Book Chapter

Novel Therapies Based on Synthetic Biology to Cure a Range of Ailments

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Abstract

Synthetic biology is a multidisciplinary field of study that tries to develop new biological components, devices, and systems, as well as remodel existing systems found in nature. It is created when engineering science meets biological science, it combines biology and engineering to create new biological systems which currently do not exist in nature. As the immune system serves as the first line of defense against infections and plays a crucial role in chronic diseases, the development of immune-based therapy involves manipulating the immune system both endogenously and exogenously. Now, synthetic biology can be used in the context of medicine, to engineer biological systems with structures and functions not found in nature to process information, maintain cell environment, even enhance human health, and discover new therapeutic targets for the treatment of various diseases so that methods based on this platform enable the design of new strategies for the treatment and diagnosis of cancer, immune diseases, metabolic disorders, and infectious diseases. However, challenges loom at every stage of the process in synthetic biology, from part characterization to system design and assembly. Herein, we will describe the recent synthetic biology-based developments as novel therapeutics for cancer, bacterial drug resistance, natural drug synthesis, autoimmunity, neurodegenerative, and infectious diseases, highlighting the challenges faced in immune based therapies and synthetic biology.

Keywords

Introduction

Everything living or nonliving is just chemicals made from atoms. Fish are just potages of atoms, but what makes the fish alive is how the atoms are structured, by a special kind of molecule, the DNA. Every living creature has a code that makes it develop, reproduce, and converge. 20 years ago, scientists learned to read a creature's entire genome. As biologists got better at this, as Stanford professor Drew Endy, a new kind of science was born, called "Synthetic Biology" [1]. Some of the most challenging global health issues require novel, technology-enabled alternatives to traditional approaches for developing novel human health solutions, where solutions must combine ground-breaking science with being useful, affordable, and accessible to those in need [2]. Synthetic biology is a recent field that is currently growing quickly. This prompts the query of what distinct contributions synthetic biology makes that cannot be met by other means. A synthetic biology method tries to solve issues through the creation of new models rather than only through research and observation, which sets it apart from a purely biological approach. By combining biology, chemistry, and engineering, this method offers a fresh viewpoint on several long-standing biological issues.

Building and simulating biological systems aids in a deeper comprehension of pertinent biological phenomena and paves the way for the creation of more accurate, predictable methods of biological manipulation in the fields of agriculture, bioenergy production, and medicines (Figure 1). Contributing to the development of biomedical breakthroughs is one of the goals of synthetic biology. Among these difficulties is the escalating bacterial antibiotic resistance [3], the rapid introduction of novel infectious diseases [4], and the evolving cancer medication resistance [5]. Synthetic biology anticipates the creation of specially crafted, easily controlled, and secure devices that would support human immune systems and correct metabolic anomalies to solve these issues. This review focuses on current synthetic biology based potential treatments as promising future disease therapies. We will go through several synthetic biology techniques that have recently been applied to research into disease mechanisms.
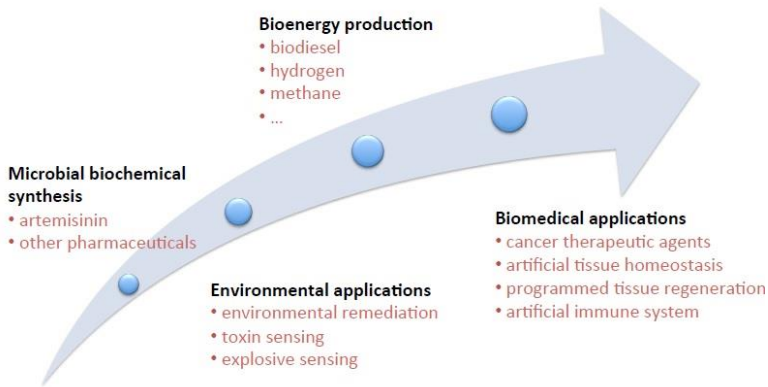
Figure 1: Synthetic biology applications. The figure is segmented into three distinct areas: Bioenergy Production, Biomedical Applications, and Environmental Applications. Bioenergy Production encompasses the generation of biodiesel, hydrogen, and methane. Biomedical Applications involve the production of pharmaceuticals such as artemisinin and cancer therapeutic agents, and the process of tissue engineering for artificial tissue homeostasis and programmed tissue regeneration. Environmental Applications focus on environmental remediation, toxin sensing, and explosive sensing, highlighting the diverse applications of the depicted processes [6].

Synthetic Biology Meets Medicine: Applications of Synthetic Biology in Developing Diagnostics and Therapeutics

Current global pandemics demonstrate that the approach to disease diagnosis, treatment, and prevention necessitates the organized and efficient use of ever-increasing amounts of biological data and bioengineering techniques to maximize responsiveness and to be prepared for future threats to human health [7]. Over the last two decades, synthetic biology has offered enormous potential in a wide range of applications. For instance, the primary goal of synthetic biology is to genetically change cells and redesign/synthesize regulatory systems to aid in disease diagnosis and treatment [8].

In the context of diagnostics, the vital first step in treating a disease is determining if it is present or not, making diagnostics a

vital component of public health. The primary goal of diagnostics development is to improve clinical performance, such as increased sensitivity, specificity, and quantification accuracy, as well as assay characteristics, such as shorter time to results, lower cost, greater portability, simplified workflow, and contaminant resistance [7]. For that synthetic biologists have begun to direct their bio-molecular engineering approaches toward this goal, yielding promising results that could lead to the development of new classes of low-cost, easily accessible diagnostics [9].
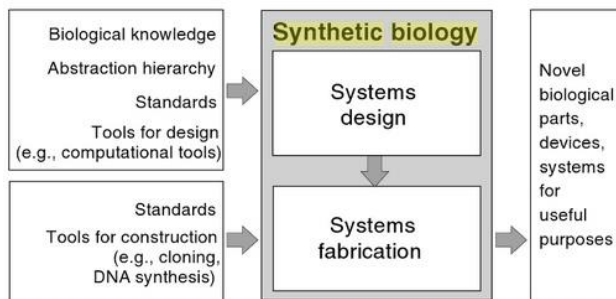

Figure 2: Synthetic Biology: Infusing Engineering Principles into Bioengineering. Synthetic biology encompasses systems design and fabrication. Each part has its specific prerequisites and inputs. Ultimately, synthetic biology delivers novel biological entities with improved functionality [6].

Synthetic biologists are applying rational engineering principles to the development of novel biosensing devices, which include a sensor that detects desired signal(s) from *in vitro* or *in vivo* environments, a processor that is a simple or multiplex synthetic circuit capable of processing received signals, integrating them with medical knowledge, and classifying patient conditions into clinical categories, and a reporter that displays assay results as chemical, biological, electronic, or a combination of these (Figure 2). These synthetic systems can be developed in a variety of ways, either as standalone diagnostic or diagnostic-therapeutic assays/devices or as components of conventional assays/devices. For instance, *in vitro* assays are made up of synthetic genes and oligonucleotides, synthetic multifunctional antibodies, or multiepitope and chimeric antigens that are printed on paper or

integrated into traditional testing platforms for probing or detection. For example, Geraldi et al. highlighted two synthetic biology-based In Vitro Diagnostics (IVD) systems, synthetic RNA-CRISPR/Cas-based biosensors that have been shown to detect and report various nucleic acid biomarkers from pathogens with high accuracy and sensitivity, relatively simple logistics, and low development and operational costs [10]. In another example, fluoromycobacteriophages are genetically altered phages that have been produced for phage-based diagnostics to identify pathogens such as Staphylococcus aureus, Listeria, E. coli, and Bacillus anthracis [11]. Moreover, Ye et al., for example, created a synthetic insulin-sensitive mammalian transcription circuit capable of detecting and correcting insulin resistance *in vivo* [12].

On the other hand, it is vital not only to detect an illness but also to employ the proper treatment. However, there are certain biomedical challenges such as increasing bacterial antibiotic resistance [3], the rapid emergence of new infectious diseases [4], and the evolution of cancer treatment resistance [13]. To overcome such challenges, synthetic biology envisions the development of specially made, easily controlled, safe devices that would support human immune system and solve metabolic disorders [14].

In this context, an example of synthetic biology-based therapeutics is the use of modified T cells, such as chimeric antigen receptor T cell immunotherapy (CAR-T), which is used to treat leukemia. Tisagenlecleucel was the first CAR-T treatment authorized by the FDA to treat cell acute lymphoblastic leukemia (ALL), a cancer that affects B lymphocytes (or B cells) in the immune system [15]. Another successful examples have been the manufacture of terpenoid chemicals in E. coli [16] and Saccharomyces cerevisiae [17] that can be employed in the manufacturing of anti-malaria medication, and synthetic mammalian gene circuit was utilized to find novel antituberculosis drugs [16]. Also, synthetic biology can be used in the design and modification of viruses to efficiently deliver healthy genes to the target tissue, facilitating precise recombination and integration of synthetic genes with the

existing genome [18]. Furthermore, it aids in the understanding of the complex traits of a disease, which paves the way to address these difficulties through personalized medicine [19].

Thus, synthetic biotics, like many other medical therapies under clinical trials, hold great promise for preventing, diagnosing, and treating diseases, as well as lowering future mortality and morbidity rates. Synthetic biology has the potential to boost the availability and efficacy of treatments as well as supply rapid diagnostic testing for bacterial illnesses. The risk of incorrect use, on the other hand, needs controls to prevent potential chemical and biological risks and ensure that this technology helps the greater good. If these novel medicines are effective in the market in the future years, they will contribute to the transformation of modern medicine.

Immunology-Related Applications of Synthetic Biology: Promising Cures for Autoimmune Disorders

The primary functions of the immune system lie in protecting the body against foreign antigens and activating repair systems to heal damaged tissues. Unfortunately, immune malfunctions can occur in several conditions, thus being a root cause of diseases in the body. Autoimmune disorders are one of the immune malfunctions where the immune system fails to distinguish between self and non-self; as a result, immune cells will attack self-antigens and destroy them [20]. Both aspects of the immune system, innate and adaptive immunity, take part in the contribute to autoimmune disorders [21]. This state of self-intolerance induces the production of autoantibodies and autoreactive T cells against self-bodies. Over 80 types of autoimmune disorders are diagnosed worldwide including multiple sclerosis (MS), systemic lupus erythematosus (SLE), type 1 diabetes (T1D), etc.. [22].

Defects in the production of mature B and T cells remain a leading etiology for the development of self-intolerance status [23]. This made B and T lymphocytes targets for therapeutic approaches against autoimmune disorders, and thus cellular

immunotherapy became a breakthrough in the treatment of autoimmunity. Immune cells are engineered to eliminate autoreactive immune effectors [24]. Monoclonal antibodies are one of the approaches for treating B cell-mediated autoimmunity, such as in the case of Rheumatoid Arthritis (RA) [25]. Rituximab is an anti-CD20 chimeric monoclonal antibody, designed against B-cells expressing CD20 antigens. It causes depletion of pre-B cells and mature B cells expressing CD20 via several mechanisms including B cell apoptosis, antibody-dependent cellular toxicity, and complement-dependent cellular toxicity [26]. This approach showed significant improvement in patients with RA [25]. Moreover, chimeric antibodies have been studied as a treatment for Systemic Lupus erythematosus. These chimeric antibodies were able to selectively target double stranded DNA-specific B cells at early stages of differentiation, causing their silencing and inactivation [27].

Equipped with chimeric antigen receptor (CAR), engineered T cells showed significant success in treating B-cell malignancies and leading to lasting remission periods [28]. This paved the way for directing CAR-T cell therapy toward B-cell mediated autoimmune diseases. CARs are synthetic receptors, having fragments of the single chain variable fragment (scFv) from the antibody, and instruct the immune cells to attack specific targets. Thus, allows taking further advantage of the specificity of autoantigens as tools for designing autoimmune therapies [29]. To study the efficacy of cell-based therapy against SLE, CD19 targeted CAR T-cells were administered to SLE mice models. Results showed depletion of CD19+ B cells, effective elimination of autoantibodies, reduction in lupus manifestations, and increased lifespan of the mice [30]. This approach helped overcome limitations seen in the course of treating SLE by monoclonal antibody technology and highlighted the superiority of CAR-T cell therapy [30]. However, depletion of B cells can raise risks of hypogammaglobulinemia, thus requiring a more pathogenic B cells specific approach [31]. Chimeric Autoantibody Receptor (CAAR) T cell therapy helped in targeting autoreactive B-cells through B cell receptor (BCR) specificity, and this technique showed promising results in cases of Pemphigus vulgaris (PV) autoimmune disease [32]. During

PV, autoantibodies are secreted against the keratinocyte adhesion protein desmoglein 3 (Dsg3). Accordingly, T-cells were engineered to express Dsg3-specific CAAR, enabling these T-cells to specifically target and destroy B-cells expressing anti-Dsg3 BCR [32]. CAAR-T cell therapy has been also applied in approaches to treat Myasthenia gravis (MG) disease, an autoimmune disorder where autoantibodies are secreted against muscle-specific tyrosine kinase (MuSK) [33]. *In vivo* results in mice showed the efficacy of MuSk CAAR T-cells in depleting B cells bearing anti-MuSK BCR [33].

CAR T-cell therapy has shown promising results in terms of efficacy and safety; however, a set of roadblocks exist. Limitations include HLA compatibility with donors, cytotoxicity, and the sophisticated manufacturing process [28]. Similarly, the production of CAAR T-cells is also difficult to standardize [34]. Thus, CAR engineering has gone beyond T cells, where other immune cells have been studied, including Natural Killer (NK) cells [35]. CAR-NK cell therapy administration in B-cell cancer patients showed significant clinical response to the treatment without adverse side effects [35]. Eventually, CAAR-NK cell therapy has been studied in the battle against autoimmune diseases. Autoantibodies against La/SSB are detected in patients with several immune diseases including Systemic Lupus Erythematosus (SLE) and Sjögren's syndrome (SS) [34]. NK92MI cell line was used to engineer La/SSB CAAR NK-cells and then co-cultured with anti-La/SSB autoantibody positive whole blood samples from SLE and SS patients. *In vitro* results showed selective killing of B-cells expressing anti-La/SSB BCR, indicating promising results for this technology [34].

The aforementioned techniques are based on the engineering of effector T cells, which can end up in critical complications in case of an askew [36]. As an alternative, regulatory T cells (Tregs) are approached as key players in the CAR technology against autoimmune diseases [36]. Tregs possess an immunosuppressive power to control autoimmunity, such as in the case of RA. Patients with RA have the citrullinated vimentin (CV) protein abundantly expressed in the extracellular matrix of

their inflamed joints, in consistency with the presence of anti-citrullinated protein antibodies (ACPA) [37].

Current therapies adopted against autoimmune diseases rely on immunosuppressive and anti-inflammatory agents, along with synthesized biologics like monoclonal antibodies. Nevertheless, cell-based therapy has shown promising treatment approaches in cancer patients, and within existing experiments against autoimmune diseases [38]. Indeed, the application of this technology in autoimmune diseases will pave the way for a new potential therapeutic option [38].

Synthetic Biology Approaches for The Degenerating Brain: Novel Treatments for Neurodegenerative Diseases

The central nervous system (CNS) is susceptible to a wide range of neurodegenerative diseases that impair neural connection and communication, which are crucial for sensory, motor, and cognitive functions like vision, hearing, movement, speech and language, memory, and others. Neurodegenerative disorders, including Alzheimer's disease (AD), Huntington's disease (HD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS), impact the brain tissue, resulting in diverse symptoms. Although the etiology, severity, and rate of progression of each neurodegenerative disease are unique, common molecular changes and mechanisms can be identified, providing potential directions for research into a variety of diseases [39].

Synthetic biology has the potential to advance therapies for neurodegenerative illnesses by introducing new tools and methods. Synthetic biology will particularly aid in the design of small molecules, proteins, gene networks, and vectors to target disease-related genes. Combining protection and repair methods will lead to the development of novel therapies, including the use of medication, the promotion of neurotrophic factor synthesis, and gene targeting [40]. Due to their master regulatory function in several signal transduction cascades in the neuroscience field, protein kinases (PKs) have come to be identified as CNS disease-relevant targets. In the context of neurodegeneration,

GSK-3, FYN, and DYRK1A are particularly important, and the deregulation of all three PKs has been connected to a variety of CNS disorders [41]. Since Alzheimer's disease (AD) has a complex etiology and a dynamic progression from preclinical to clinical stages, it poses a unique therapeutic challenge. In more than two thousand clinical trials, several prospective treatment targets and approaches for AD were investigated. Recent breakthroughs in technical developments in the design, manufacturing, and targeting of brain mRNA and microRNA with synthetic antisense oligonucleotides (ASOs) appear to present opportunities to address the problems of AD therapy. Given that over 70% of human miRNAs are expressed in the brain and that many of these miRNAs play a role in the regulation of neuroinflammation and other important pathomechanisms of AD, this novel idea of targeting entire signaling cascades using miRNA-based ASOs is further validated [42]. By targeting the mRNA for APP or its amyloidogenic processing enzymes, several ASOs attempted to reduce the amounts of toxic amyloid. ASO OL-1 was created to specifically target the 17-30 amino acid region of the APP mRNA. Two AD mouse models, transgenic Tg2576 (APPswe) and SAMP8 mice that spontaneously generate Aβ plaques with aging, both had their APP expression levels reduced by OL-1 in the brain. Despite worries caused by the observed shift towards soluble Aβ, OL-1 treated mice were characterized by enhanced cognitive function and decreased neuroinflammation [43]. In older SAMP8 mice, ASO aimed at APP processing PS1 reduced Aβ-mediated brain oxidative stress and enhanced learning and memory. Another ASO downregulated BACE1 mRNA and protein levels in the HEK293 cell line by 90% and 45%, respectively [44]. After giving ASO intracerebroventricularly to SAMP8 mice, tau phosphorylation and oxidative stress were reduced, and learning and memory were also improved [45].Using an ASO intended to promote exon 19 inclusion in ApoER2 mRNA enhanced synaptic function, memory, and learning in mice [46]. Huntington's disease (HD) is an autosomal-dominant neurological disease brought on by CAG expansion in huntingtin (HTT) exon 1. Because the mutant huntingtin (mHTT) protein is the underlying cause of Huntington's disease, oligonucleotide-based therapeutic methods

using siRNAs and antisense oligonucleotides created to specifically silence mHTT may be cutting-edge treatment options [47]. A genetic circuit for the cytomegalovirus promoter that codes for both mHTTsiRNA and a rabies virus glycoprotein tag that targets neurons was created. This circuit was able to reprogram hepatocytes to transcribe and self-assemble mHTTsiRNA into rabies virus glycoprotein-tagged exosomes after being taken up by mouse livers following intravenous injection. A rabies virus glycoprotein tag is used to direct the mHTTsiRNA to the cortex and striatum during subsequent delivery via the exosome-circulating mechanism. As a result, the amounts of mHTT protein and toxic aggregates were successfully decreased in the cortex of three mouse models of Huntington's disease that were treated with this circuit. These findings established an efficient method for siRNA self-assembly *in vivo* that could have a considerable therapeutic advantage for Huntington's disease [48]. Parkinson's disease (PD) is a multi-factorial degenerative illness that causes tremors, gait rigidity, and hypokinesia, making daily life difficult. Because this disease is typically detected in its later stages, when neurons have completely degenerated, cure is on hold, eventually leading to death due to a lack of early diagnostic techniques. As a result, biomarkers are required to diagnose the disease early on when prevention is viable [49]. The GPR55 receptor is abundantly expressed in the brain, particularly in the striatum, implying that it may have a role in motor function. Indeed, mice lacking GPR55 have impaired motor behavior, as well as dampened inflammatory responses. Abnormal-cannabidiol (AbnCBD), a synthetic cannabidiol (CBD) isomer, is a GPR55 agonist that may be used to treat inflammatory illnesses. Abn-CBD had an anti-cataleptic effect that was reversed by CBD and PSB1216, a recently synthesized GPR55 antagonist, and two other GPR55 agonists (CID1792197 and CID2440433) also had anti-cataleptic effects. These findings show, for the first time, that activating GPR55 may be beneficial in the treatment of Parkinson's disease [50]. ALS is a fatal neurological disease that causes specific degeneration of motor neurons, resulting in muscular atrophy, eventual respiratory failure, and death. A well-known variant of the disease (fALS) is linked to point mutations. The most prevalent is an extension of the C9orf72

gene's noncoding GGGGCC hexanucleotide repeat on chromosome 9p21. Chemical chaperones (CSs) are a traditional technique for discovering treatments for protein aggregation-related disorders. The FDA has approved a CS treatment for such complication in ALS; 4-phenylbutyric acid (4-PBA). However, its high active concentration dosage prevents it from being used as an effective therapeutic agent for a long time. Hence, selective targeting of 4-PBA to specific cellular compartments such as lysosomes and ER is needed to limit its effective concentration. The 4-PBA derivatives were synthesized using organic chemistry synthetic techniques and solid-phase peptide synthesis (SPPS). The effect of these derivatives was evaluated on the eye of an ALS Drosophila model expressing C9orf72 repeat expansion. Several 4-PBA derivatives had a significant biological effect on eye degeneration. They inhibited neurodegeneration in the retina at various efficacy levels. Compound 9, a peptide derivative that was ER-targeted, was the most active CS [51]. Novel CSs surpassed 4-PBA in terms of efficacy, suggesting that they could be employed as a new class of therapeutic candidates to treat ALS [52].

Synthetic Biology-Based Cancer Therapies

By manipulating the behavior of living organisms, synthetic biology tries to apply engineering ideas to biology. An emerging application of this field is the genetic modification of bacteria to program therapeutic, safety, and specificity features as a cancer therapy [53]. Bacteria can naturally localize to tumors by entering through the vast vascular of the tumor. Once inside, they can colonize the necrotic core, an immune-privileged environment that protects them from macrophages and neutrophils. This natural colonization process has the potential to be enhanced by the addition of targeting or directing mechanisms, which can potentially lower the likelihood of off-target colonization. One approach is engineering bacteria to express tumor-homing proteins or peptides on the outer membrane. Affibodies (proteins engineered to bind targets such as upregulated receptors in cancer cells (e.g., HER2)), synthetic adhesion molecules that mimic immunoglobulin fragments and identify antigen receptors, and recognized tumor-targeting

peptides such as RGD, have all been employed as targeting motifs [54]. A magnetotactic bacterium strain was developed to transport drug-loaded nanoliposomes. After injecting grafted tumors in mice. The introduction of a magnetic field was applied in the tumor-guided bacteria. after injection of grafted tumors in mice. Enhancing tumor localization, whether through creating targeted or remotely guidable bacteria, has the potential to enhance colonization efficiency and off-target effects, and may have a significant impact on attaining effective clinical usage of these medicines [55]. Animal tumor model injected with Salmonella typhimurium harboring a promoter library generating GFP, were able to discover tumor-specific promoters by sorting and sequencing GFP-expressing bacteria. In a subsequent study, these promoters were modified, and hypoxia-detecting regions were added to create a synthetic tumor-specific hypoxic promoter [56]. Recently, *in vivo,* delivery of S. typhimurium producing flagellin B, a structural component of the flagellum from Vibrio Vulnificus, resulted in macrophage mobilization and tumor regression, possibly via activation of the toll-like receptor pathways [57]. research has employed bacteria as carriers for standard chemotherapies, such as "bacteriobots" and "nanoswimmers" loaded with doxorubicin liposomes and nano-particles, respectively [58]. A bacterium that has been genetically modified, Escherichia coli MG1655, is engineered with NDH-2 enzyme (respiratory chain enzyme II) overexpression (Ec-pE), having the ability to infiltrate tumors and increase localized H_2O_2 production. As a result, magnetic Fe_3O_4 nanoparticles bond covalently to the bacteria, serving as a catalyst for a Fenton like reaction. This reaction transforms H_2O_2 into hazardous hydroxyl radicals for tumor therapy. The Fenton-like reaction happens in this bioreactor using sustainably generated H_2O_2 generated by the engineered bacteria, and thus the produced poison causes tumor death (Figure 3). These findings demonstrate that this bioreactor can achieve effective tumor colonization as well as a self-supplied therapeutic Fenton-like reaction without the use of additional H_2O_2 [59]. Using engineered cells to monitor long-term blood calcium concentration, detect the onset of mild hypercalcemia, and respond via subcutaneous accumulation of the black pigment melanin to form a visible tattoo, a synthetic biology-inspired

biomedical tattoo was developed. Cells, engineered to express ectopically generated calcium-sensing receptors rewired to a synthetic signaling cascade, stimulate the production of transgenic tyrosinase, which creates melanin in response to persistently elevated blood $Ca2+$. Tattoos were seen in all animals implanted with hypercalcemic breast and colon adenocarcinoma cells, but not in mice inoculated with normocalcemic tumor cells. Recent scientific developments have enabled the identification of tumor-specific mutations as well as the development of personalized therapeutic cancer vaccines tailored to target tumor cells rather than normal cells in individual patients, greatly enabling targeted cancer therapy [60]. Cancer vaccines, which are important components of immunotherapy, attempt to induce long-lasting antigen-specific immunity to kill tumor cells and prevent recurrence. Many cancer vaccines have been developed in the past using tumor cells, immune cells, and live attenuated or inactivated bacteria and viruses. Two examples of cancer vaccines are KLH, a glycoprotein produced from giant keyhole limpets and employed in pre-clinical investigations, and DT, a formaldehyde-inactivated diphtheria toxoid. TT is another formaldehyde-inactivated neurotoxin commonly utilized as a vaccine carrier due to its accessibility from Clostridium tetani cultures and its well-established safety profile. CRM197, derived from the Corynebacterium diphtheriae C7(197)tox- strain, is now frequently used in clinical vaccines because of its low toxicity and large-scale manufacturability. It results from a single amino acid mutation and offers a high-purity protein [61]. Synthetic mRNA is an interesting cancer vaccine technique that can be used to execute successful cancer immunotherapy. A lipid-polyethylene glycol (lipid-PEG) shelled adjuvant-pulsed mRNA vaccination nanoparticle (NP) consisted of an ovalbumin-coded mRNA and a palmitic acid-modified TLR7/8 agonist R848 (C16-R848). This mRNA vaccine NP formulation retained the adjuvant activity of encapsulated C16-R848 while also significantly improving mRNA transfection efficacy (>95%) and subsequent MHC class I presentation of OVA mRNA derived antigen in antigen-presenting cells. In comparison to the mRNA vaccine NP without adjuvant, the C16-R848 adjuvant-pulsed mRNA vaccine NP approach created an effective adaptive

immune response by significantly enhancing the expansion of OVA-specific CD8+ T cells and infiltration of these cells into the tumor bed *in vivo*. The approach resulted in effective anti-tumor immunity against OVA-expressing syngeneic allograft mouse models of lymphoma and prostate cancer, resulting in significant tumor growth prevention [62]. PRAD (prostate adenocarcinoma) is the main cause of death among males [63]. K LHL17, CPT1B, IQGAP3, LIME1, YJEFN3, KIAA1529, MSH5, and CELSR3 were identified as overexpressed and mutant tumor antigens with poor prognostic value in PRAD. The relationship between these genes and antigen-presenting immune cells was investigated. PIS1 had greater survival and immune cell infiltration, but PIS2 and PIS3 had cold tumor characteristics, a worse prognosis, and more tumor genetic instability. Furthermore, these immune subtypes were associated with immune checkpoints, immunogenic cell death modulators, and PRAD prognostic factors. These findings indicated that KLHL17, CPT1B, IQGAP3, LIME1, YJEFN3, KIAA1529, MSH5, and CELSR3 are possible antigens for PRAD mRNA vaccine development, and that patients in the PIS2 and PIS3 groups are better candidates for immunization [64].
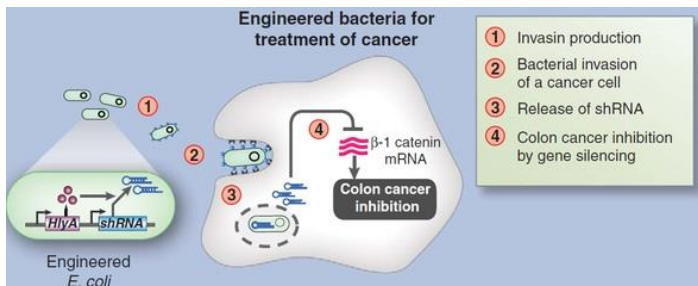

Figure 3: Engineered bacteria for treatment for cancer. A research team at UCSF engineered E. coli to penetrate mammalian cells in low-oxygen conditions, a crude indicator of a tumor. Another study enhanced this approach by programming the infiltrating E. coli to suppress a colon cancer gene (CTNNB1), thereby curbing tumor expansion. The E. coli produce a tiny RNA molecule that attaches to the CTNNB1 mRNA, signaling its breakdown. By injecting the modified E. coli, human colon cancer cells implanted beneath the skin of laboratory mice were successfully targeted, marking a fascinating initial progress [6].

Synthetic Biology in The Battle Against Infectious Diseases

Infectious diseases are a major public health concern and continue to be a leading cause of morbidity and mortality worldwide. These diseases are caused by microorganisms such as bacteria, viruses, fungi, and parasites. They can spread from person to person, through contact with infected animals or contaminated surfaces, or air [65]. Strategies for preventing the spread of infectious diseases include vaccination, proper hand hygiene, use of personal protective equipment, quarantine and isolation measures, and public health education [65]. Treatment may include antibiotics, antivirals, antifungal medications, and other targeted therapies [65]. However, a set of obstacles including late diagnosis and drug resistance prevent effective control over these diseases. Nevertheless, synthetic biology plays a significant role in disease control by providing tools for disease diagnosis and treatment [66].

If the identification of bacterial infection is delayed in diagnosing a disease, it can cause ineffective antibiotic treatment and result in early death, especially from sepsis [67]. Remarkably, the mortality rate is high, up to 30-39%, with inappropriate antibiotic treatment, whereas appropriate treatment can reduce it to 12-28%. Typically, it takes 24-48 hours or more to culture bacteria and enable appropriate antibiotic treatment [67]. Synthetic biology diagnostics can provide fast and precise identification of bacterial infections, reducing the time to diagnose [68]. For example, phages, or bacteriophages, are viruses that can eliminate bacteria. To diagnose pathogens like Staphylococcus aureus (S. aureus), Listeria, Escherichia coli (E. coli), and Bacillus anthracis, genetically engineered phages known as fluoromycobacteriophages are developed [69]. This phage-based diagnostic method can detect pathogens much faster than traditional methods, significantly reducing detection time [69]. By using this new approach, tuberculosis (TB) diagnosis, which usually takes up to 10 weeks to culture, can now be completed in as little as 24 hours [69]. The commercially available phage-based diagnostic platforms, FastPlaque TB and FastPlaque-Response assays, can diagnose TB and determine

rifampicin resistance with a sensitivity of 95% and specificity of 97% [70]. KeyPath is an FDA-approved blood culture test for methicillin-resistant S. aureus (MRSA)/methicillin-susceptible S. aureus (MSSA), which can identify S. aureus and distinguish MRSA in only 5.5 hours [71]. This is much faster than current methods of culturing S. aureus, which typically require more than 48 hours. The sensitivity and specificity of the KeyPath test are 91.8% and 98.3%, respectively. Eventually, having S. aureus as the leading sepsis etiology, the risks of sepsis are predicted to drop with this technology [71]. Moreover, a novel and affordable diagnostic tool has been created to quickly detect the Zika virus. This tool employs toehold switches, a type of RNA engineering designed for medical diagnosis, to identify the RNA genome of the Zika virus on a paper-based platform that has been freeze-dried [72]. This technique consists of a short segment of RNA that acts as a trigger (the toehold) and a larger segment of RNA that functions as the responder. When the toehold switch encounters a specific target RNA sequence, it undergoes a conformational change that activates the responder segment, allowing it to carry out the desired function, such as generating signal to indicate the presence of the target RNA [73]. Diagnostic tests based on synthetic biology are being developed to rapidly detect infectious diseases such as fungal sepsis and pneumonia, in a faster manner than traditional techniques [68].

As potential alternatives to antibiotics amid antibiotic-resistant crises, bacteriophages have been investigated as a potential therapy for bacterial infections since their initial identification as a means of treating Shigella dysenteriae in 1919 [74]. While phage therapy has been effective against a number of bacterial infections including cholera, diabetic foot ulcers, and chronic otitis, it is hindered by various limitations such as the inability of phages to penetrate cell walls, the limited host range of phages, and the development of bacterial resistance [74]. Fortunately, synthetic biology provides solutions to the limitations of phage therapy by modifying or engineering phages, which can make them safer and more effective for use in therapy [75]. One way this is accomplished is by engineering the tail component of phages to recognize multiple host strains, thus expanding the host range [76]. Using a yeast-based phage engineering system,

researchers were able to modify the host range of E. coli phages (T7, T3) by engineering the phage tail components to target Yersinia and Klebsiella bacteria. This approach succeeded in eliminating the targeted bacteria [76]. Moreover, phages have been engineered to inhibit biofilm production by expressing enzymes that degrade the biofilm matrix or by blocking communication between bacteria, which in turn leads to biofilm inhibition [76]. This strategy is highly valuable since biofilm production is a key element in disease development, as it makes bacteria resistant to drug treatment and the immune system [66]. Quorum sensing is the process through which bacteria communicate and coordinate biofilm formation. The main component of this cellular signaling is acyl-homoserine lactones (AHL), and lactonase is a molecule known for its ability to quench this quorum sensing process [77]. Based on this, scientists have developed phages that can prevent biofilm formation by quenching quorum sensing. They engineered the T7 bacteriophage to produce the AiiA lactonase enzyme upon infection, which can degrade the AHLs. As a result, the T7 phage expressing AiiAlactonase efficiently inhibited biofilm production [77].

To add more, one of the most high-profile breakthroughs in medicine from synthetic biology research is the creation of a precursor to anti-malarial drugs, artemisinic acid, from yeast [78]. Artemisinic acid can be sourced from Artemisia annua (sweet wormwood) using an extraction process, which before this discovery was in a crisis state due to unrelenting demand and limited supplies of the plant. Now, instead of relying on natural sources, scientists can produce artemisinin-based therapies (ACTs), ranked as the most effective anti-malarial treatment, which is cheaper and more reliable [78].

During the Coronavirus disease 2019 (COVID-19) pandemic, synthetic biology provided lifesaving tools for disease diagnosis, treatment, and prevention [66]. Development of several diagnostic platforms such as specific high sensitivity enzymatic reporter unlocking (SHERLOCK), DNA endonuclease-targeted CRISPR trans reporter (DETECTR), and 1 h low-cost multipurpose highly efficient system (HOLMES) helped detect

disease biomarkers with high sensitivity and efficiency [79]. Additionally, programmable toehold switch sensors have been developed to isolate viral RNA from patients' swab samples and detect SARS-CoV-2-specific genomic regions, producing a fluorescent signal [80].

As a therapeutic approach, a synthetic biology drug called carrimycin has been approved by the FDA for a Phase III trial to treat bacterial infections associated with COVID-19 [81]. This is a newly developed drug with anti-bacterial and anti-inflammatory properties, which is created through genetic engineering of Streptomyces spiramyceticus by adding a 4″-O-isovaleryltransferase gene from Streptomyces thermotolerant [81]. This modification allowed carrimycin to possess stronger antibacterial properties, where studies have demonstrated that carrimycin can effectively inhibit viral RNA synthesis, particularly post-entry replication events, without causing any major adverse effects in the treatment of severe COVID-19 cases [81].

As preventive measures, synthetic biology enabled the creation of vaccines that contain only the nucleic acid building blocks of an antigen, addressing several safety concerns [82]. This technology has been instrumental during the COVID-19 pandemic, as it has allowed for the rapid development and distribution of DNA and RNA vaccines at an unparalleled rate [83].

Finally, synthetic biology presents a promising potential to improve future rates of morbidity and mortality caused by infectious diseases through preventive, diagnostic, and therapeutic synthetic biotics (Figure 4).
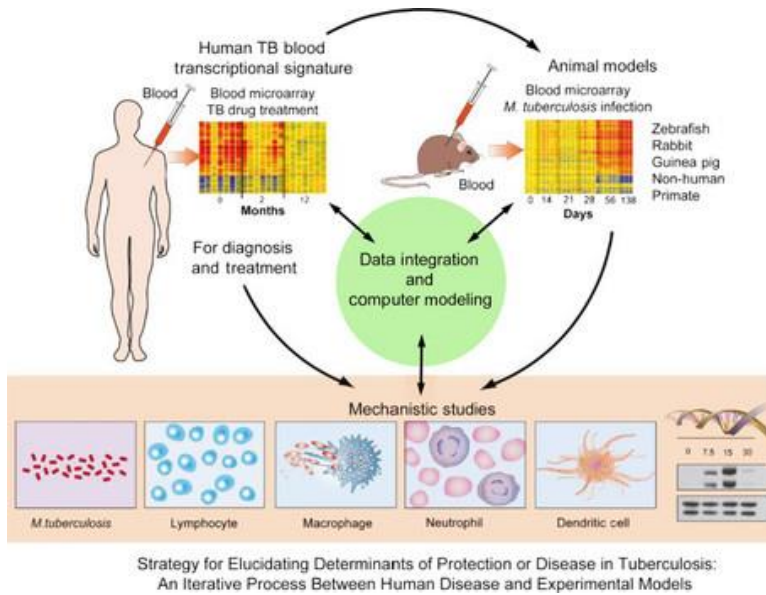
Strategy for Elucidating Determinants of Protection or Disease in Tuberculosis:
An Iterative Process Between Human Disease and Experimental Models

Figure 4: Employing a systems biology methodology in the study of infectious diseases, the figure outlines the strategy for uncovering factors that either protect against or contribute to tuberculosis. This involves a cyclical process alternating between human disease investigation and experimental model testing [84].

Tackling Bacterial Drug Resistance Utilizing Synthetic Antibiotics Assortment

The emergence and spread of bacteria resistant to the majority of known medicines increases fears of a worldwide infectious disease crisis [85]. Any solution relies on the development of novel medicines that are efficient against current bacterial diseases, although opinions differ on what methods are most effective for achieving these discoveries [86]. For decades, natural products served as both conceptual and material starting points for antibiotic discovery. However, making specific chemical modifications to structurally complex natural products (that is, semisynthesis) is inherently difficult, and the pace of drug discovery via this route has slowed significantly [87]. In an alternative approach, the development of fully synthetic platforms for the construction of tetracycline [88], macrolide

[89] group (A) streptogramin [90], and arylomycin [91] antibiotics has enabled deep structural modifications not possible with semisynthetic drugs. These tools enable chemists to imagine and evaluate an infinite number of design possibilities.

We employ component-based chemical synthesis to achieve a significant re-scaffolding of lincosamide antibiotics, yielding a broad-spectrum agent effective against a broad spectrum of multidrug-resistant bacterial infections. Lincosamides are one of the numerous ribosome-targeting groups that have proven crucial to current pharmacopeia [92]. The first member of the family, lincomycin, was discovered in 1963 from a streptomycete found in Nebraskan soil [93] and found immediate application in the management of staphylococcal, pneumococcal, and streptococcal infections. An early semisynthetic modification led to the antibiotic now known as clindamycin, a molecule with optimized pharmacokinetic properties and an expanded spectrum of activity [94] that has mainly replaced lincomycin in human medicine [94]. Considering the US Food and Drug Administration accepted clindamycin in 1970, semisynthetic and fully synthetic methods of lincosamide discovery have been investigated, leading to candidates with six- and seven-membered aminoacyl residues, each with an expanded spectrum of activity [95]. More recently, reports of changes in the aminosugar residue through semisynthesis have been made, extending coverage to some Gram-positive bacteria that are multidrug resistant [96].

Clindamycin-resistant bacteria are globally distributed and are commonly acquired through N6-dimethylation of 23S ribosomal RNA (rRNA) residue A2058 [97]. The US Centers for Disease Control and Prevention (CDC) have identified three major resistance mechanisms among its most pressing threats in their 2019 report (1): a distinct rRNA methyltransferase, Cfr (encoded by the horizontally transferrable gene CFR) [98], methylates C8 of the 23S rRNA residue A2503 and confers resistance to phenicol, lincosamide, oxazolidinone, pleuromutilin and streptogramin A [98]. (PhLOPSA) antibiotics as well as 16-membered macrolides [99]. Additionally, recent studies have elucidated a third major resistance mechanism affecting lincosamides, whereby target-protection proteins (e.g. LsaA)

bind to antibiotic-inhibited ribosomes, evicting the drug and restoring protein synthesis [100]. The prevalence of erm genes among clinical streptococcal and staphylococcal isolates has led to the rise of Clostridium difficile colitis, which challenges the continued utility of this antibiotic in patients.

To counteract the possibility of bacterial resistance, phytochemicals are thought to have a crucial role in reducing bacterial virulence. It's interesting to note that these processes allow natural phytocompounds to display their antibacterial activity through their chemical makeup and physical characteristics [101]. Because of their antimicrobial activity, bioactive-rich compounds like alkaloids, phenols, flavonoids, and terpenoids, among others, must be isolated and profiled to be used in the creation of novel and natural antimicrobial drugs. These compounds also have a specific clinical significance because of their bioactivity, which does not result in resistance. These bioactive substances are often divided into five categories: polyphenolics, alkaloids, tannins, glycosides, and steroids. Among them, polyphenols have antibacterial properties that are effective against a variety of pathogens. Polyphenolic compounds, especially flavanol and phenolic acids, have been shown to have the highest activity for a variety of scientific reasons, including attenuating bacterial virulence factors such as enzymes and toxins, decreasing extracellular polysaccharide activity, and acting as extracellular polysaccharide inhibitors. Much scientific research has clearly demonstrated that increasing the number of compounds stimulated pathogen inhibition potential [102].

Alkaloids, such as quinolone, dictamnine [103], and kokusagine [104], are a cluster of heterocyclic compounds with antimicrobial potential. These compounds show suitable antibacterial activity against a wide range of bacterial pathogens, including B. cereus, S. aureus, and K. pneumonia [105]. Piperine, which was isolated from Piper nigrum and ciprofloxacin, has been shown to inhibit type II topoisomerase enzyme and reduce the consumption of O2 against bacteria. Diterpenoid alkaloids belonging to Ranunculaceae have antimicrobial properties. The mechanism of action of quaternary

alkaloids, such as berberine and harmane, is accomplished by their ability to interpolate with DNA, leading to impairment in cell division and cell death. These compounds have antimicrobial potential against bacteria, fungi, protozoa, and even viruses by aiming at DNA intercalation, affecting RNA polymerase, gyrase, and topoisomerase, and by inhibiting cell division [106]. Comparably berberine has potent antimicrobial activity against bacteria, fungi, protozoa, and even viruses by intercalating DNA, affecting RNA polymerase, gyrase, and topoisomerase, and inhibiting cell division [107]. Berberis spp., a phytochemical substance, decreased E. coli development by inhibiting cell division, protein, and DNA synthesis [106]. The antimicrobial chemical chanoclavine, derived from Ipomoea muricata, had synergistic efficacy when combined with tetracycline, which appears to block EP and was described as efficacious and ATPase dependent [108].

Plasmid, a self-replicating, circular DNA coding for multiple gene groups, is resistant to antibiotics in bacteria. Certain phytochemicals were reported to target such plasmids [109]. 8-Epidiosbulbin-E-acetate, derived from Dioscorea bulbifera, has been shown to effectively cure antibiotic-resistant R-plasmids in clinical isolates of E. faecalis, E. coli, Shigella sonnei, and P. aeruginosa [110]. Tomatidine, a Solanaceous plant derivative, has been shown to have antibacterial action against Listeria, Bacillus, and Staphylococcus spp. Tomatidine's probable mode of action is as an ATP synthase inhibitor [111].

P. aeruginosa and S. epidermidis are resistant to allicin, an organosulfur molecule derived from Allium sativum. Allicin's antibacterial action mechanism involves the suppression of DNA synthesis, protein synthesis, and sulfhydryl-dependent enzymes [112]. The antibacterial activity of ajoene from A. sativum inhibits the sulfhydryl-dependent enzyme inhibitor of Campylobacter jejuni [113]. The application of Diplotaxis harra-derived sulforaphane as an ATP synthase inhibitor and DNA/protein synthesis inhibitor was investigated, and the results demonstrated that this molecule successfully inhibits E. coli growth. Furthermore, this compound has been shown to destroy the target pathogen's membrane [114].

Terpenes, also known as isoprenoids, are the most abundant family of substances found in essential oils and are composed of isoprene molecules [115]. Essential oils (EOs) are made up of a variety of phytochemicals and are well known for their antibacterial properties. Furthermore, because they are considered safe to consume and necessary for host tissues, they have been used as a traditional medicinal treatment to combat antibiotic resistance [116]. EOs produced from Melaleuca alternifolia improved bacterial membrane permeability [117]. Farnesol, an essential oil phytochemical, suppressed S. aureus development by damaging the cell membrane [118].

Plant flavonoids are phenolic compounds with a 2-phenyl-benzo-γ-pyrane nucleus and two benzene rings with potent antimicrobial activities. Flavonoids such as flavanols, flavanones, isoflavonoids, chalcones, and dihydrochalcones have been reported to exhibit antimicrobial properties [119]. Catechin causes membrane disruption in MRSA, leading to the accumulation of bacterial cells and increased permeability of pathogenic S. aureus and E. faecalis [120].

Numerous plant-derived bioactive substances (phytochemicals) have been studied for their ability to reverse antibiotics and kill bacteria. The bioactive potentials of such phytochemicals have been found to inhibit important virulence factors linked to resistance development, such as cell permeability, efflux pumps, DNA replication mechanisms, and other bacterial virulence-related processes, such as biofilm formation and quorum sensing. Furthermore, the synergistic effects of these phytochemicals in combination with conventional antibiotics were found to be extremely effective against antibiotic-resistant pathogenic bacteria.

Synthetic Biology Related Advances for Natural Drugs Synthesis

Synthetic biology blends classical metabolic engineering and ideas from systems biology. Recent developments in synthetic biology have strongly influenced research on herbal remedies. The characteristics of optimized and altered organisms include

reduced carbon emissions, environmental friendliness, and economic effectiveness. They can constantly and effectively synthesize specified target molecules with high yields. New biosynthetic pathways can be devised to produce numerous natural medications and analogs, eliminating the need to rely solely on conventional discovery and separation or difficult synthetic chemistry to acquire a small number of natural pharmaceuticals and analogs [121].

Plant extraction is now a way of producing natural pharmaceuticals from plants; nevertheless, traditional methods of preparing natural chemicals that rely on plant extraction have several drawbacks. Most natural plant medicines have extremely low content in the host; for example, 3 kg of bark from a 100-year-old Pacific yew may contain just 300 mg of paclitaxel, accounting for 0.01% of the bark's dry weight [122]. The first and most widely publicized application of synthetic biology for the industrial production of an important drug is the case of semi-synthetic artemisinin. This antimalarial drug was originally obtained from plant sources but can now be produced in large quantities in heterologous hosts, including Saccharomyces cerevisiae [123]. Malaria is one of the world's worst parasite infections. Malaria infected about 214 million people in 2015, killing 438,000 individuals, most of whom were children under the age of five. WHO presently considers artemisinin-based combination therapy to be the most effective technique for treating malaria [124]. Due to the unstable structure of artemisinin and the complexity of the method of purification, the manufacturing cost of artemisinin is expensive, hampering efforts to meet market needs. As a result, identifying a more efficient way to produce artemisinin is critical. The advancement of high-throughput sequencing and molecular technology has resulted in the elucidation of the artemisinin biosynthesis route, and the genes of certain essential enzymes in the artemisinin biosynthetic pathway have been cloned and described [125]. The methylerythritol-4-phosphate route (MEP) in plastids and the mevalonate pathway (MVA) in the cytoplasm of A. annua are the two pathways that give rise to the universal 5-carbon precursor isopentenyl pyrophosphate (IPP) and its double-bond isomer dimethylallyl pyrophosphate (DMAPP) [126]. The

artemisinin precursor farnesyl diphosphate (FPP) was discovered to be involved in the synthesis of different isoprenoids (artemisinin, aristolochene, caryophyllene, farnesene, and sterols) by combining two IPP molecules with a single DMAPP molecule. In artemisinin-producing organisms, farnesyl diphosphate synthase (FPS) catalyzes the synthesis of FPP [127]. The first unique sesquiterpenoid precursor of the artemisinin biosynthesis pathway, amorpha-4,11-diene (AD), is generated by cyclization of FPP catalyzed by amorpha-4,11-diene synthase (ADS) [128]. The Keasling group cloned the amorpha-4,11-diene oxidase gene CYP71AV1 from A. annua glandular hairs and inserted ADS, CYP71AV1, and CPR into Saccharomyces cerevisiae at the same time [17]. This yeast cell factory produced more than 100 mg/L of artemisinic acid. Semi-chemical synthesis can effectively and cheaply convert artemisinin acid to artemisinin. All FPP synthesis-related genes in S. cerevisiae CEN.PK2 was overexpressed in order to increase the yield of artemisinic acid [129]. These genes included ERG10, ERG13, tHMG1, ERG12, ERG8, ERG19, IDI1, and ERG20. Additionally, three copies of tHMG1 were integrated to achieve the 40 g/L fermentation level of the artemisinin. The highest concentration of artemisinic acid (25 g/L) was produced by the Keasling group [130] by combining the expression of the cytochrome b5 gene, NAD alcohol dehydrogenase, and NAD acetaldehyde dehydrogenase genes. It is still unknown whether A. annua converts dihydroartemisinic acid to artemisinin by enzymatic or nonenzymatic means during the last stages of artemisinin production. It is understood that spontaneous autoxidation, a non-enzymatic process, can transform dihydroartemisinic acid into artemisinin [131]. RNA interference technology is used for inserting antisense strands of cDNA into A. annua to downregulate the expression levels of β-farnesyl pyrophosphate synthase, β-caryophyllene synthase (CPS), and squalene synthase to block the effects of the pathways that compete with artemisinin biosynthesis [132]. The production of artemisinin in all transgenic A. annua plants rose by 70% as compared to control plants. Furthermore, because the organism is a complex membrane structure system, compartmentalization of the secondary metabolite biosynthetic process not only allows enzymes to be aggregated as functional units but also separates

the pathway from the rest of the cell. Certain types of selective advantages can be expected from the co-compartmentalization of biosynthetic enzymes. The closeness of continuous enzymes in the secondary metabolite biosynthesis pathway might increase route efficiency. This is especially critical if the products and intermediates are potentially harmful to the manufacturing cell [133].

Salvia miltiorrhiza Bunge (Danshen) is a Lamiaceae-family medicinal herb. Because the hydrophilic phenolic acids and lipophilic constituents of these roots are pharmaceutically active components, dried roots of this plant have been used in traditional Chinese medicine (TCM) for a long time [134]. Tanshinone I (TS I), TS IIA, and crypto tanshinone (CTS), which make up the majority of the lipophilic constituents, have a variety of pharmacological activities, including anticancer [135] and antibacterial effects [136], and can be effectively used to treat cardiovascular diseases [137]. The enzymes diterpene synthase copalyldiphosphate synthase (SmCPS) and kaurene synthase-like (SmKSL) cyclize GGPP, the tanshinone precursor in the cytosolic MVA pathway and plastid-localized MEP pathway, to produce miltiradiene [138], a crucial precursor for the biosynthesis of tanshinone. A mutagenesis cassette encoding SMCPS, SMKSL, ERG-20, BTS1, and HMG1 was constructed using the modularized pathway engineering methodologies, and a high yield of miltiradiene (365 mg/L) was produced in yeast [138]. Miltiradiene production was increased to 488 mg/L thanks to pathway optimization in a yeast expression system [139]. A high-yielding GGPP chassis line was created by deleting the distant genetic loci YPL062w and YJL064w and knocking out the transcriptional regulator Rox1 (repressor of hypoxia). Next, two high-efficiency catalytic enzymes (CfTPS1 and SmKSL1) were combined to create a fusion protein that can most efficiently convert GGPP to miltiradiene in yeast. Finally, miltiradiene production was increased [140].

The first-line medicine for the treatment of breast cancer and ovarian cancer is paclitaxel, a diterpenoid anticancer agent derived from Chinese yew. Yew has little paclitaxel in it, and there are not many T. chinensis plants around. The complicated and expensive complete chemical synthesis of paclitaxel makes

semichemical synthesis a significant approach for the commercial manufacturing of paclitaxel [141]. The primary pathway for paclitaxel's biosynthesis has been established, and most of the enzymes have been acquired and functionally assigned. Currently, 14 of the 19 enzymes needed for the biosynthesis of paclitaxel have been discovered; however, the sequence in which most of these enzymes, particularly P450 enzymes, engage in the pathway has not yet been established [142]. The most promising ways for producing paclitaxel right now are semisynthesis and microbial synthesis of its precursors. These technologies are expected to address the market's issues with unaffordable prices and insufficient supply of paclitaxel. The endangered T. chinensis must also be protected [143]. The basic biosynthetic pathway includes the cyclization of GGPP to taxa-4(5),11(12) diene by taxadiene synthase [144]; hydroxylation of taxane skeleton at C5, C10, C13, C2, C9, C7, and C1 by cytochrome P450 monooxygenase to generate oxetane; the creation of epoxypropane D-ring at C4 and C5; and then CoA acylation to synthesize the essential intermediate baccatin III [145]. Taxol's C13 side chain is the essential component for its anticancer properties. The side chains of baccatin III and phenylisoserine are joined at position C13, and the side chains are subsequently hydroxylated and benzoylated at positions C2 and C3 to produce paclitaxel [146]. Engels and colleagues overexpressed the genes for taxadiene synthesis, geranylgeranyl diphosphate (GGPP) synthase, HMG1 reductase (thmgr), and the transcription factor UPC2-1 in S. cerevisiae [147]. The modified microbe produced 8.7 0.85 mg/L of taxa-4(5), 11(12)-diene, and up to 33.1 5.6 mg/L of geranylgeraniol accumulated. The Scott team introduced four genes to engineer E. coli to produce taxadiene, which was a breakthrough in paclitaxel synthesis. Two P450 enzymes and an acetyltransferase enzyme were introduced into yeast to obtain taxadien-5-acetate-10-ol yield of 1 mg/L [138].

Challenges in Synthetic Biology

Despite advances in many aspects of synthetic biology, this field is facing various challenges. Challenges loom at every stage of the process in synthetic biology, from part characterization to system design and assembly. Synthetic biology aims to assemble

or build novel functions that do not exist in nature. In this regard, standard components such as amino acids, nucleotides, and other chemical concerns will be used where a biological component can range from a DNA sequence that encodes a specific protein to a promoter, which is a region that facilitates gene expression. It also takes standard parts, computer design, and software, and understanding to put together a functional system. Besides, the issue is that many sections have not been well defined. They have not always been studied to prove what they do, and even when they have, their performance might vary depending on cell type or laboratory settings. Furthermore, the full promise of synthetic biology has yet to be realized [148], primarily due to challenges associated with its integration. This integration is impeded by debates surrounding genetically modified organisms (GMOs), their potential environmental effects, and the accompanying containment and regulation problems [149]. Additionally, while cell-free systems, which mimic life-like functioning outside living cells, offer promise, debates over GMOs have influenced their adoption. However, despite these challenges, cell-free systems have demonstrated success in manufacturing essential bio-molecules, including medicines [150]. The inherent lack of understanding of fundamental biological processes is the key constraint in synthetic biology in general, not simply scaling already proven technology. Much metabolic engineering research now focuses on "mutagenesis" and "directed evolution," which involves messing with a cell's DNA and hoping for the best. So one issue is getting proteins (which are encoded by DNA) to do the exact function that we want [151]. One of the many exciting potentials that synthetic biology provides for medicine is the creation of theranostic cell lines capable of sensing disease states and producing an appropriate therapeutic response [152]. To achieve this goal, several obstacles need to be addressed. First, we must broaden the range of molecules that can be recognized as inputs by cellular "sensors," and second, we must better understand the genetic control factors that regulate gene expression in space and time so that we can engineer better activator systems. Furthermore, the circuitry is erratic. Even though the function of each element is known, the pieces may not perform as predicted when assembled. Synthetic biologists are frequently stuck in a

lengthy trial-and-error process, in contrast to the more predictable design techniques used in other modern engineering fields. Synthetic genetic circuits, once built and implanted in cells, can have unforeseen consequences for their host. For example, we can't predict how protein-DNA interactions change DNA structure, how a specific protein-DNA structure affects gene expression, how supercoiling occurs as a result of genetic circuit layout, how DNA-encoded modules perform due to the possibility of mechanical interactions, and how the location of a gene on the genome affects gene expression [153]. Even if one could mimic the entire designed cell using known software tools, the information obtained would be useless for design and verification. In truth, there is a wealth of experimental data and computational tools available to define some of these effects individually, but there is a lack of a design-oriented mathematical framework to explain these effects and their relationships. Also, our inability to address the preceding issue is primarily due to our lack of understanding of modularity and compositionality in natural biological systems. Natural biological systems, such as (unfortunately) cancer pathways, are exceedingly resistant to parameter uncertainty and external perturbations [154]. Synthetic biologists must also verify that circuits work dependably. Molecular processes inside cells are subject to random oscillations or noise, where its propagation can deteriorate circuit performance or even lead to complete circuit failure [155].

Variations in growing conditions can also influence behavior and eventually, randomly occurring genetic mutations might completely disable a circuit's function. Addressing these robustness issues may need a shift away from conventional fundamental processes in synthetic biology, such as gene expression and gene control, and toward new types of processes, such as protein-protein interactions and CRISPR/Cas-based systems. These biological tools are currently ignored, even though they may allow for faster reactions and a simpler tuning approach [156]. However, how to do circuit design employing these key techniques to obtain a desired functionality remains unknown. Moreover, synthetic biology is an example of a dual-use technology: it has many promising uses, yet it may also be harmful [157].

Conclusion

We have reached a watershed moment in the history of modified immune cell cancer therapies. The emerging field of synthetic immunology is assembling a large resource of tools that can be used to enhance or reprogram T cell function in various ways and overcome the fundamental problems in cancer treatment, how to recognize the tumor and distinguish it from normal cells, and how to mount a potent cytotoxic response that overcomes the local immunosuppression that is common in many solid tumors. Although we have concentrated on CAR T cells as an engineering T cell therapy for cancer, many of the techniques and tactics apply to other disorders such as autoimmune ones. It is also feasible that modified immune cells will become beneficial as a research tool. An immune cell with the ability to identify user-defined antigen signatures and change the microenvironment, like an antibody or medication, would enable a novel and customized form of targeted disruption. A precision-engineered cell that can participate as an active yet controlled node within the complex, *in vivo* networks would be a great tool as we learn more about the fundamental principles of tissues and systems composed of numerous cell types. In order to overcome some of the apparent drawbacks of current biological molecules, an intriguing new generation of biologics that not only disrupt the immune response but also use autologous immune cells to treat illnesses is now being developed. In conclusion, although synthetic immunology is young, it has a promising future. In preclinical and clinical trials, fascinating new treatment ideas for modifying the immune response, as well as innovative technical techniques that alter the function, activity, or Immune cell targeting are currently being developed. Modulation of the immune response based on the principles of synthetic biology has enormous potential since immune cells are implicated in the pathogenesis of a range of disorders. Synthetic biology will contribute to innovative technologies by introducing therapeutic systems based on a synthetic genome, employing an enlarged genetic code, and built for precisely tailored medication manufacturing as well as transport and activation by a pathological signal.

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