International Journal of Biomedicine 11(3) (2021) 241-249
http://dx.doi.org/10.21103/Article11(3)_RA1

INTERNATIONAL JOURNAL OF BIOMEDICINE

REVIEW ARTICLE

Biomedicine in the COVID Age: Opportunities, Responses, and Challenges

Gundu H. R. Rao, Emeritus Professor

Laboratory Medicine and Pathology
Director of Thrombosis Research, Lillehei Heart Institute
University of Minnesota, Minneapolis, USA

Abstract

According to one of the earliest definitions, *biomedicine* means "clinical medicine based on the principles of physiology and biochemistry." Clinicians for quite some time preferred the use of the term *medical research* to describe what they considered the clinical findings pertaining to various issues related to clinical studies. Since the time when the basic molecules of life, deoxyribonucleic acids, were characterized and the genetic code elucidated, there has been great excitement, anticipation, and promise for the development of precision and personalized medicine. However, the progress has been considerably slow and at times disappointing. The unprecedented coronavirus disease created a worldwide panic and exposed all our weaknesses and unpreparedness. It also demonstrated a global demand for better public health infrastructure and preparedness to combat future pandemics. This unprecedented public health crisis acted as a great stimulus for putting together a concerted effort to develop vaccines. According to the experts, the time was right and within 48 hours after the information on the SARS-CoV-2 genome was posted, Moderna scientists had on paper a workable mRNA, which would code for the spike protein. The immune engineers at Moderna as well as BioNTech were able to put together a lipid nanoparticle delivery system for safe delivery of this precious cargo to the appropriate cells. Professor Cody Meissner at Tufts University School of Medicine in Boston says, "It is absolutely astonishing that this happened [COVID Vaccine development] in such a short time—to me, it is equivalent to putting a person on the Moon." It is indeed a great achievement, and it demonstrated the power of basic science and emerging technologies. The extraordinary success of mRNA vaccines has opened new avenues for mRNA-based therapies. mRNAs, siRNAs, and non-coding miRNAs will play a very important role as novel therapeutics soon. Furthermore, this success has acted as a catalyst for ongoing work on the use of small RNAs for therapeutic purposes. Having said that, I must say that there are a great many challenges that need to be addressed.**(International Journal of Biomedicine. 2021;11(3):241-249.)**

Key Words: SARS-CoV-2 • COVID-19 • biomedicine • vaccine

For citation: Rao GHR. Biomedicine in the COVID Age: Opportunities, Responses, and Challenges. International Journal of Biomedicine. 2021;11(3):241-249. doi:10.21103/Article11(3)_RA1

Introduction

According to the Centers for Disease Control and Prevention (CDC), the 1918 influenza (H1N1) pandemic was the most severe pandemic in recent history. It is estimated that 500 million people or one-third of the global population were infected. The number of deaths was estimated to be at least 50 million worldwide. With no vaccines to protect from this virus and no antibiotics to treat the secondary bacterial infections, control efforts worldwide were limited to non-pharmacological interventions. As of this writing, global COVID-19 cases surpass 200 million with 4 million reported deaths. Compared to the 1918-1919 pandemic, we have done much better in terms of the number of deaths, which is less than 10% of what occurred with the H1N1 pandemic. We could have done much better, if only there was a global public health regulatory protocol in place, and the majority of individuals practiced public health best practices to prevent getting infected with this virus. The big difference in mortality also reflects on the advances made in biological sciences, immunology, biotechnology, and the development of a multidisciplinary approach to biomedical

Contact Information: *Emeritus Professor Gundu H. R. Rao, Laboratory Medicine and Pathology, Director, Thrombosis Research, Lillehei Heart Institute, University of Minnesota. E-mail: gundurao9@gmail.com*

sciences. In this short review, we will briefly discuss the role of biomedicine, basic sciences, and emerging technologies in the improvements made in healthcare delivery. We will also discuss the difference between the top-down approach and the hypothesis-driven approach to problem-solving strategies. The unprecedented pandemic of SARS-CoV-2 has opened great opportunities for the development of novel therapeutics. Various stakeholders of healthcare delivery have responded in a great way during this pandemic. There are several challenges ahead for overcoming this pandemic as well as for developing strategies to face future pandemics.

SARS-CoV2, a sixteen trillion-dollar killer virus, has caused unprecedented health and economic crisis worldwide.[1] The novel coronavirus (2019-nCoV, SARS-CoV-2) epidemic first broke out in December of 2019 in Wuhan, China, and has been spreading worldwide like an uncontrolled forest fire.[2] Globally, 200 million individuals have been found to be COVID-positive with 4 million COVID-related deaths. The John's Hopkins COVID-19 tracker lists the following countries as the top five, in terms of COVID-related deaths: USA (607,865), Brazil (535,838), India (411,406), Russia (142,877), and France (111,597). According to the Chinese researchers, who were the first to describe this disease, the clinical manifestation of COVID-19 is heterogeneous.[3] The new variant delta seems to mimic common cold-like symptoms and thus presents an additional complication for early detection and quarantine of the infected individuals. The most prevalent comorbidity in China was hypertension (169%), followed by diabetes (8.2%). The new variant B.1.617.2, which was first identified in India, is found to be more transmissible than the earlier variants and is spreading fast in more than 75 countries.

Why is COVID-19 so controversial and at times so very confusing? According to an article in the Atlantic, "The confusion partly arises from the pandemic's scale and pace. Worldwide, at least 3.1 million people have been infected in less than four months. Economies have nose-dived. Societies have paused. In most people's living memory, no crisis has caused so much upheaval so broadly and so quickly. "We've never faced a pandemic like this before, so we don't know what is likely to happen or what would have happened," says Zoë McLaren, a health-policy professor at the University of Maryland at Baltimore County."

The rapidity by which this killer virus rampaged various countries caused an unprecedented healthcare crisis. No country was prepared for this kind of invasion of its people by an infectious agent. The suddenness of such a great wave of infection and deaths has had a devastating effect on existing healthcare delivery. This tsunami of COVID has exposed the unpreparedness of healthcare facilities. Suddenly the countries have realized deficiencies in healthcare infrastructure, human and material resources to handle such an unprecedented tsunami of COVID. There are not enough hospital beds, trained critical care workers, pulmonologists, doctors, nurses, ICU units, ventilators, medical supplies, medical-grade oxygen for therapeutic purposes, and general guidelines for public safety.

In an unprecedented effort, hundreds of scientists, clinicians, public health workers as well as laymen/women worldwide, are in a race against time, to answer myriad questions raised by individuals who are under panic, to develop better diagnostic tools, (preservatives, reactants, and characterization technology), novel drugs, interventions (pharmacological and nonpharmacological) and vaccines for SARS-CoV-2 virus.

According to the Coronavirus Disease 2019 (COVID-19)-Associated Hospitalization Surveillance Network (COVID-NET)(COVID-NET), which includes laboratory-confirmed cases in 99 counties in 14 states, the hospitalization rate increased with patient age. Those aged 65 years and older were admitted at a rate of 13.8%, with 50-64-year-olds at 7.4%, and 18-49-year-olds at 2.5 %. Hypertension was the most common morbidity among the oldest patients, with a prevalence of 72%, followed by coronary artery disease (50.8%) and obesity (41%). Is this the common pattern of comorbidity in all geographical areas? Not necessarily. The first largest study conducted in New York City concluded that pre-existing conditions such as hypertension and diabetes were highly prevalent, and the pattern was similar, to the data reported from China.[4] According to the WHO, in 2016 more than 1.9 billion adults, 18 years or older, were overweight. Of these over 650 million were obese. Over 340 million children and adolescents, aged 5-19, were overweight or obese. These obese individuals are at a higher risk for coronavirus disease. In the first meta-analysis of its kind, published on 26 August 2020, in Obesity Reviews, researchers pooled data from scores of articles capturing 399,000 patients. They found that people with obesity who contacted SARS-CoV-2 were 113% more likely than people of healthy weight to land in the hospital, 74% more likely to be admitted to an ICU, and 48% more likely to die.[5]

The interaction between the SARS-CoV-2 spike glycoprotein(S) and the ACE2, the cellular receptor for SARS-CoV-2, seems, to be the most critical point for the entry of the virus into the host cells. The high affinity of the S protein to the human ACE2 seems to facilitate the spread of this virus in human populations. This transmembrane spike (S) glycoprotein, forms homotrimers protruding from the viral surface and comprises two functional subunits, responsible for binding to the host cell receptor (S1subunit), and fusion of the viral and cellular membranes (S2 subunit). For all viruses of this group, the S unit is further cleaved by host proteases, at the S2 site of the fusion peptide. According to experts, four important enzymes are essential for the pathogenesis: the S-protein that facilitates virus entry through the ACE2 to the host cell surface receptor, the major protease of CoV3Clpro, the papain-like protease (PLpro) involved in the assembly of new viruses, and RNA-dependent polymerase (RdPr) that facilitate CoV RNA genome replication. The proprotein convertase family (PC) is composed of nine serine-secreting proteases and is widely involved in regulating various biological processes in normal and disease states. Therefore, the PC family, especially Furin can be considered the key player that mediates the maturation of S protein processing and recognition of membrane proteins.[6]

Interventions

In the absence of a cure, the only option people have to protect from this killer virus is to hide from it at any cost.

Public health experts worldwide advocated the use of facial covering, social distancing, better detection of the infected individuals (contact tracing), and strict quarantine of infected individuals. Those countries, which enforced such public health best practices, fared well, compared to those that did not. Researchers from the UK and the USA based on the data from 149 countries found that implementation of any physical distancing intervention was associated with an overall reduction in COVID-19 incidence of 13%. Data from 11 countries suggested overall effectiveness when school closures, workplace closures, and restrictions on mass gatherings were in place.[7] When various types of non-pharmacological interventions were compared, earlier implementation of lockdown was associated with a larger reduction in the COVID-19 incidence. According to the Global Dynamic Interventions Strategies for COVID-19 Collaborative Group, non-pharmacological interventions have been the mainstay for controlling the coronavirus pandemic.[8] This multicountry analysis demonstrated that intermittent reduction of infection rate (R) below 1 through a potential combination of suppression, interventions, and relaxation can be an effective strategy for the COVID-19 pandemic control. Unprecedented public health emergencies require unprecedented measures of public health best practices. One prime example of this approach is the Olympic Games in Japan to be conducted for the first time ever with no spectators. First Lady of the United States Dr. Jill Biden is visiting Tokyo, Japan, not as a spectator but to represent the U.S in the opening ceremony.

The unprecedented pandemic also gave tremendous opportunities for the drug discovery and development industries. Professor Cody Meissner at Tufts University School of Medicine in Boston says, "It is absolutely astonishing that this happened [COVID Vaccine development] in such a short time—to me, it is equivalent to putting a person on the Moon." "This is going to change vaccinology forever."[9]

How did this tremendous effort for drug discovery and development occur? This occurred just a few weeks after the first case was discovered in Wuhan, China, and its complete genome was characterized. Less than 24 hours after the genetic sequence was released by the Chinese investigators, researchers around the world started working on vaccine candidates. The response of the global *pharmaceutical* industries was as unprecedented as the pandemic itself. Different types of vaccines work in different ways to offer protection against the pathogen in question. The majority of the vaccines prompt our immune systems to recognize and protect the system from the virus that causes the dreaded disease. mRNA vaccines contain material from the virus that provides the instructions for making harmless spike (S) proteins that are unique for viral transmission. Protein subunits used in vaccines include harmless pieces of the viral protein that are an integral part of the virus instead of the entire germ. Vector vaccines contain a modified version of a different virus than one that causes COVID-19, but once the viral vector is inside the cells, the genetic material of COVID-19 gives cells the instructions to make a protein that is unique to the virus that causes COVID-19. Currently, there are more than 1,604 global, active, unique therapies in clinical trials; 389 antiviral trials, 299 anti-inflammatory drug trials, 125 monoclonal trials, and 104 vaccine trials.

Biomedicine in the COVID Age

In a general review like this, it is important to emphasize the role of basic sciences in the rapid development of therapeutics. The response of the pharma industry to the unprecedented COVID-19 pandemic provides some useful examples to illustrate this role. As early as January 10, 2020, Chinese researchers revealed the draft genome of the virus implicated in the Wuhan pneumonia outbreak. Jeremy Farra, head of the Wellcome Trust wrote, "Sharing of data good for public health, great for those who did the work. Just needs those incentives and trust." Scientists at Moderna, a biotech specializing in messenger RNA, were able to design a vaccine on paper in 48 hours, 11 days before the US even had its first recorded case, according to Antonio Regalado in the MIT Technology Review 124 (2), 2021. Within six weeks, Moderna had doses of vaccine ready for animal testing. The first hurdle they faced was to develop RNA molecules that can avoid the cytokine storm. They were able to do this by using chemically modified building blocks of RNA. Once they had perfected this technology, they had to engineer the delivery system for these molecules with ample protective coating.

In an editorial in Science Advances, Philip Yeagle uses this example as powerful testimony to the critical role of basic science in support of great scientific discoveries.[10] A key component of these vaccines is the carrier molecules that deliver the required mRNA into the cells, where it can read the information and make peptides for coding the viral spike proteins. That carrier is lipid nanoparticles, with their own unique history for targeted delivery of drugs. Successful use of lipid nanoparticles led to an explosion of ideas on targeted delivery of therapeutic molecules by encapsulating molecules of biological interest.

Even though the vaccine manufacturers now have a delivery system for synthetic mRNA to the cells, the use of nanoparticles of lipids for targeted delivery needs further exploration. If the drug packaged in the lipid nanoparticles are introduced into the blood, they tend to end up in the liver—a self-cleaning organ of the body. Weissman seems to have found a way to target the nanoparticles so that they wind up inside the bone marrow. He has not published this method, as it is a patentable technology. What this all means according to the experts is, that the fatty particles packaged with messenger RNA may become a way to edit genomes at massive scales and at a reasonable cost. Last spring, Moderna's CEO, Stephen Bancel approached the government to pay for the vast manufacturing centers to make messenger RNA. Later that month, as part of 'Operation Warp Speed', the US effort to produce vaccines, Moderna was effectively picked as the national champion to build such centers. The US Government gave Moderna $500 million to develop its vaccine and expand manufacturing. Despite the great success of vaccines against the COVID-19, they will not solve the problem associated with the pandemic. At the time of this writing, we already have new mutants (Delta variant) that are more transmissible and deadly, spreading worldwide. As the virus replicates, there

would be more mutations and more variants. Just recently, the Lambda variant of Peru has been reported from South America. Furthermore, there are already discussions about the need for a booster shot for all the vaccinated individuals. Public health workers and politicians have no clue whatsoever, as to how to convince a large population, who are reluctant to get vaccinated.

Novel Approaches for Potentiation of Vaccine-Induced Immune Responses

Helping B cells to produce antibodies is a major function of CD4+ T Cells. The role of T follicular helper (Tfh) cells in the modulation of immune function and in a range of diseases has been the subject of several investigations in the last decade.[11,12] Immunologists at St. Jude Children's Research Hospital have identified a biological pathway that modulates immune cells called Tfh cells, which mature into functional components of the immune system. These findings offer promise for developing drugs that activate the metabolic pathway to enhance the effectiveness of vaccines. Their studies revealed an endogenous pathway, called the CDP-ethanolamine pathway that selectively regulated Tfh cells.[13] To elucidate this endogenous signaling pathway, researchers performed CRISPR-Cas9 screening using pooled guide RNA (gRNA) library, that targeted associated genes. To discover a possible key-control pathway, Chi and his associates used genetic techniques to delete the T-cell multiple enzymes known to be key factors of such metabolic pathway. Then they introduced the deletion engineered T cells into mice and followed infection with a virus. These experiments demonstrated that a key metabolic pathway called the CDP-ethanolamine pathway, selectively regulated Tfh cells. Wang and associates studied the innate immune responses in 63 individuals who had recovered from COVID-19 and had received mRNA vaccines.[14] Authors concluded, "that immunity in convalescent individuals will be very long-lasting and that convalescent individuals who receive available mRNA vaccines will produce antibodies and memory B cells that should be protective against circulating SARS-CoV-2 variants."

Development of Biologics

Studies in large populations of COVID-positive individuals have demonstrated, that antibodies to the receptor-binding domain (RBD) of the viral spike protein appear, within few days of infection with the virus.[15] Follow-up studies with monoclonal antibodies (mAbs) against RBD, have been shown to decrease the viral load in patients with recently diagnosed mild/moderate COVID-19 infection. Regeneron reports positive data from the COVID-19 antibody cocktail trial. This is the experimental cocktail that was infused to Ex-President Mr. Donald Trump, when he was found to be COVID-19 positive. Regeneron is conducting phase 11 and phase 111 clinical trials. Regeneron Pharmaceuticals has developed a monoclonal antibody cocktail (Casirivimab and Imdevimab) that is supposed to reduce the risk of COVID-19 with a single injection. Eli Lilly also is developing a similar injectable preparation of antibodies to treat COVID-positive individuals. The US FDA granted emergency use authorization (EUA) in May to Sotrovimab, a super-antibody against COVID-19. Companies are designing next-generation antibodies modeled on those taken from COVID-19 positive individuals, whose immune systems can neutralize any COVID-19 variant and related coronaviruses too.[16]

National Institutes of Health is exploring nanobodies to combat COVID-19.[17] Authors claim that the nanobodies may be delivered via inhalation. For the first time, researchers at the University of Pittsburg School of Medicine, have tested monoclonal nanobodies, which are smaller in size, more stable, and cheaper to produce for inhalation treatment against coronavirus infections in a pre-clinical model. The nanobody research presents a great opportunity for therapeutic applications against airborne viral pathogens.

Development of Antiviral Drugs

In recent years, there is a continuous emergence of viruses with epidemic or pandemic potential. Recent examples include Ebola, Zika, Middle East Respiratory Syndrome (MERS-CoV), severe acute respiratory syndrome coronavirus 1 and 2 (SARS-CoV and SARS-CoV-2). The unexpected COVID-19 pandemic forced advanced nations to come up with an effective vaccine in short order. Having said that, I must indicate that vaccines are not the ultimate cure. Moreover, we do not have effective vaccines for many of the viruses mentioned above. Therefore, the development of broad-spectrum as well as specific antiviral drugs is essential. The U.S government spent more than $18 billion last year funding drug makers to develop a COVID vaccine. The new program announced on June 18, 2021, by the Department of Health and Human Services will invest $3 billion to advance the development of antiviral pills to treat COVID-19 as well as future virus outbreaks.

An antiviral drug must act at one of the five basic steps in the viral replications cycle: 1) attachment to the receptor and entry into the cells, 2) uncoating of the virus to release virions, 3) promote the synthesis of new viral components, 4) assembly of newly formed components into a new live virus, 5) release of the virus from the host cells. Nucleoside analogs represent the largest class of small molecule-based antivirals, which form the backbone of chemotherapy of chronic infections caused by HIV, hepatitis, and herpes virus. Nucleobase and nucleoside analogs (NNA) require extensive cellular metabolism to be converted to active metabolites. Currently, there are 41 NNA drugs approved by the US FDA of which 14 and 27 are used in cancer and antiviral therapies, respectively.[18-20] Researchers of St. Jude Children's Hospital, Memphis TN, USA, and National Child Center for Child Development, Tokyo, Japan, have demonstrated that NUDT15 polymorphism influences the metabolism and therapeutic effects of acyclovir and ganciclovir. These studies suggest that pre-emptive genotyping of these variants may be clinically important to mitigate toxicities of this class of drugs.

As discussed earlier, studies have revealed the integral role of proteases in SARS-CoV-2 viral spread and infection. Proteases belonging to the proprotein convertase family, including furin and furin-like serine proteases seem to play a

ubiquitous role in viral entry and spread.[21] Researchers have been investigating the NSP5 main protease as a potential drug target because of its involvement in processing the proteins coded from viral RNAs.[22] Acquired immunodeficiency syndrome (AIDS) of humans is caused by lentiviruses and various lifestyle behaviors led to a pandemic in the early 1980s.[23] AIDS the fatal disease which had no cure was spreading fast and to check its rapid growth and replication novel pharmacological approaches were developed. The interventional drugs tested belonged to five different categories with varied mechanisms of action.[24] They are 1) reverse transcriptase inhibitors, 2) protease inhibitors, 3) fusion inhibitors, 4) viral entry inhibitors, and 5) integrase strand transfer inhibitors. Despite the current emphasis on global vaccine development, such therapeutic virus management approaches will be researched and investigated for SARS-CoV-2 management also.

We already have discussed the role of Spike proteins (S) in the attachment of the virus to the ACE2 receptors through subunit S1 interaction. The host protein CatB/L transmembrane protease serine 2 (TMPRSS2) has been shown to be involved in the viral entry process. There is considerable interest in screening protease inhibitors targeting the viral or host factors involved in this essential process. The replicate/transcriptase complex (RTC) is composed of different enzymes and cofactors involved in post-translational polyprotein processing, RNA synthesis, maturation, and virus assembly and egress. Therefore, these steps seem to constitute ideal targets for novel drug discovery and development. Currently, there is a tremendous opportunity for drug repurposing for the management of virus infection, entry, replication, and overall management of the disease.[25] Another important area of interest is a rapid screening of antiviral drugs for repurposing in the COVID-age. Korean researchers developed a virtual screening assay for drug repurposing for COVID-19 by a screening of 6,218 drugs using a cell-based assay.[26] They developed an advanced virtual screening technique with pre-and post-docking pharmacophore filtering. They found seven compounds capable of inhibiting SARS-CoV-2 replication in Vero cells. Some of these promising inhibitory drugs included emodin, omipalisib/remedesivir, tipifamib/omipalisib, and tipifarnib/remedesivir.

Drug Discovery and Development in the COVID Age

The success of mRNA vaccines for COVID management has opened lots of opportunities for the therapeutic applications of synthetic mRNA. At the time of this writing, five people connected to the Moderna and BioNTech are now billionaires. In 2018, in the USA as well as in Europe, two RNA-based therapies have been approved for hereditary amyloidosis. Many RNA therapies are in the developmental stage and about a dozen are being tested in clinical trials. According to the experts, RNA therapies can be sorted into three categories: those that target nucleic acids, those that target proteins of importance, and those that encode proteins.[27] The major hurdle to RNA therapy has been delivering RNA to the correct target in the correct cells—simply put, targeted delivery. Intellia Therapeutics is testing a treatment protocol that packages CRISPR into RNA, and then into a nanoparticle for delivery to the liver for the treatment of inherited diseases such as sickle-cell disease and HIV. Since the lipid nanoparticles have been successfully used for packaging RNA for safe delivery to cells, it looks like a very simple and easy method. However, according to Professor Drew Weissman of Perelman School of Medicine at the University of Pennsylvania, it took him testing 40 different carriers before finding the ideal delivery vehicle—nanoparticles made from a mixture of fats.

Successful use of mRNA for therapeutic purposes has inspired a host of ideas about how to harness RNA for use in medicine. Rapid developments in this technology culminated in the 2018 approval, in both the USA and Europe, of two RNA-based therapies for the treatment of hereditary ATTR amyloidosis, a progressive and potentially fatal disorder in which abnormal proteins build up in nerves and in organs, such as the heart. The latest development in the use of this technology resulted in the completion of a trial between the biotech companies Intellia and Regeneron, in treating a rare disease with an IV infusion of the gene-editing technology CRISPR as the first delivery of the medicine to a human body. In this study, six people with a rare and fatal condition called transthyretin amyloidosis received a single treatment with gene-editing therapy. All experienced a drop in the level of a misshapen protein associated with the disease. Such emerging technologies are still in their infancy and are very expensive.[27] In late 2019, the US National Institutes of Health and Bill and Melinda Gates Foundation announced a $200 million grant for developing affordable gene therapies for use in sub-Saharan Africa. The target diseases were HIV and sickle-cell disease. How can such an emerging technology be developed at an affordable cost? Antonio Regalado writes in MIT Technology Reviews, Dr. Drew Weissman told me how they would make such cutting-edge treatments cheap and easy to use; the plan may depend on using gene-editing tools like CRISPR on a person's body, making permanent changes to the genome.[28]

Dr. Weissman says he intends to use this technology to try to cure sickle-cell disease by sending new instructions into the cells of the body's blood factory. He seems to be working in the monkey model, where T cells can be engineered to seek and destroy HIV and cure AIDS. Dr. Weissman thinks that RNA packaged in fatty acid nanoparticles may become a way to edit the genome on a massive scale, and on the cheap. According to experts, breakthrough CRISPR gene therapy could be a 'one and done' injection. CRISPR gene editing earned two of its discoverers the Noble Prize in 2020. A growing number of clinical trials are beginning to test gene therapies in humans. The therapy is made up of three parts. A tiny vesicle made up of lipid nanoparticles for delivery carries a payload of CRISPR machinery: a strand of guide RNA and a sequence of mRNA coding for the Cas9 protein. This approach, if successful, would be a one-time treatment, targeting the genes, to silence the defective mechanism permanently.

Challenges for Drug Discovery and Development

There are limitations to what vaccines can do. Furthermore, large populations of the world have no access to these vaccines. "None of us is safe until we all are safe" is a

common motto about COVID-19, and it is the idea behind the COVAX program to provide global access to vaccination. The member states of WHO have been divided into two groups. One is made up of 98 more affluent countries which are funding subsidized free vaccine supplies to 92 resource-poor countries. Germany is one of the COVAX program's biggest benefactors, providing almost $1.2 billion. During her recent visit to the USA German Chancellor, Angela Merkel requested US President Joe Biden to support the COVAX efforts. According to the World Health Organization, to provide vaccines to all the countries, COVAX needs 45 billion dollars. A high-level independent panel of the G20 Nations, which is currently meeting in Italy, has urged the launch of a 'global deal' to prevent catastrophic costs of future pandemics. New York Times, July 9th, 2021, reported that the board of the International Monetary Fund (IMF) on Friday, July 7, 2021, approved a plan to distribute $650 billion in reserve funds to help poorer nations with their vaccine rollouts and pandemic recovery efforts. The plan must be approved by the IMF's board of governors. We hope that the governors of the board of IMF approve this global relief effort.

Even if the funds are made available, there are not many more vaccine doses available, because the EU and the USA have already secured a large majority of them. Whereas, in a country like the USA, which has plenty of vaccines available, a large population is reluctant to get vaccinated. Dr. Nadav Davidovitch, the head of Israel's association of public health physicians, said he believes people have an obligation to get vaccinated, particularly given the evidence that vaccine not only prevents the worst outcome but also may reduce the spread of the virus. Despite such warnings, large populations are reluctant to get vaccinated. In view of this situation, we will not be able to eradicate the COVID-19 pandemic any time soon. In the USA, Pfizer one of the vaccine developers announced that they are making booster shots. US Food and Drug Administration and the CDC say that there is no need for booster shots currently. Israel on the other hand is recommending booster shots, considering the dangers posed by the delta variant. The COVID-19 pandemic may remain with us for some time to come, and chances are eventually people may get herd immunity over a period. But till that happens, we will see a lot of suffering, economic loss, and mounting, preventable deaths worldwide. Even in an advanced country like the USA, where vaccination is free and is available for everyone, only about 47% of the eligible population is vaccinated with the two doses. Already Pfizer maker of one of the mRNA vaccines is planning a booster shot to combat the delta variant. Despite the protection offered by the modern vaccines against COVID-19, we cannot totally rely on the vaccines to eradicate this virus.

Biomedical Research and Healthcare

In an earlier article titled "Biomedical Research and Healthcare" in this journal, I discussed biomedical research innovations.[29] I also discussed President Barack Obama's billion-dollar precision medicine initiative. In the 1950s, Francis Crick and James Watson together at the University of Cambridge, England explored the structure of proteins. In 1962, these pioneer scientists were awarded the Noble Prize in Medicine for their work in determining the structure of DNA—the genetic code. The Human Genome Project, which began in October of 1990 and was completed in April of 2003, has provided the ability for the first time, to read nature's complete genetic blueprint for building a human being. National Human Genome Research Institute (HGRI) of the National Institutes of Health (NIH) has an extensive database on a variety of topics related to human diseases. From the time HGRI was initiated in the 90s, there is great expectation and excitement, about its possible contributions to improvements in healthcare. It is important to recognize the difference between genetics and genomics. Genetics is the study of single genes and their effects whereas genomics is the study not of single genes, but of the functions and interactions of all the genes in the genome. Despite the extensive database that HRG has created on a variety of 'omics' data, we are of the opinion that a well-thought-out hypothesis-based investigation will yield more valuable information than a 'Top down' approach like a precision medicine initiative. We also have advocated in our earlier articles, that treatment of the disease itself is better than focusing on the management of risk factors for a disease or cluster of diseases.[29-33]

In the following, few paragraphs we will try to provide some examples in support of our views on this topic. Let us consider coronavirus disease as an example as we have discussed this extensively in the last few months.[34-43] In News Feature in Nature July 8, 2021, Ewen Callaway writes, "Genome studies have discovered some genetic risk factors for disease — and could point to treatments."[44]

Since last March when SARS-CoV-2 became a pandemic worldwide, researchers around the world have scouted the genomes of more than 100,000 people with COVID-19, hoping to find genetic clues to who will be hit hardest by an infection with the virus SARS-CoV-2. According to Callaway, what has emerged from this global effort is a dozen or so genetic variants that have a strong statistical association with a person's chances of developing COVID-19 and becoming gravely ill with the disease.[44] Alessandra Renieri, a geneticist at the University of Siena, Italy, and an early member of the HGI (COVID-19 Host Genetics Initiative), says that each new genetic finding is like a piece of a puzzle. "Several pieces are coming together. I'm sure that the picture will be much more clear in the very near future." Having said that, I must emphasize the importance of gene expression in immune modulation. From a different perspective, if we analyze the same cohort with a different parameter for severity, then it becomes evident that the severity of the coronavirus disease was highly correlated with the presence or absence of underlying risk factors such as hypertension, excess weight, obesity, diabetes, and vascular diseases.[34-43]

Obesity is a recognized risk factor for severe COVID-19, possibly related to chronic inflammation that disrupts immune and thrombogenic responses.[45] Obese people diagnosed with COVID-19 were more than twice likely to be hospitalized, 74% more likely to need an intensive care unit, and 48% more likely to die, according to a study from the University of North Carolina Research Group. According to a report from

China based on a systematic meta-analysis, comorbidities, including, obesity, hypertension, diabetes, cardiovascular disease, cerebrovascular disease (Metabolic Diseases), are clinical risk factors for severe are fatal outcomes associated with COVID-19, with obesity being the most prevalent.[46] In a series of articles, we and others have described the relationship between the underlying metabolic diseases and the severity of coronavirus disease.[33-42] We also have indicated that patients with metabolic diseases have a compromised vascular endothelium and hence the coronavirus diseases severity is much more severe than those who do not have any underlying conditions.[33-42] Thakur and associates did a systematic review and meta-analysis of data on 120 studies with 125,446 COVID-19 patients. The most prevalent comorbidity was hypertension (32%), obesity (25%), diabetes (18%), cardiovascular disease (16%). They also found that the association of comorbidities and severity of the disease varied in different geographical locations.[47]

Discussion

One can easily say that discovery of DNA structure and elucidation of the genetic code half a century ago, heralded the 'golden era' of biomedical research and innovation.[29] If one were to list the ten greatest medical milestones, then this discovery will be one of them. Then comes the flood of research in the human genome project—humanity's biggest research endeavor. Technology did not catch up with human aspirations and the quest for immediate cures for incurable diseases.

Then came the announcement of an ambitious project by the then US President, Barack Obama, the Precision Medicine Project. Dr. Francis Collins announced a billion-dollar program, "All of Us' with two main goals; a near-term focus on cancers and diabetes, and a longer-term aim to generate knowledge applicable to the whole range of health and disease. We have described such ambitious programs as 'top-down approaches' to find solutions. Whereas we and others have advocated a hypothesis-based approach to problems. When considering such approaches, the return on investment plays a big role. On the other hand, we cannot put a price on human lives. The speed at which we provide preventable or protective interventions could save millions of lives worldwide.

In brief, gene expression means manufacturing its corresponding protein, and this is a process that follows a specific set of events. In the primary step, the information in the DNA is transferred to a messenger RNA (mRNA) molecule by a process known as transcription. During this phase, DNA of the gene serves as a template for complementary base-pairing. The RNA polymerase catalyzes the formation of a pre-mRNA molecule, which is then processed to form a mature mRNA. The resulting mRNA is a single-stranded copy of the gene. "The development of RNA vaccines is a great boon to the future of treating infectious diseases," says Lynne Maquat, the J. Lowell Orbison Distinguished Service Alumni Professor in biochemistry and biophysics, oncology, and pediatrics at Rochester and the director of Rochester's Center for RNA Biology. Although these are the first mRNA vaccines to be approved for human use, the story of mRNA vaccines starts more than 30 years ago.

Dr. Kizzmekia Corbett of NIH was the inspiring researcher who helped create COVID-9 mRNA vaccines. In an exclusive interview with the Director of NIH, Dr. Francis Collins (June 17th, 2021), she explains how they went about developing a vaccine for COVID-19 so fast. She says, "messenger RNA technologies have been in development from a basic science perspective for over 15 years. Vaccines are basically to teach your immune system how to find the virus protein and attack. Dr. Corbett and associates have described the design, testing, and development of mRNA1273, which encodes SARS-CoV-2 spike proteins in the perfusion state.[48] In the MIT Technology Review, the author Regalado writes, "Scientists at Moderna were able to design a vaccine on paper 48 hours post announcement of the information of SARS-CoV-2 genome, 11 days before the US had its first COVID positive case. In the NIH interview posted on June 17th, 2021, Dr. Corbett says, "The cool thing about this type of technology is you don't even need the lab to design the vaccine." However, according to Dr. Corbett, RNA technologies have been in progress for over a decade. It took several months to scale up the process, do the clinical trials and manufacture doses of vaccines that could be authorized for emergency use.

Despite the availability of knowledge about the exact sequence of nucleotides needed to provide the information to the immune cells to make antibodies to Spike proteins of SARS-CoV-2, all attempts to deliver this messenger RNA into humans would have failed. The human immune system would have recognized the "foreign' molecule and destroyed it. Credit for the development of an appropriate delivery system with lipid nanoparticles goes to Katalin Kariko (now at BioNTech) and Drew Weisman of the University of Pennsylvania for the discoveries the pair made two decades ago.[49]

Various versions of lipids such as ionizable lipid nanoparticles can be used to safely deliver the mRNA to target cells. Özlem Türeci, German biotechnology company BioNTech's chief medical officer, and her colleagues optimized a therapy with what she describes as "different liposomal formulations to make RNA fit for the respective purposes like an intramuscular or intravenous injection and targeting specific cell types." BioNTech found that for anti-cancer vaccines based on liposomally formulated mRNA, for instance, the antigen is expressed mainly in the dendritic cells in lymphatic compartments. These cells specialize in setting off antigen-specific immune responses.

Each improvement made through emerging basic sciences and applied technologies improves the formulation, and offers less inflammation, enhanced expression, protected delivery of the mRNA molecules, and thus allows the immune-engineers to build better mRNA vaccines as therapeutics for viral diseases as well as for cancer.[50-52]

Conclusion

Biomedical innovations and technological advances have contributed significantly to our understanding of the mechanisms that induce dysfunction of various systems and

initiate the development of risk factors or promote diseases. As we have mentioned earlier, from discovery to the application of the knowledge for practical use takes considerable time. However, an unprecedented pandemic of coronavirus exposed the weakness of global medical emergency management. This unprecedented pandemic also promoted the warp-speed development of effective vaccine candidates. The FDA has approved 3 vaccines for emergency use, as well as some experimental drugs, such as the monoclonal antibody cocktail of Regeneron.

We have discussed how the breaking of the genetic code half a century ago and understanding the role of nucleotide sequences in the synthesis of appropriate, specific proteins of biological importance accelerated the studies on RNAs in general as well as on microRNAs and messenger RNAs. For the first time in half a century, scientists and biotechnologists were able to utilize the various pieces of information available and package the appropriate sequence of mRNA, which can code for the spike proteins of SARS-CoV-2 and develop a safe vaccine against COVID-19. This success, to a great extent, is the result of extensive studies on mRNAs of other pathogenic viruses. Credit also goes to the extensive studies that developed needed technologies for packaging of biological molecules in the appropriate lipid nanoparticles for targeted delivery to the desired tissue, cells, or organs.

The extraordinary success of mRNA vaccines has opened new avenues for mRNA-based therapies. mRNAs, siRNAs, and non-coding miRNAs will play a very important role as novel therapeutics soon. Furthermore, this success has acted as a catalyst for ongoing work on the use of small RNAs for therapeutic purposes. For instance, siRNAs have become an exciting tool not only in molecular biology but also in molecular medicine. According to the experts, miRNAs regulate more than a third of all cellular mRNAs, and bioinformatic data indicate that each miRNA can control hundreds of gene targets. Small non-coding RNAs could emerge as novel therapeutics soon. The success of mRNA vaccines has opened new avenues for genetic information coders, synthetic chemists, immunologists, virologists, biotechnologists, experts in engineering targeted delivery systems, and various stakeholders of drug discovery and development. Having said that, I must emphasize that there are many challenges and abundant opportunities.

References

1. Cutler DM, Summers LH. The COVID-19 Pandemic and the $16 Trillion Virus. JAMA. 2020 Oct 20;324(15):1495-1496. doi: 10.1001/jama.2020.19759.
2. Zhu H, Wei L, Niu P. The novel coronavirus outbreak in Wuhan, China. Glob Health Res Policy. 2020 Mar 2;5:6. doi: 10.1186/s41256-020-00135-6.
3. Guan WJ, Liang WH, Zhao Y, Liang HR, Chen ZS, Li YM, et al.; China Medical Treatment Expert Group for COVID-19. Comorbidity and its impact on 1590 patients with COVID-19 in China: a nationwide analysis. Eur Respir J. 2020 May 14;55(5):2000547. doi: 10.1183/13993003.00547-2020.
4. Richardson S, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW; the Northwell COVID-19 Research Consortium, Barnaby DP, Becker LB, Chelico JD, Cohen SL, Cookingham J, Coppa K, et al. Presenting Characteristics, Comorbidities, and Outcomes Among 5700 Patients Hospitalized With COVID-19 in the New York City Area. JAMA. 2020 May 26;323(20):2052-2059. doi: 10.1001/jama.2020.6775. Erratum in: JAMA. 2020 May 26;323(20):2098.
5. Wadman M. Why COVID-19 is more deadly in people with obesity-even if they are young? Science 2020; doi:10.1126/science.abe7010
6. Rao GHR. SARS-CoV-2 biochemistry, Transmission, Clinical Manifestations and Prevention. International Journal of Biomedicine. 2020;10(4):303-311.
7. Islam N, Sharp SJ, Chowell G, Shabnam S, Kawachi I, Lacey B, Massaro JM, D'Agostino RB Sr, White M. Physical distancing interventions and incidence of coronavirus disease 2019: natural experiment in 149 countries. BMJ. 2020 Jul 15;370:m2743. doi: 10.1136/bmj.m2743.
8. Chowdhury R, Heng K, Shawon MSR, Goh G, Okonofua D, Ochoa-Rosales C, Gonzalez-Jaramillo V, Bhuiya A, Reidpath D, Prathapan S, Shahzad S, Althaus CL, Gonzalez-Jaramillo N, Franco OH; Global Dynamic Interventions Strategies for COVID-19 Collaborative Group. Dynamic interventions to control COVID-19 pandemic: a multivariate prediction modelling study comparing 16 worldwide countries. Eur J Epidemiol. 2020 May;35(5):389-399. doi: 10.1007/s10654-020-00649-w.
9. Ledford H. Six months of COVID vaccines: what 1.7 billion doses have taught scientists. Nature. 2021 Jun;594(7862):164-167. doi: 10.1038/d41586-021-01505-x.
10. Yeagle P. The potent power of basic research. Sci Adv. 2021 Jun 30;7(27):eabj8363. doi: 10.1126/sciadv.abj8363.
11. Crotty S. T Follicular Helper Cell Biology: A Decade of Discovery and Diseases. Immunity. 2019 May 21;50(5):1132-1148. doi: 10.1016/j.immuni.2019.04.011.
12. Ciabattini A, Pettini E, Fiorino F, Pastore G, Andersen P, Pozzi G, Medaglini D. Modulation of Primary Immune Response by Different Vaccine Adjuvants. Front Immunol. 2016 Oct 17;7:427. doi: 10.3389/fimmu.2016.00427.
13. Fu G, Guy CS, Chapman NM, Palacios G, Wei J, Zhou P, et al. Metabolic control of T_{FH} cells and humoral immunity by phosphatidylethanolamine. Nature. 2021 Jul;595(7869):724-729. doi: 10.1038/s41586-021-03692-z.
14. Wang Z, Muecksch F, Schaefer-Babajew D, Finkin S, Viant C, Gaebler C, et al. Naturally enhanced neutralizing breadth against SARS-CoV-2 one year after infection. Nature. 2021 Jul;595(7867):426-431. doi: 10.1038/s41586-021-03696-9.
15. Khoury DS, Cromer D, Reynaldi A, Schlub TE, Wheatley AK, Juno JA, Subbarao K, Kent SJ, Triccas JA, Davenport MP. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nat Med. 2021 Jul;27(7):1205-1211. doi: 10.1038/s41591-021-01377-8.
16. Dolgin E. 'Super-antibodies' could curb COVID-19 and help avert future pandemics. Nat Biotechnol. 2021 Jul;39(7):783-785. doi: 10.1038/s41587-021-00980-x. Erratum in: Nat Biotechnol. 2021 Jun 29.
17. Ezzikouri S, Nourlil J, Tsukiyama-Kohara K, Kohara M, El Ossmani H, Windisch MP, Benjelloun S. Nanobodies: an unexplored opportunity to combat COVID-19. J Biomol Struct Dyn. 2020 Nov 10:1-3. doi: 10.1080/07391102.2020.1845801.
18. Esparza TJ, Martin NP, Anderson GP, Goldman ER,

Brody DL. High affinity nanobodies block SARS-CoV-2 spike receptor binding domain interaction with human angiotensin converting enzyme. Sci Rep. 2020 Dec 22;10(1):22370. doi: 10.1038/s41598-020-79036-0.
19. Jang WD, Jeon S, Kim S, Lee SY. Drugs repurposed for COVID-19 by virtual screening of 6,218 drugs and cell-based assay. Proc Natl Acad Sci U S A. 2021 Jul 27;118(30):e2024302118. doi: 10.1073/pnas.2024302118.
20. Nishii R, Mizuno T, Rehling D, Smith C, Clark BL, Zhao X, et al. NUDT15 polymorphism influences the metabolism and therapeutic effects of acyclovir and ganciclovir. Nat Commun. 2021 Jul 7;12(1):4181. doi: 10.1038/s41467-021-24509-7.
21. Seth S, Batra J, Srinivasan S. COVID-19: Targeting Proteases in Viral Invasion and Host Immune Response. Front Mol Biosci. 2020 Oct 9;7:215. doi: 10.3389/fmolb.2020.00215.
22. Zhang L, Lin D, Sun X, Curth U, Drosten C, Sauerhering L, Becker S, Rox K, Hilgenfeld R. Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors. Science. 2020 Apr 24;368(6489):409-412. doi: 10.1126/science.abb3405.
23. Sharp PM, Hahn BH. Origins of HIV and the AIDS pandemic. Cold Spring Harb Perspect Med. 2011 Sep;1(1):a006841. doi: 10.1101/cshperspect.a006841.
24. Ray S, Fatima Z, Saxena A. Drugs for AIDS. Mini Rev Med Chem. 2010 Feb;10(2):147-61. doi: 10.2174/138955710791185145.
25. Cannalire R, Cerchia C, Beccari AR, Di Leva FS, Summa V. Targeting SARS-CoV-2 Proteases and Polymerase for COVID-19 Treatment: State of the Art and Future Opportunities. J Med Chem. 2020 Nov 13:acs.jmedchem.0c01140. doi: 10.1021/acs.jmedchem.0c01140.
26. Jang WD, Jeon S, Kim S, Lee SY. Drugs repurposed for COVID-19 by virtual screening of 6,218 drugs and cell-based assay. Proc Natl Acad Sci U S A. 2021 Jul 27;118(30):e2024302118. doi: 10.1073/pnas.2024302118.
27. Payne D. RNA therapies. Nature. 2019 Oct;574(7778):S1. doi: 10.1038/d41586-019-03067-5.
28. Regalado A. Messenger RNA Vaccines. MIT Technology Reviews. 2021;124(2):29-34.
29. Rao GHR. Biomedical Research and Healthcare: Opportunities, Expectations and Limitations. International Journal of Biomedicine. 2018;8(4):273-279.
30. Rao GHR. Predictive, Preventive, and Precision Medicine: The future of healthcare. Viewpoint. United J. Int. Med. 2018;1(1):1-3.
31. Rao GHR: Prevention of Acute Vascular Events: Precision Medicine, Personalized Medicine, and Conventional Medicine. EC Clin. & Medical Case Reports.2020;3(2):01-03.
32. Rao GHR: Importance of studying disease and disease process. Editorial. Developments in Clin. Med. Pathol. Crimson Publ. 2018;1:1.
33. Cohn JN, Hoke L, Whitwam W, Sommers PA, Taylor AL, Duprez D, Roessler R, Florea N. Screening for early detection of cardiovascular disease in asymptomatic individuals. Am Heart J. 2003 Oct;146(4):679-85. doi: 10.1016/S0002-8703(03)00499-X.
34. Rao GHR. COVID-19 and Cardiometabolic Diseases. Guest Editorial. EC Cardiol. 2020;7(6):08-12.
35. Rao GHR. Coronavirus Disease and Acute Vascular Events. Clin Appl Thromb Hemost. 2020 Jan-Dec;26:1076029620929091. doi: 10.1177/1076029620929091.
36. Rao GHR. Coronavirus (COVID-19), Comorbidities, and Acute Vascular Events; Guest Editorial. ECCMC EC Clinical Case Reports. 2020;3(6):87-91.
37. Rao GHR Coronavirus Disease (Covid-19), Comorbidities, and Clinical Manifestations. Guest Editorial. EC Diab Met Res. 2020; 4(6):27-33.
38. Rao GHR. Coronavirus Disease (Covid-19): A Disease of the Vascular Endothelium. Series Cardiology Res. 2020;2(1):23-27.
39. Rao GHR: Coronavirus Transmission, Vascular Dysfunction and Pathology. J Cardiol Res Rev & Reps. 2020;1(3):1-4.
40. Rao GHR. Excess weight, Obesity, Diabetes and Coronavirus Disease. Arch Diab & Obesity.2020;3(1). MS ID.000152. doi: 10.32474/ADO.2020.03.000152.
41. Rao GHR. Clinical manifestation of coronavirus disease as it relates to cardio-vascular health. Front J Cardiol & Vasc Med. 2020;1(1). IDFJCCM-20-103.
42. Rao GHR. Syndemic of Coronavirus Disease and Metabolic Diseases: Global Perspective. EC Endocrinol Met Res. 2021;6(4):28-32.
43. Rao GHR. Twindemic of Coronavirus Disease (COVID-19) and Cardiometabolic Diseases. International Journal of Biomedicine. 2021;11(2):111-122. doi. org/10.21103/Article11(2)_RAI
44. Callaway E. The quest to find genes that drive severe COVID. Nature. 2021 Jul;595(7867):346-348. doi: 10.1038/d41586-021-01827-w.
45. COVID-19 Host Genetics Initiative. Mapping the human genetic architecture of COVID-19. Nature. 2021 Jul 8. doi: 10.1038/s41586-021-03767-x.
46. Zhou Y, Yang Q, Chi J, Dong B, Lv W, Shen L, Wang Y. Comorbidities and the risk of severe or fatal outcomes associated with coronavirus disease 2019: A systematic review and meta-analysis. Int J Infect Dis. 2020 Oct;99:47-56. doi: 10.1016/j.ijid.2020.07.029.
47. Thakur B, Dubey P, Benitez J, Torres JP, Reddy S, Shokar N, Aung K, Mukherjee D, Dwivedi AK. A systematic review and meta-analysis of geographic differences in comorbidities and associated severity and mortality among individuals with COVID-19. Sci Rep. 2021 Apr 20;11(1):8562. doi: 10.1038/s41598-021-88130-w.
48. Corbett KS, Edwards DK, Leist SR, Abiona OM, Boyoglu-Barnum S, Gillespie RA, rt al. SARS-CoV-2 mRNA vaccine design enabled by prototype pathogen preparedness. Nature. 2020 Oct;586(7830):567-571. doi: 10.1038/s41586-020-2622-0.
49. May M. After COVID-19 successes, researchers push to develop mRNA vaccines for other diseases. Nat Med. 2021 Jun;27(6):930-932. doi: 10.1038/s41591-021-01393-8.
50. Miao L, Zhang Y, Huang L. mRNA vaccine for cancer immunotherapy. Mol Cancer. 2021 Feb 25;20(1):41. doi: 10.1186/s12943-021-01335-5.
51. Ryther RC, Flynt AS, Phillips JA 3rd, Patton JG. siRNA therapeutics: big potential from small RNAs. Gene Ther. 2005 Jan;12(1):5-11. doi: 10.1038/sj.gt.3302356.
52. Rossbach M. Small non-coding RNAs as novel therapeutics. Curr Mol Med. 2010 Jun;10(4):361-8. doi: 10.2174/156652410791317048.