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Review article

Systems Thinking for Medicinal Chemists

Robert E. Smith[1*], Kevin Tran[1], Kristy[1], M. Richards[1]

[1]*U.S. FDA, 11510 W 80th St., Lenexa, KS 66214, USA*

**Corresponding author: Dr. Robert E. Smith, U.S. FDA, 11510 W 80th St., Lenexa, KS 66214, USA,*

Tel: 913-852-2191; Email: Robert.smith@fda.hhs.gov

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Abstract

Systems thinking has become an essential part of modern medicinal chemistry and new drug development. It is based on the concepts that complex problems almost always have multiple causes and that there are often connections that have not always been obvious. In contrast to reductionist thinking, systems thinking uses network theory and nonlinear mathematics to prevent, diagnose, treat and even cure diseases. Systems thinking is an integral part of predictive, preventive, personalized and participatory (P4) medicine. It takes not just a holistic but also a quantitative and mathematical approach to practicing medicine. This includes using networks of professionals. That is, in the 21st century doctors, mathematicians, physicists, chemists, biologists, systems engineers and many others have been working together to develop new pharmaceutical compounds and medicines. Modern drug development is being done through collaborative initiatives between governments, universities, research institutes, hospitals and industry. The U.S. Food and Drug Administration (FDA) has a Critical Path Initiative that intends to modernize drug development by incorporating recent scientific advances, such as genomics and advanced imaging techniques into the process of developing new drugs and medicines. There are also Biomarker-Integrated Approaches for Targeted Therapy for Lung Cancer Elimination (BATTLE) and a Cancer Genome Anatomy Project (CGAP). The National Cancer Institute (NCI) also has a Cancer Genomics' Cancer Genome Characterization Initiative, or CGCI. Together, these efforts are ushering in a new era of personalized health care that will be able to prevent, diagnose and properly treat diseases with the help of patients and their caregivers.

Keywords: Systems Thinking; Medicinal Chemistry; Personalized Medicine; Allostery

Abbreviations

AIDS: Acquired Immune Deficiency Syndrome;
BATTLE: Biomarker-Integrated Approaches for Targeted Therapy for Lung Cancer Elimination;
BCR-ABL: Breakpoint Cluster region-Abelson gene;
CGAP: Cancer Genome Anatomy Project;
CGCI: Cancer Genome Characterization Initiative;
cGMP: current Good Manufacturing Practices;
CGAP: Cancer Genome Anatomy Project;
CGCI: Cancer Genome Characterization Initiative;
cDNA: complementary DNA;
CGAP: Cancer Genome Anatomy Project;
CGEMS: Cancer Genetic Markers of Susceptibility;
c-KIT: cellular proto-oncogene Kit;
c-Myc: cellular Myelocytomatosis oncogene;
CQDM: Québec Consortium for Drug Discovery;

DNA: Deoxyribonucleic Acid;
EGF: Epidermal Growth Factor;
EGFR: EGF Receptor;
ENCODE: Encyclopedia of DNA Elements;
ES: Embryonic stem cells;
EV: Expression Variance;
FDA: Food and Drug Administration;
fMRI: functional Magnetic Resonance Imaging;
GLP: Good Laboratory Practices;
GWAS: Genome-Wide Association Studies;
HAT: Histone Acetyl Transferase;
HDAC: Histone Deacetylase;
HIV: Human Immunodeficiency Virus;
HSV: Herpes Simplex Virus;
ICG: Initiative for Chemical Genetics;
IL-2: interleukin-2;
JMJD2: Jumonji Domain-Containing Protein 2;
IL-2RαIL-2 receptor;
iPSCs: induced Pluripotent Stem Cells;
Klf4: Kruppel-like factor 4;
L3MBTL3: Lethal(3)malignant brain tumor-like protein;
lncRNA: long non-coding RNA;
MGC: Mammalian Gene Collection;
mRNA: messenger RNA,
MEMo: Mutual Exclusivity Modules in cancer;
miRNA: microRNA;
NCATS: National Center for Accelerating Translational Science;
NCCAM: National Center for Complementary and Alternative Medicine;
NCI: National Cancer Institute;
NIH: National Institutes of Health;
NIMH: National Institute of Mental Health;
NSCLC: Non-Small Cell Lung Cancer;
Oct4: Octamer-binding transcription factor 4;
OCG: Office of Cancer Genomics;
ORF: Open Reading Frame;
PDB: Protein Data Bank;
PDGF-R: Platelet-Dpendent Growth Factor -Receptor;
PET: Positron Emission Tomography;
PPPs: Public–Private Partnerships;
RPCI: Roswell Park Cancer Institute;
RNA: Ribo Nucleic Acid;
SCNP: Single Cell Network Profiling;
SNPs: Single Nucleotide Polymorphisms;
SOP: Standard Operating Procedure;
Sox2: Sex Determining Region 2

Introduction

Systems thinking

Systems thinking is a term that was coined in 1987 and has been defined in many ways since then [1,2]. It was defined recently as, "a set of synergistic analytic skills used to improve the capability of identifying and understanding systems, predicting their behaviors, and devising modifications to them in order to produce desired effects. These skills work together as a system" [2]. In systems thinking, one is encouraged to use a holistic approach to look for interactions and hidden connections between objects in a whole unit [3-5]. For example, new drugs are being developed that bind to a protein in one part of the cell that interacts with other proteins in other cells, causing the therapeutic response. Also, the prescription drug vorinostat binds to the enzyme histone deacetylase, affecting the interactions between DNA and transcription factors on cancer cells, making them recognizable by natural killer cells in the patient. This is a type of immunostimulation based on nonlinear processes. That is, the whole is more than the sum of its parts, which can interact in a nonlinear way to produce unexpected effects [3]. Some interactions are synergistic and others are inhibitory [6]. Moreover, whole new properties can emerge when atoms, ions and molecules are organized to form organelles, cells, tissues, organisms, communities and ecosystems.

Systems thinkers don't look at life as if it were a machine. Diseases are not thought of as defects in a predictable machine. Instead, they are an imbalance, a lack of ease, and may be written as a hyphenated word, dis-ease. Preventing diseases is more important than curing them. Good health requires a balance. When this balance is disturbed, a disease can occur. Instead of looking for a single root cause of a problem or a disease, systems thinkers try to re-establish a healthy balance. Traditional remedies are based on systems thinking. If there is an ache or pain, it is because the whole body and/or soul are thought to be out of balance. Systems thinkers realize that few, if any, difficult problems (such as diseases) have single causes or simple solutions. So, there are many things that one must do to stay healthy and to prevent diseases. Once a disease is contracted, there are usually several things that must be done to cure it [6].

The human body is an ecosystem

It is also important to realize that the human body is an ecosystem, consisting of not just human cells, but also bacteria, viruses and fungi that are in the microbiome [6]. The bacteria in our guts help us to digest our food and interact with our innate immune system to help us distinguish between good, symbiotic bacteria and exogenous, pathogenic bacteria. The gut microbiome helps to regulate intestinal function and interacts with the rest of the body to maintain health. So, the holistic view of health that comes from systems thinking [3] must include the microbiome [6].

Viruses and remnants from ancient viral genomes may be even more important than bacteria in making us human [7-14]. By applying graph theory to inclusive similarity networks from data on over 3000 viral genomes, it was shown that there is a large, complex network of mobile genetic elements (mobilome) that remodels genetic material and moves base se-

quences throughout different kinds of organisms in the biosphere [7]. The vast majority of these mobile genetic elements are transposons and retrotransposons that were probably derived from DNA and RNA viruses, respectively [1,7,8]. They appear to be especially important in human embryology, neurology and carcinogenesis [7-12]. It is quite likely that there are ancient viral origins of cognition [10]. One transposon called Sleeping Beauty is not only important in human embryology but can be used to reprogram cells and do targeted insertions into induced pluripotent stem cells (iPSCs) [12]. Moreover, viruses may have been extremely important in evolution [13,14]. They enable non-gradual or punctuated evolutionary changes [13]. Moreover, horizontal gene transfer from viruses and bacteria to human cells is just as significant as vertical gene transfer from parents to children. So, viruses and bacteria in the environment had and continue to have important effects on the human body. We are part of the environment-not separate from it.

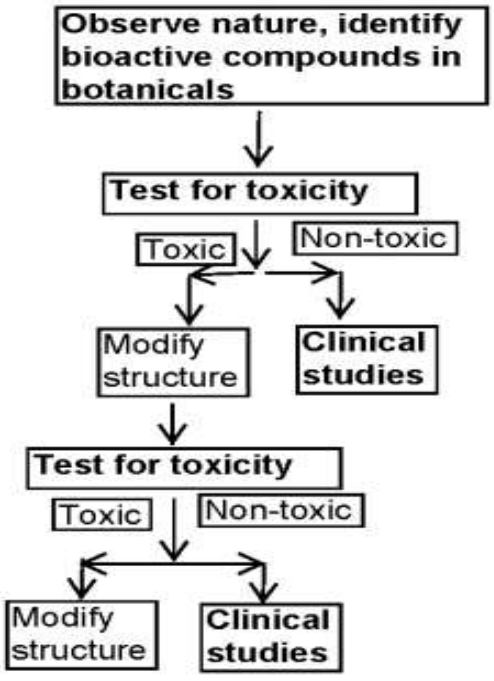
The roots of systems thinking

Moreover, the roots of systems thinking are found in traditional medicine, in which humans were thought to be a part of nature, not separate from it and should not be in conflict with it [6]. "Traditional healers try to heal the whole patient and restore balance. Thus, the term holistic medicine can be used. Also, many foods and herbs were identified as having healing properties. Unlike modern, western medicine, no attempt was made in traditional societies to find a single active ingredient. Instead, entire portions of an herb (or herbs) were given, and often several herbal remedies were given at the same time. Similarly, it was considered better to test the possible medicinal properties and toxicities of mixtures of chemicals that occur in a whole natural product, and not just pure compounds, since whole new properties can emerge with mixtures" [6].

Traditional medicine provided people with willow bark (*Salix alba*) to treat headaches and fever [4]. The bark of trees in the *Cinchona* genus as well as the leaves, stems and inflorescences of quinghao (*Artemisia annua*) were used to treat malaria [6]. The anticancer agents, paclitaxel, camptothecin, podophyllotoxin and adriamycin were obtained from *Taxus brevifolia, Podophyllum peltatum, Vinca rosea* and *Streptomyces peucetius*, respectively. The venom from a lancehead viper contains peptides that lower the blood pressure of the prey [7]. This and other lead compounds have been used by medicinal chemists to develop similar, new anti-hypertensive drugs. So, scientists at Bristol-Myers Squibb developed a small molecule that mimics one of them and it became the first ACE (angiotensin-converting enzyme) inhibitor, captopril, which is still used today [6]. There is also a drug called eptifibatide, which is a cyclic heptapeptide that was modeled on venom from the southeastern pygmy rattlesnake. It has antiplatelet activity, so it is used to reduce the risk of heart attacks in patients with unstable angina. Another example is Ziconotide, a peptide in cone snail venom for chronic pain and exenatide from the saliva of a venomous Gila monster, which has become a blockbuster drug for type-2 diabetes. These successes inspired a project called VENOMICS, which is producing a library of venom peptides that can be screened for their therapeutic potential [15].

In the meantime, the US FDA has approved a topical ointment (sinecatechins 15%, Verege ®) for the treatment of external genital and perianal warts caused by the human papillomavirus. The active ingredients are catechins that are extracted from green tea. They are immunomodulators that inhibit major viral functions. Also, at least eight different herbal medicines are undergoing clinical trials using Good Laboratory Practices (GLP) as defined by the U.S. FDA [16]. So, even the FDA is permitting complete plants and herbs to be tested for safety and efficacy and not just individual new molecular entities (NMEs). This is based on systems thinking. Figure 1 shows the loop of systems thinking in successful drug development.

Figure 1. Loop of systems thinking in successful drug development.



However, in the past there were problems with getting approval to evaluate the pharmacokinetics of traditional medicines. The active ingredients in many of them have not been completely identified. So, unlike prescription drugs, analytical chemists may not know which analyses to look for in the blood and tissues of the test subjects. Moreover, the active ingredients may not target the diseased organ directly. To address these problems, reverse pharmacokinetics can be done

on traditional medicines whose clinical benefits have been established. That is, the effects of increasing doses on different organs are evaluated. In some cases, like curcumin in the spice called turmeric, the health effects may be due to interactions between the diseased organ (such as the heart, brain or carcinoma) and the protein to which curcumin binds. Now that systems thinking is being used to discover and develop new drugs and therapies, the pharmacokinetics of more traditional medicines can be evaluated.

The placebo effect

In clinical trials that test the effects of prescription drugs on humans, another aspect of systems thinking emerges, the placebo effect [15]. That is, some patients get better, even if given a placebo, which contains no drug and is often simply a sugar pill. For the placebo effect to work, the patient must believe that the placebo will be beneficial. On the other hand, the opposite of the placebo effect often occurs. This is called the nocebo effect, in which patients get worse, just because they are told that they are susceptible to a disease [15]. Worrying and a feeling of hopelessness can be very bad for one's health. As a result, almost all diseases have a mental aspect and can be thought of as being at least partially psychosomatic [3]. For example, in the Framingham Heart Study, women who believed they were prone to heart disease were four times as likely to die as women with similar risk factors who didn't think that they were prone to heart disease [17]. On the other hand, if patients and their care givers can actively participate in finding the best treatments for the individual, it can help prevent the extra damage to one's health that depression can cause.

There was even a time when physicians thought that worrying could cause ulcers [6]. This attitude changed when it was discovered that ulcers are often caused by a type of bacteria, *Helicobacteria pylori*. Doctors stopped trying to cure ulcers with laughter or psychotherapy, and switched to the antibiotic amoxicillin. However, we now know that people can make themselves sick by self-induced stress, which can encourage the growth of *H. pylori* [6]. This is consistent with the idea that there is a gut-microbiota-brain axis that is important in psychiatric disorders and possibly even immune dysregulation [18]. This can't be predicted from the basic physical and chemical properties of the atoms and molecules some consider to be the basic building blocks of all matter, including living cells. Instead, the placebo and nocebo effects are emergent properties that only occur when atoms, ions and molecules are organized into the human and bacterial cells as well as viruses that interact with each other and make up the ecosystem that is a person's human body [6].

Even though systems thinking is very important for modern medicinal chemistry and new drug development, it has its limits [6]. In the early 1900s, before reductionist thinking was used very much, there were no cures for any infectious diseases. Traditional remedies, grandmother's recipes, and traveling salesmen were the only options for treatment. People died of diphtheria, pertussis, tetanus, Black Death, influenza, tuberculosis, Yellow Fever, typhoid fever, syphilis, rheumatic fever and cholera; all caused by bacteria. Viruses killed and maimed people by causing smallpox, polio, measles, mumps and other diseases. Appendicitis was often fatal, as doctors were unable to operate under sterile conditions. A simple puncture wound could cause death by tetanus. Men often had to marry several times, as one wife after another died during childbirth. Women who were lucky enough to survive often had many children, in hope that one or two might survive infancy. Unfortunately, many died in the first year of life. Children who did survive often suffered from common diseases, such as influenza, whooping cough, rheumatic fever, smallpox, chicken pox, polio, measles, and mumps [6].

The problem was made worse by an unregulated system for producing food and drugs [6]. Meat packing plants were notorious [6,19]. Food was often contaminated and caused food poisoning. To treat the sick, anybody could sell anything to an uneducated, desperate public. If the "snake oil" had enough alcohol, opium or cocaine in it, the patient might feel better, but the disease would usually get worse. Producers of such "elixirs" or "snake oils" did not have to list the ingredients on the bottle if they filed for a patent, so such things were called patent medicine. Eventually people started to see that cocaine was bad, not good. So, in 1906, the US Congress passed the Pure Food and Drug Act, which paved the way for the formation of the Food and Drug Administration, or FDA. In 1938, Congress passed the more comprehensive Food, Drug and Cosmetic Act. It gave the FDA the authority to oversee the safety of food, drugs and cosmetics. In time, a system of controls (Good Laboratory Practice, or GLP) was established to ensure that potential drugs are tested in a scientific manner and that approved drugs are manufactured in a clean, safe, and well-documented way (current Good Manufacturing Practice, or cGMP) [6].

This greatly improved human health, as scientists discovered antibiotics to kill bacteria and developed vaccines to prevent viral infections and even learned to put fluoride in drinking water to prevent tooth decay, periodontal disease, chronic inflammation and heart disease [6]. "As the 20th century progressed, agricultural revolutions emerged and farmers were able to feed many more people. Many of us would not be alive today if it weren't for these some of these discoveries. These successes led many to believe strongly in the power of modern western medicine and reductionist thinking" [6].

Reductionist thinking and DNA

This is in contrast to reductionist thinking, in which the whole is equal to the sum of its parts [6]. In this type of thinking, one must find the root cause of a disease and fix it. Also, DNA is thought to be the blueprint of life. All genes were considered

to be pieces of DNA that coded for mRNA, which coded for proteins, the "machinery of life". So, many drugs were developed with a single protein as the therapeutic target. Information was thought to flow from DNA to mRNA to proteins. Drugs acted by either binding to the active site of an enzyme or to the ligand binding site in a protein receptor. There was even a "one gene one protein hypothesis" in which each gene was thought to code for a single protein [6].

In contrast, it is now well known that DNA is not the blueprint of life [6]. "The properties of DNA and the messages it conveys depend on the environment of the DNA and the cell or organism. Proteins, RNA and even ions like Ca^{2+} and Mg^{2+} affect the production of DNA. It is often said that every type of human cell (except blood platelets and mature red blood cells) has the same genetic code (DNA), but that the code is read differently by different cells in different tissues and different environments. Also, availability of the code can be affected by epigenetics that attach methyl groups and other moieties that affect the accessibility of genes to transcription into mRNA. That means that we are coupled to the environment. Not only do we change the external environment, but it can also affect our internal environment. One way that this is done is for some pieces of DNA to move around to other places on the same or different chromosomes. These mobile genetic elements are active in the brain and help cause important differences in monozygotic ("identical") twins. There are also many epigenetic changes that can affect the internal and external environments of our cells" [6].

In contrast to older thinking, recent advances in DNA sequencing of single cells have shown that there is some heterogeneity or mosaicism in cellular DNA [19]. This was already well known in cancer cells, which can become polyploid, and in healthy Kupffer cells in the liver [6]. However, mosaicism is now known to occur in healthy skin, brain and white blood cells, as well as in induced pluripotent stem cells (iPSCs) [20-22]. There are also important differences in the transcriptome, epigenome, metabolome and exposome of cells [19]. "Recently, the whole genome, transcriptome, sites of DNA methylation, protein-DNA interactions, three-dimensional genome structure and B and T cell repertoires of a single person were determined. Also, the expression ratio of viral infection to healthy states was measured. Polysomal mRNAs were purified by genetic targeting and the functional consequences of genetic variations were determined. This produced an integrated personal-omics profile. It found a link between elevated blood glucose concentrations and viral infections. The subject changed his lifestyle to lower his blood glucose concentration. Subsequently, his whole genome methylation pattern was evaluated at several time points, his microbiome was analyzed and biosensors were used to monitor his physical activity and heart rhythm. Finally, he was able to find members of his extended family who had smoldering inflammation and unrecognized glucose intolerance. This caused several of them to change their medical care" [19]. So, much more than just the base sequence of one's DNA affects one's health.

Also, there is not a strict hierarchy in which one piece of DNA (gene) makes RNA, which causes proteins to be made. Some pieces of DNA are transcribed into long, pre-mRNA that contains insertion elements (introns) and exons. The introns are cut out and removed, while exons are spliced together. Exons can be mixed and matched in different ways and pre-mRNA modified further to make different mRNAs that are translated into different proteins. There are also other types of RNA (such as long non-coding RNA, lncRNA, and micro RNA, miRNA) that can affect the ability of a gene to be transcribed or an mRNA to be translated into a protein [22]. Moreover, proteins that bind to RNA can control how DNA is transcribed [6]. So, many RNA molecules play central roles in gene expression and the phenotypic complexity. We now know that most genes are made of RNA, not DNA. Only a small portion of the DNA in cells actually codes for mRNA and proteins. About 76% of human DNA is transcribed into different types of RNA. People working on a project called ENCODE have constructed an "Encyclopedia of DNA elements" [23]. It found that 80% of human DNA has a biochemical purpose. Not only does a small portion of our DNA code for pre-RNA, which will become mRNA and proteins (20,867 protein-coding genes), but other parts of DNA provide binding sites for regulatory proteins (which influence gene activity). Some of the DNA also codes for different types of RNA that have many diverse roles. Also, some parts of DNA can be modified (by the addition of methyl groups or acetyls), causing genes to be silenced. That is, it has been shown that the regulation of gene translation and mRNA transcription in humans is more complicated than previously thought. Gene transcription can be controlled by several stretches of DNA located both near and far (*cis* and long-range regulatory elements) and by non-coding RNA [6,23]. Moreover, histone proteins and ribonucleoproteins are integral parts of chromosomes that can be modified and affect gene transcription [6].

Once proteins are made, they can be modified by the addition of other types of ions and molecules, such as phosphate, acetyl groups, sugars, and lipids, which are not coded for by DNA [6]. As a result, there is no single code or blueprint of life. For example, some genes are expressed quite differently as a newly fertilized egg develops into a fetus, then a baby, and even more as development progresses. Also, the same gene can do very different things in different organisms. So, the properties of genes depend on the environment in which the genes find themselves. Also, there are epigenetic changes that can be caused by environmental pollutants, such as endocrine disruptors, and these changes can be passed down to future generations [6]. This is consistent with systems thinking.

Examples of systems thinking in biology and medicine

Changes in the definition of genes

We now know that most diseases are not caused by defects in a single gene, but by several genes and/or gene products [6]. There is now much network data on proteins, protein-protein interactions and DNA-protein interactions. The prescription drugs, tamoxifen, bicalutamide and all-*trans* retinoic acid target interactions between DNA and transcription factors (estrogen, androgen and retinoic acid receptors). Also, azacitidine targets DNA methyltransferase, while vorinostat and romidepsin target histone deacetylase. There are also descriptions of how cells are organized networks of metabolic and signal transduction cascades. There are also many -omics, including genomics, epigenomics, transcriptomics, proteomics, lipidomics, metabolome and the glycome, as well as the exposome. They are collections of all the genes, epigenetic modifications, transcription products, proteins, lipids, metabolites and glycans (carbohydrates) in a cell or organism, as well as the effects of exposure to environmental chemicals. Moreover, there are websites with data on interactions (interactome, integrome) and even an omnisciome that aims to be all the "knowledge about a cell, organism or system" [6,24].

Before the emergence of all this network data, drugs were developed based on their effect on a disease phenotype or their ability to affect a single molecular target [6]. This was based on a linear cause and effect model for many diseases and the drugs that affected them. However, this is being replaced by a multidimensional, non-linear view of diseases. For example, we now know that types 1 & 2 diabetes, coronary artery disease, and glioblastoma all result from small defects in many genes, rather than large defects in a few genes [25]. "Instead of looking at the effects of a test compound on a single gene or gene product, the effect of a perturbagen on the entire network system is evaluated. This allows investigators to probe the pattern of interactions in a disease model and identify underappreciated pressure points that could be novel drug targets. The effects of test compounds on several different cell types and variations in gene networks that define disease, toxicity or other relevant phenotypes can be evaluated. This can lead to predictive models that define networks for a particular disease subtype or for toxicity. These models can be used to construct gene expression-based assays for high-throughput screening. The effects of test compounds on the network of interest can be assayed in cell-based systems that reflect the states of the networks of interest. Such assays can be developed by identifying nodes in the networks of interest that reflect network states. High-throughput screening of compounds that affect such nodes can identify drugs that affect disease networks in favorable ways, and identify toxins that affect networks associated with adverse side effects. Compounds can be identified that target specific subtypes of disease without affecting networks that can lead to toxicity or adverse events. Drugs specific to a particular disease subtype can be matched with patients so that the right drug is given to the right person at the proper time" [25].

Network based drug discovery

Ideally, the network based drug discovery process will identify all the molecular nodes that affect a disease network [25]. "For example, the variations in DNA or genes in a population can be considered to be perturbations that affect RNA levels and/or protein states. Those changes can affect higher order phenotypes, such as disease or drug response. Instead of identifying a single target in the web of genes and proteins, large classes of compounds should be screened to identify those compounds (or combinations of compounds) that perturb the disease networks in ways that return the network to its pre-disease state. Test compounds that have favorable effects on networks that are associated with the disease can be selected and optimized, so that effects on metabolism and toxicity can also be optimized" [25]. This has been described in more detail [25].

A major advantage of the disease network approach is the construction of networks that predict specific forms of diseases that represent different phenotypes. Drugs can be screened against these phenotypes by monitoring thousands of variables in thousands of contexts, such as the presence of different perturbagens. New combinations of drugs that treat different cancers have been developed by screening compounds against the expression of many genes [26]. Combination therapy has also proven successful in anti-malaria and anti-HIV therapies. Even drugs that were approved based on their binding to one target are now known to bind to multiple targets [27]. This includes Gleevec, which was initially developed to specifically inhibit the abnormal tyrosine kinase BCR-ABL and is used to treat chronic myeloid leukemia. It is now known to target several other kinases simultaneously, including c-KIT and platelet-dependent growth factor receptor (PDGF-R). Similarly, celecoxid was developed based on its ability to inhibit cyclooxygenase, but it is now known to inhibit carbonic anhydrase [28]. The atypical antipsychotic drug clozapine binds to many targets in the central nervous system at nanomolar concentrations. So, computational chemogenomics is being used to systematically analyze and predict biological activities of test compounds against a wide range of biomolecular targets [28].

As a result, researchers are looking for similarities in drug binding sites [27]. There is a database, Cavbase, which stores the three-dimensional property descriptors that encode the physicochemical characteristics of each of the protein cavities obtained from structures in the protein databank (PDB), and ranks the similarity according to the match of the property descriptors [27]. In addition to facilitating the prediction of unknown protein function, Cavbase can also be used to extract protein targets exhibiting similar ligand binding properties.

The modularity of biological networks

It is also important to consider the modularity of biological networks [29]. Many previous studies have dealt with network modules as if they were static. These studies focused on the topological properties of the networks. However, biological networks can be dynamic. Expression levels of different types of RNA and proteins can depend on internal and external environmental factors and signals. Dynamic modules are closely regulated, whereas static modules are not [29]. So, the variance in expression levels, or expression variance (EV) of proteins, was determined in a recent study [30]. "An EV of zero indicated the lowest EV (static), while an EV of one was assigned to genes with the largest variance (most dynamic). Genes with low EV values are expressed at lower levels than genes with high EV values. There was a strong correlation between the EVs of proteins and their neighbors in a module. That is, the protein interaction network is enriched for sub-networks that are primarily composed of either static proteins or dynamic proteins, but not both. Proteins that have more interaction partners in the network are segregated into distinct network neighborhoods that are characterized by low and high EVs. Also, dynamic proteins, in contrast to static proteins, are more functionally homologous to their neighbors when they are in dynamic neighborhoods. They also have higher Pearson correlation coefficients, which measure how well proteins in a neighborhood are co expressed with each other" [30].

Also, it was found that the some hubs that had been called date hubs are found within static modules, where there is also no statistical correlation of expression among neighbors [30]. "So, it was proposed that static hubs interacting with static proteins within static modules should not be considered to be date hubs, but instead should be called 'family' hubs, since they are always present in the network and interact with their neighbors constitutively. Therefore, family and party hubs form static and dynamic modules, respectively, whereas date hubs organize the network. Date hubs contain signal transducing and signal regulating proteins such as protein kinases, phosphatases, small G-proteins and molecular chaperones" [30].

Dynamic modules can be modified, so they are mainly responsible for regulating cell behavior, depending on environmental conditions. They tend to be co-expressed together. On the other hand, static modules help make cells robust and resistant to genetic changes and differences in the levels of protein expression. Static modules helped develop the concept of robustness-related module criticality. That is, perturbations of self-organized systems are neither dampened nor amplified, but are propagated over long temporal or spatial scales. This enables information to propagate over time from one part of the system to another with a high degree of specificity and sensitivity. Criticality enables the coordination of network behavior to maintain an optimal balance between stability and adaptability [31]. For example, the human brain has broadband criticality that helps to optimize information processing and balance excitation and inhibition [31]. Moreover, diseases have modified modules.

A cascade of network modules can define cancer progression and help influence the diagnosis, prognosis and treatment. Some modules show significant changes in cancer-related signaling through the tumor suppressor proteins, p53, retinoblastoma protein (pRb), phosphatidyl inositol 3-kinases (PI3Ks) and receptor protein kinases. Module maps help to understand gene expression profiles in different tumors. Specific network modules consisting of sets of genes that act together to perform specific functions can be evaluated. Some of the modules may be activated in particular types of cancer [29]. Also, specific treatments have been suggested by examining the network of genes that are expressed in a disease. For example, the human genome atlas project found that breast cancer can be thought of as four different diseases that may be treatable by different approaches [32]. Primary breast cancers were analyzed for genomic DNA copy number, DNA methylation, exome base sequences, mRNA, microRNA (miRNA) sequences and proteins. They used a program called mutual exclusivity modules in cancer (MEMo) to analyze the data and identify the different disease network modules [32].

Autopoietic theory of life

Another good example of systems thinking in biology is the autopoietic theory of life [4,33-35]. In it, living organisms are recognized as being self-making (autopoietic) and self-maintaining systems. This is contrast to reductionist thinking that considers life as being a predictable machine. Machines do not make themselves. They are made by humans (or chimpanzees). On the other hand, almost all the cells in our bodies, and almost everything inside them, are continually being broken down and re-made. That is, organisms and cells in multi-cellular organisms have an outer layer (cell membrane, skin) that is continuously being broken down and re-made. Our memories, structures, shapes and forms stay almost the same, even though the molecules have changed. Somehow, people can remember things for over 100 years, even though none of the molecules in their bodies last for more than 10-20 years, and most last just a few days or weeks. Brain cells are continuously interacting and changing their connections with other brain cells. The turnover of blood cells in a man weighing 70 kg is close to 1 trillion cells per day, including 200 billion erythrocytes (red blood cells) and 70 billion neutrophilic leukocytes. This remarkable cell renewal process is supported by a small population of bone marrow cells called hematopoietic stem cells. So, billions of human cells undergo programmed cell death, also known as apoptosis, every day. Cognition, thinking and the mind are not defined by molecular events, but by in-

teractions between cells, tissues and organs. The mind is not a thing that is located in the part of the machine called a brain, but it is process that is an integral part of life [3,4,6,33-35].

Also, life is a cyclic process that produces the components of a living system [3,4,6,33-35]. The average human cell has about 10^5 macromolecules, and each of these is destroyed and remade 10^4 times in a cell's life time [4]. The cells of our stomach lining are replaced every five days. Skin and red blood cells live for a few days to a week. Teeth and bones are regenerated every 10-15 years. Even the proteins within long-living neurons are recycled every 90 days. Still, the form and organization of the cells and the organism stay the same and under the control of that organism or cell. This is quite different from a computer or a machine, whose components are designed by humans and made by humans [4].

However, stem cells are notable exceptions to this continuous breakdown of cells, since they can last for an entire life [6,36]. They have the potential to differentiate into many different types of cells [36]. At the same time, they are unique. They can not only self-renew, but also make differentiated cells. That is, when highly differentiated human cells undergo mitosis, they produce two identical cells. On the other hand, stem cells can divide in two different ways. A stem cell can produce two identical stem cells to maintain a reservoir of stem cells for the future. Alternatively, they can divide to produce one stem cell plus a more differentiated cell. Embryonic stem (ES) cells can make any human cell type. They can divide to produce one ES cell plus a slightly differentiated adult stem cell that is specific for each tissue. The ES cells are totipotent, since they can make any human cell type in the body, while adult stem cells are pluripotent, because they can make many (but not all) human cell types [6,36].

Dynamic systems view of stem cell biology

This leads to a dynamic systems view of stem cell biology [37]. "It represents different cellular states as points in multidimensional state space. Each axis in that space represents the abundance of genes, proteins and metabolites. Perturbations of the interactions between them can cause the state of a cell to shift from a stem cell to a differentiated cell. Stem cells and differentiated cells not only must be able to remain in the proper state when perturbed slightly by noise-generated fluctuations, but also be able to differentiate further when perturbed by additional appropriate signals. This balance must be maintained to ensure proper development during childhood and good health in adults. This is done by fluctuating and oscillatory gene expressions" [37].

Through important research and systems thinking, Japanese scientists were able to convert mouse skin cells into ES cells [38]. "They only had to insert four genes (Oct4, Sox2, Klf4, and c-Myc) into the adult cells, using retroviruses as transduction vectors. This produced induced pluripotent stem (iPS) cells, or iPSCs. This may prove to be an excellent way to mass produce one's own stem cells, which could be further stimulated to produce different organs for organ transplants to one's self. This would eliminate the current difficult problem of organ rejection when organs are donated by someone else" [38]. So, it is noteworthy that a vascularized and functional human liver was made from liver buds produced from iPSCs [39]. More recently, it was found that the transposon called Sleeping Beauty can be used to reprogram cells and do targeted insertions into induced pluripotent stem cells (iPSCs) without having to insert Oct4, Sox2, Klf4, and c-Myc genes [12].

Until recently, it was not known whether iPSCs could ever be able to be reprogrammed so that they can make any human cell, like ES cells can [40]. However, Japanese scientists were able to cause female germ cells to develop and produce fertile offspring. They intend to do the same thing with other mammals, including humans [40].

It is also possible that iPSCs could transform drug discovery and development [41]. "They can be used instead of animal models to evaluate the toxicity and clinical efficacy of drugs. That is, expensive and controversial pharmacokinetic and toxicokinetic studies are currently done on animals-usually mice. However, the results from animals often are not able to predict what will happen in humans. It has been estimated that the attrition rate of drug candidates is about 96% and that 2644 people died during clinical trials for 475 new drugs between 2005 and 2012. Moreover, only about one in 10000 new chemical entities are eventually approved and enter the market. To improve on this, patient-specific iPSCs can be prepared and used to prepare 'mini-livers', 'mini-brains' and 'mini-lungs' that exhibit the properties of whole organs. They can be used to test the efficacies of new drugs and to look for toxicities, as well as to build human disease models on a chip. This would be much cheaper, faster and productive than animal models. It may also be possible to treat or even cure diseases by permanently reversing the disease condition in a patient's iPSCs and re-injecting them back into the patient [41].

Personalized therapy

In a different twist, the FDA named the therapy called CTL019 as the breakthrough therapy of the year for treating acute lymphoblastic leukemia (ALL) [42]. "It is a personalized cellular therapy in which a patient's T-cells are removed by aphereis. They are genetically modified so that they have a chimeric antigen receptor that couples an anti-CD19 single chain Fv domain with intracellular T-cell signaling domains of the T-cell receptor. They were put back into the patient's blood, resulting in a 90% remission rate for up to two years in the 30 children and adults who received it" [42]. Finally, an international effort

is being made to produce a bank of chemically-induced iPSCs that can be used for drug discovery [41].

So, stem cell technology and personalized cellular therapy may transform personalized medicine. They will help emphasize that we are all unique, unlike machines. However, reductionist thinking and Western medicine continue to play important roles in modern biology and medicinal chemistry [6,42].

Predictive, preventive, personalized and participatory (P4) medicine

Principles of P4 medicine

Perhaps nothing epitomizes this fusion of traditional and western medicine more than predictive, preventive, personalized and participatory (P4) medicine [42,43]. It takes not just a holistic but also a quantitative and mathematical approach to practicing medicine. At the same time, systems medicine emphasizes prevention and individual participation in one's own health care. It recognizes the important human need for patients and care givers to be actively involved in preventing and curing their diseases. Mathematics, the foundation of reductionist thinking, is used to quantify huge datasets from patients, while physicists, chemists, biologists and engineers develop the analytical tools needed to generate the data. All of this can be linked through the internet and used in mobile healthcare applications [38,39]. So, systems medicine is "the application of systems biology to the study of human disease" [43]. It can use data about biomarkers taken from many different people and tissues within each individual to help everyone work with their physicians to make their own medical decisions [33,34].

Personalized medicine has been defined as, "the ability to customize medicine using molecular information to more accurately understand disease patterns and diagnose disease, as well as to tailor preventive and therapeutic intervention more effectively with fewer side effects" [44]. "It includes not only prescribing medicines, but also maintaining the mental and physical well-being of the patient and care givers. Pre-emptive genome-based testing of adults and children in personalized health care is becoming very helpful, especially when studying diseases with Mendelian inheritance. Diagnostic tests are now available for over 2000 Mendelian conditions. These tests are changing the paradigms for screening and diagnosing rare conditions. Personalized medicine can help identify patients who are more susceptible to certain diseases or disease-related symptoms or are pre-symptomatic. It will identify patients who will respond to preventive treatments differently or whose diseases or symptoms may progress differently when compared with others in the general population. Just as important, personalized medicine engages patients and helps them prevent diseases, decide treatments and monitor recovery. As we continue to personalize healthcare, the public is expressing their desire to participate actively in healthcare decision-making that is based on analyzing their genomes" [44].

History of personalized medicine

Actually, personalized medicine has been used for over 100 years to analyze blood types, to ensure that transfusions don't cause hemolytic reactions [42]. Also, the genetic basis for the selective toxicities of fava beans and an antimalarial drug (primaquine) was discovered over 50 years ago. Their toxicities are caused by a deficiency in glucose-6-phosphate dehydrogenase (G6PD), an enzyme that is important in metabolism. Subsequently, in 1977, different isozymes of cytochrome P450 2D6 (CYP450 2D6) were shown to cause the effects of the anti-hypertensive drug debrisoquine to be exaggerated and last longer than in others. So, genetic differences can cause several pharmacokinetic parameters, including the very important area under the curve, or AUC. Genetic differences are the basis of pharmacogenomics, or the study of how variations of DNA and RNA characteristics affect responses to drugs. It has been a crucial part of personalized medicine for decades [42].

Goals of P4 medicine

The goal of P4 medicine is to prescribe appropriate, individualized drugs and medical devices for people with different types of nutrition, environment, genes, mRNA, miRNA, epigenetics and/or proteins [42]. Such treatments should be designed for the patient's specific anatomy (size), physiology and environment (home, hospital, ICU). Diagnostic devices can monitor vital signs, blood glucose, oxygen or other small molecules. They can monitor brain and heart activities with electroencephalography (EEG) or electrocardiography (ECG or EKG) and do diagnostic imaging. Some can even determine part or all of the genome, epigenome, transcriptome, proteome and metabolome of the patient and/or his or her diseased cells. Also, the patient's blood or tissues can be analyzed for different types of enzymes (isozymes, like CYPs) that catalyze reactions that can metabolize drugs differently and affect their bioavailability, or ability to bind to different receptors. This approach led to the development and rapid approval of tratuzumab, or Herceptin®, for treating and curing patients who have the HER-2 gene that is involved in many cancer signaling pathways. More recently, it led to four anticancer drugs being approved by the FDA for use in patients who have specific genetic characteristics that can be identified by a companion diagnostic test [45]. Individualized medical devices are being made, as well. For example, three-dimensional printing was used to make a bioresorbable tracheal splint for an infant who was critically ill. So, advances in 3-D printing, genomics, medical imaging, and regenerative medicine, along with increased computational power and the growth of mobile and wireless capabilities are allowing patients to be treated and monitored in ways that

meet their individual needs better [45].

Evidence based medicine

This is consistent with evidence based medicine. The North American Menopausal Society (NAMS), American College of Obstetricians and Gynecologists (ACOG), U.S. Preventative Services Task Force (USPSTF), National Institutes of Health (NIH), and US Food and Drug Administration (FDA) use evidence based medicine in developing their guidelines for therapeutic practices [46]. "Evidence based medicine uses individual clinical expertise with the strongest available external clinical evidence that is obtained from systematic research. It has also been described as the conscientious, explicit, and judicious use of current best evidence in making decisions about the care of individual patients" [46]. It involves reductionist thinking and systems thinking. That is, reductionist thinking is used in the basic science involved in establishing the proof of principle behind a drug's effects, improving the lead compound, pharmacokinetics and identifying diagnostic biomarkers. Systems thinking is needed when physicians use their clinical experience to make an effective diagnosis and to thoughtfully identify and compassionately consider "individual patients' predicaments, rights, and preferences in making clinical decisions about their care" [46]. So, evidence based medicine is not a "cookbook" approach, in which diagnoses and treatments are dictated from above. Instead, it is "a bottom up approach, in which the best external evidence is used with individual clinical expertise and patients' choice" [46].

Another way to help patients meet their individual needs is to develop drugs that have fewer or no harmful side effects [42]. When reductionist thinking and Western medicine predominated last century, many drugs were developed that either targeted the active site of an enzyme or the ligand binding site on a protein receptor. This was consistent with the idea that if you could find the single root cause of a disease (like a defective protein), you could cure it by targeting the root cause. This produced many useful drugs that saved lives and improved public health. However, the active sites of many enzymes and ligand binding sites of many receptors have similar structural motifs in other proteins. This means that many older drugs would also bind to proteins that were not the desired therapeutic target, causing harmful side effects. On the other hand, there are allosteric sites on enzymes and protein receptors that are being targeted by new drugs that are being developed. Moreover, they can affect protein-protein interactions and have multiple therapeutic effects that can be modulated when a drug binds to an allosteric site on one of the proteins. So, allosteric drugs are being developed [42,46-54].

Allosteric drugs and combination therapies

Changing views of allostery

Drugs are being developed that bind to allosteric sites on enzymes (as opposed to active sites), partially inhibiting them and causing fewer side effects than drugs already developed that target active sites. This is another example of systems thinking replacing reductionist thinking, in which not only DNA, but also proteins were thought to be relatively immobile. The first DNA and protein structures to be described were based on X-ray crystallography of crystalline solids. This led to the idea that there was only one conformation of an enzyme that was biologically active. This was supported by the fact that many water-soluble enzymes could be denatured and rendered insoluble by heating them in aqueous solution. In the active state, an enzyme was thought to be folded into a single structure. Once denatured, it unfolded into a random coil. However, it was also noticed that there were many enzymes that were difficult or impossible to make into crystals that could be analyzed by X-ray diffraction. Moreover, as stronger magnets were made, NMR became able to determine the structures of some protein. In contrast to X-ray crystallography, NMR was able to see proteins that were dissolved in aqueous buffers, so they could be more mobile than when they were as solid crystals.

It was found that proteins could exist in several conformations. It is now known that the structures of active enzymes and other native proteins are flexible and this is vital to their proper physiological functions [55,56]. We also know that many important proteins that are hubs in the cellular network (such as p53) can bind many different substrates or ligands to cause a wide variety of physiological effects. Such proteins have flexible portions that can be converted into many different less flexible structures after they bind different biomolecules. These proteins can be almost impossible to crystallize when no ligand is bound to them. Instead, NMR can be used. The spectra obtained can be analyzed to produce several structures. So, when one looks for the structures of proteins on the Protein Data Bank, data based on X-ray crystallography will show just one structure, while those based on NMR will show several.

That is because proteins are dynamic, robust and adaptable [57]. These three properties cause global changes in protein structure to emerge from local, dynamic changes. They can undergo structural changes without losing their function, making them robust. They are also adaptable in that new biological activities can emerge [57]. So, a new view of allostery has emerged and is described in detail by others [58-68].

Combination therapies and multi-target drugs

Systems thinking has also led to combination therapies and multi-target drugs that combine several effects, often far from

the malfunctioning protein in the cellular network [68]. "A deep understanding of how the cellular networks (interactomes and signaling networks) work, along with detailed structural knowledge of the proteins and complexes involved will be needed to find the proper drug targets. Recently, analyses of drug-target networks showed that in more than half of the known 922 drug-disease pairs, the actual disease-associated proteins are not targeted. Instead, their third or fourth nearest neighbors are. That is, the action of drugs can be thought of as a network perturbation. The drug-induced effects can either destroy the network of infectious agents or cancer cells, or shift the pathophysiological network back to a healthy state. Drugs that are used in anti-infectious or anti-cancer therapies often act at cross-roads of cellular pathways. On the other hand, in diabetes and neurodegenerative diseases, efficient drug targets are not directly involved in major cellular pathways but indirectly influence them in a highly efficient manner. So, the effects of allo-network drugs may be indirect but they can be cause specific, limited changes at the systems level. The result is fewer side-effects and lower toxicities than those of conventional drugs" [68].

This is important, because the previous paradigm for structure based drug design that was based mostly on reductionist thinking hasn't produced enough NMEs [64]. "Moreover, many of the NMEs discovered by reductionist thinking fail in the final, most expensive phases of clinical trials. Instead, systems-based drug discovery and design offer a likely solution to this problem. Biological networks as well as protein-protein interactions (PPIs), signaling and metabolic networks can be modeled and analyzed. In this new paradigm, scientists look for changes in the networks that occur in diseases, which are undesirable network states. Drug therapy should shift the network back to a healthy state. Network dynamics can be altered by changing the levels of expression of related drug targets. Then, NMEs can be found by studying not only the affinity of drug binding, but also the drug's efficacy. To assist in this, a multi-purpose program called LigBuilder has been developed for de novo drug design. It helps to determine how easy or hard it might be to synthesize a compound using an embedded chemical reaction database and a retrosynthesis analyzer. There is also a cavity detection procedure for identifying binding sites. Moreover, network simulations and network pharmacology have been used to determine how the many compounds in traditional Chinese medicines work. They can target several sites simultaneously. This is consistent with the emerging paradigm of multi-target drug design and systems thinking" [64].

Examples

For example, one way of targeting more than one protein is to administer a protease inhibitor and a non-nucleoside reverse transcriptase inhibitor in highly active antiretroviral therapy, or HAART that is used to treat AIDS [42]. Concerted pharmacological interventions that use multiple drugs are also used to treat cancer, chronic obstructive pulmonary diseases (COPD), heart failure, acne, hepatitis C, rheumatoid arthritis and type-2 diabetes [64]. "Diseases can also be prevented by vaccines that target different parts of pathogenic viruses. Natural products that are used in traditional medicine also contain several active ingredients. Another way to do systems based drug discovery is to target protein-protein interactions and design allosteric inhibitors" [64].

That is, drugs that target the active site of a single target enzyme often bind other proteins or isozymes that have similar active sites, thus causing harmful side effects [42]. "This is less likely for allosteric drugs, because they bind on the protein surface away from the active site. This makes them more specific, with fewer side effects. For example, there are drugs that bind to the pleckstrin homology (PH) domain of proteins in the phosphatidyl inositol 3-kinase-Akt (PI3K-Akt) signaling pathway. Some of them inhibit individual Akt isozymes. On the other hand, allosteric drugs don't bind to the active site, so they don't act as off and on switches. Instead, they can modulate activity at relatively low doses and have therapeutic effects without harmful side effects. Examples include modulators of phosphodiesterase 4 activity that are useful in treating neurodegenerative diseases. A classic example is valium. More recent examples include maraviroc (Selzentry™) and cinacalcet (Sensipar™), for AIDS and hyperparathyroidism, respectively. So, many drug pipelines are being dedicated exclusively to the development of allosteric drugs. This concept is being expanded to allo-network drug effects, in which binding of a drug to an allosteric site on one protein can have multiple effects on proteins to which the target protein is linked. Combination therapies and multi-target drugs are examples of allo-network drugs. They cause multiple effects, often at places distant from the malfunctioning protein in the cellular network" [42,69].

For example, a structural motif that occurs in many receptors and enzymes is the plexstrin homology (PH) domain, which occurs in proteins involved in signaling pathways, such as the phosphoinositide 3 kinase (PI3K)-Akt pathway [42,69]. "Drugs have already been developed that bind to the active site. Phosphoinositide analogues inhibit Akt, but may also bind other proteins containing the PH domain and cause unwanted side effects. On the other hand, PH domain-dependent inhibitors exhibit selectivity and potency for individual Ser/Thr kinase Akt isozymes. In addition, allosteric drugs do not block the active site, so they are not competitive with the natural substrate (ATP in this example). As a result, they do not act as an on/off switch for a receptor, but instead allow intermediate levels of activity" [42,69].

Other examples include allosteric drugs that modulate the activity phosphodiesterase 4 (PDE4), without completely inhibiting it [42,69]. "They cause fewer incidences of emesis, a

dose-limiting side effect of existing active site PDE4 inhibitors like apremilast and ibudilast that block the hydrolysis of the second messenger, cAMP and treat neurodegenerative diseases. These advantages exist in other allosteric drugs, from valium and the benzodiazepines to the more recent development of maraviroc (Selzentry™) and cinacalcet (Sensipar™), for treating AIDS and hyperparathyroidism, respectively" [42,69].

Also, there are several research projects that are dedicated exclusively to the development of allosteric drugs [42]. Recent examples of allosteric drugs include RDEA119/BAY 869766 as an allosteric mitogen-activated protein/extracellular signal-regulated kinase (MEK) inhibitor, pyrvinum, a potent allosteric inhibitor of Wnt signaling, efavirenz, an allosteric reverse transcriptase inhibitor and SCF-I2, an allosteric inhibitor of (yeast) F-box protein Cdc4 [70]. Allosteric drugs could prove to be very useful if they target proteins that are nodes in the metabolic network which are being identified by new mathematical tools and algorithms [42,71]. "They can identify nodes which are dynamic, interacting with other nodes at different times and/or conditions. Such hubs preferentially connect functional modules to each other, whereas party hubs preferentially act inside functional modules. Some of them have been identified in yeast by observing its interactome in response to perturbations. They mediated the transmission of perturbations between signaling modules and are important in network cooperation" [71]. So, multitarget drugs that partially inhibit several nodes may be needed to modify many cellular functions, with little specificity for a particular disease process.

Infrastructure supporting personalized medicine

Inter-agency infrastructure

In the 21st century, doctors, mathematicians, physicists, chemists, biologists, systems engineers and many others are working together to develop new pharmaceutical compounds and medicines [42,72]. The US government's National Institute of Health (NIH) has a division called the National Center for Complementary and Alternative Medicine (NCCAM) to work with scientists to study traditional remedies. Biologists are joining with mathematicians to understand network medicine. Together, they have made many advances.

To support this effort, the US FDA and other countries' regulatory agencies have worked with industry and academia to build an elaborate infrastructure to support personalized medicine. This has been described as the five pillars of systems medicine: cutting-edge technologies, digital infrastructure, personalized data clouds, new analytical tools and systems biology models [72]. "Cutting-edge technologies and algorithms can gather data, set up user-friendly databases and do analyses. For example, in 2003 the NIH started a project called ENCODE to identify and define the functional DNA elements that are required for normal genome function. In 2012, about 40 articles were published describing the results of what had become an international effort. The internet and mobile telecommunications are establishing data clouds-hopefully for everybody. Quantitative metrics are being established for what it means to be healthy, pre-disposed to a disease, or in various stages of a disease. The data will also help find biomarkers that can help decide on the best therapies for each individual, but also monitor the treatment process, making necessary changes when new conditions emerge" [64].

Cost effectiveness

Moreover, systems medicine is making disease care more cost effective in human and financial terms. Treatments and potential cures for previously uncurable diseases are emerging. This continues to be done by separating people and diseases into distinct subgroups [72]. Genomics and other analyses can stratify people's disease risks, reactions to drugs and other clinically relevant factors into subgroups. For example, diseases such as breast cancer, which were once classified as single diseases, are being stratified into clinically relevant subgroups based on interactions in genetic, molecular and cellular networks [73]. Prostate cancer [74] and Crohn's disease [42,75] stratifications are providing increasingly more accurate diagnoses and cost-effective interventions based on the underlying causes of disease [72]. "Surgery and other aspects of treatment will be informed by disease stratification and individual needs. By focusing on the causes rather than the symptoms of a disease, physicians and patients will be able to prevent diseases from occurring in the first place, or stop them before they can cause serious damage. Moreover, as we identify and understand the biological networks that are perturbed in diseases, systems medicine will continue to provide a stream of new drug targets for the pharmaceutical industry" [72].

The drugs will be more effective and have fewer costly side effects, since they will be more personalized [72]. "They will target specific strata of the populations of people and types of diseases. It will be cheaper for pharmaceutical companies to do clinical trials if they are done on the correct patient populations. Interventions (including, but not limited to pharmaceutical interventions) will start at an earlier stage in the disease process, often pre-symptomatically, where they are much more cost effective. The impacts of such interventions are being more accurately monitored, allowing for adjustments to be made to improve outcomes and reduce costs" [72].

New networking tools

As part of this system, the goal is for as many people as possible to have a personal data cloud. It will act as a medical record, with all of the health data for each individual - including

the genome, blood chemistry, lifestyle data (activity levels, diet and stress), transcriptome and gut microbiome [72]. "The data will be collected and analyzed to produce a stream of highly personalized information about each person's health and disease. Furthermore, actionable information can be supplied back to individuals based on the analysis of data accumulated in their personal data cloud. This will be given not just to physicians and other professionals, but also to individuals and those with whom they confide. Finally, systems biology and medicine work together to create a cycle of innovation. As new biological insight inspires the development of new analytical tools, new tools and technologies produce new data, which drives the creation of more analytic tools that advance biological insight" [72].

This has been apparent since the decision was made in 1990 to sequence the human genome in 15 years. Advances in DNA sequencing produced data faster than expected. During the early years of the Human Genome Project, bioinformatic tools were produced to sort the billions of fragmented sequences into the complete genome (shotgun sequencing). This helped the International Genome Sequencing Consortium and others to announce the complete DNA sequence in just 13 years, in 2003. Many government agencies, including the US FDA established collaborations such the Center of Excellence for Bioinformatics, Functional Genomics, and Structural Genomics, Office of In Vitro Diagnostic Device Evaluation and Safety and Voluntary Genomic Data Submission (VGDS) program were established in 2012 and were quite helpful in solving the human genome. Then, in 2004, the FDA created the Genomics and Targeted Therapy Group. Numerous other groups were formed, including the Personalized Medicine Team in the Center for Biologics Evaluation and Research (CBER) in 2011 and the Division of Systems Biology at the National Center for Toxicological Research (NCTR). Also, the FDA has issued at least 21 guidances that relate to personalized medicines. This includes guidances on pharmacogenomics data submissions, tests, definitions, considerations, applying human factors and current Good Manufacturing Practices (cGMP) for combination products [76].

International collaborations

The FDA is also collaborating with other governments, academia and industry to develop regulatory standards, reference libraries, research methods, and tools that can be used to integrate biomarker identification into drug and device development and help make clinical decisions [69]. The biomarker qualification program aims to provide a framework for scientific development and regulatory acceptance of biomarkers for use in drug development, facilitate integration of qualified biomarkers in the regulatory review process, and encourage the identification of new and emerging biomarkers. There is also a project on microarray and sequencing quality control, a genomic reference library for whole genome sequencing (WGS) platforms and a virtual physiological patient. A high performance integrated virtual environment (HIVE) for next generation sequencing analysis is being built. Moreover, high resolution human leukocyte antigen (HLA) typing systems are being developed, as well as molecular tools to facilitate blood group typing. The FDA and other governments' health care agencies are working with others to design and conduct clinical trials better. They are refining the design of clinical trial and the statistical methods of analysis to address issues such as missing data, multiple endpoints, patient enrichment, and adaptive designs that often arise when developing targeted therapeutics. They are also looking closely at clinical trials of anticancer drugs. This is complicated because many cancers are heterogeneous, each with their own specific genetic makeup. This heterogeneity is one reason why different people with cancer located in the same organ often respond differently to the same therapies. To address this problem, the I-SPY 2 trial was started. It is a collaborative initiative developed under a unique public-private partnership that includes more than 20 cancer centers. They are trying to understand better the heterogeneity and complexity of diseases to provide targeted therapies [76].

It is also essential to have adequate and robust statistical methods to analyze data. So, scientists at Booz Allen Hamilton, the FDA supercomputer center, the Genomics Evaluations Team for Safety (GETS) and the Office of Vaccines Research and Review (OVRR) in CBER are comparing different methods to analyze genomic data to predict patient outcomes and/or prognosis. CDER and CDRH are also developing new device diagnostics to improve drug safety. They are assessing new device-based algorithms and biomarkers that can distinguish between benign and malignant drug-induced QT prolongation in an electrocardiogram [76].

The National Center for Toxicological Research (NCTR) is also doing research on the biology of cancer [76]. "One research project found that many tumors carry subpopulations of KRAS mutant cells, which can contribute to acquired resistance to some cancer therapeutics. Experimental approaches are being developed to identify effective treatments to prevent drug resistance in tumors with defined genetic profiles. Researchers at CBER are identifying pharmacogenetic determinants of immunogenicity in patients with Hemophila A. They may eventually be able to predict a patient's risk of immunological response to a given protein therapy before it is used in treatment. Other researchers at CBER are trying to understand better the effects of DNA modifications on the quality of protein products. Using proteins that are involved in blood clotting as models, they demonstrated that while "synonymous" or "silent" mutations do not affect the protein sequence, they may affect protein levels as well as protein folding and function. They want to know which mutations are deleterious and which may be safely em-

ployed in the design of therapeutic protein products. Their goal is to develop tools and methods to evaluate protein properties from the gene sequence. This could have many diverse implications for developing and evaluating safe and effective protein therapeutics, including biosimilar products" [76].

CBER's Office of Vaccines Research and Review (OVRR), together with the Genomics Evaluations Team for Safety (GETS) are collaborating with others to identify genetic risk factors that may be associated with adverse reactions to vaccines [76]. "Another study in collaboration with the Centers for Disease Control (CDC) and Northern California Kaiser looks at genetic risk factors of febrile seizures after MMR vaccination. Also, the Innovation Center for Biomedical Informatics (ICBI) at Georgetown University is trying to identify genes associated with vaccines, vaccine components, and several autoimmune diseases of interest. The goal is to help test the hypothesis that some autoimmune diseases might occur as adverse reactions to vaccines. Pathway models derived from this data may help predict autoimmune reactions to vaccines and other medical products in the future" [76].

Scientists at NCTR, in collaboration with scientists at the University of Liverpool (UK) and the Huashan Hospital (China), are performing whole genome sequencing and genetic analysis to identify susceptibilities to carbamazepine-induced hypersensitivity reactions [76]. "They are also collaborating with the University of Maryland to identify genetic factors that interact with common lifestyle factors in the Amish community that may contribute to heart disease. The metabolic responses of volunteers were examined after consuming certain diets and drugs that are associated with cardiovascular risk. This included blood triglyceride levels after a high fat meal, blood pressure after consuming much NaCl with a meal, and platelet aggregation response after taking aspirin or clopidogrel. The DNA from subjects who showed abnormal responses was sequenced using next-generation sequencing technology. Genetic association studies were also done. This work is ongoing, and as candidate genetic markers are discovered, they are being validated in another cohort. Identifying genetic factors that interact with drugs or certain diets to increase risks of cardiovascular disease or the efficacy of treatment will allow patients and their physicians to use personalized medicine to improve health" [76].

The NCI, the National Institute of General Medical Sciences (NIGMS), the University of Maryland and FDA are trying to see if increasing the dose of clopidogrel increases antiplatelet responses and active metabolite exposure in individuals with genetically reduced CYP2C19 metabolism compared to those with normal metabolism [76]. "Researchers in the Office of Science and Engineering Laboratories at CDRH are using new methods to analyze electrocardiograms to predict which patients will benefit from cardiovascular therapies such as cardiac resynchronization therapy. They can diagnose electrical conduction problems and to quantify scar tissue in the heart, with different criteria for women and men, since women benefit significantly more than men from cardiac resynchronization therapy. The Office of Science and Engineering Laboratories at CDRH is collaborating with George Washington University to develop a microfluidic, high-throughput microchip to test the interaction of tears with contact lenses, care products, and microbes. The goal is to provide individual testing results that can guide the prescription of lens materials and hygiene products for patients. So, from the FDA's perspective, the era of personalized medicine has arrived" [76].

FDA and personalized medicine

The FDA has a website with information on personalized nutrition and medicine by the Division of Personalized Nutrition and Medicine (DPNM) [77]. "The Division has two areas-biometry and biology. The main function of biometry is to develop biometrical methods for all aspects of the FDA's mission, goals, and objectives. A subgroup within biometry analyzes all data from the National Toxicology Program (NTP). The biology area is focusing on the broad areas of pharmacogenomics and nutrigenomics-how individuals respond to drugs and nutrients in foods. The overall goals of the DPNM are to develop and implement research strategies that will be able to account for genetic, environmental, and cultural factors that influence the expression of genetic makeups and produce knowledge for improving personal and public health. These overarching goals will be met with three parallel efforts that develop:

• Integration of omics methodologies to assess an individual's health status and, as importantly, susceptibility to specific chronic conditions influenced by environmental factors including diet

• Means to capture and assess an individual's nutritional, environmental, and activity exposures

• Classification algorithms that integrate the data from omics and environmental assessments that will result in evidence-based and validated biomedical decision making" [77].

Genomics can be used to predict whether a person is more susceptible to a disease. For example, some people have a variation of the BRCA1 gene with a higher risk of developing breast, ovarian, and possibly prostate and colon cancers. BRCA1 was the first gene found to be correlated with breast cancer. Alterations in the second gene found to be correlated with breast cancer, BRCA2, have been associated with breast, pancreatic, gallbladder, and stomach cancers. The second major aspect of pharmacogenomics, also called tumor genomics, is targeted therapy. Tumors have different genomic variations, and tests based on genomics are helping doctors to identify cancers that

are likely to respond to a particular treatment. The third aspect of pharmacogenomics includes testing for drug resistance. For example, the HIV virus genome is always changing, and resistance testing can help doctors choose the drug that will best match the virus and suppress it [77].

Critical Path Initiative

In 2004, the FDA introduced the Critical Path Initiative, with the goal of modernizing drug development by incorporating recent scientific advances, such as genomics and advanced imaging techniques, into the process [78]. "Important parts of this initiative are the identification and quantification of new biomarkers, clinical trial modernization, bioinformatics, drug manufacturing and collaborations between government, research institutes, academia and industry. The initiative began because society expects more and better drugs to be developed, but this was not happening. From 1994 to 2004, global spending on research and development of NMEs increased from about $40 billion to $60 billion, while the total number of NMEs worldwide decreased from 40 per year to a 20-year low of just over 20 NMEs" [78].

The development of new biomarkers through advanced genomic, proteomic, metabolomic and imaging technologies has a very high priority [78]. New biomarkers can improve diagnosis, define disease subsets that may differ in response, define individual variability in the drug's molecular target, and provide an early readout of response to therapy. The Biomarker Consortium (http://www.biomarkersconsortium.org) has been formed at the Foundation for NIH, or FNIH, and is funding biomarker trials for PET scanning in non-Hodgkin's lymphoma [78].

The next part of the critical initiative is the modernization of clinical trials by establishing better standards for fully automating the trials and managing the data [78]. "An important part of this is the science of bioinformatics, or the computer analysis of biological data. The FDA holds the largest set of data on animal testing of NMEs, but it is not in a user-friendly form. One improvement on this is developing digitized electrocardiograms that may help scientists evaluate NMEs for adverse cardiac events. Another aspect of bioinformatics will be to establish quantitative models of disease processes. This will use data on biomarkers, clinical outcomes and the effects of various interventions. The last part of the critical initiative is to improve drug manufacturing by incorporating new science and technology, especially the use of modern process control technologies. Process control is used widely in some industries, such as petroleum refining, production of hydroelectric energy and the manufacturing of electronics. Automated analytical instruments, such as GC, GC-MS, ion chromatography, ICP-AES, ICP-MS and LC-MS can continuously monitor parameters such as the composition of petroleum distillates, the chloride content of water and the metal content of electroplating baths. If critical parameters fall outside of control limits, the process is automatically corrected, or stopped, until it can be corrected. Similar process control analyses would be useful in drug manufacturing" [78].

Initiatives by other government agencies

It is no surprise that there is a big difference between brain cancer and cancer in other tissues. However, even for a given tissue or cell type, such as leukocyte cancer (leukemia), there are important differences. So, the NCI started a $100 million research program to determine the genomes of different cancers [79]. They are doing this in collaboration with the National Human Genome Research Institute to determine the genomes of brain, lung and ovarian cancers [80]. Also, the M.D. Anderson Cancer Center at the University of Texas had a research project called the Biomarker-Integrated Approaches for Targeted Therapy for Lung Cancer Elimination (BATTLE) [79]. They collected tissue samples (biopsies) of lung cancer cells at different stages of the disease. Tissue and cancer biopsies identified DNA biomarkers. They established the proof-of-principle that molecular-based, individualized, targeted therapy can work for lung cancer patients [79].

The NCI Office of Cancer Genome Characterization Initiative, CGCI, supports cutting-edge genomics research on adult and pediatric cancers. Researchers develop and apply advanced sequencing and other genome-based methods to identify novel genetic abnormalities in tumors. The extensive genetic profiles generated by CGCI may inform better cancer diagnosis and treatment [80]. "They completed a pilot lung cancer project. They used next generation transcriptome sequencing, as well as gene expression and epigenome profiling to study early-stage lung carcinogenesis. They discovered some changes in early-stage lung epithelial tumor cells and tested them to discover their roles in cancer development. They also compared candidate alterations between the various pathologically-determined epithelial phenotypes (normal, dysplastic, neoplastic, and malignant) to identify alterations that associate with those phenotypes. Alterations that were found to correlate with early-stage phenotypes (dysplastic and neoplastic) are likely to play a role in the initiation of lung cancer. Completion of this comparison analysis could identify a new set of regulatory pathways in lung carcinogenesis, which may be pursued in future prospective biomarker studies for risk assessment, early diagnosis, and targeted therapies" [80].

The CGCI also has a Cancer Genome Anatomy Project (CGAP) that generated a wide range of genomics data on cancerous cells that are accessible through the CGAP website (ocg@mail.nih.gov) [80]. "Part of this is the SNP500 database whose goal is to re-sequence 102 reference samples to find known or newly discovered single nucleotide polymorphisms (SNPs) which

are of immediate importance to molecular epidemiology studies in cancer. Together with the Initiative for Chemical Genetics (ICG), the CGCI established Chembank, an interactive database for small molecules. It contains data from hundreds of biomedically relevant small molecule screens of hundreds-of-thousands of compounds. Chembank also provides analytical tools to facilitate data mining. They also have a Childhood Cancer center that has much data on childhood cancers, including current treatments, clinical trials, prevention, genetics, testing, and more. There is also a Cancer Genetic Markers of Susceptibility (CGEMS) initiative. It is identifying common inherited genetic variations associated with a number of cancers, including breast and prostate. Data from these genome-wide association studies (GWAS) are available through the Division of Cancer Epidemiology & Genetics website. (http://dceg.cancer.gov/research/how-we-study/genomic-studies/cgems-summary). The CGCI also provides the scientific community with validated, full open reading frame (ORF) cDNA clones for all of the currently defined human genes. There is also a mammalian cDNA Library from the NIH Mammalian Gene Collection (MGC). It is providing full-length clones for most of the defined human and mouse genes, along with selected clones of cow and rat genes" [80].

Finally, the Office of Cancer Genomics has an SOP manual [76]. It is a set of guidelines for investigators participating in OCG projects to characterize tumors on a molecular level. The SOP provides templates and protocols that apply to all projects, as well as some that apply specifically to individual projects. Investigators must follow the protocols in the SOP when contributing samples and data. The sample and data acquisition process is explained in comprehensive detail to ensure that all materials contributed will be of sufficient quality to be utilized in the projects [80].

In 2009, researchers began examining the genomes of medulloblastoma, a brain tumor that occurs primarily in children, and B-cell non-Hodgkin lymphoma, a white blood cell cancer [80]. "Both projects revealed many genetic alterations not detected in previous studies that are potentially important in cancer onset and/or progression. Additionally, CGCI partners with the OCG TARGET initiative to help solve the genetic mysteries of some pediatric cancers that do not respond well to standard therapies. These hard-to-treat cancers include acute myeloid leukemia that is refractory to treatment and two rare kidney tumors. Findings from some or all of these projects may translate into improved "survival and quality-of-life for patients afflicted with these diseases. They have begun projects to better characterize cancers from HIV-positive patients, early-stage lung cancer, and Burkitt lymphoma. Data is publicly available through NIH and NCI databases" [80].

International collaborations

In December 2012, British Prime Minister David Cameron announced a project to sequence the genomes of 100,000 Britons (100,000 Genome Project or 100kGP) affected with cancer or rare diseases [81]. "In July 2013, the Department of Health announced that this initiative will be coordinated by the newly-established, government-owned company Genomics England (London, UK). They will try to incorporate whole-genome and whole exome sequencing into clinical studies. They also plan to develop strategies for the effective and safe use of whole-genome sequencing. This will require excellent patient communication, given the breadth and value of information it can generate" [81].

The Sheik Khalifa Bin Zayed Al Nahyan Institute for Personalized Cancer Therapy at the MD Anderson Center supports preclinical research and clinical trials in which a patient's tumor biopsy is assayed for abnormal genes and gene products to select the best therapy with agents targeting the product of those particular abnormal genes [82]. "This program integrates research into clinical trials. Its goal is to implement personalized cancer therapy and improve patient outcomes. The seminal BATTLE trial in lung cancer demonstrated the practicality of this approach. They did a series of Phase I/II trials. Experimental drugs were assigned based on biomarkers that were detected in the patients' cancers. Several clinical trials based on genetic and molecular biomarkers in patients' cancers are now underway. They significantly improved response rates in combined targeted therapy/chemotherapy Phase I clinical trials in multiple disease sites. For example, patients treated with a drug targeting the PI3K pathway had a response rate of 35% in patients with mutations, compared to a response rate of 4 to11% that is generally observed in phase I trials" [82].

Academic collaborations

Stanford University and a company called Nodality started looking at leukemia [79]. "They prepared fluorescent-labeled antibodies that bind to specific phosphoproteins, which are important in signaling pathways in cancer cells. They used a cell flow technology in which cells flowed past a fluorescence detector. Those that had phosphoproteins fluoresced and were detected. This established their amount, which controlled the "extent to which the signaling pathways are activated in cancer cells" [79]. Nodality recently described a Single Cell Network Profiling (SCNP) technology that characterized signaling and drug sensitivity and resistance profiles in mast cell leukemia bone marrow mast cells [83]. They also showed quantitative measurements of EGFR pathway signaling and modulation from non-small cell lung cancer (NSCLC) patients. The epidermal growth factor (EGF) binds to its receptor (EGFR), which is also a tyrosine kinase. It catalyzes the phosphorylation of some of its own tyrosine residues, which activates downstream

signaling cascades [83].

The Harvard Partners Center for Genetics and Genomics was founded in 2001 with the specific goal of accelerating the realization of personalized medicine. Its name changed in 2008 to the Center for Personalized Genetic Medicine [84]. "The change reflects an emphasis on translational medicine. That is, they are trying to translate laboratory results into clear medical treatments and cures. The Personal Genome Project was announced by George Church in 2006. It will publish full genome sequences and medical records of volunteers to enable research into personalized medicine. The Laboratory for Personalized Molecular Medicine was founded in 2007 to identify specific mutations in genes linked to clinical outcomes in patients with leukemia and lymphoma. Identifying the presence or absence of these mutations is becoming a standard of care for patients with acute myeloid leukemia. LabPMM also develops patient-specific molecular tests from patient tumor DNA samples. The ultra-sensitive tests are used by leading cancer treatment centers world-wide to monitor residual disease and treatment" [84].

The Mayo Clinic also has a Center for Individualized Medicine [85]. "About 100,000 people in the USA die each year from adverse reactions to medications and another two million are hospitalized. Hopefully, research at the intersection of pharmacology and genetics (pharmacogenomics) will make it possible to predict who is likely to have an adverse reaction to a drug before it is given to them. It may also be able to predict whether a patient will respond well to a medicine" [85]. There are also Centers for Personalized Medicine at the Roswell Park Cancer Institute (RPCI) [86], Duke [87], Stanford [88], the University of Pennsylvania (Penn) [89], the Sheikh Khalifa Bin Zayed Al Nahyan Institute for Personalized Cancer Therapy [82] and many others.

Importance of human and social networks

There are also human and social networks that aid in medical research. There is a downloadable program called Fight AIDS at Home [90]. It can calculate the enthalpy of interaction between a test compound and target protein for many different conformations of the drug and its targeted receptor or enzyme. It is part of the World Community Grid distributed computing network. It has been used to look for new chemical entities that might bind to not just the wild-type HIV protease, but also as many mutant forms as possible [91]. Programs like this that enable patients and/or their caregivers to participate in finding proper treatments can help give them a positive attitude and avoid depression.

Collaborative networks have also emerged between academia and industry. There is a European Consortium called SILVER for developing antiviral drugs for priority and neglected viruses [92,93]. There is a Biomarkers Consortium that focuses on validation of biomarkers for application in translational research and drug development [94], an Alzheimer's Disease Neuroimaging Initiative that uses proteomics to validate a panel of markers using plasma and CSF samples, NEWMEDS for developing animal models with standardized paradigms for fMRI and PET imaging, an open access consortium model, Arch2POCM, that offers a venue to extend partnerships between government, academia, and the private sector to identify compounds that explore the proof of clinical mechanism for selected diseases and targets and a Québec Consortium for Drug Discovery (CQDM) that serves as a communication channel between the needs of the industry on one hand, and the creativity of the academic world and small businesses on the other, with partners in academia and pharmaceutical companies (AstraZeneca, Merck, Pfizer, Boehringer Ingelheim, Eli Lilly and GlaxoSmithKline) [91-98]. Moreover, the Structural Genomics Consortium and Sage Bionetworks are building a precompetitive partnership to optimize the clinical validation of new therapeutic targets [97]. "They are removing data-access restrictions to eliminate redundant discovery programs and reduce the overall cost of research and development. Also, the Archipelago to Proof of Clinical Mechanism (Arch2POCM) hopes to improve the efficiency and lower the costs of drug development by generating a portfolio of small molecules that hit new therapeutic targets and by carrying out early clinical work - up to Phase II clinical trials" [97].

The National Institute of Mental Health (NIMH) is building networks and investing resources to develop improved, personalized drugs for disorders of the central nervous system [99]. "HTS, structural biology and targeted approaches are used to target signaling molecules, cell phenotypes and protein-protein interactions (PPIs). The NIH Molecular Libraries Program has implemented 600 HTS assays and generated more than 200 chemical probes. The NIMH has also issued initiatives to develop new in vitro assays of neuronal and glial function and to explore epigenetic changes during brain development and neurodegenerative diseases. They also encourage research into iPSCs, which can be grown in culture to study diseases, predict drug toxicology and discover biomarkers. The NIMH is trying to standardize protocols for neuroimaging, physiological and genomic analyses that can be used by many sites, to promote data sharing. This includes biomarkers for depression and risk factors for developmental disorders. Moreover, the new National Center for Accelerating Translational Science (NCATS) and the NIH-FDA Leadership Council are encouraging multi-target and combination therapies. They hope to introduce novel classes of multi-target drugs with fewer adverse effects and less toxicity by targeting cellular function rather than single proteins. Pre-competitive public–private partnerships (PPPs) exist for finding biomarkers and for methods development to support innovation in drug discovery. The Biomarkers Consortium focuses on the validation of biomarkers for

drug development. One neuroscience project aims to validate a panel of biomarkers using samples of blood plasma and cerebrospinal fluids from the Alzheimer's Disease Neuroimaging Initiative. The Innovative Medicines Initiative supports collaborative research projects and builds networks to enhance pharmaceutical innovation in Europe. One project, NEWMEDS, focuses on development of animal models, standardized paradigms for fMRI and PET imaging, pharmacogenetic markers and trial designs for developing new medications for depression and schizophrenia" [99]. Moreover, a new, open access consortium model, Arch2POCM, is extending partnerships between government, academia, and the private sector to identify compounds that explore proof of clinical mechanism for selected diseases and targets [97]. Drugs that are antibody antagonists are one of the fastest-growing areas of drug development [42]. There are two databases that contain small molecule protein-protein inhibitors, the TIMBAL database [100] at the University of Cambridge and the 2P2IDB database [101] at CNRS in France. Tirofiban and maraviroc are two drugs that target protein-protein interactions that are clinically available. They target integrins to treat cardiovascular disease (tirofiban) and HIV gp120 interaction with the CCR5 receptor to block HIV viral entry (maraviroc).At least 19 other small molecules that disrupt protein-protein interactions are known. This includes five that disrupt Bak-BH3/BclxL, three that disrupt LFA/ICAM-1, two that disrupt IL2-IL2R, MDM2/p53, and one each that disrupts Tcf4/beta catenin, CRM1/NES, IL1/IL1-R, EPO/EPOR, HIV/CCR5, NGF/p75, and CD4/mHCclassII, Myc/Max interactions. Still, only 50% are covered by the diversity space of three commercial databases: Maybridge, Asinex and Chemical Diversity Database International Diversity Collection [102]. The infrastructure and collaborations are summarized in Table 1.

Table 1. Infrastructure and Collaborations for Personalized Medicine

Infrastructure or Collaboration	Goal/Achievement
NCCAM	Work with scientists to study traditional remedies.
FDA	Work with industry and academia to build an elaborate infrastructure to support personalized medicine.
ENCODE	Identify and define the functional DNA elements that are required for normal genome function.
Human Genome Project	Identify the entire sequence of bases in the human genome and the genomes of other organisms.
International Genome Sequencing Consortium	Sequence the entire human genome.
Center of Excellence for Bioinformatics	Develop bioinformatics tools.
Functional Genomics	Determine the functions of different genes.
Structural Genomics	Determine the effects of genes on biopolymer (protein, RNA) structures.
Office of In Vitro Diagnostic Device Evaluation and Safety	Evaluate the safety and efficacy of medical devices.
Voluntary Genomic Data Submission (VGDS)	Provide a mechanism for scientific exchange between FDA and external scientists.
Genomics and Targeted Therapy Group	Works to advance the application of genomic technologies in the discovery, development, regulation, and use of medications.
Personalized Medicine Team	Work to develop personalized medicines.
Division of Systems Biology	Develop and evaluate new technologies and the identification of new biomarkers (disease indicators)
cGMP	FDA rules for manufacturing prescription drugs
National Center for Toxicological Research (NCTR)	Perform toxicological studies and study the biology of cancer.
Office of Vaccines Research and Review	Identify genetic risk factors that may be associated with adverse reactions to vaccines
Genomics Evaluations Team for Safety	Identify genetic risk factors that may be associated with adverse reactions to vaccines
Innovation Center for Biomedical Informatics	Try to identify genes associated with vaccines, vaccine components, and several autoimmune diseases of interest.
University of Liverpool (UK), the Huashan Hospital (China) and NCTR	Perform whole genome sequencing and genetic analysis to identify susceptibilities to carbamazepine-induced hypersensitivity reactions.
Office of Science and Engineering Laboratories	Develop new methods to analyze electrocardiograms to predict which patients will benefit from cardiovascular therapies.
Office of Science and Engineering Laboratories and George Washington University	Develop a microfluidic, high-throughput microchip to test the interaction of tears with contact lenses, care products, and microbes.
Division of Personalized Nutrition and Medicine	Develop biometrical methods and analyze all data from the National Toxicology Program (NTP).
FDAs Critical Path Initiative	Modernize drug development by incorporating recent scientific advances, such as genomics and advanced imaging techniques, into the process.

The Biomarker Consortium	Funds biomarker trials for PET scanning in non-Hodgkin's lymphoma.
NCI and National Human Genome Research Institute	Determine the genomes of brain, lung and ovarian cancers
M.D. Anderson Cancer Center	Perform Biomarker-integrated approaches for targeted therapy for lung cancer elimination.
Cancer Genome Characterization Initiative (CGCI)	Supports cutting-edge genomics research on adult and pediatric cancers.
Cancer Genome Anatomy Project	Generate a wide range of genomics data on cancerous cells.
Initiative for Chemical Genetics and the CGCI	Established Chembank, an interactive database for small molecules.
Cancer Genetic Markers of Susceptibility (CGEMS) initiative	Identify common inherited genetic variations associated with a number of cancers.
Division of Cancer Epidemiology & Genetics	Provide data on genome-wide association studies.
Cancer Genome Anatomy Project	Generate genomics data on cancerous cells.
CGCI and Initiative for Chemical Genetics (ICG)	Establish Chembank, an interactive database for small molecules.
Cancer Genetic Markers of Susceptibility (CGEMS) initiative	Identify common inherited genetic variations associated with cancers.
Mammalian Gene Collection	Provide a mammalian cDNA library.
SOP Manual at the Office of Cancer Genomics	Provide set of guidelines for investigators participating in OCG projects to characterize tumors on a molecular level.
United Kingdom's Department of Health	Incorporate whole-genome and whole exome sequencing into clinical studies.
Sheik Khalifa Bin Zayed Al Nahyan Institute for Personalized Cancer Therapy	Do preclinical research and clinical trials in which a patient's tumor biopsy is assayed for abnormal genes and gene products to select therapy with agents targeting the product of those particular abnormal genes.
Nodality	A company that studies leukemia and develop a Single Cell Network Profiling (SCNP) technology.
Harvard Center for Personalized Genetic Medicine	Try to to translate laboratory results into clear medical treatments and cures.
Personal Genome Project	Publish full genome sequences and medical records of volunteers to enable research into personalized medicine.
Laboratory for Personalized Molecular Medicine	Identify specific mutations in genes linked to clinical outcomes in patients with leukemia and lymphoma.
Mayo Clinic's Center for Individualized Medicine	Do research at the intersection of pharmacology and genetics (pharmacogenomics) will make it possible to predict who is likely to have an adverse reaction to a drug before it is given to them.
Fight AIDS at Home	A software package for calculating the enthalpy of interaction between a test compound and target protein for many different conformations of the drug and its targeted receptor or enzyme.
European Consortium called SILVER	Develop antiviral drugs for priority and neglected viruses.
Biomarkers Consortium	Validate biomarkers for application in translational research and drug development.
NEWMEDS	Develop animal models with standardized paradigms for fMRI and PET imaging.
Arch2POCM	Provide a venue to extend partnerships between government, academia, and the private sector to identify compounds that explore the proof of clinical mechanism for selected diseases and targets.
Québec Consortium for Drug Discovery	Serve as a communication channel between the needs of the industry, academia and small businesses.
Structural Genomics Consortium and Sage Bionetworks	Build a precompetitive partnership to optimize the clinical validation of new therapeutic targets.
Archipelago to Proof of Clinical Mechanism (Arch2POCM)	Improve the efficiency and lower the costs of drug development by generating a portfolio of small molecules that hit new therapeutic targets and by carrying out early clinical work - up to Phase II clinical trials.
National Institute of Mental Health	Build networks and investing resources to develop improved, personalized drugs for disorders of the central nervous system.
National Center for Accelerating Translational Science (NCATS) and the NIH-FDA Leadership Council	Encourage multi-target and combination therapies.
TIMBAL and 2P2IDB databases	Provide databases of small molecule protein-protein inhibitors.

Protein-protein interactions

One complication is that protein-protein interfaces are broad and flat, so scoring functions designed for small ligands in tight pockets may be inapplicable for judging the quality of ligands targeting protein-protein interactions [102]. "However, there are usually 'hot spots' that contribute most of the free energy of binding. They make good targets for fragment-based drug design. The fragment based ligand design approach has been particularly successful in the case of designing inhibitors of the interactions between interleukin-2 (IL-2) and its receptor (IL-2Rα). Another new chemical entity (NCE), Ro26-4550, disrupts this interaction. Another nanomolar inhibitor of this interaction is Sp4206. There is also a NCE, ABT-263, which disrupts interactions between B cell lymphoma dimers of Bck2 and Bcl XL, and through lead optimization was developed into ABT-263. Navitoclax is a new Bcl-2/Bcl-xL inhibitor currently in Phase I clinical trials for hematologic and solid tumors and Phase II for chronic lymphocytic leukemia. HDM2 (human protein double minute 2) is a cancer drug target that blocks interactions between the p53 protein and HDM2, which binds to p53 and blocks its transcriptional activity. Other drugs, including Sp304, disrupt the interactions between the human papilloma virus (HPV) E2 viral transcription factor and the viral helicase E1. There are many other examples of drugs in development that target protein-protein interactions and databases that categorize and classify protein-protein interactions" [102].

Computational methods

There are also computational methods that try to identify specific druggable 'hot spots' in protein-protein interfaces. This includes FTMAP, FTFLEX, FastContact 2.0, ANCHOR, PocketQuery, a program that predicts protein-protein binding rate constants, GridDock, and another program that for predicting and disrupting protein-protein interactions in bacterial outer membrane and eukaryotic mitochondria beta barrel membrane proteins [102].

So, network medicine includes not just networks of ions, molecules, cells, diseases and organisms, but also people and professional networks.

FDA Critical Path Initiative

The FDA and other governments' agencies evaluate applications for new medical devices and drugs. So, the following goals were described in a recent report from the FDA [78].

"Personalized medicine seeks to reduce the burden of disease by targeting prevention and treatment more effectively. With the help of personalized medicine, the health care management paradigm will focus on prevention, moving from illness to wellness, and from treating disease to maintaining health. By improving our ability to predict and account for individual differences in disease diagnosis, experience, and therapy response, personalized medicine offers hope for diminishing the duration and severity of illness, shortening product development timelines, and improving success rates. At the same time, it may reduce healthcare costs by improving our ability to quickly and reliably select the effective therapy for each patient while minimizing the costs associated with ineffective treatment and avoidable adverse events" [78].

Cancer Genome Characterization Initiative, CGCI

The NCI has an initiative (the CGCI) that supports cutting-edge genomics research on adult and pediatric cancers [42,103]. "Advanced sequencing and other genome-based methods are being developed and applied for identifying new genetic abnormalities in tumors. The extensive genetic profiles generated by CGCI will make it easier to improve cancer diagnosis and treatment. They completed a pilot lung cancer project by using next generation transcriptome sequencing, as well as gene expression and epigenome profiling to study early-stage lung carcinogenesis. They discovered several changes in early-stage lung epithelial tumor cells and tested them to discover their roles in cancer development. They also compared possible changes in the many pathologically-determined epithelial phenotypes (normal, dysplastic, neoplastic, and malignant) to identify alterations that associate with those phenotypes. Some were found to correlate with early-stage phenotypes (dysplastic and neoplastic) that are important in the initiation of lung cancer. This comparison analysis is being used to identify a new set of regulatory pathways in lung carcinogenesis. This will probably be pursued in future prospective biomarker studies for risk assessment, early diagnosis, and targeted therapies" [42,103].

The CGCI also has a Cancer Genome Anatomy Project (CGAP) that is producing much genomics data on cancerous cells that are accessible through the CGAP website (ocg@mail.nih.gov) [42]. "Part of this is the SNP500 database that has the goal of resequencing 102 reference samples to find known or newly discovered SNPs. These SNPs are quite important to molecular epidemiology studies of cancer. The CGCI, together with the Initiative for Chemical Genetics (ICG), established Chembank, an interactive database for small molecules. It contains data from hundreds of biomedically relevant small molecule evaluations of many of compounds. Chembank also provides analytical tools to facilitate data mining. They also have a Childhood Cancer Center that has important information about childhood cancers, including current treatments, clinical trials, prevention, genetics, testing, and more. There is also a Cancer Genetic Markers of Susceptibility (CGEMS) initiative that aims to identify commonly inherited genetic variations that are associated with a number of cancers, including breast and prostate. Data from these genome-wide association studies (GWAS) are available through the Division of Cancer Epidemiology & Genetics website.(http://dceg.cancer.gov/research/how-we-study/genomic-studies/cgems-summary). The CGCI is also providing validated, full open reading frame (ORF) cDNA clones for all of the currently defined human genes. Moreover, there also a

mammalian cDNA Library from the NIH, called the Mammalian Gene Collection (MGC). It provides full-length clones for most of the defined human and mouse genes, along with selected clones of cow and rat genes" [42].

Conclusion

In conclusion, systems thinking has become an essential part of modern medicinal chemistry and new drug development. In contrast to reductionist thinking, systems thinking uses network theory and nonlinear mathematics to prevent, diagnose, treat and even cure diseases. Systems thinking is an integral part of predictive, preventive, personalized and participatory (P4) medicine. It takes not just a holistic but also a quantitative and mathematical approach to practicing medicine. Modern drug development is being done through collaborative initiatives between governments, universities, research institutes, hospitals and industry. The U.S. FDA has a Critical Path Initiative that intends to modernize drug development by incorporating recent scientific advances, such as genomics and advanced imaging techniques into the process of developing new drugs and medical. There are also Biomarker-Integrated Approaches for Targeted Therapy for Lung Cancer Elimination (BATTLE) and a Cancer Genome Anatomy Project (CGAP) project. The NCI also has a Cancer Genomics' Cancer Genome Characterization Initiative, or CGCI. Together, these efforts are ushering in a new era of personalized health care that will be able to prevent, diagnose and properly treat diseases with the help of patients and their caregivers.

This work should not be taken as reflecting FDA policy or regulation.

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