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Bringing next-generation therapeutics to the clinic through synthetic biology

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Recent advances in synthetic biology have created genetic tools with the potential to enhance the specificity, dynamic control, efficacy, and safety of medical treatments. Interfacing these genetic devices with human patients may thus bring about more efficient treatments or entirely new solutions to presently intractable maladies. Here we review engineered circuits with clinical potential and discuss their design, implementation, and validation.

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Introduction

Advances in synthetic biology have enabled the genetic engineering of microbes into an expanding array of living sensors, actuators, and chemical factories. The development of such tools for mammalian systems has been comparatively slower, in large part due to the increased complexity of mammalian cells compared to protozoans. However, efforts to address these challenges and implement synthetic biological approaches in mammalian cells and toward clinical applications offer the potential for a new generation of therapeutics that complement or even address shortcomings of traditional small molecule and protein biologics. As one example, small molecule drugs are typically administered orally or intravenously and enter the systemic circulation, which in contrast to localized delivery could yield untoward side effects. However, synthetic biology could ultimately create living 'smart drugs' that can sense a pathological signal or state in a complex, noisy environment and actuate an appropriately tuned, localized therapeutic response *in situ*.

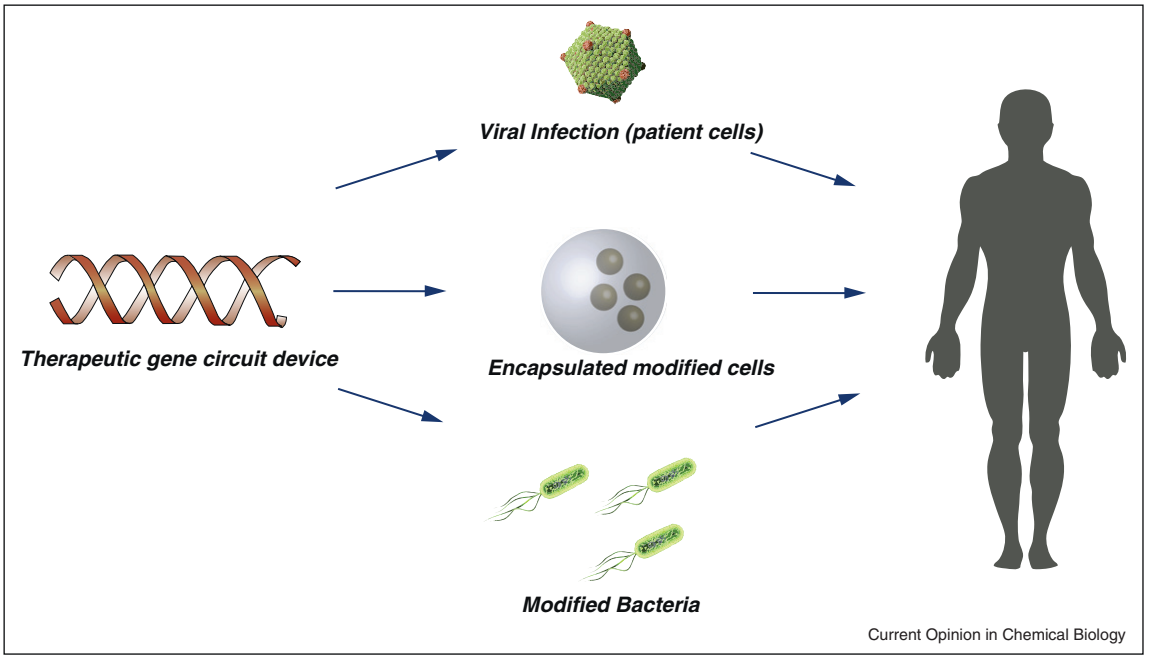
Synthetic biology has been applied to healthcare in numerous ways, from yeast engineered for the cost-effective production of the anti-malarial artemisinin [1] to mosquitoes designed to propagate dominant-lethal genetic circuitry throughout Dengue-transmitting mosquito populations [2]. These and other examples have been detailed in several elegant reviews [3–5]. Here, we focus on clinical applications in which synthetic genetic systems are being engineered for direct administration to patients, including strategies to directly modify host cells, to implant encapsulated genetically modified cells, and to administer genetic devices contained in a bacterial vector (Figure 1).

Devices for direct host cell modification

Genetic modification of a patient's cells offers a direct means to treat chronic cell and tissue dysfunction, and efforts in viral gene therapy have enjoyed increasing success in establishing the feasibility and efficacy of this approach [6–14]. Synthetic biology can potentially augment traditional gene therapy strategies by enhancing control of the therapeutic gene to be expressed, for example through circuit architecture involving environmental sensing or feedback, or through the use of inducible promoters responsive to orally administered small molecule pills.

Initially developed decades ago, small molecule control over mammalian gene expression could be considered an early example of synthetic biology in mammalian cells [15]. In such a system, a transgene of interest is placed downstream of a promoter that is induced by a small molecule. Depending on specific circuit components or 'logic,' the small molecule induces transcription factor association to or dissociation from the promoter region, thereby modulating transgene expression. Small molecule control of transcription has been extended to a variety of inducers, including antibiotics [15–17], steroid hormones [18], food components [19,20••], and even photons [21•]. Because of their modularity, reversibility, and responsiveness to readily deliverable molecules, inducible promoters offer transgene expression control and therefore potentially enhanced safety in therapeutically relevant gene circuits. The first therapeutic inducible transgene system has already entered into clinical trials [Trial of an Intratumoral Injections of INXN-3001

Figure 1



Interfacing synthetic biological therapeutics with patients. Therapeutic genetic circuits must be interfaced with human patients in a manner that maximizes safety and efficacy of a specific treatment. These devices can be administered via: (1) DNA modification of the host-cell genome; (2) delivery of encapsulated cells modified with the device; and (3) delivery of bacteria transformed with the DNA device. The specific application will dictate the appropriate mode of delivery for a particular device.

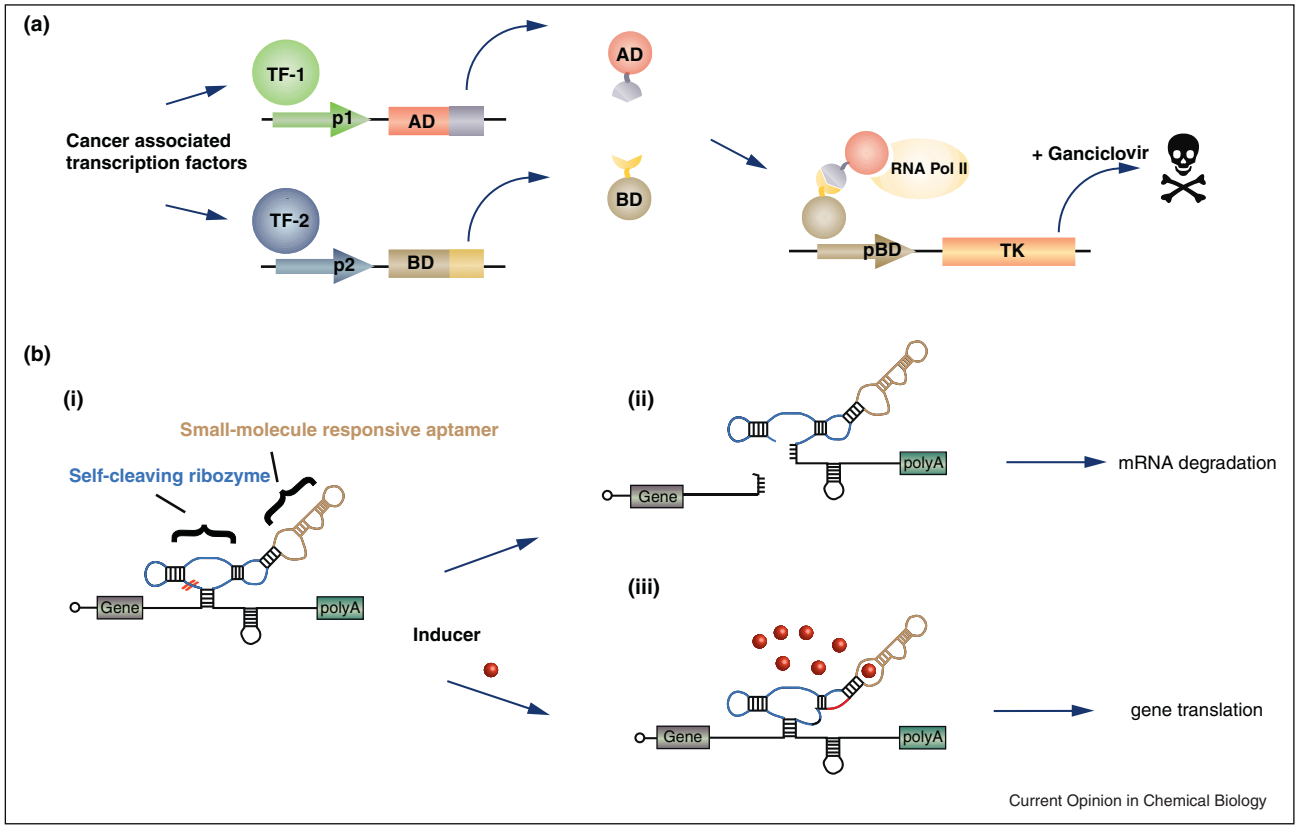
in Subjects With Stage III or IV Melanoma; URL: http://clinicaltrials.gov].

Cancer is an attractive target for therapies involving introduction of gene circuits to a host cell genome, since treating diseased cells specifically and sparing healthy neighboring tissue is essential. Genetic elements have been developed to enhance this specificity by sensing the cancer state of a particular cell. In one instance, researchers created an AND-logic gate that relies on the activity of two promoters highly active in cancer to express both halves of a two-hybrid transcription factor, which in turn drives the expression of the apoptosis-inducing thymidine kinase 1 (TK1) (Figure 2a) [22]. Similarly, Xie and colleagues developed a six-input miRNA-based device that senses the high/low state of up to six cancer-related miRNAs within a cell and initiates transcription of the apoptosis gene hBax within the correct miRNA window [23•]. Tuned to recognize HeLa cells, the device was able to selectively sense and kill these cells over other cancer cell types. Such proofs of concept demonstrate an additional level of cell-type specificity afforded by sensor-actuator circuits.

RNA-based devices are therapeutically attractive since they lack potential immunogenicity associated with heterologous protein expression. Additionally, RNA devices can be activated or inactivated more rapidly than DNA devices, since system control takes place downstream of DNA transcription and is thus rate-limited only by protein translation and folding. As an example, Chen and colleagues modified T cells with a tunable RNA-based controller of cell proliferation that enabled rapid, drug-inducible expression of interleukin-15 (IL-15) — a cytokine important in T cell survival and proliferation — in mice (Figure 2b) [24]. Implementation of this system resulted in controlled and enhanced T cell survival and proliferation both *in vitro* and in a mouse model. Similar outcomes in humans could enhance adoptive transfer therapy, where safe, long-term persistence of implanted T-cells is critical.

In general, as highly promising engineered circuits are explored for introduction into a patient's genome, the increased capabilities that accompany the incorporation of additional parts should be considered in light of the potential ways in which increasingly complex systems could fail. For instance, in contemplating such devices for human therapeutic use, multiple avenues should be considered to validate and enhance their safety, including using targeted gene delivery vehicles, validating orthogonality or lack of unintended interactions between engineered and host circuitry, designing systems that can withstand mutational loss or even gain of function, and implementing failsafe measures to remove the

Figure 2



Autonomous and inducible gene expression devices. **(a)** A cell autonomous AND-gate cancer sensor. Transcription factors upregulated in cancer (TF-1 and TF-2) drive the expression of two halves of a two-hybrid transcription complex, where an activating domain (AD, orange) and a DNA binding domain (BD, brown) are fused to two interacting protein domains (purple and yellow). Upon high TF-1 and TF-2 activity in cancer cells, the two protein halves are expressed and form a complex, which then binds to the BD recognition site upstream of a cytotoxic gene (TK). The AD then recruits polymerase to transcribe the gene, leading to cancer-cell specific death. **(b)** An RNA device for small molecule-inducible gene expression. A self-cleaving ribozyme fused to a small-molecule binding RNA (an aptamer) is encoded downstream of a gene of interest (i). In the absence of the small molecule (ii), the ribozyme cleaves itself (at the double red line in (i)), removing the polyA tail from the gene and inducing mRNA degradation. Upon binding of a small molecule (iii), the aptamer changes conformation and inactivates the ribozyme cleavage, retaining mRNA stability and inducing transcription of the gene of interest. TF-1, TF-2: transcription factor 1, 2; p1, p2: promoters binding TF-1 or TF-2. AD: activation domain; BD: binding domain, pBD: promoter binding BD; TK: thymidine kinase.

devices or their carrier cells. It is therefore arguable that future therapeutic development should start with genetic devices that offer the potential for efficacy with minimal complexity, since fewer components may offer fewer potential modes of failure.

Devices for implanted encapsulated cells

Early cell implantation therapies involved pancreatic islet cell administration into diabetic patients to sense blood glucose levels and secrete insulin [25]. To prevent immune clearance, the cells were microencapsulated in a material (originally porous alginate-poly-L-lysine, though many others have since been investigated) that allowed diffusion of small molecules and proteins yet prevented macromolecule transport necessary for immune reactivity. Encapsulation technologies for therapeutic cell implantation have subsequently been expanded to kidney cells [26], parathyroid cells [27], hepatocytes [28] as well as to standard cell lines expressing heterologous proteins, as previously reviewed [29].

Implantation of genetically modified autologous, allogeneic, or xenogeneic cells presents an alternative method for therapeutic gene circuit delivery that offers several advantages compared to direct gene delivery: first, cells can be screened *ex vivo* for optimal properites, for example for safe-harbor genetic integration; second, the encapsulation effectively sequesters modified cells from host tissues, which provides a safety mechanism in the case of unforeseen therapeutic side-effects; third, physiological interference of the synthetic device with the implanted cell may be acceptable so long as the engineered functionality remains; fourth, concerns over genetic delivery to off-target cells *in vivo* are eliminated.

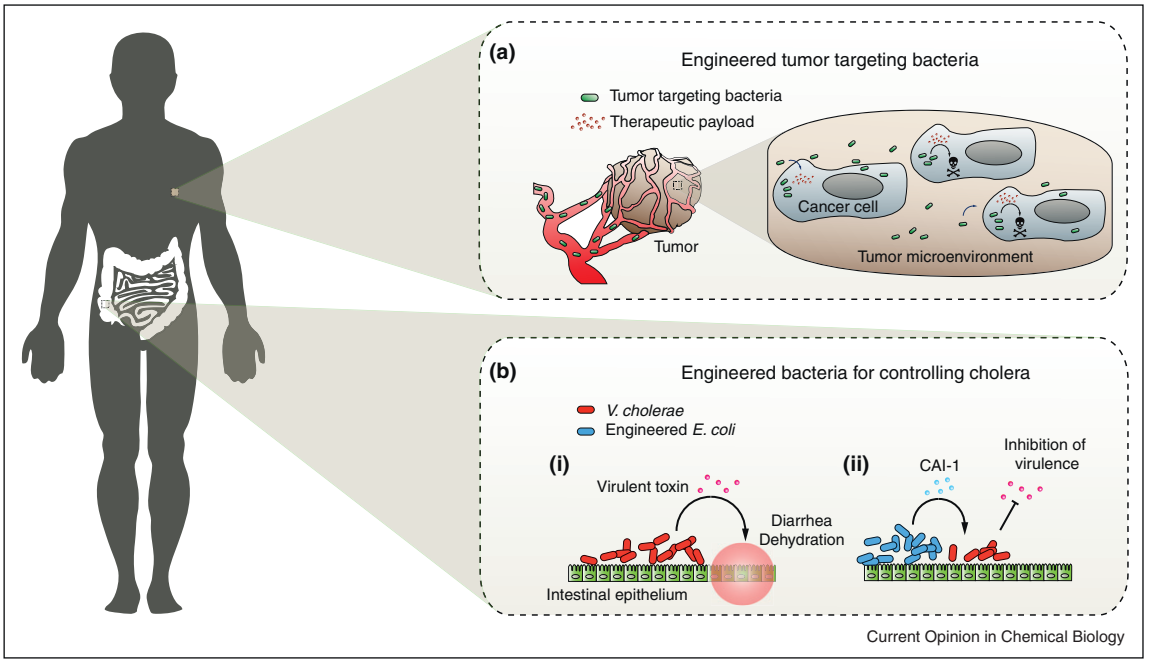
Though not without its problems, encapsulation technology has thus far proven safe for the implantation of cells carrying natural sensor-actuator systems, and many synthetic biology systems could benefit from analogous strategies. For example, cancer treatment often results in tumor lysis syndrome, where systemic uric acid release from dying cells leads to gout. To treat this, microencapsulated HEK 293 cells modified with an engineered uric acid-responsive transcription factor that promotes the expression of a secreted urate oxidase enzyme were implanted and were able to maintain serum urate at subpathological levels [30••]. In another example, encapsulated cells expressing the photoreceptor melanopsin were implanted subcutaneously into a type II diabetes mouse model, and exposure to blue light initiated a signaling cascade that led to the expression of a glucagon-like peptide (GLP1) transgene that regulated glucose homeostasis [31••]. Optical methods present an exciting possibility of allowing highly localized drug dosing by illuminating (activating) a therapy specifically within diseased tissue.

Such examples highlight the opportunity of using synthetic biology to link novel inputs (e.g. light) to functional outputs from implanted cells. In another demonstration, Gitzinger and colleagues developed a phloretin-repressible gene expression system which can be controlled transdermally through application of a skin cream containing phloretin, a flavonoid common in fruits and routinely consumed by humans [20••]. Development of therapies activated through non-oral delivery routes can offer more control over drug delivery through both localized drug administration and bypassing first-pass hepatic metabolism.

Devices for delivery via bacterial vectors

In some instances, genetically engineered bacteria themselves may be delivered as therapy. It has been well documented that several bacterial genera including *Escherichia*, *Clostridium*, and *Salmonella* naturally target and accumulate within tumors when injected intravenously [32•]. Though the homing mechanisms remain incompletely understood, bacterial tumor targeting relies on increased blood flow at the inflamed tumor site, bacterial entrapment in the turbulent tumor vasculature, and chemotaxis toward tumor-specific and necrotic compounds [32•]. Bacteria possess unique properties that make them an intriguing alternative to traditional chemical therapeutics in cancer treatment: first, they are easy to manipulate genetically, for example for delivery of anti-tumor

Figure 3



Synthetic devices for therapeutic bacterial delivery. **(a)** Engineering bacteria to invade and kill tumors. Researchers have utilized the natural ability of bacteria to home specifically to tumors to create bacteria that invade tumor cells and deliver a cytotoxic or imaging payload, enabling specific treatment and/or visualization of tumors. Decreasing immunogenicity and increasing cancer-cell specificity of the vectors may yield an effective strategy for targeted cancer cell therapy. **(b)** Engineering commensal bacteria for disease control. *Vibrio cholerae* in the gut secrete toxins that act on the intestinal epithelium and cause symptoms typical of cholera. Once *V. cholerae* senses its own critical density through the quorum-sensing CAI-1, toxin production is inhibited, and the *V. cholerae* are cleared from the gut. Commensal *E. coli* engineered to express CAI-1 and allowed to precolonize the gut before *V. cholerae* ingestion were able to inhibit *V. cholerae* colonization and virulence and significantly improved survival in mice. CAI-1: cholera autoinducer-1.

agents, and numerous modular control networks have been implanted in a bacterial chassis; second, they target tumors specifically, offering the potential for localized release of anti-tumor agents; and third, they are motile, allowing deeper tumor penetration than allowed by passive diffusion, particularly to hypoxic regions inaccessible to blood.

An ideal bacterial cancer therapeutic would specifically invade a tumor and deliver either a cytotoxic agent, an immunostimulatory cytokine, or the genetic material to enable host-cell production of these molecules [32•] (Figure 3a). As an example of this strategy, tumor-infiltrating *Salmonella* have been engineered to deliver plasmid DNA encoding siRNA against bcl-2 [33], STAT3 [34], and β-catenin [35], proteins with anti-apoptotic and pro-proliferative activity. Successful knockdown of protein levels through this approach decreased tumor size 2–10-fold over controls in mouse xenograft models.

Bacterial tumor targeting may also be employed for tumor detection. Bacterial-aided delivery of imaging agents has allowed tumor visualization through PET [36,37], MRI [38–40], fluorescence [41,42], and bioluminescence [43–47]. These capabilities may yield future therapies that allow simultaneous treatment and noninvasive visualization of tumor progression and metastasis. Additionally, targeted fluorescent protein delivery to tumors has been shown to aid surgeons in operative resectioning, enhancing tumor removal and survival outcomes in animal models [48•]. Tumor-specific bacterial delivery of these (and other) imaging proteins may allow for delivery to a broader range of tumors, with higher signal-to-noise between tumor and nontumor tissue, and may expand the imaging modalities available to aid surgeons in optimal removal of cancer tissue.

Despite their possible promise, the safety of tumor targeting bacteria remains a primary issue. To date, three clinical trials in patients with advanced cancer have demonstrated tolerance of attenuated *Salmonella* up to a dose of 3×10^8 cfu/m^2 [49–51], though toxicity at this maximal dose was observed with detectable tumor targeting in only 12–66% of patients, depending on the trial. Strategies to decrease bacterial virulence or enhance targeting efficiency will be critical for therapeutic relevance. Promisingly, nonpathogenic, probiotic *Escherichia coli* have been shown to target tumors as well as or better than *Salmonella* [52]. In addition, Anderson and colleagues have engineered nonpathogenic *E. coli* to invade tumor cells upon sensing hypoxic environments or high bacterial density [53]. Combining natural bacterial properties with synthetically designed capabilities may yield effective and safe next-generation cancer therapies.

In addition to acute cancer, chronic conditions may also benefit from bacterial therapy. In the human body, bacterial cells are thought to outnumber human cells 10:1, and a majority of these exist symbiotically in our intestinal tracts [54]. Thus, the human gut offers a hospitable niche for therapeutically engineered bacteria. Duan and colleagues have engineered probiotic *E. coli* to secrete glucagon-like peptide-1 (GLP1) and a cell-permeable pancreatic and duodenal homeobox gene 1 (PDX1), both of which stimulate intestinal epithelial cells to produce insulin in response to glucose [55]. Successful implementation *in vivo* could provide an alternative to insulin injections for type I diabetes patients. In another example, Duan and March developed an *E. coli* strain to prevent *Vibrio cholerae* virulence [56••] (Figure 3b). *E. coli* were engineered to secrete the *V. cholerae* specific quorum-sensing molecule CAI-1, which inhibits production of pathogenic cholera toxins at high densities. In mice, intestinal colonization of these *E. coli* increased survival rate 48 hours after *V. cholerae* challenge from 0% to 92%. Such prophylactics may be particularly well suited as inexpensive yet effective solutions to public health concerns in the developing world where cholera is most prevalent.

Conclusion and perspectives

The past decade has witnessed an impressive rise in the capabilities of the synthetic biology field, spurred by rapid advances in DNA sequencing and synthesis. Though most work to date has focused on microbial engineering for industrial processes, developing and applying synthetic biological tools for biomedical application holds substantial promise. Novel treatments may provide prophylactics, therapeutics, and diagnostics not possible with current technologies, and tangible successes would enhance public acceptance, understanding, and support of this nascent field. Fostering public education and support of the science will be a key component as our capabilities to design and define living organisms become more powerful.

In light of the potentially significant public and scientific gains, a clinically successful product of synthetic biology should be a focus for the field. The speed of implementing such new therapeutics must be balanced, however, with extreme caution. Unforeseen and adverse clinical outcomes may have irreparable consequences for future development. With clinical gene therapy trials of the late 1990s serving as a lesson [57,58], we must remember that biological systems are complex, often beyond our understanding, and we would do well to minimally disrupt host genomic and protein systems in achieving our desired outcome. Simplicity in device design will be paramount. In particular, regulatory evaluation and approval of genetic devices as therapies will be a challenge, and in this respect, devices with fewer components present fewer modes of failure or dysfunction, increasing safety and the likelihood of approval. Already, the first clinical tests of synthetic biology tools are underway. Initial small-molecule inducible devices controlling

interleukin-12 production for the treatment of cancer have received Investigational New Drug (IND) approval from the FDA, and preliminary results of Phase I trials in advanced melanoma patients show positive host safety and tolerance of the treatment (DJ Schwartzentruber *et al.*, abstract #2540, 2011 Annual meeting of the American Society of Clinical Oncology, Chicago, IL, June 2011). Results of these pioneering trials will set the stage for realizing the clinical potential of synthetic biology.

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- •• of outstanding interest

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