

Concise Original Report

Synthetic biology: at the crossroads of genetic engineering and human therapeutics—a Keystone Symposia report

Jennifer Cable,¹ Joshua N. Leonard,² Timothy K. Lu,^{3,4} Zhen Xie,⁵ Matthew Wook Chang,⁶ Luis Ángel Fernández,⁷ José M. Lora,⁸ Howard L. Kaufman,⁹ Francisco J. Quintana,¹⁰ Roger Geiger,¹¹ Cammie F. Lesser,¹² Jason P. Lynch,¹² David L. Hava,³ Virginia W. Cornish,¹³ Gary K. Lee,⁴ Breanna DiAndreth,¹⁴ Michael Fero,¹⁵ Rajkamal Srivastava,¹⁶ Tim De Coster,¹⁷ Kole T. Roybal,^{18,19} Owen J. L. Rackham,²⁰ Samira Kiani,²¹ Iowis Zhu,²² Rogelio A. Hernandez-Lopez,²³ Tingxi Guo,²⁴ and William C. W. Chen²⁵

¹PhD Science Writer, New York, New York. ²Department of Chemical and Biological Engineering, Center for Synthetic Biology, Interdisciplinary Biological Sciences Program, Chemistry of Life Processes Institute; and Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Evanston, Illinois. ³Research Lab of Electronics, Massachusetts Institute of Technology, Cambridge, Massachusetts. ⁴Senti Biosciences, South San Francisco, California. ⁵MOE Key Laboratory of Bioinformatics and Bioinformatics Division, Center for Synthetic and System Biology, Department of Automation, Beijing National Research Center for Information Science and Technology, Tsinghua University, Beijing, China. ⁶Synthetic Biology Translational Research Program and Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore; NUS Synthetic Biology for Clinical and Technological Innovation (SynCTI), National University of Singapore, Singapore. ⁷Department of Microbial Biotechnology, Centro Nacional de Biotecnología, Consejo Superior de Investigaciones Científicas (CNB-CSIC), Madrid, Spain. ⁸Intergalactic Therapeutics, Cambridge, Massachusetts. ⁹Division of Surgical Oncology, Massachusetts General Hospital, Boston, Massachusetts. ¹⁰Ann Romney Center for Neurologic Diseases, Brigham and Women's Hospital, Harvard Medical School, Boston and The Broad Institute of MIT and Harvard, Cambridge, Massachusetts. ¹¹Institute for Research in Biomedicine, and Institute of Oncology Research, Università della Svizzera italiana, Bellinzona, Switzerland. ¹²Department of Microbiology, Blavatnik Institute, Harvard Medical School and Center for Bacterial Pathogenesis, Division of Infectious Diseases, Department of Medicine, Massachusetts General Hospital, Boston, Massachusetts. ¹³Department of Chemistry, Columbia University, New York, New York. ¹⁴A2 Biotherapeutics, Agoura Hills, California. ¹⁵TeselaGen Biotechnology, San Francisco, California. ¹⁶Biophysics and Structural Genomics Division, Saha Institute of Nuclear Physics, Homi Bhabha National Institute (HBNI), Kolkata, India. ¹⁷Laboratory of Experimental Cardiology, Department of Cardiology, Leiden University Medical Center, Leiden, the Netherlands. ¹⁸Department of Microbiology and Immunology and Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, San Francisco, California. ¹⁹Chan Zuckerberg Biohub; Parker Institute for Cancer Immunotherapy, Gladstone-UCSF Institute for Genomic Immunology; and UCSF Cell Design Institute, San Francisco, California. ²⁰Program in Cardiovascular and Metabolic Disorders, Duke-National University of Singapore Medical School, Singapore. ²¹Division of Experimental Pathology, Department of Pathology, School of Medicine; and Pittsburgh Liver Research Center, University of Pittsburgh, Pittsburgh, Pennsylvania. ²²University of California, San Francisco, San Francisco, California. ²³Cell Design Institute, Department of Cellular and Molecular Pharmacology; and Center for Cellular Construction, University of California San Francisco, San Francisco, California. ²⁴Massachusetts Institute of Technology, Cambridge, Massachusetts. ²⁵Research Laboratory of Electronics and Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge; and Cardiovascular Research Center, Massachusetts General Hospital, Boston, Massachusetts

Address for correspondence: annals@nyas.org

Synthetic biology has the potential to transform cell- and gene-based therapies for a variety of diseases. Sophisticated tools are now available for both eukaryotic and prokaryotic cells to engineer cells to selectively achieve therapeutic effects in response to one or more disease-related signals, thus sparing healthy tissue from potentially cytotoxic effects. This report summarizes the Keystone eSymposium “Synthetic Biology: At the Crossroads of Genetic Engineering and Human Therapeutics,” which took place on May 3 and 4, 2021. Given that several therapies engineered using synthetic biology have entered clinical trials, there was a clear need for a synthetic biology symposium that emphasizes the therapeutic applications of synthetic biology as opposed to the technical aspects. Presenters

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discussed the use of synthetic biology to improve T cell, gene, and viral therapies, to engineer probiotics, and to expand upon existing modalities and functions of cell-based therapies.

Keywords: bacterial engineering; biosensor; CAR T cells; cell therapy; cellular engineering; CRISPR; gene therapy; genetic engineering; logic gating; oncolytic virus; receptor engineering; synthetic biology; tumor-associated antigen

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Introduction

Synthetic biology tools and principles have advanced significantly over the past decade. Sophisticated tools are now available for both eukaryotic and prokaryotic cells to engineer the ability for cells to sense their environment, compute a decision based on one or more inputs, and translate that decision into a desired output.

On May 3 and 4, 2021, experts in synthetic biology across academia and industry met virtually for the Keystone eSymposium “Synthetic Biology: At the Crossroads of Genetic Engineering and Human Therapeutics.” While many meetings on synthetic biology focus on technological aspects, the goal of this symposium was to emphasize therapeutic applications of synthetic biology.

There are several parallels between cell-based and molecule-based therapies. Molecule-based therapies consist of small molecules or biologics whose goal is typically to inhibit or activate a protein target. By contrast, synthetic biology-based therapies typically consist of engineered bacteria; viruses; or implantable, circulating, or tissue-resident cells that are armed with the ability to secrete effector molecules, perform complex enzymatic transformations, or activate cellular activities that are based on signals from the environment. Synthetic biology therapeutics thereby offer the potential for increased specificity, as well as tunability, that can improve their therapeutic effectiveness and safety profile relative to molecule-based therapies.

Synthetic biology is a powerful approach to rationally design combinatorial therapies with applications in diverse therapeutic indications, including regenerative medicine, immunology,

oncology, genetic diseases, and neuroscience. During the symposium, presenters discussed the use of synthetic biology to incorporate gene circuits into chimeric antigen receptor (CAR) T cell therapies or oncolytic viruses to enable them to better distinguish between tumor and healthy cells, thus limiting their cytotoxic activity to areas of disease. Other presentations focused on the use of synthetic biology to engineer bacteria and yeast to create probiotic therapies that specifically recognize diseased states and generate therapeutic activity.¹ Therapies developed using synthetic biology have been extensively studied in preclinical models, with some being evaluated in clinical studies in areas, such as cancer,^{2–4} gastrointestinal (GI) disorders,⁵ and metabolic diseases.^{6–9} In addition, several speakers presented work on developing modular platforms consisting of synthetic receptors, gene circuits, and effectors that can be mixed and matched to generate a variety of desired outcomes.

Keynote address: new tools for engineering mammalian cells

Joshua N. Leonard from Northwestern University presented his team’s work on developing tools for engineering systems within mammalian cells, focusing on designing synthetic biological sensors and genetic processors. Leonard’s group has created a synthetic receptor sensor called modular extracellular sensor architecture (MESA), which mimics several aspects of native signaling mechanisms to sense soluble ligands and alter cellular states. In brief, ligand binding to an extracellular domain, such as a cytokine receptor, antibody fragment, or

engineered protein, induces receptor dimerization and brings together two pieces of a protease that liberates a sequestered molecule, such as a transcription factor (TF), chromatin modifier, or nuclease.^{10,11} Leonard's group in collaboration with Srivatsan Raman at the University of Wisconsin recently developed a computational approach to design split proteases to make MESA more robust to variations in expression levels. Split Protein Optimization by Reconstitution Tuning (SPORT) uses the protein design program Rosetta to design split proteins that are active upon dimerization and associate less readily to reduce background noise. SPORT was able to design high-performing receptors that outperformed other synthetic and natural receptors.¹²

Leonard also described his group's work on developing tools to aid in signaling processing. Composable Mammalian Elements of Transcription (COMET) is a library of engineered TFs and promoters based on modular zinc finger motifs; Leonard has published rigorous quantitative evaluation of these parts to enable researchers to choose the TF/promoter pair that yields the desired level of gene expression.¹³ His group has developed mathematical models about why COMET works that have revealed quantitative design rules for building and implementing transcriptional control. Leonard showed that these rules enable predictive design of mammalian genic programs without tuning or refinement.¹⁴

As computational modeling becomes increasingly indispensable in synthetic biology, it is important to have a standardized approach to evaluate models. Currently, modeling is challenging and fairly bespoke within applications and groups. To address this need, Leonard's group has created Generation and Analysis of Models for Exploring Synthetic (GAMES), a rigorous workflow that makes model development more objective and improves rigor and reproducibility (Dray, Mangan, Bagheri & Leonard unpublished).

Incorporating logic gating into T and natural killer cell therapies

One promising application of synthetic biology is improving cell-based immunotherapies. By incorporating CARs that recognize tumor-associated antigens (TAAs) into a patient's T cells, researchers have been able to unleash specific cytotoxic T cell

activity against tumors. CAR T cell therapies have demonstrated high response rates and durable effects against hematological malignancies; however, relapse due to antigen loss is a concern. In addition, it has been challenging to develop CAR T cell therapies against solid tumors. This is because of many factors, including the immunosuppressive nature of the tumor microenvironment (TME); difficulties in trafficking T cells to the tumor; T cell exhaustion, which limits the durability of response; and the lack of clean, targetable antigens that enable T cells to discriminate between tumor and healthy cells.¹⁵

Several speakers spoke about how engineering gene circuits into cell-based therapies that incorporate logic gating can improve the ability of engineered T or natural killer (NK) cells to comprehensively target cancer cells while sparing healthy cells.

Modular approaches for engineering T cell and NK cell therapies

Timothy K. Lu from Senti Biosciences and Massachusetts Institute of Technology described how Senti Biosciences has created a gene circuit technology platform that includes smart sensors, logic gating, regulator dials, and multiarming that can be used to program numerous cell and gene therapy products across several modalities, such as NK cells, T cells, and viral vectors.

By rationally combining any of the four gene circuit platform technologies, Senti Biosciences has the potential to address indications that conventional small molecule, protein and cell, and gene therapies cannot.

For one, incorporating smart sensors can allow cell and gene therapies to detect and become active only in disease environments. Senti Biosciences has established, and continues to scale, a Design-Build-Test-Learn, or DBTL, development engine to generate therapeutic gene circuits. As an example, synthetic promoters can act as smart sensors that activate a gene circuit only upon recognition of disease-associated marker(s). Synthetic promoters may be able to improve the efficacy and safety of gene therapies by recognizing when and where the transgene should be expressed, thus enhancing selectivity for target tissues, increasing potency, and reducing cost. Lu also showed how promoters can selectively activate in cancer cells to express cytotoxic genes while remaining inactive in noncancer cells.

Lu described how Senti Biosciences is incorporating logic gating to improve the specificity of cell-based therapies. For example, incorporating a NOT GATE, in which engagement of a TAA triggers cytotoxicity, while engagement of a safety antigen abrogates killing, can increase specificity for tumor cells and thus improve the therapeutic windows of TAA targets.¹⁶ In addition, gene and cellular therapies can be dynamically regulated *in vivo* using Food and Drug Administration (FDA)-approved small molecules. For example, hepatitis C virus protease inhibitors can be used to control protease activity fused to a TF that can drive the expression of a desired gene in a dose-dependent fashion. And gene circuits can be designed to target multiple disease pathways to overcome disease heterogeneity; for example, designing a single cell to express multiple drug payloads and engage multiple pathways can trigger more robust antitumor activity.

One of the key challenges to bringing cell therapies to the clinic is manufacturing. Lu ended by highlighting Senti Bioscience's work in developing a robust manufacturing process to reproducibly produce allogeneic CAR-NK cells at scale. Senti Bioscience's lead product candidates utilize allogeneic CAR NK cells outfitted with gene circuit technologies in several oncology indications. Lu showed that NK cells can be efficiently isolated from healthy donor peripheral blood collections at high viability¹² and that transduction can achieve robust CAR expression with high viability. Finally, the company has tested CAR-NK formulation conditions, demonstrating that off-the-shelf cryopreserved CAR-NK cells retain viability and functionality post thawing *in vitro* and *in vivo*.

Gary K. Lee, also from Senti Biosciences, described the use of an OR/NOT GATE in an engineered allogeneic CAR-NK cell therapy for acute myeloid leukemia (AML). One of the challenges for developing an effective AML therapy is to comprehensively target both the AML blast cells and the leukemic stem cells (LSCs). LSCs have similar features as healthy hematopoietic stem cells (HSCs) and are difficult to target without also incurring toxicity against healthy HSCs. Single-target treatments often target AML blast cells while leaving a population of LSCs that can eventually lead to relapse. Less discriminating treatments, however, run the risk of killing healthy cells.

Senti Biosciences's engineered CAR-NK cell-based therapy incorporates an OR/NOT GATE to target both leukemic blast cells and LSCs while sparing healthy HSCs. NK cells are engineered to target the TAA CD33, which is highly expressed on AML mature blast cells, as well as FLT3, which are more broadly expressed on AML LSCs as well as on healthy HSCs. To protect healthy HSCs, a NOT GATE prevents activation upon engagement with a safety antigen that is only expressed on HSCs. Senti Biosciences has identified a group of TAA/safety antigen pairs that can be leveraged to build cancer therapeutics. The safety antigen used for AML-targeted therapy is endomucin (EMCN), which is expressed on healthy HSCs but not LSCs. Lee believes that this approach has the potential to drive toward a cure for AML without the need for a bone marrow transplant by enabling killing of diverse AML cells while sparing HSCs that regenerate the blood and immune systems.

Lee showed internal data demonstrating that CAR-NK cells that target single antigens (FLT3 or CD33) can efficiently kill specific leukemia cell lines that overexpress each antigen but were less effective in killing cell lines that do not express the targeted antigen at a high level. Targeting either FLT3 or CD33 with an OR GATE led to comprehensive killing of AML cells expressing either antigen in leukemic cell lines. FLT3 or CD33 CAR-NK cells significantly suppressed tumor growth and extended survival in an AML xenotransplant model in mice. Incorporating the EMCN-specific NOT GATE significantly reduced cytotoxicity against EMCN⁺ cells while preserving NK cell-mediated killing of FLT3⁺EMCN⁻ cells.

On the basis of these preclinical studies, Senti Biosciences is currently developing a product candidate for clinical studies that is designed to target and eliminate AML cells while sparing healthy bone marrow.

Targeting antigen loss with T cell therapies

Breanna DiAndreth from A2 Biotherapeutics described a different approach for selectively targeting cancer cells with cell-based therapies. While most tumor-targeting approaches focus on the gain of TAAs due to mutation or upregulation, A2 Biotherapeutics is engineering T cell therapies that differentiate between normal and tumor cells by taking advantage of the loss of antigens in tumor

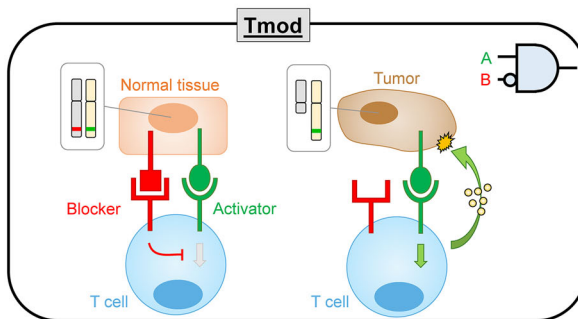


Figure 1. The T cell module (Tmod) system, which incorporates a NOT gate that is activated only on normal cells.

cells. The typical tumor loses approximately 20% of the genome. Much of this loss of genomic information is believed to occur with the founding tumor clone. Therefore, most cells in a tumor will have lost the same genetic material; the loss of an antigen is as much a definitive marker of tumor cells as the gain of neoantigens. A2 is exploiting this genetic difference using a dual-control therapeutic T cell.

Their T cell module (Tmod) incorporates a NOT GATE in which an activator receptor responds to an activator antigen present on both normal and tumor cells. A blocker receptor responds to a blocker antigen expressed on normal tissue that has been lost in tumor cells. Lack of engagement of the blocker receptor enables T cell killing of the tumor cell (Fig. 1).

The blocker module consists of the intracellular domain of LIR-1 fused to an extracellular domain specific to the desired target. DiAndreth showed that LIR-1 inhibits both CAR and T cell receptor (TCR) activation in a ligand-dependent manner in cells.

To test the ability of Tmod cells to selectively target tumor cells and spare healthy cells, A2 developed CAR T cells containing an activating CD19 CAR and an HLA-specific blocker. Coculture of the engineered T cells with engineered cells that mimic normal cells (expressing both antigens) or tumor cells (expressing only CD19) demonstrated robust, selective killing of the tumor mimic cells. A2 has also tested their Tmod cells in mouse models.¹⁷

DiAndreth argued that Tmod opens the door to a so-far untapped approach—targeting the loss of genetic material in tumors rather than the gain of mutations/targets.

Increasing T cell specificity with synNotch receptors

Kole T. Roybal from University of California, San Francisco presented their work on increasing the specificity of CAR T cell therapies by using an AND GATE that controls CAR expression upon activation of a synthetic Notch receptor (synNotch). In this system, activation of a synNotch receptor by a TAA upregulates a CAR specific for a second tumor antigen. By integrating multiple inputs, T cell activity is better localized to sites of disease.^{18,19} Roybal hopes that requiring the presence of two antigens will facilitate development of T cell therapies against solid tumors, which rarely express a single clean, targetable antigen.

Roybal's lab has identified ALPPL2 as a highly specific tumor antigen found on mesothelioma, ovarian cancer, and other solid tumors. While ALPPL2 is highly specific for tumor cells, its expression is heterogenous within tumors. Developing a CAR T cell therapy against ALPPL2, therefore, would likely leave many tumor cells intact. Roybal's group has engineered a regulated CAR T cell in which recognition of ALPPL2 by a synNotch receptor induces expression of a CAR against a TAA that is broadly expressed across the tumor, such as MCAM, mesothelin, or HER2. Since these TAAs are also found on normal tissue, targeting them directly often leads to on-target toxicities. Roybal showed that regulated CAR T cells exhibit paced elimination of tumor cells compared with CAR T cells, which rapidly deplete tumor cells. In a mouse model of mesothelioma, regulated MCAM CAR T cells resulted in better tumor shrinkage and longer term efficacy than either MCAM CAR

T cells or ALPPL2 CAR T cells. Treatment with the regulated CAR T cells led to higher levels of tumor-infiltrating lymphocytes (TILs) and a higher proportion of functional cells. While MCAM CAR T cells had signatures of T cell exhaustion, this was not observed in the regulated CAR T cells. Similar results were seen with regulated HER2 CAR T cells in a mouse model of ovarian cancer. In these models, mice treated with regulated CAR T cells were resistant to rechallenge, and the cells persisted in the periphery, whereas HER2 CAR T cells did not.²⁰

The preclinical studies suggest that regulated CAR T cells show increased persistence compared with CAR T cells. Expression of CARs leads to tonic signaling that drives cells to a more terminal effector state. Because regulated CAR T cells do not express CARs until they encounter the tumor, the T cells are longer lived, reducing exhaustion and maintaining T cell stemness. SynNotch-regulated CAR T cells, therefore, offer the potential for increased tumor-specificity by requiring two signals for activation and demonstrate increased T cell stemness and decreased T cell exhaustion, which may reduce the risk of relapse and result in longer term efficacy.²⁰

Iowis Zhu from Roybal's lab noted that synNotch receptors can be difficult to humanize and are incompatible with a range of TFs. Therefore, Zhu has used synthetic design to maximize the use of human proteins and minimize immunogenicity, achieve high expression, and enable tunable gene regulation in a compact receptor. Essentially, the receptors can be broken down into three components—an extracellular (ECD) domain, a transmembrane domain (TMD), and an intracellular juxtamembrane domain (JMD). By determining which characteristics are responsible for ligand-dependent activation and screening ECDs, TMDs, and JMDs for expression and signaling intensity, Zhu has developed a modular synthetic receptor system dubbed SyNthetic Intramembrane Proteolysis Receptor (SNIPR). By mixing and matching different ECDs, TMDs, and JMDs, the transcriptional output of the SNIPR can be tuned to a desired level. Zhu showed that tuning the transcriptional output can also tune the phenotypic effect. For example, tuning cytokine expression leads to tunable effects on cell proliferation.²¹

Engineering tunable T cell therapies

Rogelio A. Hernandez-Lopez from Wendell Lim's lab at the University of California, San Francisco presented work on engineering T cell therapies to sense antigen density. Current CAR T cell therapies cannot distinguish between tumor cells, which express high levels of TAAs, and normal cells, which express lower levels of TAAs, leading to on-target toxicities.^{22–24}

Hernandez-Lopez has engineered CAR T cells that exhibit a sigmoidal response to antigen levels, thereby enabling T cells to discriminate between target cells on the basis of antigen density. The circuit consists of a synNotch receptor that controls expression of a CAR, similar to that described by Roybal.^{18,19,25} However, in Hernandez-Lopez's system, the synNotch receptor is designed to have low affinity for the antigen, while the CAR has high affinity (Fig. 2). In this way, the synNotch receptor acts as a high-density prefilter, while the CAR acts as an amplifier. If the T cell encounters a target cell with low antigen density, the low affinity of the synNotch receptor precludes it from activating CAR expression. On the other hand, if it encounters a target cell with high antigen density that is sufficient to engage the synNotch receptor, CAR expression is induced, resulting in a positive feedback loop.²⁶

Hernandez-Lopez has built a series of cell lines with varying levels of HER2 spanning a 100-fold range, which is comparable to the levels found in different cancer cell lines and normal breast tissue. Coculturing T cells with the low-affinity synNotch plus a high-affinity CAR circuit with cells of varying HER2 expression achieved a sigmoidal response curve. The activity can be tuned by altering the affinities of the synNotch and/or the CAR; however, altering the affinity of a constitutively expressed CAR did not achieve a sigmoidal response curve. The low-to-high circuit enabled T cells to efficiently discriminate between high- and low-density tumor cells *in vitro* and *in vivo*. Similar results were seen in cells targeting EGFR.²⁶

Hernandez-Lopez hopes that the ability to tune T cell therapies on the basis of antigen density can help to develop therapies to treat solid tumors, in which TAAs are often overexpressed but typically not absolutely unique.

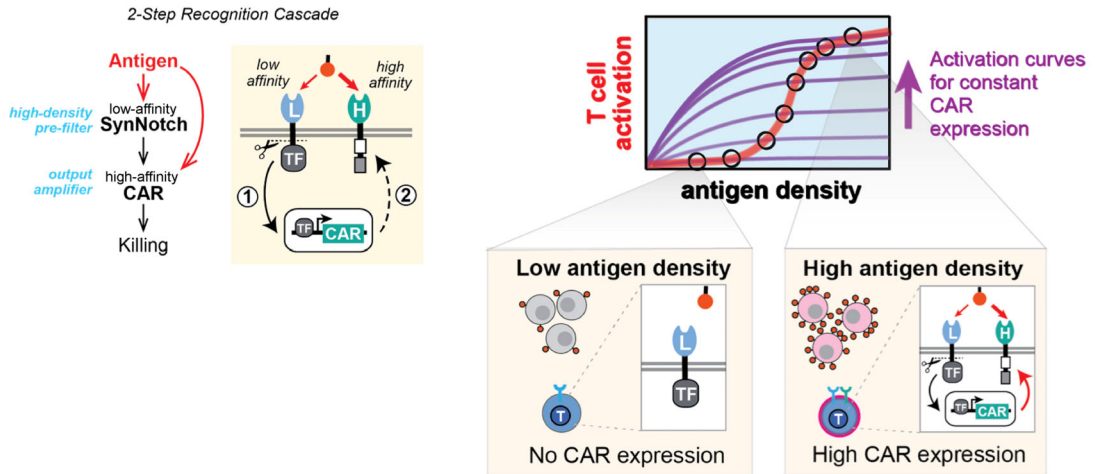


Figure 2. A two-step low-to-high affinity recognition circuit yields ultrasensitive antigen-density sensing.²²

Engineering oncolytic viruses

Another anticancer treatment modality that may benefit from synthetic biology is oncolytic viruses. Oncolytic viruses are attenuated, replication-competent viruses that can selectively replicate in and cause cancer cell lysis. Oncolytic viruses can exert anticancer effects through several mechanisms. They can directly replicate in and kill cancer cells, induce immunogenic cell death by triggering the host immune response, or shut down the vasculature that delivers vital nutrients to tumors. Possibly, one of the more interesting features of oncolytic viruses is that they may have the potential to turn immunologically cold tumors hot, reversing the immunosuppressive nature of the TME and potentially improving the efficacy of checkpoint inhibitors. As multiple virotherapies have been shown to be safe, their potential to be used in combination with other immunotherapies is very attractive.^{27,28}

Despite their potential, only a few oncolytic viruses have been approved for clinical use. In 2004, Rigvir[®] was approved for use in Latvia for the treatment of melanoma and was subsequently approved for use in the Commonwealth of Independent States.²⁹ In 2006, the Chinese FDA approved the first adenovirus therapy for head and neck squamous cell carcinoma. In 2015, the U.S. FDA approved talimogene laherparepvec (T-VEC) for melanoma. T-VEC consists of a modified HSV-1 virus that encodes the human GM-CSF gene to boost the host immune response.³⁰

Improving the efficacy of T-VEC

Howard L. Kaufman from Massachusetts General Hospital presented some of the preclinical and clinical data supporting the use of T-VEC in melanoma. In cell culture, T-VEC demonstrated a lytic effect and induced immunogenic cell death against human melanoma cells.^{31,32} It also induced infiltration of tumor antigen-specific TILs, supporting the notion that oncolytic viruses may be able to turn immunologically cold viruses hot and induce many with long-term immunity.³³ In clinical studies, T-VEC improved progression-free survival and overall survival compared with GM-CSF in patients with stage III/IV melanoma. The benefit persisted for at least 4 years and was more pronounced in patients with less advanced disease.^{34,35}

Kaufman's group is working to improve T-VEC efficacy with combination therapies. Combining T-VEC with the BRAF inhibitor vemurafenib, which is approved for patients with BRAF-mutated melanoma, augmented T-VEC-mediated oncolysis in BRAF- mutant melanoma cell lines but not in wild-type cells. Combining T-VEC with the MEK inhibitor trametinib, which is approved for patients with melanoma, augmented T-VEC-mediated oncolysis independent of BRAF status in human melanoma cell lines. In xenograft and syngeneic mouse melanoma models, trametinib plus T-VEC enhanced T cell activation and melanoma antigen-specific T cell responses, reprogrammed the TME to increase PD-1 and PD-L1 expression, and induced a tumor immune inflammatory

signature associated with a better response to checkpoint blockade. Adding an anti-PD-1 agent to trametinib and T-VEC improved survival in mice more than any of the doublet combinations.³⁶

There is limited evidence on the combination of T-VEC and checkpoint inhibitors in humans. A phase 1 clinical trial of T-VEC and pembrolizumab in melanoma showed that the combination induced T cell recruitment and upregulated PD-L1 in the TME. Several patients with immunologically cold tumors at baseline experienced a complete response, suggesting that T-VEC combination may restore sensitivity to checkpoint blockade in an otherwise insensitive tumor.²⁸ In a second study, Kaufman's group showed that T-VEC plus ipilimumab increased response rates compared with ipilimumab alone without increasing toxicity in patients with advanced melanoma.³⁷ While limited, these data suggest that T-VEC may improve responses to checkpoint inhibitors without increasing toxicity. A randomized phase III clinical trial of T-VEC and pembrolizumab versus pembrolizumab alone has been completed but study results have not yet been reported.

Kaufman also discussed emerging data on predictive biomarkers to help select patients for T-VEC treatment. A recent study showed that JAK mutations confer resistance to checkpoint blockade but enhance oncolytic virus-mediated tumor cell killing.³⁸ Kaufman's group is looking at STING as a potential biomarker for T-VEC efficacy. Murine cancer cell lines with low levels of STING show no response to anti-PD-1 therapy but do respond to T-VEC. The level of STING expression correlates with T-VEC-mediated lysis; knocking out STING showed improved T-VEC sensitivity.³¹ Taken together, these data suggest that HSV-1 replicates better in JAK-mutated tumors or those with low STING expression, while tumors with intact JAK signaling and STING expression may be more amenable to checkpoint blockade (Fig. 3).³⁹ More research will hopefully shed light on whether these features can serve as useful biomarkers in the clinic.

Engineering oncolytic viruses

Zhen Xie from Tsinghua University presented work on using synthetic biology to improve two main features of oncolytic viruses—the ability to discriminate between tumor and healthy cells and to strengthen the host immune response. Single-

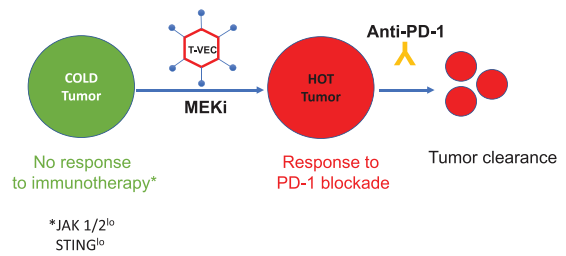


Figure 3. Example of how oncolytic viruses, such as T-VEC, preferentially replicate in JAK 1- and 2-mutated and STING^{lo} immune-deserted tumors—antitumor activity that is enhanced by MEK inhibition, which increases PD-1/PD-L1 expression and increases sensitivity of the tumor to PD-1 blockade.

cell sequencing of patients treated with oncolytic virotherapy shows that the virus does not have high selectivity for cancer cells and is able to replicate in both normal and tumor cells.⁴⁰ To improve the cancer-targeting ability of an adenovirus oncolytic virus, Xie's group incorporated a synthetic gene circuit that controls viral replication and expression of immune effectors, such as GM-CSF or IL-2, by sensing one promoter and two microRNA inputs, one of which is expressed at high levels in healthy cells (miR-a), while the other is expressed in tumor cells (miR-b). The sensory switch enables expression of E1A, a protein necessary for viral replication, and immune effectors if all three of the following conditions are met: the cancer-specific promoter is activated, miR-a levels are low, and miR-b levels are high.⁴¹ As a proof-of-principle, Xie's group created a circuit using α -fetoprotein (AFP) as a marker of hepatocellular carcinoma (HCC) cells to activate the cancer-specific promoter, miR-21 as the tumor-specific microRNA, and miR199a-3p as a marker for normal liver cells.⁴¹

Xie showed that the synthetic oncolytic virus (SynOV) selectively inhibits HCC tumor growth in mice and human cell lines. Biomarker data indicated elevated effectiveness from activated T cell response and other immune modulators encoded in the viral genome. In addition, tumors did not grow after rechallenge, indicating that the therapy elicits a memory T cell response.

Xie's group is testing their oncolytic virus, SynOV1.1, as a monotherapy and in combination with atezolizumab in a phase 1/2a clinical trial in patients with AFP⁺ advanced/metastatic solid tumors that are feasible for local injection.

Engineering microbes to treat disease

Engineering microbes to act as therapeutics has potential in a variety of disease states, including infection, immune disorders, and cancer. The goal of engineered microbial therapy is to enable microbes to both detect disease conditions and exert cellular functions that lead to therapeutic activities. The most common bacterial chassis used for therapeutic purposes is *E. coli* Nissle (EcN). EcN is a nonpathogenic commensal bacterium that was originally identified in 1916 from a soldier who survived dysentery from *Shigella* infection. It has several inherent antibacterial and anti-inflammatory properties that can be leveraged for therapeutic purposes. In addition, EcN can be safely administered; unengineered strains are used in Europe and Canada to prevent inflammatory bowel disorder (IBD) flares. EcN has also been studied as a treatment for GI disorders.⁴²

The use of engineered EcN as therapeutics primarily focuses on diseases of the gut and epithelial tissues, as these are readily colonized by bacteria. Synthetic biology approaches can, therefore, benefit from these natural capacities of bacteria to engineer therapeutic microbes.

Engineering bacteria to improve antitumor immune responses

José M. Lora from Intergalactic Therapeutics presented work done while he was at Synlogic on engineering EcN to engage the host immune system to improve antitumor immunity. The TME is an immunosuppressive environment that can promote insufficient T cell priming. Lora's goal is to engineer EcN to enhance a durable antitumor T cell response by improving T cell priming by activating tumor innate immune cells, such as dendritic cells, to produce proinflammatory cytokines like interferons (IFNs). Type I IFN-related transcripts correlate with T cell infiltration in tumors and are essential for the efficacy of cancer immunotherapy. Type I IFN is the end result of a number of nucleic acid-sensing pathways, including the STING pathway, which is activated by cyclic-di-AMP.

Lora's group has engineered EcN to engage the STING pathway by catalyzing the transformation of two ATP molecules to cyclic-di-AMP under conditions of hypoxia, a common feature of the TME. The group first identified a hypoxia-inducible promoter, *P_{hns}*. Next, they identified an enzyme, *dacA* from

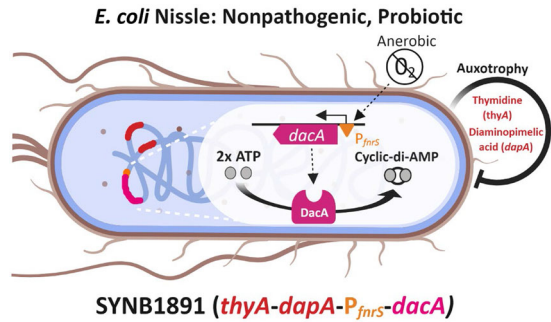


Figure 4. SYNBI1891, an EcN strain engineered to engage the STING pathway in the tumor microenvironment.

Listeria, that could convert two ATP molecules to cyclic di-AMP. Under control of a TET promoter, *dacA* drove robust antitumor activity characterized by activation of an early innate immune response and a late T cell-related immune response. These two functionalities were combined to produce the EcN strain SYNBI1891 (Fig. 4). Lora showed that internalization of SYNBI1891 by dendritic cells and macrophages induces IFN production via the STING pathway both in mice and human cell lines. In mice, SYNBI1891 elicited robust and durable antitumor activity and drove long-term immune memory, preventing tumor growth after rechallenge.⁴

SYNBI1891 is being investigated in a phase 1 clinical trial in patients with advanced solid tumors, both as a monotherapy and in combination with atezolizumab. Interim results presented at the American Association for Cancer Research 2021 showed that SYNBI1891 is well tolerated and exhibits evidence of target engagement and clinical activity in this heterogeneous, heavily pretreated population. The trial is ongoing.

Roger Geiger from the Institute for Research in Biomedicine presented their work on engineering bacteria to modulate the TME to combat cancer. Several lines of evidence suggest that arginine metabolism may play a role in antitumor T cell activity. Geiger previously showed that T cells with high arginine levels have higher antitumor activity.⁴³ In addition, many tumors are infiltrated by myeloid-derived suppressor cells that express Arg1, an enzyme that degrades arginine.⁴⁴ Geiger's group is working to restore arginine levels in the TME to improve the antitumor T cell response. Oral administration of arginine showed

synergistic activity with an anti-PD-L1 agent in mice; however, the dose of arginine required for an effect was not feasible for clinical applications.⁴³ Geiger presented work done in collaboration with David Hava's group at Synlogic. They developed a probiotic L-arginine-producing EcN strain that colonizes tumors, continuously synthesizes arginine, and releases it into the TME.⁹⁰ The group co-opted the endogenous arginine biosynthesis pathway, which converts ammonia, a metabolic waste product found at high levels in the TME, to arginine. The engineered probiotic has been modified to negate the endogenous negative feedback loop that normally inhibits arginine biosynthesis in the presence of high levels of arginine and to bypass the arginine repressor in the arginine operon, which regulates the expression of genes involved in arginine synthesis. Geiger showed that the engineered probiotic upregulates enzymes throughout the arginine biosynthesis pathway and produces high levels of arginine in culture compared with wild-type strains.

In mice, colon tumors injected with the engineered probiotic had high levels of arginine and increased numbers of TILs. The arginine-producing bacteria also showed synergistic activity with PD-1 blocking agents in terms of reducing tumor growth and improving survival, and mice were refractory to rechallenge, suggesting that the bacteria induce T cell memory.

Geiger's work shows that engineered bacteria can alter the TME in favor of an effective antitumor immune response.

Co-opting bacterial secretion systems to deliver therapeutic proteins

Several speakers presented work on incorporating the Type III secretion system (T3SS) into EcN. T3SS is a natural bacterial delivery system found in the inner and outer membranes of Gram-negative bacteria that acts as a nanosyringe to inject proteins into mammalian cells.

Luis Ángel Fernández from the National Center for Biotechnology–CSIC presented work on engineering *E. coli* to selectively inject proteins into tumor cells. Fernández's group is focused on treating epithelial tumors. As bacteria naturally associate with epithelial mucosal surfaces, they can be locally administered without the need for systemic exposure, and tumors dysregulate and mislocalize cell

surface proteins that can act as tumor markers. To engineer bacteria to target and combat epithelial tumors, Fernández's group is incorporating several modular functionalities into bacteria, including the ability to colonize the tumor niche, to adhere to tumor cells, and to deliver therapeutic molecules in a controlled manner.

To engineer bacteria to selectively bind to cancer cells, Fernández's group created synthetic adhesins targeted to bind the extracellular domain of proteins expressed on the surface of tumor cells, such as EGFR.^{45,46} They also created a synthetic injector system *E. coli* strain (SIEC) by transferring genes encoding the T3SS of enteropathogenic *E. coli* (EPEC) into a nonpathogenic *E. coli* K-12 strain under an IPTG-inducible promoter.⁴⁷ Fernández showed unpublished data indicating SIEC assemble functional T3SS that can transport a number of heterologous proteins to tumor cells, including single domain antibodies.^{48,49} Fernández also presented unpublished results on how to combine adhesion and T3SS into *E. coli* to selectively translocate cytotoxic proteins into colon cancer cells *in vitro* and *in vivo*.

Cammie F. Lesser from Massachusetts General Hospital has reengineered the T3SS (type III secretion system) of pathogenic bacteria to deliver therapeutic payloads into the intestinal lumen, with the aim of blocking inflammation or infection. The group first captured the operons that encode the structural components of the *Shigella* T3SS onto a plasmid via recombineering. Lesser showed that the introduction of this plasmid into *E. coli* results in a strain with a functional T3SS.^{50,51} By modifying the secretion system, they have developed variants of *E. coli* that secrete proteins into their surroundings (Fig. 5). In collaboration with Chuck Shoemaker's group at Tufts University, Lesser's group is designing camelid VHH to be secreted as therapeutic payloads. VHHs are small, stable heavy chain-only antibodies that can target both extracellular and intracellular antigens.⁵² Shoemaker's group has shown that systemic administration of VHH that targets pathogenic antigens can protect against infection in preclinical models.^{53–55} Lesser showed that modified VHHs can be recognized and secreted by the T3SS and maintain their activity in the extracellular milieu. **Jason P. Lynch**, from Lesser's lab, gave a separate presentation in

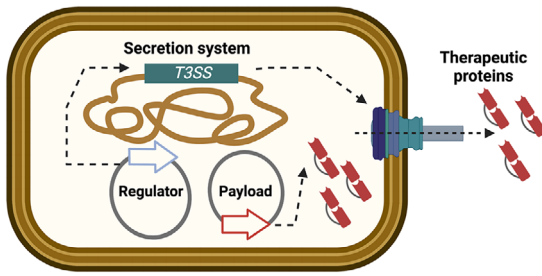


Figure 5. Schematic of *E. coli* equipped with a modified T3SS, its regulator, and a therapeutic payload engineered to be recognized as a secreted substrate.

which he described unpublished work on applying VHH-secreting variants of the modified *E. coli* strain in preclinical settings of inflammation.

Modulating intestinal metabolites for therapeutic applications

Matthew Wook Chang from the National University of Singapore presented work on engineering EcN to treat diseases. Earlier work by Chang showed that genetic sensors and circuits can be introduced into *E. coli* that allow them to detect Quorum-sensing molecules released by the pathogen *P. aeruginosa* and that produce and release the antimicrobial peptide pyocin in mice. Treatment with the engineered probiotic cleared 80% of colonized pathogens, while pretreatment attenuated gut colonization by >98% in mice.⁵⁶

Chang showed that EcN can be engineered to convert intestinal metabolites into drugs for cancer treatment. In this system, three new cellular functions were engineered into EcN: the ability to selectively bind to colon cancer cells, the ability to convert the intestinal metabolite glucosinolate into the anticancer compound sulforaphane, and the ability to secrete that sulforaphane. To promote selective binding to cancer cells, EcN was engineered to express HlpA, which binds to heparan sulfate proteoglycan (HSPG), which is highly produced during carcinogenesis. To convert glucosinolate to sulforaphane, EcN was engineered to express the enzyme myrosinase. In a model of colon cancer, mice fed the engineered microbes exhibited tumor regression and reduced tumor recurrence. Immunohistochemical staining showed that probiotics localized on HSPG regions of colon cancer.⁵⁷ Chang also presented unpublished work on engineering probiotics to modulate intestinal metabolism and limit the pathogenesis of *C. difficile*.

Taken together, Chang's work shows the potential of microbes to modulate the intestinal environment and host–microbiome interactions to treat diseases.

David L. Hava from Synlogic discussed the company's efforts to engineer bacteria to target metabolic diseases. Synlogic has developed a library of reusable genetic parts for engineering EcN, including molecular pumps to transport molecules across the cell membrane, genetic switches to control expression of effectors, and auxo-tro-phies to prevent growth with the host organism. These parts can be mixed and matched to rapidly and iteratively develop rationally designed strains. Hava stressed that these bacterial therapeutics are meant to be transient and dosed regularly, similar to small molecules or biologics.

A major focus of Synlogic's pipeline is to engineer EcN to consume toxic metabolites in the gut. Toward this goal, Synlogic has engineered EcN strains that consume phenylalanine to reduce its toxic effects in people with phenylketonuria (PKU)⁹ and have shown that this strain is active in healthy volunteers and patients with PKU.⁷ Hava described in more detail the company's efforts to develop a probiotic that consumes oxalate in people with enteric hyperoxaluria. Enteric hyperoxaluria occurs from hyperabsorption of oxalate, a compound found in foods like spinach, chocolate, and nuts, in the GI tract. It is generally triggered by an underlying insult to the bowel, such as IBD or bariatric surgery. Patients develop chronic, recurrent kidney stones that can lead to chronic kidney disease and kidney failure. Other therapies in development, including oral enzymes and the bacteria *Oxalobacter formigenes*, are only active in the upper GI or colon, respectively. Synlogic's goal is to develop a probiotic therapy that consumes and eliminates oxalate across the entire GI tract. They introduced the oxalate consumption pathway from *Oxalobacter* into EcN. The pathway consists of a transporter to bring oxalate into the cell in exchange for formate, and two enzymes that convert oxalate to formate through oxalyl-CoA and formyl-CoA. Because EcN does not contain a mechanism to generate oxalyl-CoA, a yeast enzyme that converts oxalyl-CoA from oxalate was also added to kick-start the oxalate-consuming pathway. Through the optimization of this pathway and additional genetic engineering, a clinical candidate strain, called SYN8802, was generated.

In an *in vitro* gut simulation assay that simulates the conditions of the stomach, small intestine, and colon, the engineered bacteria, SYN8802, was active and consumed oxalate in each compartment. In preclinical models, orally administered SYN8802 lowered urinary oxalate levels in mice fed labeled oxalate as well as in nonhuman primates fed a high oxalate diet, indicating that the strain is active *in vivo*. To support continued development, Synlogic also built mathematical models of strain activity and performed *in silico* simulations to translate SYN8802 *in vitro* activity to predicted clinical activity. These simulations predicted clinically meaningful lowering of urinary oxalate in humans.⁵⁸ On the basis of preclinical data, SYN8802 was advanced into clinical development. In a phase 1 clinical study of healthy volunteers fed a high oxalate diet, orally administered SYN8802 was safe and well tolerated, and dose dependently decreased urinary oxalate levels. The phase 1 study is continuing in patients with enteric hyperoxaluria.

Francisco J. Quintana from Harvard Medical School presented their work on engineering yeast to recognize and modulate inflammation in the gut. Quintana's group has engineered yeast to sense extracellular ATP (eATP), a marker of inflammation in IBD. While ATP is often considered as the main source of energy for the cell, it also plays a role in inflammation. In IBD, both immune cells and gut microbiota release ATP into the extracellular space. Indeed, eATP has been shown to promote IBD pathology and is increased at sites of gut inflammation in IBD as a result of genetic polymorphisms, immune activation, and dysbiotic commensal flora.^{59–63} eATP can also drive inflammation by activating innate immune cells and boosting T cell effector differentiation.^{64–69}

Quintana's group reasoned that eATP can serve as a biomarker of inflammation and that removing eATP may be a therapeutic strategy to relieve inflammation. The role of the microbiome in IBD pathogenesis supports the development of probiotic-based therapies; however, probiotics have shown limited therapeutic effects. Synthetic biology offers the potential to engineer probiotics optimized for therapeutic immune modulation via a well-defined mechanism of action.

Quintana's group co-opted the yeast mating pathway to create an eATP-responsive anti-

inflammatory yeast probiotic. To sense eATP, they incorporated the human P2Y2 eATP receptor, using directed evolution to increase its sensitivity. Quintana and coworkers showed that local eATP levels activate P2Y2-driven responses in engineered yeast probiotics in a mouse model of colitis. Expression of a fluorescent reporter gene was activated in regions of the gut with high eATP levels and not in regions without eATP, demonstrating that the system is sensitive to detect eATP levels that accumulate at sites of inflammation in the gut.⁷⁰

To reduce inflammation, Quintana's group designed the yeast to express apyrase upon eATP sensing. Apyrase is an enzyme that converts eATP to adenosine, which is anti-inflammatory. Quintana showed that eATP induces dose-dependent apyrase activity in engineered yeast.⁷⁰

The engineered yeast probiotics were evaluated in three preclinical models of IBD-TNBS-induced colitis, which is characterized by T cell-driven inflammation of the large intestine; anti-CD3-induced enteritis, which is characterized by T cell-driven inflammation of the small intestine; and dextran sulfate sodium-induced colitis, which is driven by innate immunity. In all three models, the yeast probiotics significantly improved markers of inflammation and disease pathology. These effects were comparable to, or even better than, those seen with therapies used to treat IBD. Importantly, these improvements were not seen with a yeast strain that constitutively expresses apyrase, indicating that spatial control over eATP degradation is important for mediating anti-inflammatory effects.⁷⁰

Quintana's group has expanded upon this yeast platform to develop a modular platform with a number of sensors and therapeutic effectors to expand and combine various sensing and therapeutic mechanisms and apply this approach to other inflammatory diseases targeting the GI tract and other tissues.

Engineering a yeast-based biosensor

Virginia W. Cornish from Columbia University presented their work on developing a yeast-based biosensor. Cornish's group has developed tools to engineer yeast to select for enzyme catalysis of chemistry not natural to the cell.⁷¹ They have used this directed evolution approach to select for

complex chemistry, such as glycosynthase enzymes,⁷² and cellulase enzymes for biomass conversion.⁷³ They also developed mutagenesis technologies that exploit the high efficiency of homologous recombination to make libraries via transformation of oligonucleotides.⁷⁴ Cornish showed that they can use this technology to build up large pathways⁷⁵ and to cross libraries via sexual reproduction.⁷⁶

Cornish's lab has leveraged mutagenesis and selection technologies to engineer yeast to act as a biosensor using the production of lycopene, a red compound often used as a biomarker for genetic assays as a readout. For the sensor, the system uses peptide G protein-coupled receptors (GPCRs), which detect mating peptides during yeast mating. They used their directed evolution approach to generate GPCRs that detect a target ligand, such as a pathogen peptide. Cornish showed that GPCRs can be developed to recognize a range of purified pathogen peptides as well as detect the presence of different pathogens in culture.⁷⁷

Cornish's group has built a prototype for an at-home test in which the yeast biosensor is functional in a lateral flow format, similar to an at-home pregnancy test. They hope that this yeast-based biosensor could offer an affordable, scalable test for point-of-care or in-home diagnostics.

A machine learning-based approach to improve synthetic designs

Michael Fero from TeselaGen Biotechnology presented the company's machine learning-enabled platform for engineering biological systems. Fero noted that designing, building, testing, and scaling up production of large molecules or modified organisms require a cross-disciplinary approach that incorporates scientists, technicians, automation, and data science. TeselaGen has rebuilt the product development and formulation framework as an artificial intelligence platform that incorporates automation and machine learning. The platform has the potential to bring the time from designing experiments to target discovery from several years to a few months.

The platform consists of four parts: design, build, test, and discover. In the design phase, the platform designs experiments that expand the space of possible solutions to data-driven problems. The build phase orchestrates experimental workflows,

the test phase directly imports data from analytical instruments, and the discover phase implements machine learning models that feedback into design. TeselaGen has also developed robust integration modes to integrate with existing bioinformatics systems so that they can collaborate with various companies and academic groups.

Fero described an example in which TeselaGen's platform was used to optimize tryptophan production in yeast.⁷⁸ Taking into account all the potential steps involved in tryptophan synthesis leads to a very high-dimensional design space. Using prior knowledge and mechanistic modeling can shrink that space into something more tractable. In this case, they chose to focus on five genes in the glycolysis and pentose phosphate pathways. The researchers then built a combinatorial library of approximately 7000 combinations of genes and promoters. They used TeselaGen's platform as well as a second algorithm to build, train, model, and recommend changes. The predicted constructs led to increased tryptophan synthesis. Fero stressed that the concept is generalizable to many biological problems. At a high level, the platform can be thought of as an iterative process consisting of spanning a genotypic space, measuring an associated phenotype, building a model based on the data, and providing recommendations about perturbations that lead to the desired phenotype.

Engineering reversible logic gates

Rajkamal Srivastava from Sangram Bagh's lab at the Saha Institute of Nuclear Physics presented unpublished work on engineering reversible logic in *E. coli* using synthetic gene circuits. Logical reversibility has never been implemented in living systems. Instead, irreversible logic systems are implemented. In irreversible logic, two different inputs can lead to the same output, and there is no additional information to trace a given output back to its original input. However, in reversible logic, outputs can be traced back to a specific input, thus preserving the information on the input that led to a given output. Srivastava described two types of reversible logic gates with *E. coli*, a Feynman gate (2 input–2 output gate) and a Fredkin gate (3 input–3 output gate) by using an artificial neural network-type architecture made from a mixed population of engineered bacteria, where bacteria work as artificial neurons and perform complex chemical

computations based on environmental inputs.⁷⁹ The framework takes a minimalistic approach that does not require an electronic circuit diagram and does not follow a traditional hierarchical logic circuit approach. Besides this multicell approach, in order to expand the capability of reversible logic circuits in living cells, Srivastava also described the design and implementation of a reversible Feynman gate in a single *E. coli* cell and showed its integration with a mammalian cell for intercellular information transfer.⁸⁰

Engineering an arrhythmia-gated ion channel

Tim De Coster from Daniël A. Pijnappel's lab of Experimental Cardiology at the Leiden University Medical Center presented their work on developing antiarrhythmic ion channels to detect and ameliorate cardiac arrhythmias. Currently, cardiac arrhythmias are treated with an implantable cardioverter defibrillator that delivers a high-voltage shock to the heart when it detects an irregular rhythm. This shock resets all the cells in the heart and restores rhythmicity. Given that the heart itself is a source of electricity, De Coster is interested in intrinsic strategies that can reset cardiac rhythmicity. To achieve this, the group has taken advantage of the fact that most ion channels in the heart are voltage gated, that is, they open and close to allow the flow of ions across the cell membrane in response to the transmembrane voltage.⁸¹

De Coster and colleagues have engineered a frequency-gated ion channel that is activated by an arrhythmia, thus allowing cardiac tissue to discriminate between normal rhythm and an arrhythmia. Altering the opening and closing transition rates leads to an accumulation of open channels. Upon activation, opening of the channel prolongs the action potential and restores a normal cardiac rhythm. Introducing a nonlinearity into the closing rate allows the channel to close rapidly once the rhythm has been reset.⁸¹

In virtual cardiac monolayers and 3D whole atria simulations, introducing the ion channel generates an ionic current to terminate a detected arrhythmia and restore normal excitation rhythm. The ion channel has also been validated in human cardiac cells. Injecting a current obeying the gating properties of the frequency-gated channel as a surrogate for a real ion channel into human atrial

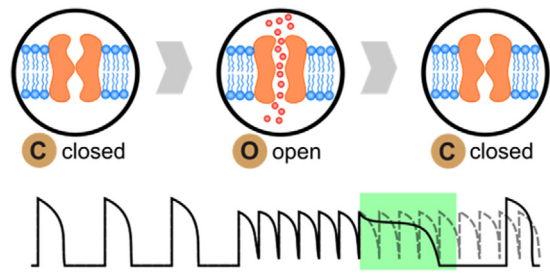


Figure 6. A frequency-gated ion channel was designed that allows discrimination between normal rhythm and arrhythmia. Upon activation, opening of the channel prolongs the action potential to restore normal cardiac rhythm.

cells was able to terminate fast-pacing rhythms. De Coster's work shows that heart rhythm can be regulated through expression of customized ion channels, and this can be leveraged to self-reset a dysregulated heart rhythm (Fig. 6).⁸¹

Expanding the capabilities of synthetic biology

Broadening engineerable cell types

Owen J.L. Rackham from Duke-NUS described their work on developing data-driven approaches to broaden the scope of cell therapies. Currently, many cell therapies make use of the cytotoxic functions of NK or T cells. Several speakers throughout the symposium discussed work on engineering these cells with CARs and logic gates to process logic on the basis of the environment so that they can initiate therapeutic functions. Rackham, however, is interested in broadening the cell types that can be engineered.

Achieving this requires the ability to both make the cell type of interest and to culture that cell type *in vitro*. Rackham's group has developed an algorithm, Mogrify, that predicts the TFs required to convert from one cell type to another. Mogrify was developed using gene expression data for approximately 300 cell types. Mogrify takes input sequence data from two or more cell states and will predict the TFs that can drive a conversion between the two states. In brief, Mogrify calculates a differential expression score for every gene to ascertain how specific that gene is for a given cell type. Next, Mogrify estimates the sphere of influence for each TF to identify TFs that will have a large effect on the intended genes upon overexpression. Finally, Mogrify identifies a set of TFs with

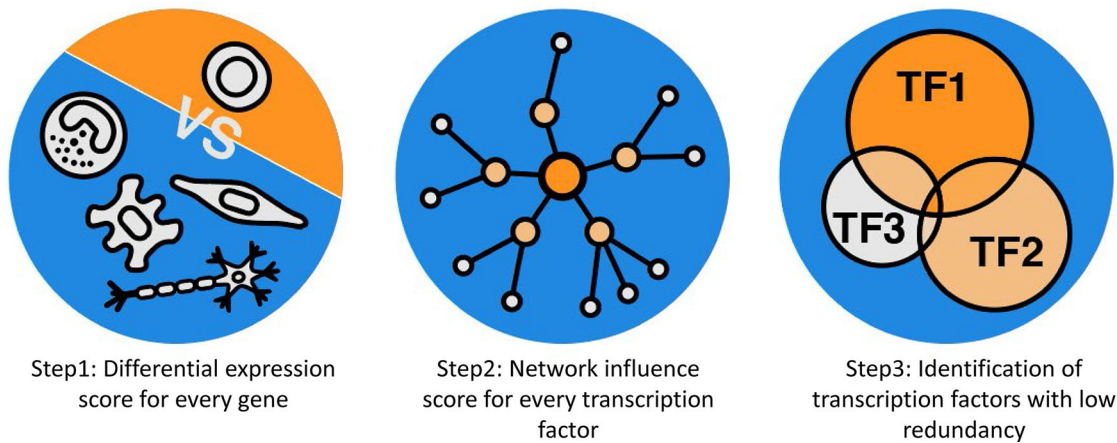


Figure 7. Steps by which the algorithm Mogrify predicts a set of transcription factors required to convert a cell from one type to another.

low redundancy (Fig. 7). The algorithm outputs a set of influential, nonredundant TFs that control expression of genes important for cell identity. Rackham showed that Mogrify successfully predicted TFs for several known cell-state conversions as well as predicted TFs for previously unpublished conversions, such as keratinocytes to microvascular endothelial cells and dermal fibroblasts to keratinocytes.⁸²

Rackham's group has developed a second algorithm, EpiMogrify, that predicts the cell culture conditions needed to culture any cell type. Given the number of cell types in humans, the number of cell types that can be cultured *in vitro* is small, and the number that can be cultured in GMP-compliant conditions necessary for manufacturing cell therapies is even smaller. Rackham showed that genes important for cell identity can be determined by looking at the breadth of H3K4me3 peaks in ChIPSeq data. Very broad H3K4me3 peaks are typically observed near promoters of genes important for cell identity. EpiMogrify uses H3K4me3 ChIPSeq data to identify receptor–ligand pairs important for cell identity. Including these ligands in the cell culture medium should, therefore, promote that identity *in vitro*. Rackham showed that EpiMogrify can predict culture conditions that promote maintenance and differentiation of several cell types *in vitro*.⁸³

Rackham hopes that these two tools will broaden the types of cells that can be engineered to create new cell therapies in new areas, a vision shared by

Mogrify Ltd, a biotech company based in Cambridge, UK, that is utilizing these technologies and others for the development of cell therapies, as well as for the pioneering of a novel class of *in vivo* reprogramming therapies.

Identifying new inducible promoters

Tingxi Guo from Timothy K. Lu's lab at MIT presented their work on expanding the types of responses that can be achieved with T cell therapies. CAR T cell therapies have enabled researchers to elicit T cell responses to non-MHC antigens, thus expanding the types of inputs that can elicit T cell cytotoxicity. Guo argued that to fully realize the potential of T cell therapies, advances in broadening the types of responses that can be achieved are needed as well.

Toward that goal, Guo is developing CAR T cells that can deliver an antigen-inducible payload, such as a proinflammatory cytokine, to improve anti-tumor efficacy. During their talk, Guo focused on identifying appropriate promoters to control gene expression of the payload. An antigen-inducible promoter derived from nuclear factor of activated T cells (*NFAT*) has been previously developed for research uses and repurposed to achieve targeted payload expression in CAR T cells.⁸⁴

Guo has identified alternatives to the *NFAT* promoter that may be more optimal for antigen-induced expression. They tested these alternatives at a panel of promoter constructs in which response elements are targeted by TFs downstream of TCR

signaling or upregulated by activation of TCR signaling. In primary human T cells, promoters based on *NR4A* and *API* showed inducible expression of a reporter gene with low background expression. The promoters were nontoxic and reversibly activatable. Guo showed that a novel *NR4A* promoter induced higher responses with suboptimal CAR stimulation than the commonly used *NFAT* promoter, and the former could deliver cytokines to improve suboptimal CAR response.⁸⁵

Facilitating programmable gene expression

William C.W. Chen from MIT presented work on developing a synthetic transcription system that enables programmable transgene expression. Chen's work is part of an alliance between MIT and Pfizer to use synthetic biology for therapeutic applications. Key goals for the alliance include developing toolkits for industry, increasing product yield, increasing cell stability, and developing next-generation expression engineering strategies.

Chen's presentation focused on achieving predictable protein expression. Available mammalian promoters often exhibit different behaviors in different cell types or conditions and can be hard to predict and control. Chen has developed a CRISPR-based synthetic transcription system that offers a continuous spectrum of expression levels. CRISPR-based transcriptional activators (CRISPRa) have previously been used to upregulate endogenous gene expression.⁸⁶ Chen has used CRISPRa systems to create a modular synthetic transcription system for programmable protein expression, called PRECISE. They showed that PRECISE enables predictable control of gene expression in multiple cell types.⁸⁷

Improving gene therapies with CRISPR

Samira Kiani from the University of Pittsburgh School of Medicine discussed using CRISPR-based gene circuits to modulate the host immune response and improve the efficacy and safety of gene therapies. One major challenge for gene therapies is immunogenicity of the viral vector, often adeno-associated virus (AAV), used to deliver the transgene. AAV can initiate an antiviral adaptive and innate immune response against the target organ cells that express the transgene. This limits the efficacy of gene therapies and prevents readministration to patients with preexisting immunity.

While several approaches are being investigated to modulate the immune response, many do not target multiple pathways of innate and adaptive immunity, and they lack spatiotemporal control.

Kiani's group is investigating CRISPR-based gene circuits to modulate the immune response to AAV-based therapies. CRISPR has been used as a transcriptional reprogramming tool for other applications. In general, a TF is fused to a catalytically dead Cas9, which directs the TF to the gene of interest to transiently modulate gene expression. In Kiani's approach, a catalytically competent Cas9, disabled guide RNA, and MS2 repressor domains are used to simultaneously accomplish gene and epigenetic editing. Kiani showed that this system can be used to selectively repress several genes in the human genome.⁸⁸ To target immune response, they used this CRISPR-based system to repress *Myd88*. *Myd88* is activated downstream of most TLRs and is a key node in both innate and adaptive immunity.⁸⁹ Kiani believes that *Myd88* may be a universal gene to target for control of an antiviral immune response.

In mice, AAV delivery increases the expression of *Myd88* in various tissues. Kiani showed that their CRISPR-based treatment reduced AAV-mediated *Myd88* expression in blood, lung, and bone marrow. Both innate and adaptive immunity were affected. The CRISPR-based treatment downregulated the expression of proinflammatory genes downstream of *Myd88*, such as *Icam1* and *Tnf*, as well as IgG-related genes. *Myd88* suppression enhanced cargo transgene expression, suggesting that suppressing the antiviral immune response may improve the efficacy of gene therapies. While no toxicity was observed with *Myd88* suppression, it remains unknown whether it may affect immunity in response to infection.⁸⁸

Kiani's work shows that CRISPR can be used as a transcriptional repressor to reprogram immune homeostasis *in vivo* and can achieve efficient downregulation of *Myd88* expression. This leads to a decreased immune reaction to AAV, which may improve the efficacy of AAV treatment and allow for redosing. Their group is also working to embed synthetic biology-based safety switches to achieve spatiotemporal control over immune modulation.

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Competing interests

R.G. received research funding from Synlogic Inc.

S.K. is a founder and CEO of GenexGen Inc.

L.A.F. is an inventor on patent and patent application related to the use of T3SS for antibody protein translocation into mammalian cells (US 8,623349 B2, EP20383142).

References

- Charbonneau, M., V. Isabella, N. Li, *et al.* 2020. Developing a new class of engineered live bacterial therapeutics to treat human diseases. *Nat. Commun.* **11**: 1738.
- Beijing Syngentech Co., Ltd. 2020. *An open-label phase I/IIa study to evaluate the safety, tolerability and efficacy of SynOV1.1 recombinant oncolytic adenovirus injection as monotherapy or in combination with atezolizumab in participants with locally advanced or metastatic hepatocellular carcinoma*. clinicaltrials.gov. Accessed Sep. 15, 2021.
- Synlogic. 2021. *A phase 1, open-label, multicenter study of SYNBI891 administered by intratumoral injection to patients with advanced/metastatic solid tumors and lymphoma alone and in combination with atezolizumab*. clinicaltrials.gov. Accessed Sep. 15, 2021.
- Leventhal, D., A. Sokolovska, N. Li, *et al.* 2020. Immunotherapy with engineered bacteria by targeting the STING pathway for anti-tumor immunity. *Nat. Commun.* **11**: 2739.
- Synlogic. 2021. *A double-blind, randomized, placebo-controlled study to assess the safety, tolerability, and pharmacodynamics of SYNBI8802 in healthy volunteers and in patients with enteric hyperoxaluria*. clinicaltrials.gov. Accessed Sep. 15, 2021.
- Synlogic. 2021. *An open-label study of the efficacy and safety of SYNBI1618 in patients with phenylketonuria (SynPheny-1)*. clinicaltrials.gov. Accessed Sep. 15, 2021.
- Puurunen, M., J. Vockley, S. Searle, *et al.* 2021. Safety and pharmacodynamics of an engineered *E. coli* Nissle for the treatment of phenylketonuria: a first-in-human phase 1/2a study. *Nat. Metab.* **3**: 1125–1132.
- Kurtz, C., Y. Millet, M. Puurunen, *et al.* 2019. An engineered *E. coli* Nissle improves hyperammonemia and survival in mice and shows dose-dependent exposure in healthy humans. *Sci. Transl. Med.* **11**: eaau7975.
- Isabella, V., B. Ha, M. Castillo, *et al.* 2018. Development of a synthetic live bacterial therapeutic for the human metabolic disease phenylketonuria. *Nat. Biotechnol.* **36**: 857–864.
- Daringer, N., R. Dudek, K. Schwarz, *et al.* 2014. Modular extracellular sensor architecture for engineering mammalian cell-based devices. *ACS Synth. Biol.* **3**: 892–902.
- Schwarz, K., N. Daringer, T. Dolberg, *et al.* 2017. Rewiring human cellular input–output using modular extracellular sensors. *Nat. Chem. Biol.* **13**: 202–209.
- Dolberg, T., A. Meger, J. Boucher, *et al.* 2021. Computation-guided optimization of split protein systems. *Nat. Chem. Biol.* **17**: 531–539.
- Donahue, P., J. Draut, J. Muldoon, *et al.* 2020. The COMET toolkit for composing customizable genetic programs in mammalian cells. *Nat. Commun.* **11**: 779.
- Muldoon, J., V. Kandula, M. Hong, *et al.* 2021. Model-guided design of mammalian genetic programs. *Sci. Adv.* **7**: eabe9375.
- Dana, H., G. Chalbatani, S. Jalali, *et al.* 2021. CAR-T cells: early successes in blood cancer and challenges in solid tumors. *Acta Pharm. Sin. B* **11**: 1129–1147.
- Garrison, B., H. Deng, G. Yucel, *et al.* Precise targeting of AML with OR/NOT logic-gated gene circuits in CAR-NK cells. ASGCT – Abstract #77. May 12, 2021.
- Hamburger, A., B. DiAndreth, J. Cui, *et al.* 2020. Engineered T cells directed at tumors with defined allelic loss. *Mol. Immunol.* **128**: 298–310.
- Roybal, K., J. Williams, L. Morsut, *et al.* 2016. Engineering T cells with customized therapeutic response programs using synthetic Notch receptors. *Cell* **167**: 419–432.e16.

19. Roybal, K., L. Rupp, L. Morsut, *et al.* 2016. Precision tumor recognition by T cells with combinatorial antigen-sensing circuits. *Cell* **164**: 770–779.
20. Hyrenius-Wittsten, A., Y. Su, M. Park, *et al.* 2021. SynNotch CAR circuits enhance solid tumor recognition and promote persistent antitumor activity in mouse models. *Sci. Transl. Med.* **13**: eabd8836.
21. Zhu, I., R. Liu, A. Hyrenius-Wittsten, *et al.* 2021. Design and modular assembly of synthetic intramembrane proteolysis receptors for custom gene regulation in therapeutic cells. *bioRxiv*. <https://doi.org/10.1101/2021.05.21.445218>
22. Lamers, C., S. Sleijfer, S. van Steenbergen, *et al.* 2013. Treatment of metastatic renal cell carcinoma with CAIX CAR-engineered T cells: clinical evaluation and management of on-target toxicity. *Mol. Ther. J. Am. Soc. Gene Ther.* **21**: 904–912.
23. Morgan, R., J. Yang, M. Kitano, *et al.* 2010. Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. *Mol. Ther. J. Am. Soc. Gene Ther.* **18**: 843–851.
24. Thistlethwaite, F., D. Gilham, R. Guest, *et al.* 2017. The clinical efficacy of first-generation carcinoembryonic antigen (CEACAM5)-specific CAR T cells is limited by poor persistence and transient pre-conditioning-dependent respiratory toxicity. *Cancer Immunol. Immunother.* **66**: 1425–1436.
25. Morsut, L., K. Roybal, X. Xiong, *et al.* 2016. Engineering customized cell sensing and response behaviors using synthetic Notch receptors. *Cell* **164**: 780–791.
26. Hernandez-Lopez, R., W. Yu, K. Cabral, *et al.* 2021. T cell circuits that sense antigen density with an ultrasensitive threshold. *Science* **371**: 1166–1171.
27. Chaurasiya, S., Y. Fong & S. Warner. 2021. Oncolytic virotherapy for cancer: clinical experience. *Biomedicines* **9**: 419.
28. Ribas, A., R. Dummer, I. Puzanov, *et al.* 2017. Oncolytic virotherapy promotes intratumoral T cell infiltration and improves anti-PD-1 immunotherapy. *Cell* **170**: 1109–1119.e10.
29. Rigvir. RIGVIR Group. Accessed October 1, 2021. <https://www.rigvir.com/products/rigvir.php>.
30. Bommareddy, P., A. Patel, S. Hossain, *et al.* 2017. Talimogene laherparepvec (T-VEC) and other oncolytic viruses for the treatment of melanoma. *Am. J. Clin. Dermatol.* **18**: 1–15.
31. Bommareddy, P., A. Zloza, S. Rabkin, *et al.* 2019. Oncolytic virus immunotherapy induces immunogenic cell death and overcomes STING deficiency in melanoma. *Oncoimmunology* **8**: 1591875.
32. Liu, B., M. Robinson, Z.-Q. Han, *et al.* 2003. ICP34.5 deleted herpes simplex virus with enhanced oncolytic, immune stimulating, and anti-tumour properties. *Gene Ther.* **10**: 292–303.
33. Kaufman, H., D. Kim, G. DeRaffele, *et al.* 2010. Local and distant immunity induced by intralesional vaccination with an oncolytic herpes virus encoding GM-CSF in patients with stage IIIc and IV melanoma. *Ann. Surg. Oncol.* **17**: 718–730.
34. Andtbacka, R., F. Collichio, K. Harrington, *et al.* 2019. Final analyses of OPTiM: a randomized phase III trial of talimogene laherparepvec versus granulocyte-macrophage colony-stimulating factor in unresectable stage III–IV melanoma. *J. Immunother. Cancer* **7**: 145.
35. Andtbacka, R., H. Kaufman, F. Collichio, *et al.* 2015. Talimogene laherparepvec improves durable response rate in patients with advanced melanoma. *J. Clin. Oncol.* **33**: 2780–2788.
36. Bommareddy, P., S. Aspromonte, A. Zloza, *et al.* 2018. MEK inhibition enhances oncolytic virus immunotherapy through increased tumor cell killing and T cell activation. *Sci. Transl. Med.* **10**: eaa0417.
37. Chesney, J., I. Puzanov, F. Collichio, *et al.* 2018. Randomized, open-label phase II study evaluating the efficacy and safety of talimogene laherparepvec in combination with ipilimumab versus ipilimumab alone in patients with advanced, unresectable melanoma. *J. Clin. Oncol.* **36**: 1658–1667.
38. Nguyen, T.-T., L. Ramsay, M. Ahanfeshar-Adams, *et al.* 2021. Mutations in the IFN γ -JAK-STAT pathway causing resistance to immune checkpoint inhibitors in melanoma increase sensitivity to oncolytic virus treatment. *Clin. Cancer Res.* **27**: 3432–3442.
39. Kaufman, H. 2021. Can biomarkers guide oncolytic virus immunotherapy? *Clin. Cancer Res.* **27**: 3278–3279.
40. Ramelyte, E., A. Tastanova, Z. Balázs, *et al.* 2021. Oncolytic virotherapy-mediated anti-tumor response: a single-cell perspective. *Cancer Cell* **39**: 394–406.e4.
41. Huang, H., Y. Liu, W. Liao, *et al.* 2019. Oncolytic adenovirus programmed by synthetic gene circuit for cancer immunotherapy. *Nat. Commun.* **10**: 4801.
42. Wassenaar, T. 2016. Insights from 100 years of research with probiotic *E. coli*. *Eur. J. Microbiol. Immunol.* **6**: 147–161.
43. Geiger, R., J. Rieckmann, T. Wolf, *et al.* 2016. L-arginine modulates T cell metabolism and enhances survival and anti-tumor activity. *Cell* **167**: 829–842.e13.
44. Bronte, V. & P. Zanovello. 2005. Regulation of immune responses by L-arginine metabolism. *Nat. Rev. Immunol.* **5**: 641–654.
45. Piñero-Lambea, C., G. Bodelón, R. Fernández-Periáñez, *et al.* 2015. Programming controlled adhesion of *E. coli* to target surfaces, cells, and tumors with synthetic adhesins. *ACS Synth. Biol.* **4**: 463–473.
46. Salema, V., C. Mañas, L. Cerdán, *et al.* 2016. High affinity nanobodies against human epidermal growth factor receptor selected on cells by *E. coli* display. *mAbs* **8**: 1286–1301.
47. Ruano-Gallego, D., B. Álvarez & L. Fernández. 2015. Engineering the controlled assembly of filamentous injectisomes in *E. coli* K-12 for protein translocation into mammalian cells. *ACS Synth. Biol.* **4**: 1030–1041.
48. Salema, V. & L. Fernández. 2017. *Escherichia coli* surface display for the selection of nanobodies. *Microb. Biotechnol.* **10**: 1468–1484.
49. Blanco-Toribio, A., S. Muyldermans, G. Frankel, *et al.* 2010. Direct injection of functional single-domain antibodies from *E. coli* into human cells. *PLoS One* **5**: e15227.
50. Reeves, A., W. Spears, J. Du, *et al.* 2015. Engineering *Escherichia coli* into a protein delivery system for mammalian cells. *ACS Synth. Biol.* **4**: 644–654.
51. Mou, X., S. Souter, J. Du, *et al.* 2018. Synthetic bottom-up approach reveals the complex interplay of *Shigella* effectors

- in regulation of epithelial cell death. *Proc. Natl. Acad. Sci. USA* **115**: 6452–6457.
52. Bannas, P., J. Hambach & F. Koch-Nolte. 2017. Nanobodies and nanobody-based human heavy chain antibodies as anti-tumor therapeutics. *Front. Immunol.* **8**: 1603.
 53. Schmidt, D., G. Beamer, J. Tremblay, *et al.* 2016. A tetraspecific VHH-based neutralizing antibody modifies disease outcome in three animal models of *Clostridium difficile* infection. *Clin. Vaccine Immunol.* **23**: 774–784.
 54. Moayeri, M., J. Tremblay, M. Debatis, *et al.* 2016. Adenoviral expression of a bispecific VHH-based neutralizing agent that targets protective antigen provides prophylactic protection from anthrax in mice. *Clin. Vaccine Immunol.* **23**: 213–218.
 55. Sheoran, A., I. Dmitriev, E. Kashentseva, *et al.* 2015. Adenovirus vector expressing Stx1/Stx2-neutralizing agent protects piglets infected with *Escherichia coli* O157:H7 against fatal systemic intoxication. *Infect. Immun.* **83**: 286–291.
 56. Hwang, C.-Y., Y.-J. Chen, M.-W. Lin, *et al.* 2012. Cancer risk in patients with allergic rhinitis, asthma and atopic dermatitis: a nationwide cohort study in Taiwan. *Int. J. Cancer* **130**: 1160–1167.
 57. Ho, C., H. Tan, K. Chua, *et al.* 2018. Engineered commensal microbes for diet-mediated colorectal-cancer chemoprevention. *Nat. Biomed. Eng.* **2**: 27–37.
 58. Renaud, L., D. Lubkowitz, S. Ghoshal, *et al.* 2020. Development of a synthetic biotic, SYN8802, for the treatment of enteric hyperoxaluria. <https://www.prnewswire.com/news-releases/synlogic-presents-synb8802-for-enteric-hyperoxaluria-at-the-american-institute-of-chemical-engineers-conference-on-microbiome-engineering-301187531.html>.
 59. Atarashi, K., J. Nishimura, T. Shima, *et al.* 2008. ATP drives lamina propria T(H)17 cell differentiation. *Nature* **455**: 808–812.
 60. Blander, J., R. Longman, I. Iliev, *et al.* 2017. Regulation of inflammation by microbiota interactions with the host. *Nat. Immunol.* **18**: 851–860.
 61. Proietti, M., L. Perruzza, D. Scribano, *et al.* 2019. ATP released by intestinal bacteria limits the generation of protective IgA against enteropathogens. *Nat. Commun.* **10**: 250.
 62. Huang, B., Z. Chen, L. Geng, *et al.* 2019. Mucosal profiling of pediatric-onset colitis and IBD reveals common pathogenesis and therapeutic pathways. *Cell* **179**: 1160–1176.e24.
 63. Mager, L., R. Burkhard, N. Pett, *et al.* 2020. Microbiome-derived inosine modulates response to checkpoint inhibitor immunotherapy. *Science* **369**: 1481–1489.
 64. Takenaka, M., S. Robson & F. Quintana. 2016. Regulation of the T cell response by CD39. *Trends Immunol.* **37**: 427–439.
 65. Mascanfroni, I., A. Yeste, S. Vieira, *et al.* 2013. IL-27 acts on DCs to suppress the T cell response and autoimmunity by inducing expression of the immunoregulatory molecule CD39. *Nat. Immunol.* **14**: 1054–1063.
 66. Mascanfroni, I., M. Takenaka, A. Yeste, *et al.* 2015. Metabolic control of type 1 regulatory T cell differentiation by AHR and HIF1- α . *Nat. Med.* **21**: 638–646.
 67. Rothhammer, V., I. Mascanfroni, L. Bunse, *et al.* 2016. Type I interferons and microbial metabolites of tryptophan modulate astrocyte activity and central nervous system inflammation via the aryl hydrocarbon receptor. *Nat. Med.* **22**: 586–597.
 68. Takenaka, M., G. Gabrieli, V. Rothhammer, *et al.* 2019. Control of tumor-associated macrophages and T cells in glioblastoma via AHR and CD39. *Nat. Neurosci.* **22**: 729–740.
 69. Badimon, A., H. Strasburger, P. Ayata, *et al.* 2020. Negative feedback control of neuronal activity by microglia. *Nature* **586**: 417–423.
 70. Scott, B., C. Gutiérrez-Vázquez, L. Sanmarco, *et al.* 2021. Self-tunable engineered yeast probiotics for the treatment of inflammatory bowel disease. *Nat. Med.* **27**: 1212–1222.
 71. Lin, H., W. Abida, R. Sauer, *et al.* 2000. Dexamethasone—methotrexate: an efficient chemical inducer of protein dimerization *in vivo*. *J. Am. Chem. Soc.* **122**: 4247–4248.
 72. Lin, H., H. Tao & V. Cornish. 2004. Directed evolution of a glycosynthase via chemical complementation. *J. Am. Chem. Soc.* **126**: 15051–15059.
 73. Peralta-Yahya, P., B. Carter, H. Lin, *et al.* 2008. High-throughput selection for cellulase catalysts using chemical complementation. *J. Am. Chem. Soc.* **130**: 17446–17452.
 74. Pirakitikulr, N., N. Ostrov, P. Peralta-Yahya, *et al.* 2010. PCR-less library mutagenesis via oligonucleotide recombination in yeast. *Protein Sci.* **19**: 2336–2346.
 75. Wingler, L. & V. Cornish. 2011. Reiterative recombination for the *in vivo* assembly of libraries of multigene pathways. *Proc. Natl. Acad. Sci. USA* **108**: 15135–15140.
 76. Romanini, D., P. Peralta-Yahya, V. Mondol, *et al.* 2012. A heritable recombination system for synthetic Darwinian evolution in yeast. *ACS Synth. Biol.* **1**: 602–609.
 77. Ostrov, N., M. Jimenez, S. Billerbeck, *et al.* 2017. A modular yeast biosensor for low-cost point-of-care pathogen detection. *Sci. Adv.* **3**: e1603221.
 78. Zhang, J., S. Petersen, T. Radivojevic, *et al.* 2020. Combining mechanistic and machine learning models for predictive engineering and optimization of tryptophan metabolism. *Nat. Commun.* **11**: 4880.
 79. Sarkar, K., D. Bonnerjee & S. Bagh. 2020. A single layer artificial neural network with engineered bacteria. *ArXiv200100792 Phys. Q-Bio*.
 80. Srivastava, R., K. Sarkar, D. Bonnerjee & S. Bagh. 2021. A synthetic genetic reversible Feynman gate in a single E. coli cell and its application in bacterial to mammalian cell information transfer. *bioRxiv* 2021.08.16.456494.
 81. Majumder, R., T. De Coster, N. Kudryashova, *et al.* 2020. Self-restoration of cardiac excitation rhythm by anti-arrhythmic ion channel gating. *eLife* **9**: e55921.
 82. Rackham, O., J. Firas, H. Fang, *et al.* 2016. A predictive computational framework for direct reprogramming between human cell types. *Nat. Genet.* **48**: 331–335.
 83. Kamaraj, U., J. Chen, K. Katwadi, *et al.* 2020. EpiMogrify models H3K4me3 data to identify signaling molecules that improve cell fate control and maintenance. *Cell Syst.* **11**: 509–522.e10.

84. Zimmermann, K., J. Kuehle, A. Dragon, *et al.* 2020. Design and characterization of an “all-in-one” lentiviral vector system combining constitutive anti-GD2 CAR expression and inducible cytokines. *Cancers* **12**: E375.
85. Guo, T., D. Ma & T. Lu. 2021. Sense-and-respond payload delivery using a novel antigen-inducible promoter improves suboptimal CAR-T activation. *bioRxiv*. <https://doi.org/10.1101/2021.04.02.438280>.
86. Chavez, A., M. Tuttle, B. Pruitt, *et al.* 2016. Comparison of Cas9 activators in multiple species. *Nat. Methods* **13**: 563–567.
87. Chen, W., L. Gaidukov, M-R. Wu, *et al.* 2020. A synthetic transcription platform for programmable gene expression in mammalian cells. *bioRxiv*. <https://doi.org/10.1101/2020.12.11.420000>.
88. Moghadam, F., R. LeGraw, J. Velazquez, *et al.* 2020. Synthetic immunomodulation with a CRISPR super-repressor *in vivo*. *Nat. Cell Biol.* **22**: 1143–1154.
89. Wang, J., Y. Jeelall, L. Ferguson, *et al.* 2014. Toll-like receptors and cancer: MYD88 mutation and inflammation. *Front. Immunol.* **5**: 367.
90. Canale, F.P., C. Basso, G. Antonini, *et al.* 2021. Metabolic modulation of tumours with engineered bacteria for immunotherapy. *Nature*. <https://doi.org/10.1038/s41586-021-04003-2>.