Theranostic cells: emerging clinical applications of synthetic biology

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Abstract | Synthetic biology seeks to redesign biological systems to perform novel functions in a predictable manner. Recent advances in bacterial and mammalian cell engineering include the development of cells that function in biological samples or within the body as minimally invasive diagnostics or theranostics for the real-time regulation of complex diseased states. Ex vivo and in vivo cell-based biosensors and therapeutics have been developed to target a wide range of diseases including cancer, microbiome dysbiosis and autoimmune and metabolic diseases. While probiotic therapies have advanced to clinical trials, chimeric antigen receptor (CAR) T cell therapies have received regulatory approval, exemplifying the clinical potential of cellular therapies. This Review discusses preclinical and clinical applications of bacterial and mammalian sensing and drug delivery platforms as well as the underlying biological designs that could enable new classes of cell diagnostics and therapeutics. Additionally, we describe challenges that must be overcome for more rapid and safer clinical use of engineered systems.

Off-target effects
Unintended therapeutic consequences that occur when a drug binds to molecules in the body that are not the intended target of the drug.

Synthetic biology
A multidisciplinary field of biological research that seeks to design and engineer biology akin to other engineering disciplines. Goals of the field are the development of molecules, cells and even organisms with novel functions.

Current methods for diagnosing and treating disease are hampered by their inability to respond locally and dynamically to disease states. Many diagnostic approaches necessitate invasive biopsies and subsequent pathological analysis1–7. Therapeutics face the challenge of administration without real-time knowledge of the internal diseased state. Despite recent advances in targeting different diseases, tissues or cell types of interest, many biological-based therapeutics act systemically, thereby increasing the risk of off-target effects and potentially reducing patient compliance8.

Synthetic biology, a field that strives to engineer biology to perform user-defined functions, is well poised to meet the need for new classes of diagnostics and therapeutics. Early advances in synthetic biology led to the creation of prokaryotic cells capable of performing complex computations, whereby they produce differential output based on external signals9. Combining synthetic biology with concurrent advances in protein engineering led to the creation of cells that could use synthetic receptors to activate native pathways10. These systems laid the groundwork for building ‘theranostic’ cells, which can serve as both diagnostic tools and therapeutic delivery systems. Theranostic cells are engineered to express sensors that detect the presence of a disease marker (for example, a cell surface receptor that targets a ligand) and signalling machinery that precisely controls a cellular response (for example, therapeutic protein expression or cell killing11). Relative to small molecules and biologics, which generally act systemically and in an untimed manner, these therapies enable more precise control as they should only activate upon sensing the target biomarker.

A major milestone in the field of theranostic cell engineering was the 2017 FDA approval of tisagenlecleucel (Kymriah), the first gene therapy to be approved in the USA. Tisagenlecleucel is a chimeric antigen receptor (CAR) T cell therapy. It consists of immune cells taken from the patient, which are then engineered to express receptors that target B cell precursor acute lymphoblastic leukaemia. Since then, three other CAR T cell therapies — axicabtagene ciloleucel (Yescarta), brexucabtagene autoleucel (Tecartus) and lisocabtagene maraleucel (Breyanzi) — have been approved to treat different types of blood cancer. These therapies all demonstrate the potential of cell-based therapies as a new treatment modality. Building on this success, many academic laboratories and companies are developing cell therapies that are more effective, safe and applicable to a wide variety of diseases.

Our increased understanding of how cells function, combined with technological advances over the past decade, has expedited cell diagnostic and therapeutic development. For instance, research into the gut microbiome has illuminated the integral and complex role that microorganisms play in regulating physiology12, and advances in microbial engineering have enabled the creation of cells that can dynamically regulate this internal microbial ecosystem13. Similarly, genome editing techniques, such as CRISPR–Cas technologies, have led to more precise and potentially safer methods to introduce targeted...
Chimeric antigen receptor (CAR). A synthetic receptor that combines the specific, extracellular antigen-binding ability of an antibody and the T cell-activating abilities of the T cell receptor (intracellular stimulatory domains) to redirect immune cell action towards a disease antigen of interest.

Cell-free systems
Mixtures of proteins, nucleic acids, salts and metabolites that resemble the cytoplasm of cells and can be used to enact genetic circuits.

Probiotics
Beneficial microorganisms that are used to promote health. Probiotics can be naturally occurring microorganisms or microorganisms that have been engineered to sense and respond to a specific condition.

Genetic circuits
DNA systems in which the presence of different combinations of signals leads to differential gene expression. Circuits usually consist of an input (that is, surface receptor binding to a ligand) and an output (that is, expression of therapeutics) that is enacted only after the input condition is satisfied.

Transcription factors
Proteins that control the expression of a gene, generally either by activating or repressing its expression.

Quorum-sensing systems
Cell to cell communication systems, prominent in prokaryotes, that use extracellular small molecules as signalling cues.

Bacterial diagnostics
The earliest work in synthetic biology used well-studied systems to engineer microorganisms to respond predictably to environmental changes. Since then, a plethora of engineered sensors and more advanced genetic circuits have expanded the scope of compounds that microorganisms can sense and the computations that they can perform, resulting in microorganism-based systems with industrial, health and environmental applications. More recently, bacterial sensors have been engineered to function in biological samples (for example, serum and urine) and even within the body, enabling them to serve as low-cost, minimally invasive diagnostics and theranostics that produce a therapeutic output upon sensing a diseased state. We differentiate between ex vivo diagnostics, which are used outside the body, and in vivo diagnostics, which are used inside the body.

Ex vivo diagnostics
Current ‘gold-standard’ methods to analyse compounds in the body such as ions, metabolites and peptides require the use of advanced machinery and extensive sample processing. By harnessing microorganisms’ natural sense and respond machinery, biosensors offer a low-cost and potentially more accessible testing alternative. Microorganisms can be engineered to sense target compounds and produce visibly coloured changes in response, serving as a ‘litmus test’ for disease. Such sensors could enable fast and low-cost diagnoses, potentially at the point of care.

Whole-cell diagnostics
Nearly all ex vivo microbial diagnostics produce an easily detectable output — either a fluorescent protein or a visible pigment — upon recognition of a target signal. Multiple groups have engineered Escherichia coli cells to sense and respond to analytes such as micronutrients and sugars by harnessing transcription factors that naturally sense these molecules to control expression of colour-based reporters. For example, the zinc-responsive transcription factors Zur and ZnR can be used to control production of visible pigments, such that cells change to a different colour based on the zinc concentration in serum.

In this Review, we address recent advances in the applications of bacterial and mammalian cell diagnostics and therapeutics. Whereas previous reviews have focused on these areas separately, here we provide a broad overview across bacterial and mammalian systems and discuss systems that have been engineered for safer and more effective clinical use. We focus mainly on cellular applications but briefly touch on cell-free systems and viral therapies. First, we discuss bacterial diagnostics and therapeutics, focusing on engineering approaches that have enabled cells to function in the body over extended time periods, and give examples of engineered probiotics that have recently advanced to clinical trials. Then, we explore recent advances in mammalian cell engineering, focusing on ways that chimeric receptors can be engineered to create theranostic cells that modulate the immune system. We conclude by offering our outlook on the challenges that engineered cell diagnostics and therapeutics still face and the advances required for engineered cells to become a new pillar of modern diagnostics and therapeutics.
and produce results within minutes of sample addition (FIG. 2A). These have been used to detect viral biomarkers, such as nucleic acids derived from Ebola, Zika and SARS-CoV-2 (REF. 41) as well as small molecules such as zinc (which reflects nutrition levels) or quorum-sensing molecules secreted from pathogenic bacteria (which indicate the degree of infection).

An advantage of both microbial and cell-free systems for ex vivo analysis is their ability to function in diverse environments and to produce easily detectable outputs.
Sensors can be lyophilized and stored at ambient temperatures for long periods of time, and upon reconstitution with a biological sample they can produce visibly coloured reporters. This supports the use of these diagnostics in low-resource settings, as they can be shipped to remote regions of the world or easily sold from a pharmacy, then used and interpreted with minimal or no equipment. The safety and logistical considerations to such use will be discussed in the later part of this Review.

**In vivo diagnostics**

As bacteria naturally live in symbiosis with the human body, they can be harnessed to serve as in vivo diagnostics, reporting on internal biomarkers in a minimally invasive fashion. Current in vivo diagnostics have been used to detect cancer and inflammation and to monitor gut function and regulation in real time.

**Microbiome diagnostics.** The gut microbiome has become an engineering hotspot, as the growing pool of microbiome research has revealed its critical role in maintaining proper immune and digestive function and in drug metabolism. As bacteria naturally colonize the gastrointestinal tract (termed the gut), they have the potential to serve as stable and long-term reporters of its state. Gut inflammation is a hallmark of diseases such as inflammatory bowel disease and Crohn’s disease, but real-time monitoring of inflammation has been difficult, in part, because of a lack of reliable biomarkers in easily accessible samples: traditional markers of inflammation such as CRP (analysed from blood samples) and calprotectin (analysed from stool) are not specific to inflammation of the gut and have high variability. Biomarkers indicative of the reactive oxygen species (ROS) produced in the gut during inflammation would be more valuable, but indicators of ROS, such as tetrathionate, are transient and cannot be detected without invasive procedures. To monitor inflammation in the mouse gut in a non-invasive way, a commensal murine strain of *E. coli* (NGF1) was engineered to internally sense and record tetrathionate exposure. This engineering approach connects a tetrathionate sensor to a transcriptional element that then continually produces the reporter β-galactosidase. When stool samples from mice that have ingested engineered bacteria are collected and plated, they show β-galactosidase activity based on mouse gut inflammation (Fig. 28a).

Information on the time course of disease progression could be valuable both for better understanding the pathogenesis of gut inflammation and for developing more efficient treatments. To this end, the repressor, a fundamental synthetic biology tool, was harnessed to create a ‘bacterial clock’ that provides information on cellular activity in the gut. The repressor function by using three orthogonal promoter–repressor pairs to control three differentially fluorescent proteins; expression of each protein turns on in a controlled and predictable fashion. When fed to mice and subsequently analysed, these engineered bacteria can report on cellular growth rate and abnormal conditions (such as gut inflammation) that can disrupt standard transcriptional oscillations. Similar systems could be used to dynamically modulate the gut microbiome; the resulting theranostics are described in subsequent sections.

**Real-time reporting.** The interplay between nanotechnology and biotechnology has led to the development of devices that can transmit signals from inside the body, generating real-time health reports. For example, bacteria were engineered to produce luciferase upon detection of clinically relevant biomarkers, such as haemoglobin, thiosulfate and molecules indicative of specific bacterial strains. These engineered bacteria were embedded in an ingestible electronic capsule that processed the light produced from the bacteria and transmitted the information via radio waves to a phone or computer outside the body (Fig. 28b). The capsule can safely migrate through the digestive tract, providing real-time information on the insults encountered through the capsule’s journey. This approach has been successfully used to assess blood in the gastrointestinal tract of a pig but has yet to be tested in humans.

**Bacterial therapeutics**

Naturally occurring bacteria have been used extensively as probiotics for years, and synthetic biology has enabled the creation of engineered probiotics that can treat specific diseases or conditions. Bacteria can be programmed to release therapeutics upon sensing a target compound. In this manner, bacteria have been used to modulate cancer progression, metabolic disorders and microbiome dysbiosis. Several bacteria-based therapeutic systems have advanced to clinical trials.

**Cancer therapeutics**

**Bacteria for tumour targeting.** Bacteria have long been explored as potential cancer treatments: in the 1800s, an injection of streptococcal bacteria shrunk a malignant tumour, and in the 1970s bacillus Calmette–Guérin, an
Bacterial diagnostics for ex vivo diagnosis of zinc deficiency. Production of different pigments (lycopene, violacein and β-carotene) is controlled by zinc-responsive transcription factors. Cells can be lyophilized, rehydrated and, after incubation at body temperature, turn different visible colours. Ab | Cell-free systems for ex vivo diagnosis of target nucleic acid sequences. Reactions consist of bacterial proteins, added reagents and the sensor plasmid. Upon addition of a sample, the reactions turn purple to indicate the presence of the target sequence.

Bacterial diagnostics report on internal inflammatory markers. A | Ex vivo diagnostics. Aa | Bacterial diagnostics for ex vivo diagnosis of zinc deficiency. Production of different pigments (lycopene, violacein and β-carotene) is controlled by zinc-responsive transcription factors. Cells can be lyophilized, rehydrated and, after incubation at body temperature, turn different visible colours. Ab | Cell-free systems for ex vivo diagnosis of target nucleic acid sequences. Reactions consist of bacterial proteins, added reagents and the sensor plasmid. Upon addition of a sample, the reactions turn purple to indicate the presence of the target sequence.

In vivo diagnostics.

B | In vivo diagnostics. Ba | Toggle switch for analysis of gut inflammation. In the absence of an inflammatory stimulus (pink), the circuit is ‘off’ and no β-galactosidase (lacZ) is expressed from P\textit{r}, a promoter that is repressed by cl. Upon exposure to an inflammatory stimulus (green), the protein Cro is expressed from an inflammatory-responsive promoter (P\textit{inflam}), Cro represses cl expression from the promoter P\textit{r} and ‘flips’ the genetic switch into the ‘on’ state, leading to expression of β-galactosidase. A positive feedback loop ensures that the circuit stays in the ‘on’ state, even if the inflammatory stimulus is removed. The engineered bacteria can be orally administered to mice, and stool analysis reveals whether the mice have internal inflammation. Bb | Real-time monitoring of gut activity through bacterial-electronic systems. Bacteria are engineered to sense some internal signal (that is, blood) and then produce luciferase from a haem-responsive promoter (P\textit{haem}). These bacteria are embedded in a small electronic device that can be orally delivered to large mammals. The electronic device detects luciferase produced by the sensor bacteria and transmits the signal in real time via radio waves to electronic devices outside the body.

Attenuated strain of Mycobacterium bovis, was approved to treat bladder cancer. More recently, Salmonella typhimurium has gained attention because it preferentially colonizes necrotic and hypoxic tumour microenvironments. The oxygen-deprived, immune-privileged environment is conducive to anaerobic bacterial growth, which subsequently induces host immune responses to target the bacteria and tumours in a cancer antigen-independent fashion. In the past, S. typhimurium has been involved in numerous phase I trials to treat cancers such as melanoma. However, the treatments were ineffective in humans, and failures were attributed to poor tumour targeting and dose-related toxicity. More recently, treatments utilizing S. typhimurium in combination with chemotherapy drugs have been investigated, and one that targets pancreatic cancer has advanced to a phase II clinical study (NCT04589234). Additional genetically tractable obligate and facultative anaerobes, including Bifidobacterium, Escherichia and Clostridium, were genetically modified to increase tumour specificity by, for example, expressing tumour-targeting peptides or antibodies on the cell surface.

Bacteria can deliver various anticancer effectors upon sensing a diseased state. In general, these therapies function by placing the gene encoding an effector molecule under the control of a promoter that responds to a tumour-specific signal (Fig. 3A). S. typhimurium was engineered to produce a cytolysin protein HlyE upon sensing hypoxia, which resulted in reduced tumour volume when tested in vivo. Similarly, E. coli strains have been engineered to produce antitumour proteins upon sensing a specific cell density, low oxygen levels or decreasing glucose gradients. These sensors have been coupled to additional effector molecules such as prodrug-cleaving enzymes or short interfering RNAs that suppress tumour growth. A phase I clinical trial (NCT01562626) is currently testing whether Bifidobacterium longum that expresses the prodrug-converting enzyme cytosine deaminase enhances the efficacy of flucytosine-based treatment of solid tumours; the cytosine deaminase is expected to convert flucytosine into the standard chemotherapy drug 5-fluorouracil at the site of the tumour. Although these therapeutic systems are relatively straightforward,
autolytic expression
AHL-controlled

P . aeruginosa
L-Phe

Engineered pathways

\textbf{b} Defective host metabolism
L-Phe
\rightarrow
L-Tyr
Phenylpyruvate

\textbf{c} DspB (biofilm distribution)
P . aeruginosa

\textbf{d} Plasmid delivery via conjugation

\begin{itemize}
  \item Enable biocontainment by removing \textit{dpaA} to generate a diaminopimelate auxotroph
  \item Anaerobic-inducible promoter driving expression of \textit{L-Phe} degrading cytosolic and periplasmic enzymes
\end{itemize}

\begin{itemize}
  \item Pyocin S5 (antibacterial)
  \item E7 lysis protein (release of effectors)
  \item P \textit{luxR} Nissle \textit{ΔalrΔdadX} expressing LasR
\end{itemize}

\begin{itemize}
  \item Plasmid encoding RNA-guided nuclease
  \item CRISPR–Cas-mediated removal of antibiotic resistance gene
\end{itemize}

**Fig. 3** | Bacterial therapeutics for treating diseases in vivo. a | Facultative anaerobic bacteria have been engineered to colonize tumour environments, which may include tumour-specific microbiome communities (represented as blue and green rectangles), by sensing various tumour-specific signals, such as hypoxia or decreasing glucose concentrations. This triggers activation of host immune defences, which facilitate destruction of tumour cells (step 1). Additional circuits have been employed to specifically trigger autolysis when a certain bacterial density is reached (step 2). This allows constitutively expressed effector molecules to be delivered within the tumour microenvironment. b | As a proposed treatment for phenylketonuria disorders caused by defects in phenylalanine-metabolizing enzymes, \textit{Escherichia coli} has been metabolically engineered to increase assimilation of \textit{L-phenylalanine} (\textit{L-Phe}) to form \textit{trans-cinnamate}, lowering blood \textit{L-Phe} levels in mouse models\(^{66}\). Expression of the periplasm-associated enzyme \textit{L-amino acid deaminase} also lowers blood \textit{L-Phe} levels by converting \textit{L-Phe} into phenylpyruvate. This provided the basis of a clinical trial that investigated the application of using engineered bacteria to treat phenylketonuria disorders in patients with defects in \textit{L-Phe}-metabolizing enzymes. c | \textit{E. coli} Nissle was demonstrated to decrease viability of pathogenic \textit{Pseudomonas aeruginosa} in a co-culture experiment and to impair pathogenic \textit{P. aeruginosa} colonization in the mouse gut\(^{67}\). In the presence of the target-derived quorum-sensing molecules, this ‘sense-and-kill’ strain expresses several effector genes placed under the control of a \textit{P}_{\text{luxR}} promoter. Specifically, DspB disrupts the target biofilm. The antibacterial agent pyocin S5 is produced and released into the environment after the \textit{E7} lysis proteins lyse the host cell. For biocontainment, the engineered strain requires exogenous \textit{D-alanine} for growth owing to deletions of the alanine racemase genes \textit{air} and \textit{dadX}. d | \textit{E. coli} was engineered to deliver a plasmid encoding RNA-guide nuclease to cleave an antibiotic-resistance gene in enterohaemorrhagic \textit{E. coli}. In this particular study, a type II CRISPR–Cas system was used to cleave the target DNA sequence\(^{51}\). AHL, acyl-homoserine lactone.

**tuning activity** is an ongoing challenge, as it is critical that they respond to the appropriate signal threshold and generate appropriate levels of effector molecules.

**Dynamic delivery of anticancer drugs.** To effectively and safely treat cancer, bacteria must be able to deliver the anticancer payload in a controlled fashion and to autoregulate their replication rates. Inducible autolysis has been explored as a strategy to both release a drug and maintain a stable bacterial population\(^{35,69}\). This approach harnesses quorum-sensing systems. When the concentration of \textit{acyl-homoserine lactone} (AHL), a quorum-sensing molecule, is low, the cells divide and produce an anticancer drug. As the bacterial density increases, the concentration of AHL reaches a threshold that activates autolysis, releasing the anticancer protein into the tumour microenvironment (FIG. 3a). Mice injected with these engineered cells showed significant reduction of tumour volume compared with effector alone and cell-only controls\(^{68}\).

In conclusion, the preliminary bacteria-based anticancer treatments discussed here hold promise to specifically target and kill cancerous cells. Although therapies that use more advanced genetic circuits are still in preclinical development, many bacteria-based cancer therapies have advanced through phase I clinical trials (TABLE 1).

**Limitations to bacterial cancer therapies.** Bacteria-based treatments that yield effective results in humans, especially strains with complex, engineered gene networks,
**Dysbiosis**

An imbalance of the gut microbiome community. Disruption to gut bacterial homeostasis has been associated with human diseases such as inflammatory bowel diseases and irritable bowel syndrome.

**Chassis**

The organism or cell line that houses a genetic circuit and is engineered to perform specific tasks.

remain limited. Balancing the fitness of the bacteria, maintaining stability of the introduced gene circuit, attenuating virulence and increasing target specificity in vivo remain grand challenges to developing bacteria-based cancer therapies. Furthermore, bacterial treatment of cancers (such as leukaemia) that do not form solid tumours conducive to bacterial colonization would likely be ineffective and dangerous, as such treatments would require high concentrations of bacteria in the bloodstream. Treatments in these cases would likely rely on employing engineered mammalian cells, such as those discussed in subsequent sections. Finally, in some cases it is known that tumours contain their own natural microbiome that influences cancer progression. These tumour-specific microbial communities are highly variable between patients\(^{70,71}\) and their potentially different effects on therapeutic performance must be taken into consideration during strain selection and engineering\(^{72}\).

**Gut therapeutics**

Engineered microorganisms can modulate the gut microbiome by sensing biomarker levels, providing potential treatments for gut dysbiosis, inflammation and metabolic diseases\(^{76}\). Most current therapies require ingestion of engineered bacteria, but efforts are being made to modify microorganisms in vivo\(^{73-75}\), which could expand the scope of therapeutic applications.

**Gut modulation with engineered bacteria.** The gut is a prime target for bacterial therapeutics because bacteria naturally colonize the gut and because the gut microbiome plays an important role in modulating diseases such as obesity, diabetes, inflammatory diseases and cancer\(^{76}\). *E. coli* Nissle 1917 is a popular chassis for therapeutic engineering because it is non-pathogenic and easy to engineer, and has a naturally positive effect on the gut microbiome. Other strains, such as *Lactobacillus*,

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**Table 1 | Recent theranostic cell clinical trials of particular interest**

| Clinical trial   | Disease                              | Treatment or intervention                                                                 | Phase |
|------------------|--------------------------------------|------------------------------------------------------------------------------------------|-------|
| NCT04589234      | Metastatic pancreatic cancer         | *Salmonella typhimurium* expressing human IL-2 in combination with either FOLFIRINOX or gemcitabine | II    |
| NCT03751007      | Type 1 diabetes mellitus             | *Lactococcus lactis* in combination with teplizumab                                         | I/II  |
| NCT01562626      | Advanced/metastatic solid tumours    | *Bifidobacterium longum* expressing cytosine deaminase in combination with the drug flucytosine and growth enhancer 10% maltose | I/II  |
| NCT04167137      | Metastatic solid neoplasm, lymphoma  | *Escherichia coli* Nissle engineered to express STING agonist in combination with atezolizumab | I     |
| NCT04534842      | Phenylketonuria                       | *E. coli* Nissle engineered to metabolize L-phenylalanine                                    | II    |
| NCT03016377      | r/r ALL                              | CD19 CAR T cells that express a suicide gene (iCasp9) allowing CAR T cell destruction after rimiducid administration | I/II  |
| NCT03085173      | r/r CLL                              | CD19 CAR T cells 'armoured' with expression of an additional co-stimulatory ligand (4-1BBL) that also express a suicide gene (EGFRt) allowing CAR T cell destruction after cetuximab administration | I     |
| NCT0437932       | r/r GPC3+ solid tumours              | GPC3 CAR T cells 'armoured' with expression of IL-15 that also express a suicide gene (iCasp9) allowing CAR T cell destruction after rimiducid administration | I     |
| NCT04230265      | r/r AML, r/r B-ALL, BPDCN            | Modular, uniCAR T cells in combination with a recombinant antibody derivative targeted towards CD123 | I     |
| NCT0463148       | Renal cancer, transitional cell cancer of the renal pelvis and ureter, prostate cancer, non-small-cell lung cancer, breast cancer, colorectal cancer | Modular, uniCAR T cells in combination with a recombinant antibody derivative targeted towards PSMA peptide | I     |
| NCT04150497      | r/r B-ALL                            | Allogeneic CD22 CAR T cells                                                               | I     |
| NCT04093596      | r/r MM                               | Allogeneic BCMA CAR T cells                                                               | I     |
| NCT03056339      | r/r acute and CLL                    | Cord blood-derived natural killer cells with CD19 CAR, iCasp9, IL-15                      | I/II  |
| NCT03896658      | Glioblastoma                         | Mesenchymal stem cells producing oncolytic adenovirus                                        | I     |

**Notes:**

- ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; B-ALL, B cell acute lymphoblastic leukaemia; BCMA, B cell maturation antigen; BPDCN, blastic plasmacytoid dendritic cell neoplasms; CAR, chimeric antigen receptor; CLL, chronic lymphocytic leukaemia; GPC3, glypican 3; iCasp9, inducible caspase 9; MM, multiple myeloma; PSMA, prostate-specific membrane antigen; r/r, relapsed or refractory; uniCAR, universal CAR.
Clostridium and Bacteroides, have also shown promise in therapeutic development.

Metabolic diseases are a prime target for dynamic modulation, as bacteria can be readily engineered to process the accumulated metabolite. However, these efforts have had mixed results. Hyperammonaemia is a disease characterized by excess ammonia accumulation in the blood, resulting from defective enzymes in the urea cycle. An E. coli Nissle strain was engineered to assimilate ammonia and sequester the nitrogen into the amino acid l-arginine. Administration of this engineered bacteria to mice with hyperammonaemia reduced blood ammonia levels and improved survival. It completed phase I clinical trials (NCT03179878), but was terminated owing to ineffectiveness in lowering blood ammonia in humans. A similar strategy was used to address phenylketonuria, a genetic disease caused by an inability to metabolize l-phenylalanine (l-Phe) (Fig. 3b). E. coli engineered to convert l-Phe into other metabolites resulted in increased l-Phe metabolism in monkeys, a strategy that recently passed phase I clinical trials (NCT03516487) and is on track for testing in phase II trials.

Engineered bacteria could also be used to control the composition of the gut microbiome and eliminate pathogenic bacteria. Commensal E. coli Nissle were engineered to target Pseudomonas aeruginosa, a bacterium that can cause serious infection. The E. coli cells contain a genetic circuit encoding antimicrobial peptides and a biofilm-degrading enzyme. Upon detecting the P. aeruginosa quorum-sensing compound, the engineered cells produce the peptide and enzyme (Fig. 3c). Co-culture of the two strains reduces P. aeruginosa viability and biofilm content. In a mouse infection model, administering the engineered E. coli led to ~70% reduction of P. aeruginosa colonization, providing a viable antimicrobial strategy to combat antibiotic-resistant pathogens.

Gene delivery and gene expression modulation. Gut therapeutics can function by delivering gene circuits to bacteria that are already present in the gut, which can enable precise editing and modification of the gut microbiome. For example, gut bacteria have been engineered to deliver CRISPR-based tools into recipient pathogenic cells to reduce host drug resistance or deactivate virulence genes (Fig. 3d). This strategy could be used to create novel antibiotics, as it can eliminate pathogenic bacteria or decrease their pathogenic effects. Alternatively, phages can be used to modulate bacterial gene expression in the gut. Non-lytic, temperate phages can deliver catalytically inactive (‘dead’) Cas9 (dCas9) and CRISPR RNAs in situ, which alters gene expression of infected bacteria. This strategy could enable the development of phage therapy to modulate pathogen gene expression by, for example, suppressing the expression of virulence factors.

Engineered bacteria can also control gene expression in mammalian cells. For example, commensal bacteria were engineered to modify mammalian cells that over-express cyclooxygenase 2 (COX2), which is characteristic of inflammatory diseases such as Crohn’s disease and ulcerative colitis. These bacteria invade cells in the colon mucosa and transfer plasmids expressing short interfering RNAs that downregulate expression of COX2. This strategy has been demonstrated to attenuate the inflammatory responses in mouse models, but the general approach still faces challenges to clinical translation; primarily, there is currently little control over specific entry into mammalian cells, which could cause detrimental off-target effects if bacteria were to centre healthy cells. Additionally, it is difficult to control the rate of circuit delivery into recipient cells, which could lead to non-uniform levels of gene knockdown.

Biocontainment and safety of engineered bacteria

Using engineered bacteria in a safe and contained way is a top priority in therapeutic development and is required to obtain regulatory approval. For example, in both the European Union and the USA, regulatory agencies require extensive demonstration of the bacteria’s safety, genome stability, colonization time and ability to be removed. To effectively engineer bacteria to meet these criteria, it is critical to attenuate pathogenicity, control bacterial survival and replication, and minimize the risk of mutation.

Engineering safe and containable strains

Various engineering strategies can be used to make bacteria safe and to ensure that they do not survive outside their intended environments. To ensure safety, virulence genes can be readily removed via standard gene editing approaches. Further, ‘suicide genes’ can be incorporated so that engineered bacteria can be selectively removed from the population. A quorum-sensing system that prompts self-destruction upon reaching a certain density threshold is one example of an effective suicide gene, but other strategies can offer more external control. For instance, the use of auxotrophic bacteria allows growth only when an exogenous nutrient (for example, an unnatural amino acid) is supplied, enabling easy removal of engineered bacteria through withholding of the amino acid.

Maintenance of genetic stability

Another safety concern for engineered bacteria is ensuring that they do not mutate over time. This can happen through mutations in the sensor-encoding or effector-encoding genetic circuit, which can reduce treatment effectiveness or cause unwanted adverse effects. Circuits with minimal burden on engineered cells have been shown to be genetically stable in the gut environment, and engineering approaches can further stabilize systems. For instance, synthetic communities composed of multiple bacterial strains engineered to sense and replace a mutating subpopulation have increased circuit stability. Bacterial cells are also subject to horizontal gene transfer, whereby genetic material from other cells or viruses centre and can alter cell function in an unpredictable fashion. To prevent horizontal gene transfer, bacteria can be genetically re-coded to impair expression of viral proteins or replication of foreign plasmids, minimizing the risk of major mutations.
Safety

Ba Suicide genes
iCasp9 HSV-TK

Efficacy

Bd Combinatorial logic
SynNotch-gated CARs

Be Avidity tuning
AvidCARs

Bf Antigen switching
SUPRA CARs

A

Tumour antigen
Antigen-recognition domain
Spacer
Transmembrane domain
Signalling domains (T cell activation, co-stimulation)

1st generation 2nd generation 3rd generation 4th generation

CAR T cell

Ba

Small-molecule administration

Bb

Inhibitory receptors
iCARs

Bc

Expression-level modulation
CAR LID

LID domain

Small-molecule administration

Bf

Antigen switching
SUPRA CARs

B

Tumour cell

Healthy cell

Tumour cell

Tumour antigen
Tumour killing

Actuation and cytotoxicity

Actuation and cytotoxicity

Tumour antigen

No activation

Cytokine production

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**Mammalian diagnostics**

Compared with bacterial engineering, mammalian synthetic biology faces the added complexity of eukaryotic cell biology and associated gene regulation, but recent technological advances have improved our ability to control eukaryotic cell output at the transcriptional, translational and post-translational level. Although bacterial cells have been the primary chassis for whole-cell diagnostics, a handful of mammalian cell diagnostics have been developed. These show potential for diagnosing conditions with biomarkers that mammalian cells can recognize more easily than bacterial cells, such as inflammatory molecules produced by the immune system.

**Ex vivo mammalian diagnostics**

One prominent example of an ex vivo mammalian diagnostic is a whole-cell sensor for personalized, precise profiling of allergies. Allergen profiling is normally done with intrusive skin pricks that expose patients to allergens and induce immune reactions in the skin. As an alternative, HEK293 cells were engineered to robustly respond to histamine, a compound secreted by immune cells that indicates an allergic reaction. When a blood sample taken from a patient is exposed to an allergen, immune effector cells secrete histamine as usual, and the engineered sensor cells can detect and score the amount of histamine produced. These cells could be the basis of a high-throughput assay for allergic responses, which could replace the traditional skin prick test.

**In vivo mammalian diagnostics**

In vivo mammalian cell diagnostics are less common, primarily because most mammalian cells engineered to respond to diseases in vivo also serve as therapeutics, which we discuss in detail in the next section. However, one example of a purely diagnostic mammalian cell system is a sensor for hypercalcaemia. High levels of calcium are a result of hormone-mediated dysregulation of bone resorption and are associated with asymptomatic cancers. HEK293 cells were engineered to detect and respond to histamine, a compound secreted by immune cells. When injected under the skin of mice, these aptly named HEK-human cells function well as calcium reporters. However, they have not been tested in humans, presumably because of the immunogenicity associated with cell implantation if the cells were to leak out of the alginic capsules.

**Mammalian therapeutics**

The primary focus in the field of mammalian synthetic biology has been the creation of theranostic cells that can simultaneously recognize a diseased state and respond to it in vivo. These systems harness the innate ability of mammalian cells to respond to a wide variety of stimuli, and thus show promise for real-time regulation of complex diseases. In this section, we explore recent progress in the field of mammalian theranostic cell therapies. We first discuss engineered T cells as prime examples of theranostic cell engineering, focusing mainly on CAR T cells. We describe new protein engineering approaches to build CAR cells with improved specificity and safety profiles. Finally, we briefly describe ways that other types of theranostic cell are being engineered for diverse applications.

**Synthetic TCR T cell therapeutics**

T cell receptor (TCR)-modified T cells were first developed as a strategy to harness the potent therapeutic effects of cytotoxic T cells for anticancer therapies. TCRs recognize antigen peptides displayed on major histocompatibility complex (MHC) proteins, and they can be engineered to target specific, researcher-defined antigen peptides. These antigen peptides can originate from membrane-bound or intracellular proteins, giving researchers a wide range of potential targets. However, TCRs can only recognize certain peptide–MHC complexes, and MHC genes are highly polymorphic in the general population, which limits these therapies to patients expressing a given MHC haplotype. Nevertheless, clinical trials of TCR-engineered T cells have been successful in treating myeloma and melanoma, and many others are currently underway.

**CAR T cells for cancer treatment**

Similar to TCR cell therapies, CAR T cells were engineered to sense cancer biomarkers and elicit a downstream cytotoxic response. Unlike TCRs, CARs (previously referred to as TCRs) are artificial receptors that combine the antigen-binding specificity of an antibody and the T cell-activating signalling domains of the TCR without MHC restriction. The extracellular domain of a CAR is a single-chain variable fragment (scFv), which
**Autologous**
A term to denote cells or tissue derived from a patient for the treatment of that same patient.

**Cytokine release syndrome**
A potentially deadly adverse effect of chimeric antigen receptor (CAR) T cell therapy, resulting from the mass release of pro-inflammatory cytokine expression from activated T cells.

**Cytokines**
Secreted proteins that bind to receptors on other cells that induce a change in that target cell, generally categorized as pro-inflammatory or anti-inflammatory.

**Boolean logic gates**
Systems of binary, on/off signals used to generate complex behaviours in a system (for example, an AND-gated circuit necessitates the presence of two inputs before expression of the output).

Confers antigen-binding specificity, and the intracellular domain contains elements that activate T cell signalling. Upon extracellular target recognition, the intracellular domain activates the T cell response, which produces co-stimulatory signals necessary for T cell function, proliferation and persistence, leading to killing of cells that have the targeted receptor. Multiple generations of CARs have been created to stimulate the optimal combination of intracellular signalling. T cell activation and T cell persistence. Currently approved CAR T cell therapies are autologous cell therapies. T cells harvested from the patient are first expanded and engineered ex vivo with a viral vector encoding the CAR protein for long-term expression; the engineered T cells are then infused back into the patient, where they home to tumours expressing an antigen of interest. Patients with previously non-responsive B cell cancers have experienced complete remission upon treatment with CAR T cells, and they are currently being developed for the treatment of many other cancer types, including solid tumours.

Despite the clinical success of CAR T cells in treating haematological cancers, there are still major obstacles to safe and efficacious CAR T cell treatment of diverse cancer types. In both haematological and solid tumours, there is a dearth of tumour-specific antigens, and on-target off-tumour killing of healthy cells can occur. Tumour cells can also downregulate expression of the antigen targeted by CAR T cells, a process known as antigen escape, allowing the tumour to grow again unchecked by the immune response. Additionally, many patients experience major adverse effects during treatment, such as neurotoxicity and cytokine release syndrome, which results from constitutive CAR activation. Ongoing efforts to modify CARs aim to overcome the above challenges, and thus improve treatment safety and efficacy. Specifically, research has demonstrated ways that CARs can be designed to enhance tumour specificity and to control the spatio-temporal profile of inflammatory cytokines. Novel modifications to CARs should both improve treatment safety and maximize the on-target immune response.

**Improving CAR T cell safety.** Unlike traditional small-molecule drugs where the dose is controlled during administration, the activity and proliferation of CAR T cells is largely uncontrollable once the therapy is administered. Thus, much research has focused on engineering control systems that allow in vivo CAR T cell modulation to improve the safety of the therapy. Many of these systems use small molecules to modulate T cell function. One approach is the development of cells engineered to have inducible suicide function so that they self-destruct upon addition of a small-molecule regulator. Two suicide genes that have been effectively used are an inducible caspase 9 (iCasp9) that initiates downstream apoptotic pathways once activated by a dimerizing small molecule, and herpes simplex virus thymidine kinase (HSV-TK), which inhibits DNA synthesis when activated with the small molecule ganciclovir. A second approach to improving safety is expression of co-receptors that inhibit CAR T cell action against healthy cells. Antigen-specific inhibitory CARs contain an scFv directed to antigens expressed on healthy cells fused to the signalling domains of T cell inhibitory receptors, CTLA4 and PD1 (Fig. 4Bc). When bound to antigens indicative of healthy cells, CAR T cell action is inhibited. However, this approach is limited, as it is challenging to find cell surface markers that are unique to healthy cells. A third approach to controlling function is to modulate levels of functional CAR proteins at the cell surface. A prime example of CAR surface expression control is the development of CAR T cells that can be reversibly paused after administration of a small-molecule ligand. These T cells express second-generation CARs fused to a ligand-induced degradation (LID) domain. Binding of the ligand to the LID domain induces the release of a cryptic degron, which results in selective CAR degradation; however, the T cells themselves still remain, unlike inducible suicide gene systems. Thus, the CAR T cells can resume activity when the ligand is removed, enabling precise and reversible control of CAR T cell function in a ligand concentration-dependent manner.

**Improving CAR T cell efficacy.** The ideal CAR T cell will only be active upon recognition of cancer-specific antigens, but the lack of tumour-specific antigens and antigen-independent activation of CARs (termed tonic signalling) can lead to unwanted CAR T cell activation. Additionally, tumours can lose expression of antigens targeted by CARs, rendering the treatment useless. One approach for overcoming antigen specificity challenges is the use of cells that can recognize multiple antigens simultaneously, for example, using SynNotch-gated CARs. These comprise AND-gated Boolean logic gates, which means they are only activated after binding two tumour antigens. Binding of one scFv to its targeted tumour antigen triggers the translocation of a synthetic transcription factor to the nucleus, which causes expression of a CAR directed to a second tumour antigen.

Affinity or avidity tuning of chimeric proteins is another form of AND-gating in both receptor and ligand design that can diminish the targeting of healthy antigen-presenting cells. CARs dimerize to effect their signalling in the cell; however, tonic signalling, whereby CARs dimerize without the presence of antigen, is an issue that currently approved CARs face. One example of avidity tuning is the AvidCAR T cell platform, which prevents unwanted dimerization and cell activation. This system employs monomeric CARs with low-affinity, single-domain antigen-binding domains (instead of an scFv) that rely on bivalent antigen engagement for dimerization and activation. Reduced affinity of the single-domain antigen-binding domain prevents constitutive CAR dimerization, and CAR signalling and effector function are only active when antigens are co-expressed on the same cell. This platform is thus an easily controllable and combinatorial system that better targets tumour cells co-expressing antigens rather than healthy surrounding tissue. Aside from AND gates, other logic gates, such as OR and NOT gates, have been engineered to recognize different combinations of...
surface antigens to increase tumour targeting over the targeting of healthy cells\textsuperscript{107,108,123}.

Finally, an alternative approach to combat antigen escape is the development of universal CAR T cells, which can be altered to detect different antigens without having to entirely re-engineer the CAR\textsuperscript{124–127}. These systems split the antigen-recognition domain and the co-stimulatory domains of conventional CARs into separate components. The first component is an engineered T cell expressing a universal CAR construct consisting of intracellular signalling domains and an extracellular adapter (instead of an scFv directed to the antigen of interest). The second component is a complementary adapter molecule that confers antigen-binding specificity and the modularity of the platform. One example of such an approach is the split, universal and programmable CAR (SUPRA CAR) platform\textsuperscript{128}, which concurrently addresses specificity, safety and ease of design [FIG. 4B]. The SUPRA CAR system consists of a T cell that only expresses the T cell signalling domains of a CAR linked to an extracellular leucine zipper domain (zipCAR) and an adapter molecule, which is an scFv linked to a complementary leucine zipper (zipFv). The zipFv confers the antigen specificity of the CAR T cell, and different scFv leucine zippers can be easily injected into a patient without reinfecting cells. This modularity allows combinatorial logic and inhibition of CAR function depending on the zipCARs and zipFvs used.

**CAR T cells for solid tumours.** Although this review focuses on CAR T cells engineered to treat haematological cancers, CAR T cells are also being explored to treat solid tumours\textsuperscript{407}. These approaches have not been as successful in generating remission\textsuperscript{149}, and there are no currently approved CAR T cells that target solid tumours. Beyond the challenges discussed above, these therapies must overcome the immunosuppressive tumour microenvironment and impaired trafficking of CAR T cells into the tumour mass\textsuperscript{407}. Examples of CAR T cell modifications to circumvent these challenges are the expression of inflammatory cytokines, such as IL-12, that improve CAR persistence and activity and the co-expression of chemokine receptors, such as CCR4 [REF.\textsuperscript{150}]. Additionally, CARs can be used in combination with oncolytic viruses, which can cause direct tumour cell lysis or can generate an inflammatory immune response\textsuperscript{412,129}, but these systems are beyond the scope of this Review.

**Other immunomodulatory CAR cells.** The majority of recent T cell engineering has focused on developing novel cancer therapeutics, yet CARs also have the potential to treat autoimmune disorders or to regulate ageing cells. By targeting cells that secrete inflammatory cytokines, CARs can dampen an overactive immune response. Recently, ‘senolytic’ CAR T cells were used to recognize and eliminate senescent cells\textsuperscript{130}. Eliminating senescent cells reduces inflammation and tissue damage and increases the healthspan\textsuperscript{132,133}, suggesting that senolytic T cells could be a potential anti-ageing therapeutic.

Additionally, introducing CARs into immune cells beyond T cells leverages the diversity of effector functions found across immune cell types (BOX 1). The resulting therapies could overcome some of the current limitations of CAR T cells and also broaden the scope of CAR cell applications to other immune disorders.

**Theranostic cells for other applications**

Whereas recent mammalian theranostics have centred on therapeutic T cells, mammalian theranostic cells have also been developed for an array of other diseases\textsuperscript{95,99,134,135}. Although these have not yet been developed for clinical use, they have shown great potential in preclinical studies, particularly for the treatment of autoimmune diseases.

**Cells to modulate the immune system.** To engineer cells with novel functions, synthetic biologists can piece together genetic elements from diverse cell types. A prime example of such engineering is the development of HEK293 cells for the treatment of psoriasis. These cells express anti-inflammatory cytokines upon recognition of psoriasis-specific inflammatory cytokines\textsuperscript{136} [FIG. 5A]. The cytokines TNF and IL-22 are characteristic of psoriasis, but HEK293 cells only endogenously express one-half of the IL-22 receptor (IL-10RB). To endow HEK293 cells with the ability to recognize IL-22, the endogenous TNF-responsive pathway was engineered to control production of the other half of the IL-22 receptor (IL-22RA)\textsuperscript{137}. Then, binding of IL-22 to the expressed receptor triggers the IL-22 signalling cascade. This pathway was rewired to control production of the two anti-inflammatory cytokines IL-4 and IL-10 [FIG. 5A]. The resulting cells successfully reduce inflammation upon sensing the psoriatic phenotype, and in mouse models they prevent onset of psoriatic flares and attenuate acute psoriasis\textsuperscript{138}. Thus, the researchers rewired an endogenous pathway (TNF signalling) to express a component (IL-22 receptor) from a different cell type to generate novel responses in the theranostic cell. Using similar engineering tactics, other theranostic cells have been developed to treat diverse diseased states such as diabetes\textsuperscript{137,138}, methicillin-resistant *Staphylococcus aureus* allergy\textsuperscript{139} and inflammation\textsuperscript{410,140}.

**Engineered stem cells for regenerative medicine.** Genetically engineered mesenchymal stem cells are powerful tools for tissue regeneration and gene therapy, and using synthetic biology approaches to control their activity could expand their regenerative applications. For example, engineering cells to overexpress IL-1 receptor antagonist (IL-1RA) can dampen an overactive, inflammatory immune response, and simultaneously expressing vascular endothelial growth factor (VEGF) promotes angiogenesis, both critical components of tissue regeneration\textsuperscript{142}. Whereas delivery of VEGF-encoding DNA produces angiogenic effects\textsuperscript{143–145}, stimulation of the native VEGF promoter triggers more robust angiogenesis\textsuperscript{146}. This is thought to be due to post-transcriptional processing leading to multiple splice variants of VEGF, which provide a more comprehensive set of angiogenic stimuli. In line with these results, transgenic expression of survivin, an enhancer of VEGF production, accelerates myocardial healing post infarction\textsuperscript{147}. Because mammalian cells have
**Box 1 | Other CAR immune cells and their effector functions**

**Natural killer cells**

Similar to cytotoxic T cells, natural killer cells, which are innate immune cells that do not require prior activation, also induce receptor-mediated cell death. Unlike chimeric antigen receptor (CAR) T cells, which need to be patient-derived to avoid immune rejection, natural killer cells derived from unrelated, HLA-mismatched donors were well tolerated in phase I and II clinical trials. Although the mechanism for this is still unclear, cytotoxicity of donor natural killer cells against a patient’s alloreactive T cells seems to mediate this tolerance. By redirecting natural killer cell activity with antibodies or single-chain variable fragment (scFv) domains against CD19, the tumour-associated antigen mesothelin and HIV gp160, CAR-engineered natural killer cells have been shown to be effective for targeting lymphoma, solid ovarian tumours, and HIV-infected cells, respectively (see the figure, part a).

**Macrophages**

Macrophages are phagocytic cells that can engulf and neutralize pathogens. They can infiltrate the solid tumour microenvironment and bridge the gap between the innate and adaptive immune systems by presenting engulfed antigens to B cells and T cells of the adaptive immune system. Macrophages use cytokines to activate or inhibit cells of the adaptive immune system, including T cells, making them a potent target for CAR engineering. Recently, engineered CAR macrophages were shown to not only infiltrate tumours but also overcome the immunosuppressive tumour microenvironment and maintain a pro-inflammatory M1 phenotype (see the figure, part b). The authors also showed that adenoviral transduction of the CAR into macrophages was sufficient for M1 polarization, and that this polarization induced pro-inflammatory gene expression in the tumour microenvironment, including converting bystander M2 macrophages into M1 macrophages. This proof-of-concept study demonstrates that CAR macrophages could become a promising new therapeutic for solid tumours, and highlights the potential of antigen-presenting cell engineering in altering the inflammatory state of the microenvironment.

**Regulatory T cells**

Regulatory T cells (T\(_{\text{reg}}\) cells) are important mediators of immune tolerance, and can dampen the immune response through interactions with cells of the innate and adaptive immune systems. T\(_{\text{reg}}\) cells suppress the inflammatory effector functions of cognate immune cells via localized anti-inflammatory cytokine secretion, although they can also perform perforin-mediated killing like their CD8\(^{+}\) counterparts. T\(_{\text{reg}}\) cells have been engineered with both T cell receptors (TCRs) and CARs to direct their immunosuppressive functions, although CAR-mediated activation has been reported as stronger than TCR-based activation of T\(_{\text{reg}}\) cells in inducing proliferation. Whereas polyclonal T\(_{\text{reg}}\) cell therapy is currently being tested in clinical trials for autoimmune diseases, these studies suggest that the next generation of targeted CAR T\(_{\text{reg}}\) cells could have even greater potency in future clinical trials.

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complex post-transcriptional processing that is difficult to control (that is, alternative RNA splicing), stimulating native cytokine expression may not be sufficient to achieve the desired phenotype; alternatively, exogenous expression of cytokines can be more rationally engineered and more tightly controlled (FIG. 5b).

Ectopic transcription factor expression (expression of transcription factors not normally present in a cell type) can also modify cell behaviour, most potently demonstrated by the use of Yamanaka factors for generating induced pluripotent stem cells. Since then, expression of other transcription factors has expanded the scope of mesenchymal stem cell therapies. For example, overexpression of HIF1\(\alpha\) enhances haematopoietic growth factor production, which could increase the success of bone marrow mesenchymal stem cell transplants. As engineered receptors, transcription factors and cytokines are connected and regulated in more complex pathways, mammalian synthetic biologists will be able to better control cell function and create new types of therapeutic cell with more potent regenerative capabilities.

**Conclusions and perspectives**

Although engineered cells show great promise for changing treatment paradigms, many challenges must be addressed to ensure their widespread clinical approval
Allogeneic
A term to describe cells or tissue derived from one person used to treat a genetically different person (allotransplantation).

and success. A primary challenge for engineered bacterial cells is the current dearth of sensors available for many physiologically relevant compounds. Most bacterial diagnostic and theranostic approaches rely on finding existing proteins that interact with and respond to the molecule of interest. This bioprospecting approach works well when sensors are available for a given target, but it is not widely generalizable. A platform that uses modular sensors such as aptamers or antibody fragments, which can be evolved to specifically bind a target molecule with a user-defined affinity, could greatly expand the scope of bacterial diagnostics and theranostics. Another challenge for bacterial theranostics is the precise control of cellular output. Bacteria can be engineered to produce and secrete small-molecule and protein drugs, but it is difficult to tightly control the amounts of drugs that are produced; such control is critical, especially for drugs that have small therapeutic windows. Cells that integrate production over time or can sense external levels of the produced drug could be used to effectively titrate drug levels. As in silico design approaches make it easier to build robust and complex circuits, it is becoming more feasible to build such complex systems.

In mammalian cell engineering, the high cost and logistical challenges associated with CAR T cell treatment, in addition to potentially lethal adverse effects, have limited their use to a ‘last-resort’ option. Manufacturing a single dose of tisagenlecleucel costs upwards of US$40,000, and other costs associated with treatment have driven the cost to $475,000 per person, making it the most expensive cancer therapy to date. The need to individually engineer each patient’s cells ex vivo is largely responsible for the high cost. A promising alternative, currently in clinical trials, is the use of allogeneic T cells, which could serve as ‘off-the-shelf’ cell therapies, eliminating the need to engineer custom therapies for each patient. However, the use of allogeneic cells increases the risk of graft-versus-host disease, necessitating further genetic engineering to limit rejection. Another potential way to reduce therapy costs and manufacturing challenges would be to directly inject genetic circuits into patients, rather than into their isolated cells, which is feasible when the tissue can be accessed directly; for example, in the case of retinal tissue, voretigene neparvovec (Luxturna) is directed directly into the eye and was recently approved as a gene therapy against vision loss. Advances in targeting adeno-associated virus vectors to specific tissues and cell types could help provide similarly specific gene delivery to tissues that are inaccessible by injections.

As theranostic cells become more potent, it will be critical to monitor their impact on the surrounding tissue. For example, incorporation of CARs into diverse immune cell types will lead to changes in the local microenvironment around the target cell, which are good for the immediate treatment goal but could have potentially detrimental long-term consequences. As these therapies become more developed, it will be critical to assess the reversibility of these changes and then develop engineering approaches to enable better control.

Fig. 5 | Theranostic cells for non-cancer applications. a | Cytokine converter cells sense the presence of two inflammatory cytokines expressed in psoriasis, TNF and IL-22, and express anti-inflammatory cytokines, IL-4 and IL-10, in response. The endogenous TNF receptor (TNFR)/NF-κB signalling pathway of HEK293 cells is rewired to activate expression of IL-22 receptor-α (IL-22RA) in the presence of TNF. When IL-22RA is expressed and IL-22 is present, IL-22RA and endogenous IL-10RB dimerize and activate the endogenous JAK–STAT pathway. STAT3 signalling is rewired to activate expression of anti-inflammatory cytokines IL-4 and IL-10 that can improve the psoriatic phenotype by calming inflammation.

b | Highlighted strategies for engineering native therapeutic pathways in mesenchymal stem cells include expression of receptor intracellular domains to induce a pro-erythropoietic phenotype (step 1), transgenic expression of vascular endothelial growth factor (VEGF) (step 2) and upregulation of the native isoforms of VEGF to promote angiogenesis (step 3).
Finally, both bacterial and mammalian cell therapies must overcome negative public opinion, which is fuelled both by the stigma of using genetically modified organisms and by previous failures and breaches of scientific ethics during the use of genetic therapies. Positive branding and public campaigns highlighting the safety and health benefits of diagnostic and therapeutic bacteria could help to alter public opinion on the use of engineered cells. Expanding mammalian cell-based treatments to non-terminal diseases will require extensive demonstration of their safety and benefits over more traditional treatments.

Taken together, recently developed cellular diagnostics and therapeutics have shown that synthetic biology has real potential to transform health-care paradigms. Cell-based therapies have rapidly progressed through clinical trials and regulatory approval, and are thus emerging as an alternative treatment modality to existing small-molecule drugs and protein biologics. The expanding cellular engineering toolbox will lead to more sensitive diagnostics and novel therapeutic approaches for previously intractable diseases.

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