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Building with intent: technologies and principles for engineering mammalian cell-based therapies to sense and respond

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Abstract

The engineering of cells as programmable devices has enabled therapeutic strategies that could not otherwise be achieved. Such strategies include recapitulating and enhancing native cellular functions and composing novel functions. These novel functions may be composed using both natural and engineered biological components, with the latter exemplified by the development of synthetic receptor and signal transduction systems. Recent advances in implementing these approaches include the treatment of cancer, where the most clinical progress has been made to date, and the treatment of diabetes. Principles for engineering cell-based therapies that are safe and effective are increasingly needed and beginning to emerge, and will be essential in the development of this new class of therapeutics.

Graphical abstract

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Introduction

Engineered cell-based therapies are a powerful and rapidly advancing frontier in medicine. Cells can perform functions that are currently inaccessible by pharmacological means, including directed trafficking within the body, sustained production of therapeutics *in situ*, and performance of complex tasks such as cell-mediated killing. In this review, we focus on a promising facet of this approach—the potential to engineer cells to carry out defined sense-and-respond behaviors in which the therapeutic interfaces with the host environment. As discussed below, the promise of this approach has been demonstrated by recent successes in the treatment of certain cancers. Thus, the need for tools and principles for designing and engineering cells to perform increasingly sophisticated and robust functions represents a pressing challenge in biomedical engineering and in mammalian synthetic biology.

The expanding suite of genetic parts and their applications for engineered cell therapies have been extensively reviewed elsewhere [1–3], and thus here we endeavor to provide a conceptual framework for considering and ultimately designing new therapies. First, we survey recent progress in the field, focusing on examples that illustrate distinct modes by which cell therapies can be engineered to sense and modulate host physiology. We highlight how each application utilizes native functions, novel functions, or both. Second, we draw upon experience gained from these examples and others to summarize current understanding of principles and challenges for engineering cell-based therapies that may ultimately achieve clinical benefits in diverse applications.

Potentiating, controlling, and recapitulating native functional modalities

Enhancing the T cell response to cancer

A forefront example of modifying cells to potentiate and enhance a native functionality is engineering T cells for cancer therapy [2,4]. The chimeric antigen receptor (CAR) is an engineered T cell receptor (TCR) that confers programmable antigen specificity and enhanced activation and persistence to T cells upon antigen recognition. CAR T cell therapies have improved survival in an increasing number of cancer clinical trials [5]. The modular CAR structure renders these receptors readily modifiable to recognize tumor

antigens, modulate signaling, and increasingly implement sophisticated refinements upon the control of T cell activation to address specific clinically-observed challenges; these topics are reviewed extensively elsewhere [2]. A distinct recent approach utilized native transcriptional regulation to address challenges associated with CAR therapies: using Cas9 DNA editing to express the CAR from the native TCR locus minimized antigen-independent signaling and promoted recovery to basal levels after antigen-induced receptor internalization, thereby reducing the problem of T cell exhaustion [6]. The success of CAR T cells in clinical trials [5], recent advisory panel recommendation for FDA approval of an anti-CD19 CAR T cell for B-cell acute lymphoblastic leukemia [7], and substantial commercialization of CAR technologies have all paved the way for cell-based therapies with functionalities beyond cell-mediated killing.

Controlling blood sugar in diabetes

Diabetes is a disorder of blood sugar regulation that results from either a deficiency of insulin-producing beta cells or an insensitivity to insulin. The ultimate cure for type 1 diabetes is replacement of beta cell function, but pancreas or islet cell transplant is still limited to select patients [8]. Transplantation of differentiated beta cells has been investigated as an alternative to transplantation of the pancreas or islet cells, however obtaining physiologic glucose-sensing and insulin production remains a challenge [9]. One approach that could address the need for beta cells is to differentiate human induced pluripotent stem cells (iPSCs) into beta-like cells. Toward this end, a synthetic lineage-control gene expression network was developed to induce the sequential expression of multiple pancreatic transcription factors (TFs) under external control of vanillic acid provided in the medium. This strategy generated iPSCs that released insulin in a glucose-responsive manner, matching the necessary human physiological input/output ranges [10].

An alternative strategy to transplanting beta cells is expression of the components required for glucose-responsive insulin secretion in non-beta cells. Early work included constitutive insulin expression in a hepatic cell line [11] and inducible insulin expression using nanoparticle-mediated local heating to induce a calcium-responsive promoter via a heat-responsive ion channel [12]. More recently, genetic circuits have been developed that place the expression of a glucagon-like peptide 1 (GLP-1) analogue, which enhances insulin secretion, under control of blue light [13] or oleanolic acid, which may be orally administered [14]. Notably, these circuits rely upon external or open-loop control to regulate blood sugar, rather than the closed-loop control achieved by natural beta cells. However, closed-loop glycemic control was recently demonstrated by engineering non-beta HEK293T cells to produce insulin in response to glucose [15]. Microencapsulation and implantation of these cells, which precluded cellular infiltration and exfiltration from the capsule while allowing diffusive transport, restored diabetic mice to normoglycemia. Closed-loop control has also been implemented using a combination of biological and electronic technologies. Insulin production was coupled to LED implant-generated far-red light under the control of a computer (or smartphone) that uses a glucometer to achieve real-time feedback control. If desired, this setup allows for human intervention as a safety precaution [16]. In a novel strategy for the treatment of type 2 diabetes, a signaling pathway was engineered to produce an adiponectin derivative, a hormone that increases insulin sensitivity, in response to

hyperinsulinemia [17]. These studies show how synthetic recapitulation and augmentation of natural functions can address specific clinical needs.

Building new therapeutic functional modalities

A unique prospect for engineered cell therapies is the potential to go beyond enhancing and controlling native functions to construct novel therapeutic modalities that cells do not otherwise exhibit. In this section, we first discuss recent progress in building novel functions by repurposing natural biological parts. We then consider recent technological advances that enable one to direct how a cell senses features of its environment in a customizable fashion using synthetic receptors.

Engineering novel cellular functions by repurposing natural parts

Many diseases may be viewed as disorders of homeostasis, with therapeutic intervention aimed at restoring a natural balance. Among these, chronic inflammatory diseases provide examples in which engineered sense-and-respond has been applied by repurposing natural parts to build cellular “devices” that counter the pathological inflammation. Psoriasis, arthritis, and inflammatory bowel disease involve dysregulated immune responses and increased expression of pro-inflammatory cytokines. Since systemic ablation of pro-inflammatory cytokines or their action can result in profound immunosuppression, a more desirable therapeutic modality is to utilize feedback control. A recent application of this approach to psoriasis leveraged native receptors and intracellular signaling cascades to sense the pro-inflammatory cytokines tumor necrosis factor alpha (TNF) and interleukin (IL-22). A downstream gene circuit was used to implement an AND gate for producing the anti-inflammatory cytokines IL-4 and IL-10 when both TNF and IL-22, markers of psoriasis-associated inflammation, were present [18]. Microencapsulation and implantation of these cells alleviated symptoms of skin lesions and restored tissue morphology in a mouse model of psoriasis.

In another example of rewiring native components to implement feedback control of inflammation, iPSCs were modified by commandeering the native TNF receptor and signaling cascade to drive the production, from a genomically integrated transgene, of anti-inflammatory molecules that antagonize the TNF receptor (a soluble TNF receptor-IgG fusion) or the IL-1 receptor (IL-1Ra, Anakinra) [19]. An appealing feature of this approach is that stem cells can be differentiated to manifest this desired feedback control in mature cell types for disease-specific applications. In a similar approach, sensing and countering pro-inflammatory cues was implemented using a synthetic gene expression program that is induced by NF- κ B (a TF activated downstream of many receptors for pro-inflammatory cues) to drive secretion of an anti-inflammatory protein [20]. The circuit was designed using network motifs that amplify the sensing event, threshold the responsiveness to NF- κ B, and provide a small molecule-inducible OFF switch.

Native component rewiring has also been employed for applications beyond immune regulation. To generate a device that recognizes and prevents liver injury, the bile-acid sensing TGR5 receptor was rewired through the cAMP/CREB pathway to produce hepatocyte growth factor (HGF) [21]. Following drug-induced liver injury, elevated serum

bile acid levels induced HGF production from engineered cells in a microencapsulated device, and mice with such a device showed normal liver histology and serum. A new treatment for a common inflammatory arthritis (gout) has been developed by engineering cells to sense uric acid, the metabolite responsible for the formation of the pathogenic crystals that lead to joint inflammation[22]. A genetic circuit was constructed in mammalian cells, comprising parts from other organisms, to produce urate oxidase in the presence of uric acid. Following implantation into mice, this device controlled hyperuricemia to a degree similar to that achieved with the standard pharmaceutical treatment, allopurinol. Each of these examples highlights the potential to link existing genetic components in new ways to generate novel, therapeutically useful functions.

Technologies for orthogonal cell sensing

A recent advance that further enables bioengineers to compose new functions is the development of synthetic receptors that direct cells to recognize and respond to specified features of their environment. SynNotch utilizes components of the Notch receptor to transduce the sensing of surface-presented target (*e.g.*, an antigen expressed on the surface of a target cell) into the proteolytic release of a TF to regulate target genes [23]. This modular receptor can be modified to recognize new inputs by exchanging the external ligand-binding domain, and to utilize new intracellular mediators by exchanging the TF. SynNotch receptors have been used to implement multiple therapeutic functions, including engineering T cells to express the immunostimulatory cytokine IL-2 in response to sensing the B cell antigen CD19 [24] and increasing the specificity of CAR T cell activation by placing the expression of a CAR recognizing mesothelin under the control of a synNotch receptor recognizing CD19 [25]. Another novel receptor, which enables the sensing of soluble extracellular cues, is the modular extracellular sensor architecture (MESA) [26]. Each MESA receptor comprises two separate membrane-spanning chains, one with a TF and the other with a protease. Upon the binding of the ectodomain of each chain to the extracellular cue, the chains dimerize, leading to *trans*-cleavage and release of the TF. MESA was recently used to construct a non-native immune response in T cells, which may address a challenge in the immunotherapy of solid tumors—local immunosuppression at the tumor site. Toward this goal, T cells were engineered such that MESA-mediated detection of the immunosuppressive cytokine VEGF induced the expression of pro-inflammatory IL-2 [27]. Like synNotch, MESA can be adapted to utilize novel inputs and outputs by exchange of modular receptor domains. A key advantage of these self-contained receptor-signal transduction systems is that they are essentially orthogonal to endogenous signaling and regulation, and thus may be amenable to multiplexed sensing, implementation in diverse cell contexts, and quantitative tuning of performance characteristics.

Principles for engineering cell-based therapies

This final, forward-looking section draws upon the examples above and lessons learned to date to present a contemporary view of key choices, challenges, and considerations that will impact cell-based therapies. We explore how experiences from related fields could inform the design of future therapies that are safer and more effective.

Design priorities and trade-offs

The initial phase for designing a cell-based therapy entails a prioritization of multiple competing objectives. This prioritization, whether explicit or implicit, affects the development of prototypes in ways that shape eventual clinical outcomes. Although synthetic biology research involves an iterative process driven by improving the performance of a device in the laboratory, it is not yet clear *how* this initial optimization funnels the space of biomolecular designs and constrains outcomes in subsequent preclinical and clinical evaluations. This is in part because choices that improve a device's performance within a carefully controlled and idealized laboratory setting do not necessarily guarantee robustness[28]—the ability to perform a function under the wide-ranging conditions the device would encounter in a human recipient.

Further trade-offs include the accuracy versus rapidity with which a device responds to a stimulus [29], and the sensitivity of a device to the stimulus versus the specificity with which the device responds only to that particular stimulus. Each of these trade-offs can shift the balance between a device's safety and efficacy (Figure 1A). These concepts have already proven useful for improving CAR T cell therapies: small-molecule inducible kill switches [30] and activation-ON switches [31] for improved safety; requiring multiple cues to trigger an activation event in order to confer improved specificity [32]; and varying the cell dose or route of administration, or the affinity of the receptor for the target ligand, to achieve decreased *on-target off-tissue* activation [33]. New therapies will benefit from a better understanding of the relationships between initial priorities, prototype evaluations, and success in translation.

Extracellular sensor properties

Sense-and-respond programs can be engineered using natural or synthetic cell surface receptors, as discussed in *Building new therapeutic functional modalities*. In each case, key choices include the type of ligand-binding domain employed (*e.g.*, native receptor domain, single-chain variable fragment, or nanobody [34]) and its concomitant size, valency, stability, orientation, and ligand-binding mechanism and kinetics (Figure 1B). Experience from CAR T cells indicates that tuning ligand-binding affinity can dramatically improve how and under what conditions the device is activated [33]. A challenge for device development is that although physiologically relevant ligand concentrations can be estimated for some species *in vivo* [35], the local concentration of ligand that a device would encounter *in vivo* is generally less understood and likely varies widely between milieus.

Consequences of intercellular variation

There now exist various methods to implement genetic programs in cells, and elucidating the relationship between implementation method and cellular device performance is an area of active investigation. A key choice is whether to integrate a genetic payload genomically or to maintain it extra-genomically and potentially transiently. Each delivery method has advantages and disadvantages. Lentiviral and retroviral vectors carry payloads up to ~10kb, integrate pseudo-randomly in the genome, and may be silenced in certain cell types depending on the integration site [36]. Transposon/transposase systems deliver larger payloads but are less efficient than lentiviral and retroviral vectors and integrate randomly,

exhibiting a less biased pattern of integration [37]. Recombinase-based landing pads provide site-specific integration and more homogeneous gene expression, but thus far the required insertion of the initial landing pad precludes the use of primary cells [38], and it is not yet clear whether a given safe harbor site for targeting genomic integration is safe across all applications [39]. Targeted integration by Cas9-mediated DNA editing may confer site specificity, and sgRNAs are relatively straightforward to design, but integration efficiency remains a challenge [40]. Extra-genomic artificial chromosomes are emerging vectors that are maintained at a single copy, replicate during mitosis, and carry payloads of up to many megabases [41]. Finally, plasmid transfection, RNA replicons [42], and non-integrating viral vectors are transient delivery options. Each method confers a unique profile of variation in gene expression and risk of gene silencing, and how such variation can impact device characterization and performance is a topic of ongoing investigation.

Intercellular variation in device performance can be attributed to external sources, such as sample preparation, cell density, and cell-to-cell contact, as well as internal sources like cell cycle asynchrony, unequal inheritance in cell division, and stochastic fluctuations in gene expression [43,44]. While design goals may be framed using digital metaphors like circuits, ultimately cells operate within a continuum of variation in both component doses and signals, and such variation can limit the extent to which a device reliably performs an engineered function (Figure 1C) [45]. It has been suggested that if the factors that contribute to variation are correlated, then distributions of cell behavior will be skewed by outlier cells [46]. Indeed, it was recently demonstrated that cells expressing high levels of exogenous components produced outlier responses that dominated the average behavior of the population [47]. More broadly, such effects may influence iterative tuning to bias design choices toward those in which many cells do not exhibit the desired functionality. Therefore, precise characterizations for ligand detection limits, dose-response profiles, and parameter sensitivities require individual-cell resolved analyses [48]. Rigorous consideration of how intercellular variation impacts device performance, and perhaps minimization of such variation in the manufacturing process [49], may lead to devices that perform better at translational stages.

Coordination of cellular functions

The desired function of a cell-based device is often specified as a cell-intrinsic input-output relationship. However, *in vivo*, cells operate as populations and interact with other cell types in complex and dynamic environments. As a result, it is not obvious how many cells are required to achieve a desired physiological outcome, or what fraction of an engineered population will exhibit the desired function. Engineering intercellular coordination (*e.g.*, orthogonal to native signaling) is a promising strategy to enhance the reliability or synchrony of programmed behaviors and thus mask the effects of inevitable cell variation and unexpected perturbations (Figure 1D). Negative paracrine feedback could confer adaptive responses to sustained stimuli, and positive paracrine feedback could provide more digital (“yes or no”) commitment to a decision [50] [51].

More sophisticated functional programs can be realized by multiplexing different receptors [47] or different *types* of receptors in a cell [25], or potentially by distributing the job of

sensing across multiple cell subpopulations. Recently, non-receptor components have been distributed in this way, allowing subpopulations to exchange metabolites [52] and to coordinate transgene expression in a manner dependent on the subpopulation densities and for how long the subpopulations are in communication [53]. Whether such coordination will increase or decrease the challenges of designing and implementing a therapeutic product remains to be seen, but this type of approach may eventually augment the safety and efficacy of cell-based devices.

Conclusions

In the past half-decade, the field of engineered cell therapies has matured into a discipline with powerful technologies, commercial investment, a regulatory track record, and clinical experience yielding both informative challenges and successes. This progress has been led, in large part, by pioneering work in cancer immunotherapy, which has been transformative for both clinical oncology and the field of mammalian synthetic biology. As the application space for such approaches continues to grow, the need for useful and general principles for conceiving, developing, and manufacturing engineered cell therapies will increase apace. Towards that goal, here we discussed opportunities to use conceptual tools and understanding drawn from both cell therapies and adjacent fields to facilitate the design, analysis, and refinement of novel engineered cellular functions. Ultimately, maximizing the clinical impact of engineered cells on diverse areas of medicine will require both technologies and principles that enable the safe, effective, and practical implementation of this exciting new therapeutic modality.

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Abbreviations

CAR	Chimeric antigen receptor
GLP-1	Glucagon-like peptide-1
HEK	Human embryonic kidney
IL	Interleukin
iPSC	Induced pluripotent stem cell
MESA	Modular extracellular sensor architecture
TCR	T cell receptor

TF	Transcription factor
TNF	Tumor necrosis factor
VEGF	Vascular endothelial growth factor

References

Of special interest (•); Of outstanding interest (••)

1. Black JB, Perez-Pinera P, Gersbach CA. Mammalian Synthetic Biology: Engineering Biological Systems. *Annu Rev Biomed Eng.* 2017; 19:249–277. [PubMed: 28633563]
2. Lim WA, June CH. The Principles of Engineering Immune Cells to Treat Cancer. *Cell.* 2017; 168:724–740. [PubMed: 28187291]
3. Schwarz KA, Leonard JN. Engineering cell-based therapies to interface robustly with host physiology. *Adv Drug Deliv Rev.* 2016; 105:55–65. [PubMed: 27266446]
4. Johnson LA, June CH. Driving gene-engineered T cell immunotherapy of cancer. *Cell Res.* 2017; 27:38–58. [PubMed: 28025979]
5. Holzinger A, Barden M, Abken H. The growing world of CAR T cell trials: a systematic review. *Cancer Immunol Immunother.* 2016; 65:1433–1450. [PubMed: 27613725]
6. Eyquem J, Mansilla-Soto J, Giavridis T, van der Stegen SJ, Hamieh M, Cunanan KM, Odak A, Gonen M, Sadelain M. Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection. *Nature.* 2017; 543:113–117. [PubMed: 28225754]
7. Novartis: Novartis CAR-T cell therapy CTL019 unanimously (10–0) recommended for approval by FDA advisory committee to treat pediatric, young adult r/r B-cell ALL. 2017.
8. Robertson, RP. Pancreas and islet transplantation in diabetes mellitus. WoltersKluwer; 2016. March 7, 2016
9. Rezaia A, Bruin JE, Arora P, Rubin A, Batushansky I, Asadi A, O'Dwyer S, Quiskamp N, Mojibian M, Albrecht T, et al. Reversal of diabetes with insulin-producing cells derived in vitro from human pluripotent stem cells. *Nat Biotechnol.* 2014; 32:1121–1133. [PubMed: 25211370]
10. Saxena P, Heng BC, Bai P, Folcher M, Zulewski H, Fussenegger M. A programmable synthetic lineage-control network that differentiates human iPSCs into glucose-sensitive insulin-secreting beta-like cells. *Nat Commun.* 2016; 7:11247. [PubMed: 27063289]
11. Tuch BE, Szymanska B, Yao M, Tabiin MT, Gross DJ, Holman S, Swan MA, Humphrey RK, Marshall GM, Simpson AM. Function of a genetically modified human liver cell line that stores, processes and secretes insulin. *Gene Ther.* 2003; 10:490–503. [PubMed: 12621453]
12. Stanley SA, Gagner JE, Damanpour S, Yoshida M, Dordick JS, Friedman JM. Radio-wave heating of iron oxide nanoparticles can regulate plasma glucose in mice. *Science.* 2012; 336:604–608. [PubMed: 22556257]
13. Ye H, Daoud-El Baba M, Peng RW, Fussenegger M. A synthetic optogenetic transcription device enhances blood-glucose homeostasis in mice. *Science.* 2011; 332:1565–1568. [PubMed: 21700876]
14. Xue S, Yin J, Shao J, Yu Y, Yang L, Wang Y, Xie M, Fussenegger M, Ye H. A Synthetic-Biology-Inspired Therapeutic Strategy for Targeting and Treating Hepatogenous Diabetes. *Mol Ther.* 2017; 25:443–455. [PubMed: 28153094]
15. Xie M, Ye H, Wang H, Charpin-El Hamri G, Lormeau C, Saxena P, Stelling J, Fussenegger M. beta-cell-mimetic designer cells provide closed-loop glycemic control. *Science.* 2016; 354:1296–1301. [PubMed: 27940875]
16. Shao J, Xue S, Yu G, Yu Y, Yang X, Bai Y, Zhu S, Yang L, Yin J, Wang Y, et al. Smartphone-controlled optogenetically engineered cells enable semiautomatic glucose homeostasis in diabetic mice. *Sci Transl Med.* 2017;9.
17. Ye H, Xie M, Xue S, Charpin-El Hamri G, Yin J, Zulewski H, Fussenegger M. Self-adjusting synthetic gene circuit for correcting insulin resistance. *Nat Biomed Eng.* 2017; 1:0005. [PubMed: 28480128]

18. Schukur L, Geering B, Charpin-El Hamri G, Fussenegger M. Implantable synthetic cytokine converter cells with AND-gate logic treat experimental psoriasis. *Sci Transl Med*. 2015; 7:318ra201.
19. Brunger JM, Zutshi A, Willard VP, Gersbach CA, Guilak F. Genome Engineering of Stem Cells for Autonomously Regulated, Closed-Loop Delivery of Biologic Drugs. *Stem Cell Reports*. 2017; 8:1202–1213. [PubMed: 28457885]
20. Smole A, Lainscek D, Bezeljak U, Horvat S, Jerala R. A Synthetic Mammalian Therapeutic Gene Circuit for Sensing and Suppressing Inflammation. *Mol Ther*. 2017; 25:102–119. [PubMed: 28129106]
21. Bai P, Ye H, Xie M, Saxena P, Zulewski H, Hamri GC, Djonov V, Fussenegger M. A synthetic biology-based liver-protection device preventing acute liver injuries. *J Hepatol*. 2016
22. Kemmer C, Gitzinger M, Daoud-El Baba M, Djonov V, Stelling J, Fussenegger M. Self-sufficient control of urate homeostasis in mice by a synthetic circuit. *Nat Biotechnol*. 2010; 28:355–360. [PubMed: 20351688]
23. Morsut L, Roybal KT, Xiong X, Gordley RM, Coyle SM, Thomson M, Lim WA. Engineering Customized Cell Sensing and Response Behaviors Using Synthetic Notch Receptors. *Cell*. 2016; 164:780–791. [PubMed: 26830878]
24. Roybal KT, Williams JZ, Morsut L, Rupp LJ, Kolinko I, Choe JH, Walker WJ, McNally KA, Lim WA. Engineering T Cells with Customized Therapeutic Response Programs Using Synthetic Notch Receptors. *Cell*. 2016; 167:419–432. e416. [PubMed: 27693353]
25. Roybal KT, Rupp LJ, Morsut L, Walker WJ, McNally KA, Park JS, Lim WA. Precision Tumor Recognition by T Cells With Combinatorial Antigen-Sensing Circuits. *Cell*. 2016; 164:770–779. [PubMed: 26830879]
26. Daringer NM, Dudek RM, Schwarz KA, Leonard JN. Modular extracellular sensor architecture for engineering mammalian cell-based devices. *ACS Synth Biol*. 2014; 3:892–902. [PubMed: 24611683]
27. Schwarz KA, Daringer NM, Dolberg TB, Leonard JN. Rewiring human cellular input-output using modular extracellular sensors. *Nature Chemical Biology*. 2017; 13:202–209. [PubMed: 27941759]
28. Kitano H. Biological robustness. *Nat Rev Genet*. 2004; 5:826–837. [PubMed: 15520792]
29. Mehta P, Lang AH, Schwab DJ. Landauer in the Age of Synthetic Biology: Energy Consumption and Information Processing in Biochemical Networks. *Journal of Statistical Physics*. 2016; 162:1153–1166.
30. Di Stasi A, Tey S-K, Dotti G, Fujita Y, Kennedy-Nasser A, Martinez C, Straathof K, Liu E, Durett AG, Grilley B, et al. Inducible apoptosis as a safety switch for adoptive cell therapy. *N Engl J Med*. 2011; 365:1673–1683. [PubMed: 22047558]
31. Wu CY, Roybal KT, Puchner EM, Onuffer J, Lim WA. Remote control of therapeutic T cells through a small molecule-gated chimeric receptor. *Science*. 2015; 350:aab4077. [PubMed: 26405231]
32. Zhang E, Xu H. A new insight in chimeric antigen receptor-engineered T cells for cancer immunotherapy. *J Hematol Oncol*. 2017; 10:1. [PubMed: 28049484]
33. Shi H, Liu L, Wang Z. Improving the efficacy and safety of engineered T cell therapy for cancer. *Cancer Lett*. 2013; 328:191–197. [PubMed: 23022475]
34. Kirchhofer A, Helma J, Schmidthals K, Frauer C, Cui S, Karcher A, Pellis M, Muyldermans S, Casas-Delucchi CS, Cardoso MC, et al. Modulation of protein properties in living cells using nanobodies. *Nat Struct Mol Biol*. 2010; 17:133–138. [PubMed: 20010839]
35. Kelley SO. What are clinically relevant levels of cellular and biomolecular analytes? *ACS Sens*. 2017; 2:193–197. [PubMed: 28723142]
36. Suerth JD, Schambach A, Baum C. Genetic modification of lymphocytes by retrovirus-based vectors. *Curr Opin Immunol*. 2012; 24:598–608. [PubMed: 22995202]
37. Singh H, Huls H, Kebriaei P, Cooper LJN. A new approach to gene therapy using Sleeping Beauty to genetically modify clinical-grade T cells to target CD19. *Immunol Rev*. 2013; 257:181–190.
38. Duportet X, Wroblewska L, Guye P, Li Y, Eyquem J, Rieders J, Rimchala T, Batt G, Weiss R. A platform for rapid prototyping of synthetic gene networks in mammalian cells. *Nucleic Acids Res*. 2014; 42:13440–13451. [PubMed: 25378321]

39. Papapetrou EP, Schambach A. Gene Insertion Into Genomic Safe Harbors for Human Gene Therapy. *Molecular Therapy*. 2016; 24:678–684. [PubMed: 26867951]
40. Chu VT, Weber T, Wefers B, Wurst W, Sander S, Rajewsky K, Kuhn R. Increasing the efficiency of homology-directed repair for CRISPR-Cas9-induced precise gene editing in mammalian cells. *Nat Biotechnol*. 2015; 33:543–548. [PubMed: 25803306]
41. Martella A, Pollard SM, Dai J, Cai Y. Mammalian Synthetic Biology: Time for Big MACs. *ACS Synth Biol*. 2016; 5:1040–1049. [PubMed: 27076218]
42. Wroblewska L, Kitada T, Endo K, Siciliano V, Stillo B, Saito H, Weiss R. Mammalian synthetic circuits with RNA binding proteins for RNA-only delivery. *Nat Biotechnol*. 2015; 33:839–841. [PubMed: 26237515]
43. Gough A, Stern AM, Maier J, Lezon T, Shun TY, Chennubhotla C, Schurdak ME, Haney SA, Taylor DL. Biologically Relevant Heterogeneity: Metrics and Practical Insights. *SLAS Discov*. 2017; 22:213–237. [PubMed: 28231035]
44. Loewer A, Lahav G. We are all individuals: causes and consequences of non-genetic heterogeneity in mammalian cells. *Curr Opin Genet Dev*. 2011; 21:753–758. [PubMed: 22005655]
45. Morel M, Shtrahman R, Rotter V, Nissim L, Bar-Ziv RH. Cellular heterogeneity mediates inherent sensitivity-specificity tradeoff in cancer targeting by synthetic circuits. *Proc Natl Acad Sci U S A*. 2016; 113:8133–8138. [PubMed: 27385823]
46. Beal J. Biochemical complexity drives log-normal variation in genetic expression. *Eng Biol*. 2017:1–6.
47. Hartfield RM, Schwarz KA, Muldoon JJ, Bagheri N, Leonard JN. Multiplexing engineered receptors for multiparametric evaluation of environmental ligands. *ACS Synth Biol*. in press.
48. Lin-Gibson S, Sarkar S, Elliott J, Plant A. Understanding and managing sources of variability in cell measurements. *Cell & Gene Therapy Insights*. 2016; 2:663–673.
49. Heathman TR, Nienow AW, McCall MJ, Coopman K, Kara B, Hewitt CJ. The translation of cell-based therapies: clinical landscape and manufacturing challenges. *Regen Med*. 2015; 10:49–64. [PubMed: 25562352]
50. Helikar, T., Kochi, N., Konvalina, J., Rogers, JA. *Systems biology for signaling networks*. Springer-Verlag; 2010. Decision making in cells; p. 295-336.
51. Youk H, Lim WA. Secreting and sensing the same molecule allows cells to achieve versatile social behaviors. *Science*. 2014; 343:1242782. [PubMed: 24503857]
52. Wang WD, Chen ZT, Kang BG, Li R. Construction of an artificial intercellular communication network using the nitric oxide signaling elements in mammalian cells. *Exp Cell Res*. 2008; 314:699–706. [PubMed: 18207141]
53. Bacchus W, Lang M, El-Baba MD, Weber W, Stelling J, Fussenegger M. Synthetic two-way communication between mammalian cells. *Nat Biotechnol*. 2012; 30:991–996. [PubMed: 22983089]

- Engineered cell-based therapies are a powerful, extensible medical technology
- Cellular functions can be composed using both natural and engineered biological components
- Cells made to sense and respond to host physiology enable new treatment modalities
- Principles for building safe, effective, and robust cell therapies are emerging

Figure 1. Considerations and principles for engineering mammalian cell-based therapies

(A) Designing a cell-based device involves balancing trade-offs for various characteristics, which may each be desirable but cannot all be realized simultaneously due to biophysical constraints. To illustrate, we consider a device that is intended to become activated only under a specified physiological condition, by expressing an engineered receptor for sensing an extracellular cue and producing a measurable readout in response. Fold-difference is calculated as the ligand-induced readout divided by the ligand-independent (background) value, and sensitivity and specificity are defined using classification terms (T = true, F = false, P = positive, N = negative). These performance metrics differ from robustness, which is the extent to which performance is maintained under external perturbations or inherent cell variation. While an ideal device is both high-performing and robust, in practice these characteristics may come at some expense of one another, and therefore the design process involves deciding which to prioritize. (B) The endogenous ligand imposes constraints on how a receptor can be engineered to form a productive signaling complex. For a ligand with multiple subunits, a cell-based device may realize better performance with a receptor that has a corresponding valency, oligomerizes, or undergoes cooperative binding; however, this complexity can also introduce more geometric or kinetic tuning to the design process. (C) Intercellular variation can profoundly impact the design, development, and performance of an engineered cell therapy. Such variation is closely related to how endogenous and exogenous genes are expressed, as well as other sources of biological noise. Metrics such as EC₅₀ (half maximal effective concentration of ligand) or ultrasensitivity (the steepness of the dose-response) that are derived from a mean average profile of a heterogeneous population do not represent all cells. Therefore, single-cell analysis is important for characterizing the safety and efficacy of cell-based therapies. (D) Strategies for intercellular coordination may diminish the effects of intercellular variation and enhance the robustness of engineered functions, *e.g.*, by preventing false positive responses.