majority of the therapies discussed in this review are regulated as biologics under the Public Health Services Act of the USA. There is great interest in developing targeted therapy or precision medicine therapy for monogenic diseases, organ transplant applications, and tumor management, designed to interfere with targeted molecules for cancer-causing genes to slow the spread of cancer cells. Because molecular engineering, the development of biologics, gene-editing applications, and biomanufacturing are key components of emerging therapies, a keynote series was organized at INTERPHEX in November of 2021. INTEPHEX is the premier event that offers the latest intelligence, cutting-edge technologies, and state-of-the-art innovation for product development for pharmaceutical and biotechnology platforms. In an earlier article in this journal, we described drug discovery and development in the COVID Age; this overview provides a birds-eye view of the salient findings in each emerging area of medicine—precision medicine, personalized medicine, and emerging therapies.(**International Journal of Biomedicine. 2022;12(1):70-81.**)

Key Words: biomolecules • precision medicine • personalized medicine • emerging therapies

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Abbreviations

3BP, 3-bromopyruvate; **ADAR1**, RNA-specific adenosine deaminase 1; **AT**, ataxia-telangiectasia; **ATTR**, Transthyretin amyloidosis; **BRCA**, BReast CAncer genes; **Cop 1**, constitutive photomorphogenesis protein 1; **CAD,** chronic allograft damage; **CAR-T cells,** chimeric antigen receptor T cells; **CSD2**, superoxide dismutase; **CTGF**, connective tissue growth factor; **CAFs,** cancer associated

fibroblasts; **CRISPR**, clustered regularly interspersed sort palindromic repeats; **COPD**, chronic obstructive pulmonary disease; **CPFE**, combined pulmonary fibrosis and emphysema; **DNA**, deoxyribonucleic acid; **DYRKIA**, dual specificity tyrosine-(Y)-phosphorylation regulated kinase 1A; **EMT**, epithelial-mesenchymal transition; **EGF**, epidermal growth factor; **FGF**, fibroblast growth factor; **FAM13A**, family with sequence similarity 13 member A; **GFP**, green fluorescent protein; **GRIN2B**, glutamate ionotropic receptor NMDA type subunit 2B; **HGP**, Human Genome Project; **HGF**, hepatocyte growth factor; **HER2**, human epidermal growth factor receptor 2; **HIF**, hypoxia-inducible factor; **HDR**, homology-directed repair; **IRF4**, interferon regulatory factor 4; **IGF**, insulin-like growth factor; **IL6**, interleukin 6; **IPF**, idiopathic pulmonary fibrosis; **Kras**, Kirsten rat sarcoma viral oncogene homolog; **mRNA**, messenger RNA; **miRNA**, microRNA; **MAPK**, mitogen-activated protein kinase; **MLL**, mixed lineage leukemia; **NF-kB**, nuclear factor kappa B; **NHEJ**, non-homologous end joining; **NCI**, national cancer institute; **PDGF,** platelet-derived growth factor; **PTPN2**, protein tyrosine phosphatase non-receptor type 2; **PML-RARa,** promyelocytic leukemia-retinoic acid receptor α; **PD-1**, programmed cell death protein-1; **PDL1,** programmed death ligand-1; **PI3K**. phosphoinositide 3-kinase; **Pou2af**, POU class 2 homeobox associating factor coding gene; **RNA**, ribonucleic acid; **RRM2**, ribonucleotide reductase regulatory subunit M2; **RAS-MAPK,** Ras/mitogen activated protein kinase; **SNPs,** single nucleotide polymorphisms; **Sc4mol**, gene encoding a methyl sterol oxidase; **STAT**, signal transducer and activator of transcription; **sgRNA**, single-guide RNA; **TME**, tumor microenvironment; **TFs**, transcription factors; **TOLLIP**, toll-interacting protein; **VEGF**, vascular endothelial growth factor; **XBP1**, X-box binding protein 1.

Introduction

The unprecedented pandemic of coronavirus disease has created unfathomable healthcare and economic crisis worldwide.[1-4] According to healthcare experts, over 1400 pathogens are capable of infecting humans, of which 500 are capable of human-to-human transmission of pathogens.[5] Just over two decades ago, the Nobel Laureate Joshua Lederberg wrote, "The future of humanity and microbes likely will unfold as episodes of a suspense thriller,"- that could be titled 'Our Wits and Their Genes.'[6] We did not have to wait for a distant future. It looks like the SARS-CoV-2 genes have done it. They have evolved from a simple respiratory virus to a highly transmissible killer virus. The 1918 Influenza virus epidemic was considered the 'Mother of All Pandemics,' as it caused greater than 50 million deaths.[7] Since that time, a major question has been lurking in the minds of researchers. What makes some viruses so fatal? Have some critical viral genetic events produced a virus of remarkable pathogenicity? For instance, of the various variants of coronavirus that have appeared, the delta variant so far seems to be more virulent than the others. On the other hand, the new variant Omicron with over 50 mutations seems to be more infectious than delta and relatively less dangerous. Maybe natural selection prefers the survival of the species better than the severity of the disease. The authors of a seminal article on Spanish Influenza concluded, "Even with modern antiviral and antibacterial drugs, vaccines, and prevention knowledge, the return of a pandemic virus equivalent in the pathogenicity to the virus of 1918 would likely kill >100 million people worldwide."[7] Luckily for us, this prediction has not come true so far, despite the high transmissibility and pathogenicity of the coronavirus (nCoV-2).

SARS-CoV-2 (COVID-19) pandemic is an unusual, singular disaster - it made the world realize the seriousness of this public health crisis. Since it was identified in Wuhan, China (December 2019), SARS-CoV-2 is continuously evolving into different strains and spread worldwide. Global public health experts were totally unprepared for this magnitude of spread and destruction. However, it also helped the Governments, philanthropies, academicians, pharma companies to channel huge sums of money and efforts toward COVID-19 research. The unprecedented coronavirus pandemic also gave tremendous opportunities for drug discovery and development. Professor Cody Meissner at Tufts University School of Medicine in Boston says, "It is absolutely astonishing that this happened (Operation Warp Speed; development of Covid vaccines) in such a short time—to me, it is equivalent to putting a person on the Moon." "This is going to change vaccinology forever."[8,9] In the early days of the outbreak of this virus, Chinese researchers revealed the genomic information of the virus implicated in the Wuhan pneumonia outbreak. Scientists at Moderna Biotech, specializing in messenger RNA (mRNA) research, were able to design a vaccine on paper in 48 hours, 11 days before the US even had its first recorded Covid case. Within six weeks, Moderna had doses of vaccine ready for testing in animals. It is worth mentioning here the significant contributions of the US National Institutes of Health; Dr. Graham and Dr. McLellan of Vaccine Research Center, Bethesda, Maryland; Dr. Drew Weismann of Perelman School of Medicine, University of Pennsylvania; and Dr. Katalin Kariko of BioNTech, Germany, for the eventual success of mRNA vaccine development. COVID-19 pandemic and the discovery of mRNA vaccines, to a great extent, have eclipsed the news about all other current innovative research and innovations. In this overview, we will briefly review some milestones in the development of bioactive molecules, biologics, cellular and molecular therapeutics, genetically modified molecules, cells, tissues, organs, and the use of gene-editing tools in precision medicine, personalized medicine, and emerging therapies.

RNA Therapies

Among biomolecules, nucleic acids, namely DNA and RNA, have the unique function of storing an organism's genetic code, which is critical for the sustenance of life. Researchers from the Houston Methodist Research Institute, Texas, have reviewed the limitless future of RNA therapeutics.[9] According to them, RNA therapeutics comprise a rapidly expanding category of drugs (biologics) that will change the standard care for many diseases and actualized personalized medicine. They further emphasize that the drugs are cost-effective, relatively

simple to manufacture, and can target undruggable pathways. There are several cell-based therapies, which use mRNAs for the expression of desired proteins, and have reached clinical trials. The RNAs can also be designed to serve as gene-editing tools to achieve the expression of desired proteins. RNA therapy involves the use of coding RNA such as mRNA or RNAs such as noncoding small interfering RNAs (siRNA), antisense oligonucleotides (ASO), to target mRNA and clustered short palindromic repeats (CRISPR/Cas) endonuclease to target DNA and proteins. Rapid development in this technology has resulted in the approval, in both the USA and Europe, of two RNA-based therapies, for the treatment of hereditary ATTR amyloidosis, a progressive, potentially fatal disorder. Several miRNAs have recently been found to regulate adipose tissue biology, to promote metabolic diseases, muscle biology, insulin secretion, and action. Their altered expression may play a role in the development of obesity, metabolic disorders, and their clinical complications.[10] We are interested in exploring the role of miRNAs in the development of metabolic risks, such as oxidative stress (miR34a, miR638, miR150-3p), inflammation (miR27a, miR146a, miR155), endothelial dysfunction (miR29, miR126a-3p), subclinical atherosclerosis (miR121), diabetes-related clinical complications such as peripheral neuropathy (miR146a), retinopathy (miR21, miR124, miR200), nephropathy (miR29c), various vasculopathies (miR200b, miR200c, miR503), as well as fetal reprogramming of adipose tissue biology. We and others hope that this emerging technology will rapidly develop innovations in RNA therapies, facilitate the cost-effective manufacture of therapeutic products, validate them for clinical effectiveness, and provide safe and effective therapeutics to the clinic. The ability to rapidly develop or alter the sequence of the mRNA construct for personalized treatments or to adapt to an evolving pathogen (COVID) makes them unique therapeutics of the future.

Precision Medicine

President Obama announced a research initiative that aims to accelerate progress toward a new era of precision medicine in January of 2015, with the following announcement: "Tonight, I am launching a new Precision Medicine Initiative to bring us closer to curing diseases like cancer and diabetes — and to give all of us access to the personalized information we need to keep ourselves and our families healthier." The proposed initiative has two main components: a near-term focus on cancers and a longer-term aim to generate knowledge applicable to the whole range of health and disease. The initiative is supposed to tap into converging trends of increased connectivity, through social media and mobile devices, and Americans' growing desire to be active partners in medical research.[11,12] Authors describe, "The convergence of genetics, informatics, and imaging, along with other technologies such as cell sorting, epigenetics, proteomics, and metabolomics, is rapidly expanding the scope of precision medicine by refining the classification of disease, often with important prognostic and treatment implications." Despite the growing knowledge in a variety of related areas, our ability to harness the vast amounts of new knowledge and treatment options with the framework of everyday clinical practice poses a huge challenge. The accumulation of huge amounts of data, which is collected and shared through machines and machine learning applications, must be processed by data analysts, in a way, that brings value and accuracy. There is a great need to build a multidisciplinary team to process such massive research and clinical data.

What are some concerns about such 'Top-Down" approaches? The All of Us Research Program plans to enroll a diverse group of at least 1 million persons in the United States to accelerate biomedical research and improve health.[13] The program aims to make the research accessible to all participants, and it is developing new approaches to generate, access, and make data broadly available to approved researchers. As of 2019, more than 175,000 participants have contributed biospecimens. The All of Us data repository should permit researchers to consider individual differences in lifestyle, socioeconomic factors, environment, and biological characteristics, to advance the precision diagnosis, prevention, and treatment. The major weakness of The All of Us program is it does not focus on any particular set of diseases or health status. Whereas, the investigators of this study cited in support of their approach, the success of international studies such as the U.K. Biobank, the Million Veteran Program, the China Kadoorie Biobank, and other research groups, which have shown the power of very large cohorts for biomedical discovery. The investigators accept the general concern that the translation of biological and environmental discoveries into improved health is unlikely to occur quickly. Since 2015, Congress has allocated 1.02 billion to the All of Us program. The 21st Century Cures Act has authorized an additional 1.14 billion through 2026.

A WHITE HOUSE blog dated January 30, 2015, claims that Precision Medicine is already working to cure Americans. It lists six personal stories of how precision medicine has allowed cutting-edge treatment to further individuals' health and affects the lives of everyday Americans, their families, and generations to come. Although not related to The All of Us project, the stories include that of William Elder Jr., who has been diagnosed with cystic fibrosis; Emily Whitehead, who was the first pediatric patient to be treated with a new kind of cancer immunotherapy; Melanie Nix diagnosed with breast cancer (Positive for BRCA gene mutations); Hugh and Beatrice Rienhoff, who had a defect resembling Marfan Syndrome; Kareem Abdul-Jabbar, the famous basketball legend, who was diagnosed with a form of leukemia, and also mentions the name of Professor Keith Yamamoto, University of California, one of the pioneers of precision medicine. Some of the known successes of precision medicine are the treatment of BRCA with Olaparib (86% success), EGFR with Erlotinib Osimertinib (70% success), HER2 with Lapatinib Pertuzumab (50-70% success), and KIT with Imatinib (50-80% success). An emerging trend in precision medicine is the use of artificial intelligence and machine learning approaches to improve the traditional symptom-driven practice of medicine. Precision medicine, from a clinician's perspective, is about matching the right drugs to the right needs of the patient. Personalized medicine is still in its infancy—the future holds great potential. The current understanding of precision medicine can be best summarized by the statement of Dr. Nikhil Wagle, a cancer

specialist at Dana-Faber Cancer Institute in Boston, "There are very few instances in which we can look at a genomic test and pick up a drug off the shelf and say, 'That will work.' That's our goal in the long run, but in 2018 we are not there yet." One emerging trend in precision medicine is the use of artificial intelligence and machine learning to improve the traditional symptom-driven practice of medicine.

Personalized Medicine

Human Genome Project, which began in the 1990s, developed a complete sequence of the human genome (20,500 genes) in 2003. This historic milestone had a great impact on how one studies and treats diseases. Since that time, researchers have been able to identify the genetic basis of genomic variation of thousands of diseases. Emmanuelle di Tomaso, Head of Oncology, Precision Medicine, at Bayer, says, "Advancement in genomics and the advancement of related precision therapies, that pinpoint the genomic alteration driving specific disease, are transforming clinicians' approach to treatment." A prime example would be the success story of the personalized therapy of Mila Makovec—a hyper-personalized, first individually tailored treatment of its kind. Mila was diagnosed with a devastating genetic disorder called Batten disease. Clinicians in Boston, including Dr. Timothy Yu of Children's Hospital Boston, developed a personalized medicine, 'milasen', a one-of-a-kind drug, which has been named after her. Gina Kolata of the New York Times writes, "A new drug, created to treat just one patient, has pushed the bounds of personalized medicine." Milasen is a 22-nucleotide antisense oligonucleotide with the same backbone and sugar chemistry as nusinersen.[14] Nusinersen marketed as Spinraza has been used to treat spinal muscular atrophy. Since 2016, more than 10,000 people have been treated with this drug worldwide. The rapid advance made in this case was the result of prior knowledge that antisense oligonucleotides can be customized in a sequence-specific fashion—the precedent of nusinersen, being safe and effective, against spinal muscular atrophy, and relative simplicity of manufacturing.

Ipek Kuzu became the first handful of patients to receive a hyper-personalized gene-medicine tailored to treat a unique mutation. Ipek, a three-year-old, has ataxia-telangiectasia (A-T), a disease caused by an error in her DNA. It causes the loss of brain cells and could lead to infection and cancer. The one-person drug, designed by her doctor in Boston, Dr. Timothy Yu, is named 'atipeksen' for A-T and Ipek. Most of such diseases are caused by 'genetic typos.' The clinicians change the code, reprogram the drug, and treat many genetic diseases.[15] There are several challenges facing the development of personalized gene-modulating drugs. The first and the foremost is the cost of the development of such unique drugs. The second obstacle is that insurance companies do not cover such experimental drug development and treatments. Unless such treatments become cost-effective, they will be orphan drugs. There are some moves to convince funding agencies such as the National Institutes of Health, the need to fund such projects. According to a news report in the MIT Technology Reviews (2020), the FDA is considering giving doctors leeway to modify genetic drugs to try new patients without securing permission each time. Yet another serious challenge is, that short of a true healing, it may be impossible to be sure that they really work.

Transfusion-dependent β-thalassemia (TDT), and sickle cell disease (SCD), are monogenic diseases with severe and potentially life-threatening manifestations (NEJM 2021;384:252-260). A study sponsored by CRISPR Therapeutics and Vertex Pharmaceuticals describes the first two patients, one with TDT, and the other with SCD, who were infused with CTX001(autologous CRISPR.Cas9-edited CD34+HSPCs that were genetically edited to reactivate the production of fetal hemoglobin) and enrolled in CLIMB THAL-111 and CLIMB SCD-121 clinical trials. The authors of this multi-country study concluded, "Initial results from the follow-up of the first two patients who were treated with CTX001 have shown the intended CRISPR-Cas9 editing of BCL11A in long-term hematopoietic stem cells, with durable engraftment, high levels of fetal hemoglobin expression and the elimination of vaso-occlusive episodes or need for transfusion." Gene therapy is currently treating diseases ranging from neuromuscular disorders, hematological disorders, cancer, and blindness. Even though such therapies are available, health insurance companies will not cover the cost, ranging from 400,000 to 2 million. "Completely curing patients is obviously going to be a huge success, but it is not yet an achievable aim in a lot of situations." Says Julie Cruddle, a neurologist, and gene therapy researcher, at the University of Washington. There is no field in medicine that stands to benefit more from personalized medicine than organ transplantation.[16]

In 1954, the kidney was the first human organ to be transplanted successfully at the Brigham Hospital, Boston MA. Dr. Joseph Murray was honored with the Nobel Prize in medicine in 1990 for his efforts in the development of Kidney transplantation. In first-of-a-kind kidney transplantation, the University of Alabama surgical team transplanted gene-edited kidneys of a pig into a man who was brain dead. The transplanted kidneys made urine within 23 minutes. However, these kidneys did not remove creatinine from his system. Liver, heart, and pancreas transplants were successfully performed by the late 1960s. In 1967, both heart and liver transplantation was done successfully. Almost 107,000 people in the USA are currently waiting for life-saving organ transplantation. The major reason for late allograft loss is chronic allograft damage (CAD). The underlying mechanisms of CAD are poorly understood and need to be unraveled if graft function and treatment are to be successful.[16] According to the experts, the definition and identification of valid pre-and post-transplantation biomarkers will facilitate personalized medicine, leading to long-term graft survival. Emerging therapies integrate information from multiple platforms, like genotype analyses, single nucleotide polymorphisms (SNPs), epigenetic studies, analyses of mRNA, miRNA, proteins, peptides, and metabolite profiling. This massive information must be processed by using artificial intelligence and machine learning software analytics to develop appropriate risk analysis and prediction of success rates.

We mentioned in the previous paragraph that thousands of patients are waiting for organ transplantation. To overcome chronic allograft damage of the transplanted organ, Dr. Bartley Griffith, a distinguished Professor at the University of Maryland,

performed the first-ever gene-edited pig heart transplantation to Mr. David Bennett, who was waiting for a heart transplant for several months. The genetically modified pig was created by Revivicor, a biotech company. US/FDA had authorized the surgery on December 31, 2021, under 'expanded access' or sometimes referred to as "compassionate use." To genetically modify the heart, three genes were 'knocked out' for enzymes that enable pig cells to synthesize sugars (alpha-gal) that are responsible for causing an 'antibody-mediated' rejection of pig organs in humans. Six tweaks were made in the DNA for additions of human genes; two anti-inflammatory genes, two genes that promote normal coagulation and prevent blood vessel damage, and two other regulatory proteins that help tamp down antibody response. Another one was also 'knocked out' to prevent excessive growth of the pig heart tissue. Six human genes were inserted to encourage immune acceptance of the pig heart. Xenotransplantation has seen significant advances with the advent of CRISPR-Cas9 genome editing, which has made it easier to create pig organs that are less likely to be rejected by human immune systems.

Dr. Bert W. O'Malley, President and CEO of the University of Maryland Medical Center, called the surgery a "historic, monumental step forward." For decades, we have been at the forefront of research, driving progress toward the promise of xenotransplantation, as a viable solution to the organ crisis; many believed that this breakthrough would be well into the future. I remember my days at the University of Minnesota, decades ago, when the University established a new center of excellence for "Xenotransplant Research." The University of Maryland, School of Medicine, with the help of Revivicor, Blacksburg, Virginia (United Therapeutics), has achieved a major milestone in xenotransplantation. Revivicor was also behind the successful transplantation of a kidney into a human patient last October 2021, which was the first milestone in proving the viability of xenotransplantation. Harvard scientist George Church cofounded a company, eGenesis, which is working on using CRISPR gene editing to make animal organs viable for human organ transplantation. A company in Auckland, New Zealand (NZeno), is breeding miniature pigs whose kidneys remain human-sized, without growth-hormone modifications. As in any innovation, there is always a gap between the research findings, recognition of the importance of such discoveries by the regulatory agencies, and application of such findings at clinical trials. This procedure is new highly experimental, but the technique could help reduce transplant waiting lists in the future.

In the early 80s, we at the University of Minnesota were interested in pancreatic islet cell transplantation for restoring functionalities in a diabetic animal model. We used the streptozotocin-induced diabetic rat model for these studies. The diabetic animals showed an altered arachidonic acid metabolism. They produced more of proaggregatory thromboxanes and less of vasodilatory prostacyclins (Fig 1). Thromboxanes mobilize cytosolic calcium and induce platelet activation. Whereas prostacyclins, via the action of cAMP, lower cytosolic calcium and induce normalization of platelet activity. Once the pancreatic islet cells were successfully transplanted to these diabetic animals, the rats produced normal levels of thromboxane and prostacyclin levels, suggesting that this drug-induced diabetic state could be reversed and altered physiology could be normalized.[17]

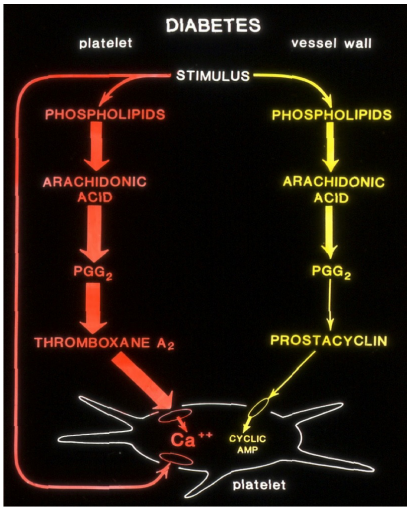

Fig 1. *Altered arachidonic acid metabolism in diabetic rats.*
(Courtesy. Dr. Jon Gerrard, Winnipeg, Canada)

Four decades after we demonstrated the beneficial effects of islet cell transplantation, Vertex Pharmaceuticals of Boston, MA, have reported the results of a trial, which infused cells grown from stem cells, like the insulin-producing pancreatic islet cells, in the first-ever human studies of this kind. Mr. Shelton was the first recipient who received the cell infusion on June 29 of this year (2021). The New York Times (November 27, 2021) published an article titled, "A Cure for Type-1 Diabetes? For One Man, It Seems to Have Worked." The challenge these researchers faced was to find out what sequence of chemical messages would turn stem cells into insulin-secreting islet-like cells.[18] The major concern that clinicians still have is "the trade-off between the burdens of diabetes and potential complications from immunosuppressive medications."

Emerging Innovative Anti-Cancer Therapies

Target specific delivery of therapeutics is a challenge when toxic drugs must be delivered to tumors. As early as in the early 80s, researchers experimented with the idea of coupling one of the most toxic biomolecules (Ricin), a glycoprotein from castor seeds to cell-specific monoclonal antibodies, for use in targeted delivery of anticancer drugs.[19] They demonstrated that a monoclonal antibody, rat IgG2b directed against Thy 1.2 antigen, provides a new binding site for the murine thymus cell surface. The authors concluded that "Ricin-monoclonal antibody hybrids of this type (could be considered a biologics or a device), combine a high degree of cell-type selectivity and toxicity, and may have pharmacological utility as antitumor agents. Viruses have been used as novel vectors for the cell-specific delivery of macromolecules, including toxins. UK researchers have shown that Ricin, a known toxin, could be encapsulated in a bacteriophage and delivered in a cell-specific

manner to the targeted tissue.[20] Yet another toxic, small biomolecule of therapeutic importance is 3-Bromo pyruvate (3BP), a potent and specific anticancer drug, which targets cancer cells' energy metabolism, both its high glycolysis and mitochondrial oxidative phosphorylation.

Professor Pederson and associates at the Johns Hopkins University, School of Medicine, report a bench side discovery that led to the effective bedside treatment of a cancer patient.[21] Targeted delivery of anticancer drugs is made difficult by a series of biological barriers that impede the drugs from reaching the target. Researchers from the University of Sciences and Technology, China, report a stimuli-responsive clustered nanoparticle to systematically overcome multiple barriers by sequentially responding to the endogenous attributes of the tumor microenvironment. They have shown that, once the cluster accumulates on the tumor cells, the intrinsic tumor extracellular acidity would trigger the discharge of platinum prodrug-conjugated poly (amidoamine) dendrimers. They claim that such structural alteration in size greatly facilitates tumor penetration and cell internalization of the therapeutics.

Tumor Microenvironment (TME)

Pancreatic cancer is a devastating disease, the 5-year survival is less than 5%, and most deaths are due to metastatic disease.[22] The pancreatic tumor environment comprises of tumor cells and a variety of stromal or non-malignant cells, including stellate cells, inflammatory and immune cells, blood vessels, extracellular matrix proteins, cytokines, growth factors, and tumor-derived exosomes.

A study of 52,728 patients with pancreatic cancer (Surveillance, Epidemiology and End Results; SEER) revealed that the rate of distant metastasis increased in a linear fashion with the increasing size of the tumor. Cancer stem cell theory proposes that solid tumors contain a small population of tumor-initiating cells or cancer stem cells that are responsible for tumor initiation. Epithelial-mesenchymal transition (EMT) involves the expression of adhesion molecules, acquisition of an invasive phenotype, which promotes cellular disassociation, degradation of the basement membrane, and acquiring drug resistance. Several transcription factors (Snail, Slug, and Twist 1) promote the activation of EMT, whereas EMT is characterized by downregulation of epithelial markers (E-cadherin, Vimentin, and Fibronectin). Inflammation seems to be a major driver of EMT in pancreatic cancer cells. Inflammation also seems to modulate Kras (one of the genes involved in the epidermal growth factor receptor pathway) and drive tumorigenesis. Furthermore, the fibroinflammatory response seems to influence the epigenome and metabolome, including Kras targets (Csf2, Rrm2, and Sc4mol). Some of the potential growth factors include connective tissue growth factor (CTGF), hepatocyte growth factor (HGF), insulin-like growth factor (IGFs), and interleukin-6 (IL-6), involved in Ras-MAPK, MYC, and STAT signaling.[22] Exosomes are vesicles that are released from the cells to provide intercellular communication, containing DNA fragments, mRNAs, and miRNAs.

Tumor Suppression

There is emerging interest in metabolic pathways to tumorigenesis. The tumor cell and tissue metabolism seem to be far greater than normal cell and tissue metabolism. Therefore, the importance of altering tumor tissue metabolism has emerged as a crucial part of the current cancer research. Many tumors can adopt a low-glucose consumption strategy by utilizing alternative energy sources such as fatty acids, amino acids, and lactate. Most cancers (90%) exhibit the "Warburg effect," showing a significant increase in glycolysis, even in the presence of oxygen. Professor Peter L. Pederson and associates at Johns Hopkins School of Medicine have demonstrated that 3-Bromo-pyruvate (3BP), a small molecule, can "trick" the cancer cells and enter like a trojan horse and deplete their energy metabolism.[23] Cancer cells that exhibit the "Warburg effect" pump out lactic acid through a transporter. The number of these transporters in cancer cells is much greater than in normal cells. Therefore, 3BP, which mimics the lactic acid in its chemical structure, enters the cancer cells preferably via this transporter and destroys them. It can be delivered to cancerous tumors via various routes. We have been exploring opportunities to test the efficacy of this therapy in India as a US-India bilateral research project.

An active tumor needs plenty of energy and nutrition to maintain accelerated growth, which is common in tumor progression. Neoangiogenesis is a key process to attain the needed vasculature in the tumor environment. Therefore, researchers are focusing their efforts on developing angiogenesis inhibitors as a desirable anti-cancer strategy.[24] Because of this focus, several regulatory and signaling molecules modulating angiogenesis are of interest, including growth factors (VEGF, PDGF, FGF, EGF), receptor tyrosine kinases, transcription factors such as HIF, as well as signaling molecules like MAPK and P13K. The ability of a tumor to induce neoangiogenesis is termed "angiogenic switch", which happens when the tumor needs extra vascularization to meet the nutritional demands of the rapidly growing tissues. During that time, pro-angiogenic growth factors bind to receptors on endothelial cells, stimulate vasodilation, permeability, and secrete matrix metalloproteinases. Once these cells are detached, they migrate and proliferate, and form new branches from the existing vasculature. Combination of angiostatin and endostatin gene transfer seems to induce synergistic antiangiogenic activity in vitro and antitumor efficacy in leukemia and solid tumors in mice. There is a great demand for pro-/anti-angiogenesis medicines to treat ischemic strokes, brain tumors, and neurodegenerative diseases. Researchers from the National Cancer Institute of NIH conclude, "Initial trials of putative anti-angiogenesis inhibitors have shown some promise in cancer, although this has not always translated to the clinic."

Personalized Cancer Therapy

Personalized, Chimeric Antigen Receptor (CAR) T-cell gene immunotherapy for aggressive pediatric blood cancers has been hailed as transformative in Pediatric Medicine. CAR-T cell therapy approach was first developed by Dr. Carl June, a Professor of Pathology and Laboratory Medicine, Perelman School of Medicine at the University of Pennsylvania. CAR-T

cell therapy genetically modifies patient's immune cells to make them seek out and kill leukemia cells. Physician-scientists of Children's Hospital, of Philadelphia (CHOP) presented their work on Kymriah ® (tisagenlecleucel, formerly CTL019)- the first-ever U. S. FDA approved personalized CAR-T cell gene therapy for aggressive blood cancers at the 60th American Society of Hematology meetings in 2018. An updated, longer-term analysis of ELIANA, the first global CAR-T cell therapy trial of Kymriah in children and young patients, showed an 82% remission rate within three months and 62% survival at 24 months. "Our unrivaled immunotherapy program treated the first, and now more patients than any other pediatric institution in the world, with this immunotherapy," says Stephen Hunger, Chief of the Division of Oncology and Director of the Center of Childhood Cancer Research at CHOP. Immunotherapy has shown success in 15 different types of cancers, including lung cancer, head and neck cancer, bladder cancer, kidney cancer, and Hodgkin lymphoma. More than 1,000 immunotherapy clinical trials are underway across the country.

Suzanne Topalian, associate director of the Bloomberg-Kimmel Institute for Cancer Immunotherapy at Johns Hopkins School of Medicine, led a team that contributed to the discovery that many cancers "put the brakes" on the body's immune cells, that would normally attack a tumor and destroy it. Dr. Topalian and associates developed a class of drugs called immune checkpoint (ICP) inhibitors that take the brakes off the immune system and give a second chance for the immune system to fight cancer. Immunotherapy helps a person's own cells to attack their cancer. Therefore, it is personal medicine approach therapy at its best. However, the real challenge is to find out why the majority of people with cancer do not respond to immunotherapy drugs. There is a great effort to find out who will and who won't respond to immunotherapy treatments. The team at Johns Hopkins has identified biomarkers in a small group of patients, which will indicate who will respond well to checkpoint blockers. Currently, we know very little about the various actors that modulate the tumor microenvironment and the pathways that modulate various immune reactions. However, cancer researchers say that the pace of immunotherapy development is' truly breathtaking', and this momentum needs to maintain in order to achieve success soon. Three decades ago, researchers at the National Cancer Institute (NCI) of NIH started working on CAR-T cell treatments painstakingly and demonstrated the benefits of this approach in the treatment of a variety of cancers. What started three decades ago as a speculative idea in these laboratories is now common practice in hospitals around the world. In a short overview like this, it is difficult to discuss all aspects of Immuno-oncology. Readers are urged to refer to original articles on this topic as well as to the extensive review articles.[25]

Despite the advances made in cancer immunotherapy, a great many challenges still exist. First and foremost is the inability to predict treatment efficacy and patient response to various customized treatments. We need to develop knowledge about additional biomarkers for risk assessment and prediction of success rates. We also need to develop clinical protocols based on the information available from large databases, to optimize the therapies and efficacy of such personalized therapies.

The fact that to date immunotherapies have demonstrated efficacy in a minority of patients indicates that we do not fully understand the multiple molecular mechanisms that modulate tumor biology, growth, and metastasis. The observed variability in patient response to immunotherapies may be attributed to a lack of information on a variety of biomarkers that modulate the tumor biology, tumor heterogeneity, tumor metastasis, variability in tumor type and stage of development, treatment history, and underlying immunosuppressive biology of the tumor.[26] Personalized drug combinations seem to be the futuristic approach to cancer immunotherapy. To achieve such a degree of success, the clinicians need information on mutation profile, genetic signature, epigenetic modifications of immune and tumor cells, antibody response, biomarker profile, immune cell characterization, and predictive genetic markers and the ability to analyze such massive data and come up with appropriate treatment protocols to enhance the success rates.

Gene Expression and Tumor Progression or Regression

When considering gene expression and tumor biology, two types of mutations modulate cancer cells; tumor suppression genes, which inhibit cell growth and division, and proto-oncogenes, which accelerate cell growth and division. Gene expression is regulated during transcription and RNA processing. The transcription control regions of protein-encoding genes include the core promoter, where RNA polymerase-11 binds the proximal and distal promoter, responsible for gene expression regulation, and the enhancers and silencers.[27] Transcription factors transduce the proliferation signals elicited by growth factors. It is well established that human oncogenes encode transcription factors, which are prevalent in neoplasias (MYC, MLL, PML-RARa). The most prominent tumor suppressors (p53) are transcription factors. Studies with carcinogens have demonstrated that changes in DNA methylation, histone acetylation and methylation, non-coding RNAs, post-translational modifications are all epigenetic promoters of tumor progression.[28] In order to use the high-throughput data available on tumor promoters, suppressors,-resulting gene expressions, modulation of transcription factors, growth factors, and various signaling mechanisms, appropriate software analytics and integrative algorithms are needed. This brings the need for a multidisciplinary approach to cancer management.

Cells become cancerous after mutations accumulate in various genes that modulate cell proliferation. In normal cells, hundreds of genes control the process of cell division and normal growth. Studies by researchers at Cancer Genome Project, Wellcome Trust Sanger Institute, UK, have demonstrated that most cancer cells possess 60 or more mutations. Growth-promoting genes like signaling protein Ras, are among the most mutated in cancer cells, promoting the formation of cells that will be strongly stimulated by growth receptors. Some anticancer drugs, for instance, work to counteract these effects by blocking the action of growth-promoting signals. The well-known breast cancer drug, Herceptin, blocks receptor tyrosine kinase (RTKs). At the same time, mutations that lead to the

suppression of cell proliferation are known as tumor suppressor genes. Many cancer cells have two copies of the gene that codes for p53, a multifunctional protein that senses DNA damage and acts as a transcription factor for checkpoint control of genes. If the checkpoints are missed, or repair genes are damaged, then the rate of damage increases in the tumor microenvironment. Tumor molecular profiling and analysis of the mutational landscape seems to have become a fundamental component of precision oncology.[29] Genomically-guided clinical trials have begun to evaluate the efficacy of approved investigational molecularly targeted therapies, for distinct tumor types with shared genetic features.

Several studies have demonstrated the role of the tumor microenvironment in the modulation of tumor progression and resistance to therapies. According to Dr. Yu Sun of Shanghai Institute of Biological Sciences, China, the TME decreases drug penetration confers genetic mutations and epigenetic changes, collectively modifying disease mortality and disturbing clinical indexes.[30-33] Intrinsic mechanisms that are responsible for this tumor resistance are enhanced drug efflux, blunted apoptotic signaling, increased metabolic activity, loss of specific oncogenes, a gain of stem cell plasticity, strengthened DNA damage machinery -promoted by mutation-selective and tumor heterogeneity. The TME comprises carcinogenetic cells, cancer-associated fibroblasts (CAFs), cancer-associated DNA (cDNAs), immune cells [T and B lymphocytes, tumor-associated macrophages (TAMs), and natural killer cells], the vascular system, and the extracellular matrix (ECM, including secreted cytokines, chemokines, metabolites, and exosomes). The transformed cancer cells seem to interact with stromal cells in the TME and promote tumor resistance. There are many signaling pathways responsible for therapeutic resistance by tumors. A well-thought-out therapy protocol should consider the multiple mechanisms involved in tumor resistance to therapy and optimize the therapies accordingly.

Gene Editing Tools and Emerging Therapies

The discovery of gene-editing tools has extended our ability to modulate genetic defects, treat diseases, and develop more accurate cellular and molecular therapies.[33] Since the discovery of CRISPR-Cas endonuclease as a programmable-guide nuclease, gene editing with engineered nucleases has rapidly developed into emerging therapies.[34] Clustered regularly interspaced short palindromic repeats (CRISPR), 'acts as molecular scissor', to find specific bits of DNA inside the cell (Fig 2). When CRISPR Cas9 protein is added to a cell, along with a piece of guide RNA (gRNA), the Cas9 proteins bind to the gRNA and move along the strands of DNA until it finds a 20 -DNA-letter sequence that matches the part of the gRNA sequence. The gRNA is made up of two units: crisprRNA (crRNA), a 17-20 nucleotide sequence complementary to the target DNA, and a tracrRNA, which serves as a binding scaffold for the Cas nuclease. Customized Cas proteins also have been developed, which do not cut the DNA, but merely turn on (CRISPRa) or off (CRISPRi). Some of the salient ongoing studies using this gene-editing tool include treating hemoglobinopathies (aiming to treat β-thalassemia and sickle cell disease with gene-edited hematopoietic stem cells), editing cells inside the body to treat genetically defined diseases, creating next-generation cell therapies for cancer, improving stem cell use for tissue engineering, transplantation, and other therapies through gene editing. The three main categories of genetic editing that can be performed with CRISPR include disruption of an unwanted segment of DNA (CAR-T Therapies), deletion of an unwanted section of DNA (treatment of hemoglobinopathies), or addition of a fragment of a DNA. Opportunities are abundant as more than 10,000 monogenic diseases are caused by single mutations in individual genes.

Precision medicine, personalized medicine, and genetic engineering are emerging new fields that have gained a lot of popularity, encouragement, and funding in recent years. CRISPR editing technology may be used in combination with Cas9 endonucleases for the development of new diagnostic and treatment strategies. The most used CRISPR system is the Type11 CRISPR-Cas system, which is made up of three main components, including the trans-activating crRNA (tracrRNA), the crisprRNA, and an endonuclease. Since the time the cancer researchers realized that changes in the DNA cause cancer, they have been exploring ways to correct those cancer-inducing mutations by manipulating the DNA. A game-changer occurred with the realization that a gene-editing tool could alter the DNA in human cells like a very precise, 'easy-to-use' pair of molecular scissors. As shown in Figure 2, the gene-editing tool consists of a gRNA and DNA-cutting enzyme (Cas9). The researchers design the guide RNA to mirror the DNA of the gene to be edited (Target). The gRNA pairs with the Cas endonuclease and leads the Cas to the target gene's DNA. When the gRNA matches up with the target gene's DNA, Cas cuts the DNA. There exist some concerns that this tool may cut DNA out of the target area (off-target). Yet another concern is, what if it starts cutting random parts of the genome? Finally, getting the gene-editing tool into the cells itself could be a challenge at times.

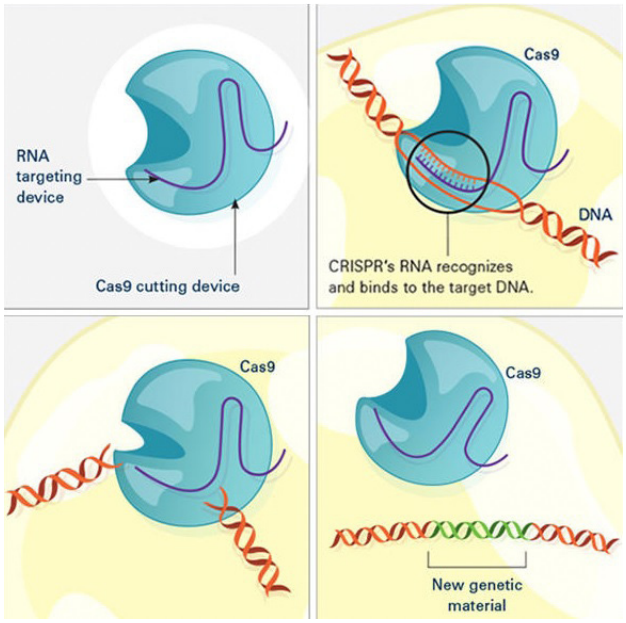

Fig. 2. *Schematic Diagram of How CRISPR Gene-Editing Tool works.*

(Credit: National Institute of General Medical Sciences, the US National Institutes of Health)

An especially exciting area of innovation in cancer therapy is cell-based therapy, a treatment that uses a patient's own immune cells, or the immune cells from another individual, to engineer potent killer cells and help fight the disease. According to Tamas Oravecz, Vice President, Cell Therapy Platform and Discovery, Janssen R & D, "Cell therapy has the potential to be a one-time, singular treatment that provides benefits throughout a patient's entire lifetime." Because "cell therapy provides the body with 'immunological memory,' which means that a person's immune system stores information about a certain stimulus, like a cancer cell, so it destroys it when encountered again." In brief, the technology involves procuring immune cells from the patients adding laboratory engineered (genetically engineered/ molecular engineering) chimeric antigen receptor (CAR) to the T cells. This modification turns T cells into CAR- T cells that attack the patient's cancer cells. A large number of CAR-T cells are produced in the laboratory and are given to the patients by infusions a few weeks after therapeutic protocols are initiated. Another area of great interest is to use immune cells from healthy donors. This approach is faster and less expensive to manufacture than CAR-T using patients' own cells. Fate Therapeutics, in partnership with Janssen R&D, uses induced pluripotent stem cells (iPSCs) to mass-produce therapeutic cells for cancer therapy. Cellular therapy is considered the therapy of the future

To discover gene targets in cancer cells whose loss enhances anti-tumor immunity, the researchers at the Dana-Farber Cancer Institute, Boston, constructed a murine lentiviral CRISPR-Cas9 knockout (MusCK) library.[35] This library included 5 sgRNAs for each of the over 4500 genes implicated in tumor initiation progression and immune modulation, which contains both custom-designed short crRNA sequences fused to the scaffold tracrRNA sequence. The principal component analyzed in these studies was an abundance of expression of sgRNA under each condition in 4T 1 cells. T cell-deficient hosts had the biggest tumors, and immune-competent hosts had the smallest. Functional Genomic screening using CRISPR-Cas9 resulted in the discovery of a novel cancer target. CRISPR screens in cancer cells co-cultured with T cells have identified genes that are; modulators of tumor immunity, novel immune-oncology targets, multiple regulators of PD-L1, Loss of Ptpn 2, and Adar1as an enhancer of tumor sensitivity to immunotherapy. These studies have demonstrated that E3 ligase Cop1 is a modulator of macrophage infiltration, secretion of chemokines, and enhancer of anti-tumor immunity. Furthermore, such studies demonstrate the effectiveness of in vivo CRISPR screens in identifying cancer-cell-intrinsic TME regulators.

To successfully apply gene-editing tools for in vivo studies, one needs an efficient and robust screening method with high levels of CAS9 and reliable single guide RNAs (sgRNA). In a recent study, German and French collaborators provide a sgRNA design tool that selects high-fidelity sgRNAs and Cas9 that expresses high levels of Cas9 in transgenic mouse lines.[36] The researchers were able to achieve average knockout efficiency of 80% in primary B Cells. The authors developed a Cas9-transgenic mouse with ubiquitous expression of Cas9 by crossing Rosa 26-with Cre-deleter mice and evaluated the expression of Cas9, in the resulting R-26-Cas9iGFP/+animals. All hematopoietic populations exhibited high GFP levels, including hematopoietic cells in the bone marrow and various lineages, such as T, B, and myeloid cells. They also studied the inactivation of transcription factors (TFs), known to be important for B cell differentiation. Expressing of sgRNA targeting for TFs, led to a strong survival disadvantage of Cas9-expressing cells, showing the decreased percentage of GFP+cells. These studies further demonstrated that the following TFs: Prdm 1, Xbp1, Irf4, Pou2af 1, and Myc are important for B-cell survival, proliferation, and terminal differentiation. To identify genes of importance for B-cell activation, they selected 83 candidate genes upregulated during plasma cell differentiation, designed sgRNA for each gene, and studied using GFP+ and CD138+ cells as readouts. Take-home lessons from these studies are that the screening system developed by these researchers leads to clear and consistent functional results, permitting the use of small-scale screens in primary mouse cells without the need for high numbers of sgRNA genes or deep sequencing.

Despite the fact the targeted nucleases are powerful tools for modulating gene alterations, there are some concerns that researchers have about the editing tool cutting 'off target' regions of the DNA. Researchers from the Massachusetts Institute of Technology (MIT) have described a set of tools for Cas9-mediated genome editing via nonhomologous end joining (NHEJ), or homology-directed repair (HDR), in mammalian cells.[37] To minimize off-target cleavage, they describe a double-nicking strategy, using Cas9 nickase mutant with paired guide RNAs. They have shown that by fusing the crRNA with tracrRNA one can develop a chimeric, single-guide RNA (sgRNA) Cas9 and can be directed toward any target of interest. They predict that by directly injecting sgRNA and mRNA encoding Cas9 into embryos, one can enable rapid generation of transgenic mice with modified alleles. In this study, they have demonstrated simultaneous targeting of two human DYRK1A and GRIN2B loci at efficiencies of 65-58% for each locus. The authors provide extensive details of the protocols used for each of their experiments, also address concerns, questions, which are frequently asked (discuss.genome-engineering.org), and answers to some of the questions from their personal experience.

The first clinical trial in the United States to test a CRISPR-made cancer therapy was launched in 2019 at the University of Pennsylvania. The study, funded by the National Cancer Institute (NCI) of NIH, is testing a type of immunotherapy in which patients' own immune cells are genetically modified to better 'see' and kill their cancer. According to NCI news of July 27, 2020, therapy involves making four genetic modifications to T cells, immune cells that can kill cancer. First, the addition of a synthetic gene gives the T cells a claw-like protein (called a receptor) that 'sees' NY-ESO-1, a molecule on some cancer cells. Then the CRISPR is used to remove three genes; two that can interfere with the NY-ESO-1 receptor and another that limits the cancer-killing abilities. The product that was developed to achieve these desired features was grown in large quantities and infused into patients. This procedure was tested in two patients (multiple myeloma and sarcoma) and found safe for use. The tumors stopped growing for a while but resumed growing later. Other clinical studies of CRISPR-made cancer treatments are underway. A few trials are testing CRISPR-engineered VAR-T cell therapies, another type of immunotherapy. One company is

testing CRISPR-engineered CAR -T Cells in people with B cell cancers and people with myeloma.

Cancer immunotherapy has started to undermine melanoma, non-small cell lung cancer, kidney cancer, head and neck cancers, and Hodgkin's lymphoma. There are tremendous opportunities in this field of research. In this overview, we have just discussed a few examples of emerging therapies. Readers are urged to consult original articles and monographs on this subject.[38-43]

There is a false belief that the availability of vast amounts of multi-omics data generated from large cohorts of the population represents a unique opportunity for the development of precision medicine. Researchers from Harvard Medical School rightly point out that it is the algorithms encoding causal reasoning domain (e.g., clinical and biological) knowledge that will facilitate the transformation of medicine to precision medicine.[43,44] In their review of this topic, they discuss principles of data science and suggest three defining tasks: 1) Association prediction, 2) Intervention, 3) Counterfactual causal interference. They conclude, "As machine learning algorithms become ubiquitous tools to handle quantitatively "big data," their integration with causal reasoning and domain knowledge is instrumental to qualitatively transform medicine, which will, in turn, improve health outcomes of patients." Mexican researchers have studied the association between genetic variants (SNPs), previously associated with COPD and IPF (FAM13A), rs2736100 (TERT), rs2076295 (DSP), 128 rs5743890, and rs111521887 (TOLLIP), and the risk of CPFE in a mestizo Mexican population.[45] They point out a differential genomic profile between COPD patients with emphysema, IPF, and CPFE that could represent different underlying mechanisms involved in the pathogenesis of the three diseases.

Like the ideas that exist about the role of precision medicine, there is a widespread belief that the underlying heterogeneity of many diseases suggests strategies for treating an individual with a disease, alternately a possibility for monitoring or preventing a disease. Because of such a belief, there is an increased expectation that treatments should be tailored or 'personalized' to that individual's unique biochemical and physiological profile.[46] We have discussed a few successful cases where drugs were developed to treat individuals with incurable conditions. The ubiquity of smartphones and smartphones 'apps' has created a concept of 'digital therapeutic' or digital healthcare.[47] Many digital 'apps' have undergone evaluation for their ability to engage users and provide benefits.[48] The US/FDA has created guidelines for registering digital therapeutics as insurance-reimbursable, approved health technologies. Dr. Kevin Dozo of Integrative Biomedical Sciences, Faculty of Health Sciences, University of Cape Town, South Africa, summarizes the progress in emerging cancer therapies in his recent review, "Although there may be successful stories to tell, evidence/data showing that cancer treatment (chemotherapy, radiotherapy, immunotherapy) itself and the involvement of tumor stromal components can result in resistance is discouraging. This is akin to unsuccessful attempts at removing rogue regimes and can cause the hardening of such regimes."[49] Researchers from the Center for Open Science University of Virginia attempted to replicate 193 experiments from 53 papers, but experienced reproducibility challenges at every phase of the research lifecycle. They also indicate none of the 193 experiments were described completely enough to design a replication protocol, without requesting clarifying details from the original authors. [50]

Conclusions

The first therapeutic protein molecule, other than antibodies (1986), is insulin (discovered in 1921), the first recombinant biopharmaceutical, approved in the USA in 1982 as an interchangeable biosimilar. Emerging innovations and advances in molecular biology, genomics, cellular and molecular engineering have dramatically increased the discovery and development of new and novel biopharmaceuticals. Big Pharma companies now have the capability to develop both small molecules as well as blockbuster biologics. Advances in biotechnology, genomics, progress made in decoding of the RNAs and DNAs, availability to gene-editing tools, stem cell, and gene therapy applications have given researchers a great opportunity to develop precision medicine as well as personalized medicine. Having said that, we must inform the readers that the development of mRNA vaccines from the time the data on the SARS-CoV-2 genome was made available to a working vaccine was nothing short of a modern miracle. Success made in the rapid development of these technologies has given researchers, pharma companies, and clinicians limitless opportunities for RNA therapeutics for use as the standard care for many diseases, especially for the development and progress of personalized medicine. Scientists have used the word 'limitless' to describe the future of these emerging therapeutics.

High-throughput analytical technologies, 'big data,' artificial intelligence, and machine learning applications have revolutionized medical research. At the time of this writing, the largest application of genetic testing in medicine occurs in newborn screening. There are suggestions and speculations that genomic sequencing could become a standard component of newborn care. Currently, we still have a limited understanding of strategies and protocols for managing genomic data, and analyzing the massive amounts of data available for developing useful information for clinical applications. This is true for other emerging areas such as genomics, epigenomics, genome-association study (GWAS), as well as for studies on microbiomes, proteomics, metabolomics, transcriptomics, and multi-Omic platforms. Despite the advances made in these areas, routine implementations of data on a population scale require advances in data acquisition, analysis, and cost-effectiveness. Clinicians would like to see the progress made in these areas to a level when they can just look at big-data banks and find specific causes for a disease (clinical decision support system), or a cluster of diseases, pick up a drug or a combination of drugs, off the shelf or find an approved safe efficacious therapy for such a disease that will work. We are not there yet.

In a Lancet Editorial (May 15, 2021) titled, '20 Years of precision medicine in oncology' the editor concludes, "But the past 20 years have been colored by advanced scientific conceptual breakthroughs, without adequate focus on the basic building blocks of implementation, and the practicalities of patient

care." In a way, this editorial stresses the need for a solution that is formulated on evidence-based observations, rather than depending upon sporadic discoveries and innovations. A century ago, the discovery of a biomolecule, 'insulin,' played an important role in understanding the role of hyperglycemia and how to control this altered glucose metabolism. The discovery of another set of biomolecules, 'antibiotics' gave us the ability to control to a great extent infectious diseases. Then came the discovery of the structure of a macromolecule of great importance to life itself, DNA. Knowledge gained over the years gave us an opportunity to reconstruct and reprogram a messenger RNA, to use as a therapeutic agent at the shortest possible time. This technology of mRNA therapy used not only a biomolecule but an 'entire cargo' of biologics to achieve safe delivery of a therapeutic messenger. The ability to decipher the biological code of the nucleotide sequence and reconstruct the desired protein/peptide messengers has increased our capabilities to develop a host of novel emerging therapeutics. The discovery of a novel 'molecular scissor,' CRISPR-Cas9, - a simpler, faster, precise, gene-editing tool, will change the way we perform cancer research as well as gene therapies.

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