Leveraging Precision Medicine to address COVID-19

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Abstract:

Precision medicine (PM) is a new paradigm in disease diagnosis, prevention, and treatment that holds great relevance in the current coronavirus pandemic. PM can be used to select specific preventive measures and biomarkers that will be beneficial in the management of disease or other respiratory viruses. Knowledge on the pathophysiology of COVID-19 has improved substantially, regarding progression of the virus, the role of gene variants (ACE2 receptors, TMPRSS2, FURIN), the types of multiorgan impacts, and the function of cytokine response. Individuals infected with COVID-19 are seen to have numerous atypical presentations of the disease, meaning that similar treatments for a large group of people would likely serve as ineffective. As much as current COVID-19 treatments are being developed, present antiviral therapies including Remdecivir, lopinavir, ritonavir and hydroxychloroquine have all been seen to ineffectively lessen mortality or length of hospital stay. Therefore, a new approach in precision medicine is needed and currently realistic, with advancements in molecular sequencing, developments in imaging and easier access to detailed information in electronic health records. Single-cell RNA sequencing is an important technology for researchers to examine the effects of vaccinations, along with genome or transcriptome content at the level of individual cells. A precision medicine approach is preventive, predictive, personalized and participatory. The three main steps in PM includes looking at its pathophysiology, the prediction or diagnosis of the virus, and finally management for prevention or treatment. The aims of this review is to discuss the precision medicine approach in regards to SARS-CoV2, and the gene-centric vision for individualized gene-targeted fixes.

Keywords: Precision medicine, Single-cell RNA sequencing

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Introduction

The outbreak of the novel coronavirus disease emerged in December of 2019 in Wuhan, China, and was declared to be a pandemic on March 11, 2020 by WHO (WHO, 2020). COVID-19 (coronavirus disease 19) is ascribed to SARS-CoV-2 (Severe acute respiratory syndrome-related coronavirus 2), which has had an expeditious spread globally, to 217 countries as of November, 2020 (CNN, 2020). In the 21st century, we have seen previous epidemics from coronavirus outbreaks, including SARS-CoV and Middle East respiratory syndrome (MERS)-CoV (Wang, Horby, Hayden, Gao, 2020). In both these cases, along with the most recent SARS-CoV-2, there has been a recurring struggle for quick, accessible and efficient diagnostic testing, ultimately hindering public health. The 1918 influenza pandemic, also known as the Spanish flu, was a highly infectious respiratory disease like COVID-19, that spread in waves. It is estimated that the flu infected approximately one-third of the planet's population - 500 million people, and killed about 20 to 50 million victims (Martini, M et al. , 2019).

Compared to past pandemics, there are newer developments in our current state, with significantly better epidemiological models and molecular diagnostic testing. The conventional way of detecting the virus is to use PCR-based tests, a molecular diagnostic approach that allows specific amplification of DNA sequences, making it possible to detect DNA from different organisms (Yang, Rothman, 2004). Currently, further advances have been made regarding personal protective equipment, antimicrobial therapies, immunotherapies, and intensive care for patients. Although there are certain developments that can be leveraged in our current state, we still face many present-day challenges. In comparison to 1918, individuals appear to be much more mobile

in the present 2020, and the pandemic has provoked considerable social unrest regarding government and economic policies. Our knowledge also indicates that SARS-Cov-2 is highly transmissible, has a high mutation rate, and affects multiple organ systems. Finally, it is known that the disease is extremely heterogeneous, meaning that it has several etiologies. With a rapid increase in the number of people testing positive for COVID-19, another major challenge to address is the classification and prioritisation of patients who need urgent care, and who can self-recover in quarantine (Lai C-C, Shih T-P, Ko W-C, et al., 2020).

(Single cell rna sequencing) With these new obstacles society has been aclimitized with today, a genetic vision of precision medicine would allow people to expect individualized gene-targeted fixes. Precision medicine would target the heterogeneous patient population, and rather than conducting randomized trials, precision studies would have a greater ability to achieve positive results.

ACE2, TMPRSS2, and FURIN Genes

In SARS-CoV-2, ACE2 which is the cell receptor, TMPRSS2 protease, and FURIN peptidase are used to invade human cells. The presence of inimical ACE2, TMPRSS2 and FURIN gene variants has the potential to modulate viral infectivity among humans, making some less vulnerable to viral infection than others. The single stranded RNA virus SARS-CoV-2 binds to the ACE2 receptor of human cells to invade the body (Hoffmann, Kleine-Weber, Schroeder, et al, 2020). This ACE2 protein plays a vital role in regulating metabolism and blood pressure, in addition to providing protection against lung, heart, brain injuries (Baig AM, Khaleeq A, Ali U, et al., 2020) As SARS-CoV-2 plays multiple roles, and is expressed at different levels in different tissues, it is important to analyze the variants of ACE2 to determine whether SARS-CoV-2 interferes with its expression.

According to new research from the Dasman Diabetes Institute in Kuwait, variations in population-wide genetics may account for a disparity of lower infection or mortality rates in the Gulf States where health conditions are usually quite prevalent. They compared genetic data for the Middle East with people from Europe, East Asia and Africa, and looked for variations in three genes considered crucial to infection of SARS-CoV-2 - ACE2, TMPRSS2, and FURIN.

ACE2 codes for a protein receptor that makes the entry of the coronavirus into human cells possible, while TMPRSS2 and FURIN code for proteins that facilitates the invasion (Al-Mulla, F., Mohammad, A., Al Madhoun, A., Haddad, D., Ali, H., Eaaswarkhanth, M., ... & Ahmad, R., 2020).

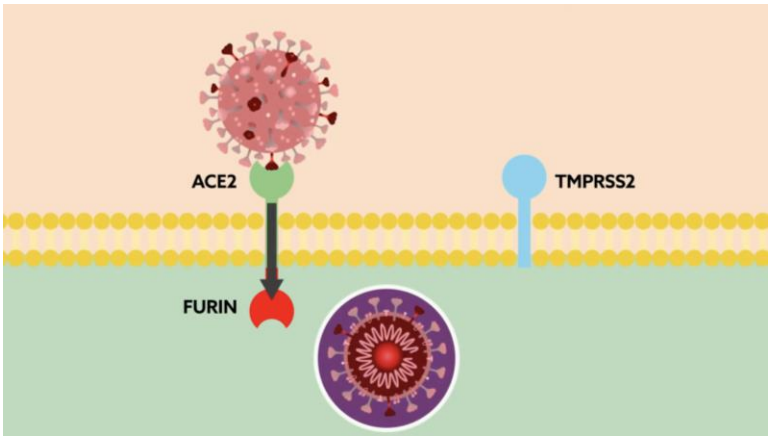

Figure 1: The role of ACE2, Furin and TMPRSS2 genes in the infection of SARS-COV2

From the research done, it was shown that one variant of ACE2, known as N720D was seen to enhance coronavirus infection among Europeans compared to those in Kuwait or Qatar.

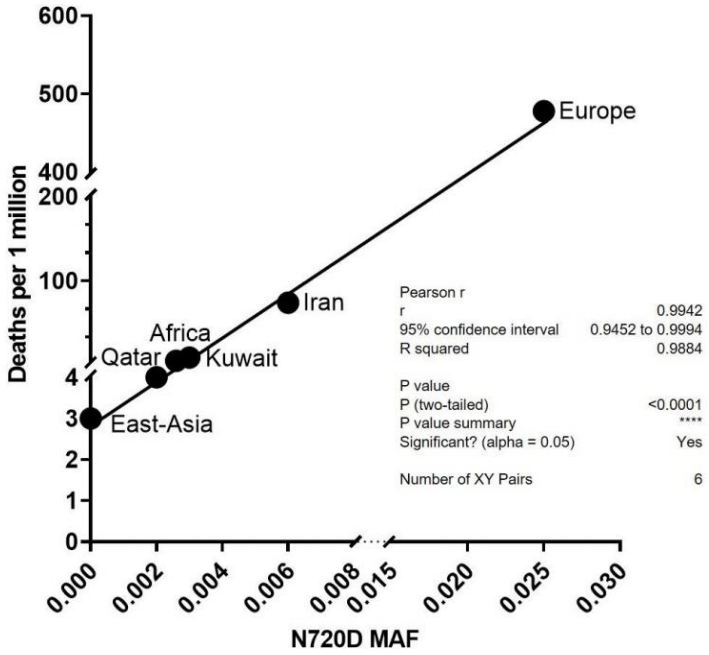

Figure 2: The plot shows the significant correlation between N720D minor allele frequency (MAF) and deaths per 1 million reported from different regions with Pearson's correlation coefficient of 99.4% ($p<0.0001$; C.I. 0.945-0.99).

A similar trend was seen with FURIN, after looking at one of its variants that could possibly weaken infection of SARS CoV-2. Their results suggest a possible protective role of FURIN gene variants against infection in the studied Middle Eastern populations. The final gene, TMPRSS2 revealed inconclusive data, however there have been alternate observations of androgens regulating TMPRSS2 expression which facilitates SARS-CoV-2 viral entry into the cell. As a result, androgen deprivation therapy is being studied as a treatment option in males infected with COVID-19. Consequently, it is crucial to study rare variants that may alter disease susceptibility with millions of people susceptible to infection.

Heterogeneity of COVID-19

Most people infected with SARS-CoV-2 develop only mild symptoms, and 70-80% don't develop any symptoms at all (Day, 2020). Asymptomatic patients are seen to have a longer median duration of viral shedding and weaker immune responses, with lower virus-specific IgG antibodies or cytokine levels (Long, QX., Tang, XJ., Shi, QL. et al., 2020). Furthermore, individuals are still able to develop mild or moderate symptoms, as well as more severe symptoms and critical illnesses. There are numerous atypical presentations of the disease with long lasting effects. Viruses like COVID-19 are heterogeneous, reflecting unique characteristics of the individual, meaning that similar treatments for a large group of people are imprecise, ineffective and costly.

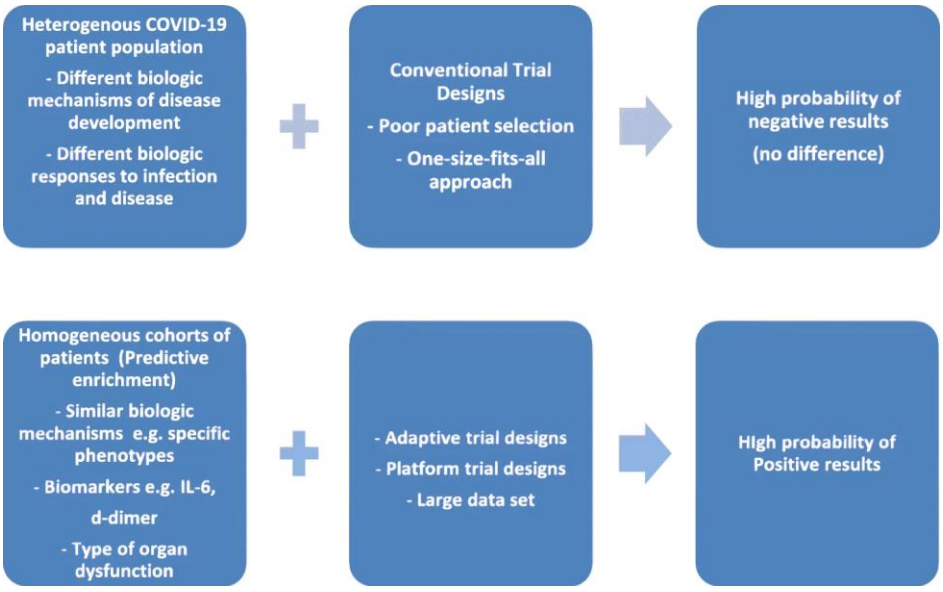

Figure 3: Heterogeneous and homogeneous patient populations and their corresponding results

Randomized Trials

Trials conducted to test antiviral therapy medications have been done by using randomized control trials (RCT). According to the WHO International Clinical Trials Registry Platform (ICTRP), more than 1000 studies addressing various aspects of COVID-19 are registered on ClinicalTrials.gov, including more than 600 interventional studies and randomized clinical trials (ICTRP, 2020). Therefore, it is also currently thought that the highest level of evidence for demonstrating the efficacy of treatment in medicine is the randomized control trial. There are several problems with RCTs that mean that they are not conclusive and fail to demonstrate a significant effect.

Randomized trials also conceal the heterogeneity of individual patient responses resulting in a narrow amount of patients eligible for a given treatment. Individuals participating in RCTs aren't generally representative of the population as a whole, and it isn't possible to tell what subset of the population actually benefited from the intervention being studied. The right treatment involves discovering an individual patient treatment effect, which is what precision medicine strives to do, compared to an average treatment effect from randomized trials.

Multisystem disease

The coronavirus was initially recognized as a respiratory virus, as an infection initially begins as a local upper respiratory tract infection. However, new evidence shows that it can spread to affect multiple organ systems resulting in kidney and liver damage, changes in the coagulation system, cardiac issues, endocrine or gastrointestinal problems, and even neurological issues. A Covid-19 infection is resulted from multiple host defence responses, inflammatory activity, vascular involvement with distinct coagulopathy, and a strong propensity to develop thromboembolic complications. A hyper-inflammatory tissue reaction, along with a weakened circulatory system, leads to sudden multiple organ dysfunction, affecting numerous body parts. In the patients most severely affected, a cytokine "storm" occurs, resulting in higher risk of multisystem failure. ACE2-receptors allow SARS-CoV-2 to infect endothelial cells and release cytokines that make them more adhesive, increasing coagulation (BMJ, 2020). Therefore, there is a lot of complexity with regards to this disease, and we will have to consider what the best way is to address it.Understanding the differences observed in biological factors such as age, sex, race, presence of comorbid conditions, as well as the host and viral genome and the roles they play in the variability in COVID-19 presentation and susceptibility may provide clues into disease

pathophysiology, therapeutic targets, and enable identification of the high-risk patient for therapeutic intervention and vaccination.

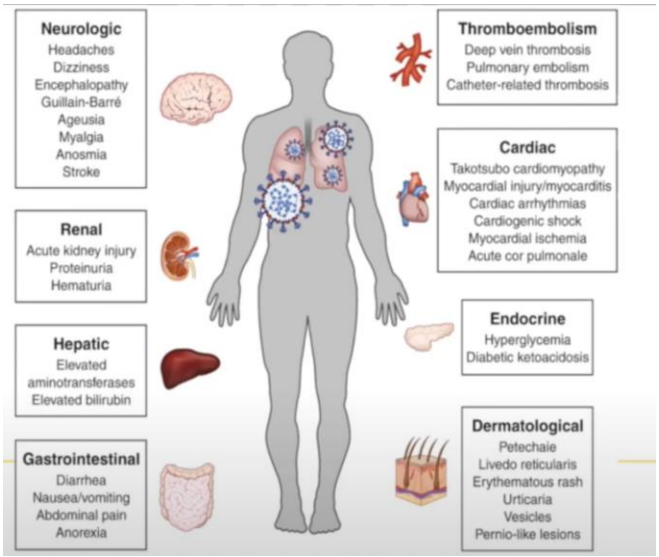

Figure 4: Diagram illustrating the areas of multi-organ failure in the human body.

Current Antiviral therapy

With few COVID-19 treatments underway, a majority of the strategies for treatment are considerably based on preclinical studies and previous experiences from SARS and MERS (Patel, A., Jernigan, D. B., 2020). This has been done by administering a number of antiviral treatments to patients with COVID-19. As of October 22, 2020, Remdesivir, an antiviral agent that inhibits viral RNA polymerases, is the only drug approved for treatment of COVID-19 (J Grein, N Ohmagari, D Shin, et al., 2020). Remdesivir is a repurposed medication that was previously developed to address Ebola. The coronavirus contains an RNA molecule that carries the virus's genetic information, which the viral polymerase makes multiple copies of (Meissner, H., 2020).

Remdesivir blocks the virus’s RNA polymerase from viral replication, in turn preventing the virus from multiplying and infecting more cells in the body (The Conversation, 2020).

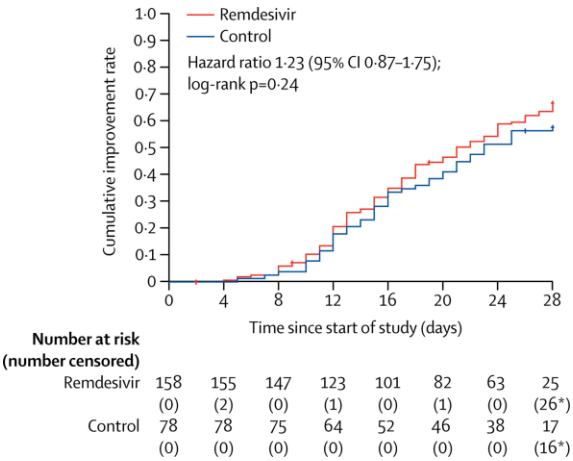

Figure 5: The time to clinical improvement for Remdesivir-treated individuals compared to a control group

The above graph representing a trial for therapy using Remdesivir, clearly shows that results for time to clinical improvement were extremely similar in both control and medication given populations.The time taken by patients for clinical improvement, mortality, or the time to clear the virus is not significantly improved by intravenous remdesivir in comparison with a placebo. When looking at the improvement rate over time after receiving medication, the treated and non-treated patients essentially have the same degree of improvement.

In addition to this, two HIV inhibitors, lopinavir and ritonavir, have conjointly been proposed as a treatment for COVID-19, on the basis of in vitro activity, preclinical experiments, and observational studies. Lopinavir is a HIV-1 protease inhibitor, which is combined with ritonavir, found to boost the half-life of lopinavir by inhibiting cytochrome P450 (Hull and Montaner, 2011). Lopinavir is also an inhibitor of the severe acute respiratory syndrome coronavirus (SARS-CoV) main protease, which is critical for replication and appears to be highly conserved in SARS-CoV-2 (The Lancet, 2020). However, a previous randomised trial of lopinavir–ritonavir among 199 patients admitted to hospital with COVID-19 demonstrated no recovery in viral load, length of hospital stay, or mortality. Another antiviral drug hydroxychloroquine that involved 150 individuals positive with SARS-CoV2 reported no significant effect of the drug on accelerating viral clearance (Tang, Wei, et al., 2020).

Precision Medicine

It seems that many aspects of current medicine regarding the coronavirus is imprecise, inefficient and costly, most likely due to the fact that the virus is intrinsically heterogeneous that reflects the unique characteristics of the individual. Precision medicine is a novel, innovative approach to health, defined as states of health and disease which are functions of the unique

characteristics of a person. It also ensures that the right treatment is delivered to the right person, at the right time. Essentially, if medicine and healthcare was looked at in an individualized manner, it would be in turn more effective and efficient. Precision medicine is now something that is realizable for numerous reasons. There have been advancements in molecular sequencing, developments in imaging, easy access to detailed information in electronic health records, and the development of artificial intelligence.

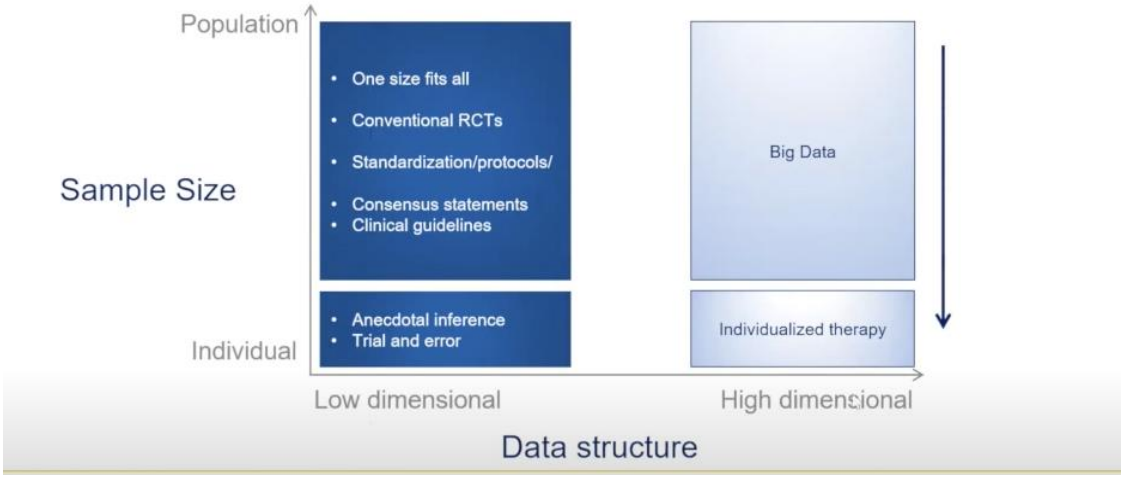

Figure 6: Graph depicting the way medicine is practised now versus. how it could be practiced with precision medicine.

A precision medicine approach is preventive, predictive, personalized and participatory. Prevention refers to taking steps that may delay the development of a disease before its symptomatic manifestations occur, and then implementing secondary prevention measures once the disease has settled. Predictive aspects take action on risk factors, lifestyle, and social determinants to anticipate the appearance of diseases. Personalization examines the genetic, molecular, and specific factors of individuals and pathogens, in order to recommend the most suitable therapeutic strategy based on their condition. Participatory refers to a combination of biomedical research, academic institutions, health professionals, and patients.

The three main steps in Precision Medicine are as follows: (1) pathophysiology: identification of molecular mechanisms of the disease and its variants; (2) prediction/diagnosis: identification of biomarkers and specific diagnostic tools; and (3) management: blocking/interfere those mechanisms for prevention and/or treatment (Hamburg MA, Collins FS, 2010).

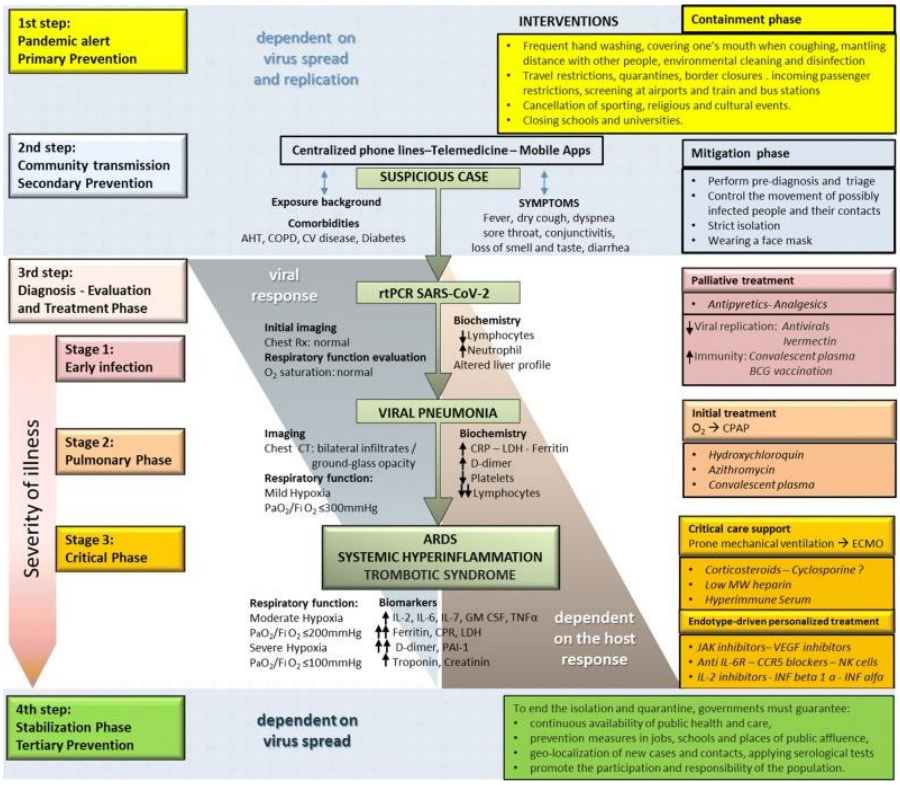

Figure 7: A precision medicine approach to SARS-CoV-2 pandemic management.

Molecular sequencing

First, we have seen great advancements in molecular sequencing. We can now sequence the genome quickly for about a couple hundred dollars, at a very affordable price. As shown in the graph below, the blue line illustrates the hypothetical cost of phenotyping.

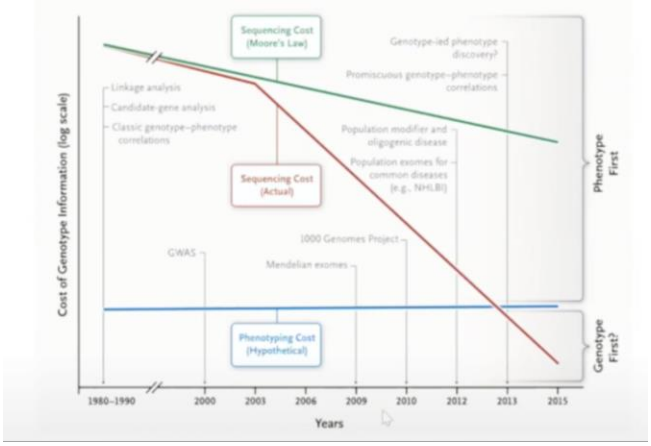

Figure 8: Declining cost of sequencing the genome from 1980 to 2015

This is essentially what the cost is to bring a patient to a clinic or into the hospital to perform physical exams, history data, EKGs or labs. All these tests would eventually cost

considerably more than it costs to perform a genome sequence of an individual. We have reached a point where our genome is now accessible, which means that we can now see the health of an individual on a multidimensional level. In addition to the genome, we can collect data from the proteome, which is the collection of proteins collectively in the bloodstream. Information can also be obtained from the microbiome, which is the diversity of various bacteria types and viruses. Social and family context enables us to collect additional information, and so we are now able to access a vast amount of data related to the health of individuals. Subsequently, using advanced statistics and machine learning, we can then generate a more precise estimate of the multidimensional nature of health.

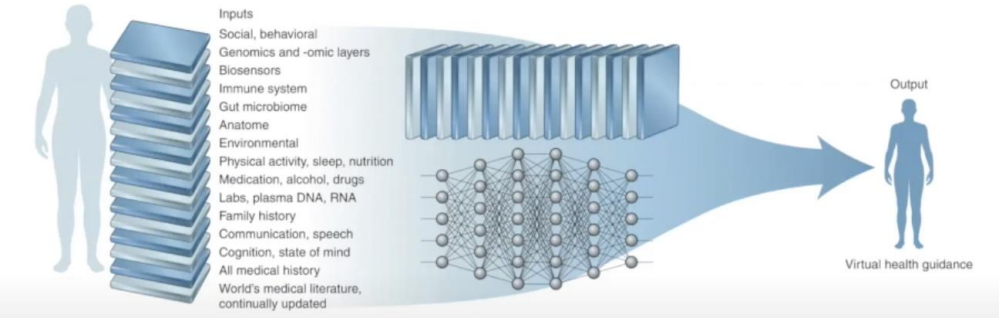

Figure 9: Multidimensional view of health

Single cell RNA sequencing

Precision medicine can be translated using Single-cell RNA sequencing to investigate unique patient biology and investigate environmental contributions. Single-cell RNA sequencing provides detailed profiles of cellular immune responses from limited samples, setting the stage for a new era in systems immunology. Currently used techniques, such as ELISAs, ELISpots, growth inhibition assays, and more, generally measure responses in the T cell after vaccination, but cannot measure differences in response between single immune cells (Kaijser, B. 1979). ScRNA-seq is performed by first isolating viable single cells from the tissue of interest. Next, lysis takes place in isolated individual cells in order to capture as many RNA molecules as possible. In order to specifically analyse polyadenylated mRNA molecules, and to avoid capturing ribosomal RNAs, poly[T] sequence primers are commonly used. The minute amounts of Complementary DNA (cDNA) are then amplified by PCR or by in vitro transcription followed by reverse transcription. Lastly, amplified and tagged cDNA from every cell is pooled and sequenced by Next Generation Sequencing (NGS).

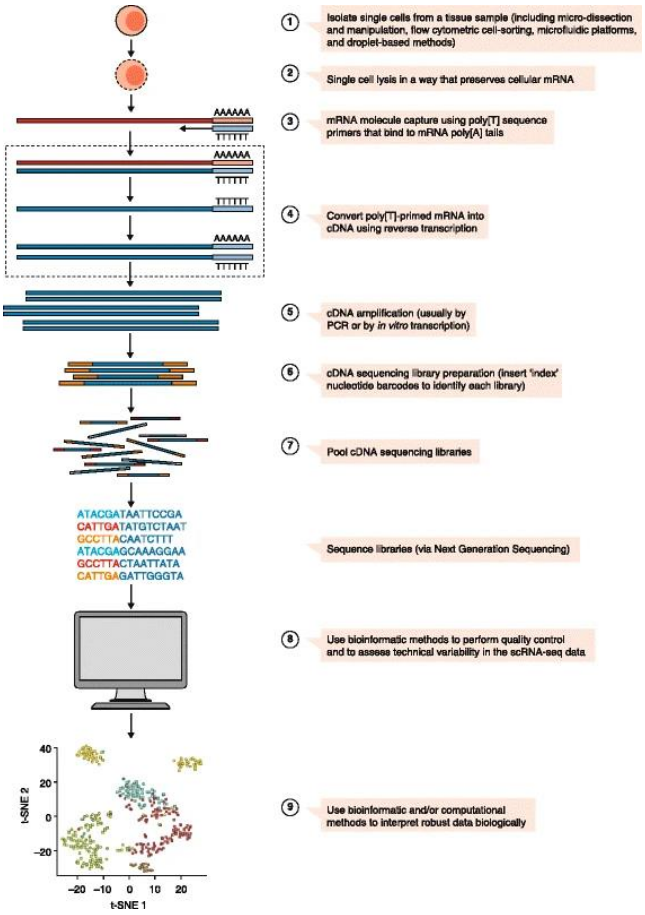

Figure 10: General workflow of single-cell RNA-sequencing (scRNA-seq) experiments. A typical scRNA-seq workflow includes most of the following steps: 1) isolation of single cells, 2) cell lysis while preserving mRNA, 3) mRNA capture, 4) reverse transcription of primed RNA into complementary DNA (cDNA), 5) cDNA amplification, 6) preparation of cDNA sequencing library, 7) pooling of sequence libraries, 8) use of bioinformatic tools to assess quality and variability, and 9) use of specialized tools to analyse and present the data.

ScRNA-seq has numerous advantages as it allows comparison of transcriptional similarities and differences among a population of cells, showing to be important in heterogeneity analysis. Assessments of transcriptional differences between individual cells have been used to identify rare cell populations that would otherwise go undetected in analyses of pooled cells, along with examination of single cells where each one is essentially unique - for example, individual T lymphocytes expressing highly diverse T-cell receptors. In addition to resolving cellular heterogeneity, scRNA-seq can also provide important information about fundamental characteristics of gene expression, including monoallelic gene expression, splicing patterns, as well as noise during transcriptional responses. Importantly, studying gene co-expression patterns at the single-cell level might allow identification of co-regulated gene modules and even inference of

gene-regulatory networks that underlie functional heterogeneity and cell-type specification (Creighton, R., Schuch, V., Urbanski, A. H., Giddalur, J., Costa-Martins, A. G., & Nakaya, H. I., 2020).

Conclusion

The coronavirus pandemic demands an urgent precision medicine approach for detecting and controlling the spread of the virus. Understanding the differences observed in biological factors, immune regulation or genetics that explain variability in COVID-19, information can be gotten from its pathophysiology or therapeutic targets. Identifying these individual differences would ultimately help in individualizing targeted therapy, hospitalization and be advantageous in the management of other respiratory viruses. Due to the ineffectiveness of current antiviral therapies, precision medicine is vital to combat this intrinsically heterogeneous virus.

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Overarching Question:

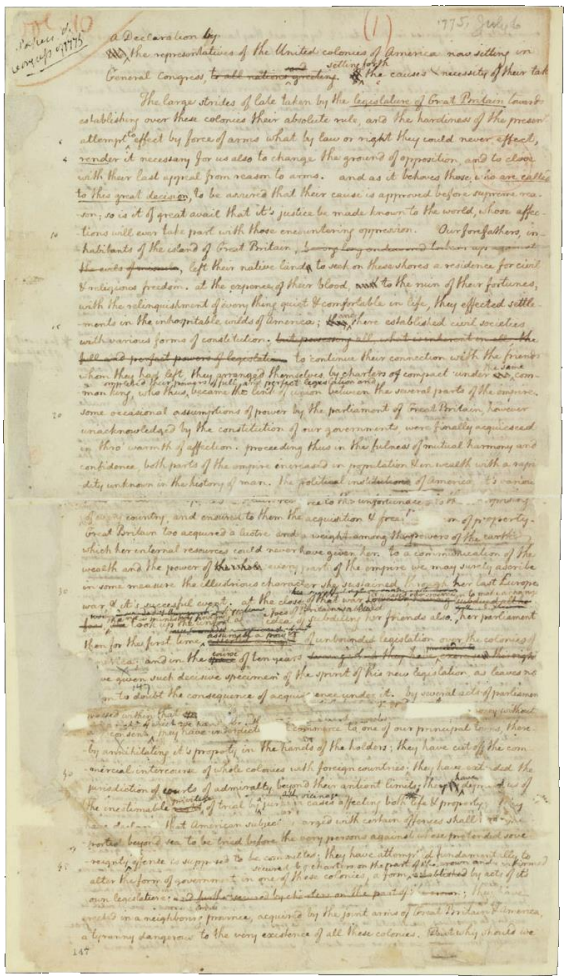
To What Extent Has America Become The Nation It Set Out to Be?

Primary Source 2: *Although many religious groups like Roman Catholics, Quakers, and Jewish refugees sought religious liberty in America, a Puritan theocracy existed that didn't withstand any religious dissent.*

In 1775, after issuing "a long and prosperous reign" in the Olive Branch Petition to King George III, Congress expressed "the causes and necessity of their taking up arms" against British

authority, declaring they were willing “to die freemen rather than live as slaves. The reasons for colonists' insurrection against the British government were discussed in the “Declaration of the Cause and Necessity of Taking Up Arms,” opposing contentious policies including taxation without representation, the Coercive Acts and the Declaratory Act. In response to having their pleas dismissed against a series of new laws, John Dickonson and Thomas Jefferson concluded that Britain’s “cruel and impolitic purpose of enslaving these colonies by violence...have thereby rendered it necessary for us to close with their last appeal from reason to arms.” Due to restrictive policies such as the Stamp Act that demanded colonists pay tax on printed material, political tensions escalated, ultimately leading to the Declaration of Independence. As depicted in the “Declaration of the Cause and Necessity of Taking Up Arms,” American colonists wished to be liberated of Britain's various restrictions on colonial economies, along with their lack of representation in Parliament. Consequently, this colonial opposition that led to the Declaration of Independence, helped colonists achieve their goals regarding the end of mercantilism,, securing economic prosperity, autonomy, and a liberal democracy.

Primary source: “Political Cartoons by Henry Payne.” Townhall, Townhall.com, 1 July 2014, https://townhall.com/political-cartoons/HenryPayne/2014/07/01/120335.



Primary source 2: *Colonists, emboldened by British policies that violated their rights as freemen, led to the American revolution and their emancipation from Britain, giving thej the economic independence and autonomy they valued.*

In 1775, after Congress issued “a long and prosperous reign” in the Olive Branch Petition to King George III, they expressed “the causes and necessity of their taking up arms” against British authority, declaring they were willing “to die freemen rather than live as slaves.These reasons for colonists' rebellion against the British government were discussed in the “Declaration of the Cause and Necessity of Taking Up Arms,” with policies including taxation without representation, the Coercive Acts, and the Declaratory Act. In response to having their pleas rejected against a series of new laws, John Dickonson and Thomas Jefferson concluded that Britain’s “cruel and impolitic purpose of enslaving these colonies by violence...have thereby rendered it necessary for us to close with their last appeal from reason to arms.” Due to restrictive policies such as the Stamp Act that required colonists to pay tax on printed material, tensions between colonists escalated, leading to the Declaration of Independence. As depicted in “Declaration of the Cause and Necessity of Taking Up Arms,” American colonists wished to be liberated of Britain's various restrictions on colonial economies, along with their lack of representation in Parliament. Therefore, this colonial opposition that led to the declaration of independence helped colonists achieve their goal of the end of mercantilism, political participation, securing economic growth, autonomy and liberal democracy.

Primary source: “Creating the United States Creating the Declaration of Independence.” *Causes and Necessity of Taking Up Arms - Creating the Declaration of Independence - Creating the United States | Exhibitions - Library of Congress*, www.loc.gov/exhibits/creating-the-united-states/interactives/declaration-of-independence/documents/enlarge5.html.