Engineering Precision Therapies: Lessons and Motivations from the Clinic
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Abstract:
In the past decade, gene- and cell-based therapies have been at the forefront of the biomedical revolution. Synthetic biology, the engineering discipline of building sophisticated “genetic software” to enable precise regulation of gene activities in living cells, has been a decisive success factor of these new therapies. Here, we discuss the core technologies and treatment strategies that have already gained approval for therapeutic applications in humans. We also review promising preclinical work that could either enhance the efficacy of existing treatment strategies or pave the way for new precision medicines to treat currently intractable human conditions.

Introduction:
Unlike small-molecular drugs or antibodies, cell-based therapies have the potential to sense various input signals and respond by initiating context-dependent treatment actions (Bailey and Maus, 2019; Lim and June, 2017). Although gene- and cell-based therapies have been seen as offering tremendous promise since the early days of recombinant DNA and virus technologies, they have only begun taking center stage in the pharmaceutical industry over the past decade (Ma et al., 2019; Naldini, 2015; Yla-Herttuala, 2019). Currently, regulatory approval of such therapies is accelerating the technological revolution in biotechnology and medicine (Mount et al., 2015), and these changes have the potential to produce tectonic shifts in the global economy and in society. For example, Glybera was released in the European market in 2012 as a gene therapy treatment designed to reverse lipoprotein lipase deficiency, but treatment costs of more
than 1 million USD per patient forced its withdrawal from the market a few years later (Yla-Herttuala, 2019). On the other hand, although official price tags for the recently approved chimeric antigen receptor T cell (CAR-T) anti-cancer therapies Kymriah (tisagenlecleucel) and Yescarta (axicabtagene ciloleucel) remain in a similar range, the National Health Service (NHS) in the UK has already made these drugs available to patients (Ilic et al., 2019), and Japan has also recently made Kymriah available under their national health insurance system (Ilic et al., 2019). Thus, these breakthrough technologies are becoming clinical realities.

At present, a clean technical distinction between the terms “gene therapy” and “cell-based therapy” remains elusive. Whereas the US Food and Drug Administration (FDA) defines gene therapy as an attempt to “modify or manipulate the expression of a gene or to alter the biological properties of living cells for therapeutic use”, the European Medicines Agency (EMA) describes it as “medicinal products administered as nucleic acids, lipid complexes, viruses, or genetically engineered micro-organisms for a therapeutic, prophylactic or diagnostic effect” (Ma et al., 2019). Cell therapies on the other hand, are based on patient-derived cells that are genetically modified \textit{ex vovo} and finally returned to the patient (Ma et al., 2019). However, in view of the increasing complexity of potential therapies that are already in (pre)clinical stages, we believe that the term “advanced therapy medicinal products (ATMPs)” introduced by EMA might be a more suitable description for all classes of “gene therapies”, “cell therapies” and “cell-based gene therapies” that have gained clinical consideration or approval (Table S1), and would also cover most next-generation precision medicines that will shape the pharmaceutical landscape in the future (Table S2). Therefore, we use this term in the present review.

\textbf{Treatment Approaches and Molecular Targets of Current ATMPs}
In principle, any ATMP therapy works by strategic manipulation of a patient’s immune tolerance, but an unbalanced intervention may result in severe adverse effects (Fig. 1). Autoimmune diseases represent a chronic state of compromised immune (self)-tolerance caused by premature T-cell activation against auto-antigens (Fig. 1A-i), while cancers result from excessive immune tolerance that has allowed tumor cells to evade timely elimination (Fig. 1A-ii) (Luo et al., 2016). Thus, therapies based on adoptive transfer of cytotoxic T lymphocytes (e.g. CAR-T cells) essentially focus on site-specific reduction of (self)-tolerance to cancer cells; specifically, activation of T-cell-mediated killing is engineered to no longer depend on the binding of native T-cell receptors (TCRs) to human leukocyte antigens (HLA) on antigen-presenting cells, but can be directly activated by tailored tumor-specific antigens (Fig. 1B-i) (Fesnak et al., 2016). In addition, some tumor cells evade leukocyte-mediated clearance by expressing immune checkpoint inhibitors (e.g. programmed cell death protein 1 (PD-1) or cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)) that block (co)stimulation of TCRs (Fig. 1A-ii). Thus, antibodies that selectively bind to PD-1 or CTLA-4 and block their binding to their cognate receptors on the T-cell have shown great clinical success in the treatment of many cancers (Brenner et al., 2018; Cassetta and Kitamura, 2018). Paradoxically, many ATMPs involve allogeneic and xenogeneic components that could trigger transgene immunogenicity upon implantation or infusion (Porteus, 2019). Stimulation of immune tolerance for the transplant occurs through antagonism of very same molecular targets used in adoptive T cell therapies, such as PD-1/CTLA-4 activation, TCR inhibition or secretion of immunomodulatory cytokines (e.g. TGF-β, IL-12, CXCL12 or CCL22) that trigger regulatory T-cell (Treg) differentiation (Fig. 1B-ii) (Luo et al., 2016). Therefore, the safety and efficacy profile of every ATMP depends directly on how selectively each therapy component suppresses or stimulates the various targets involved in the regulation of immune tolerance.
Similarly, ATMP therapies involving implantation of foreign materials (e.g. medical devices or encapsulated therapeutic cells) also need to overcome rejection mechanisms associated with immune clearance. Implanted biomaterials often trigger the host immune system to initiate a foreign body reaction, a “diverted” wound-healing process that ultimately forms a fibrotic capsule around the implanted device (Fig. 1B-iii) (Kastellorizios et al., 2015). Proinflammatory cytokines are secreted during the early phase of the foreign body reaction. The elevated cytokine level at the implantation site recruits leukocytes to the implantation site, activates macrophages, and attracts fibroblasts, which deposit collagen. The eventual formation of the fibrotic tissue triggers secretion of anti-inflammatory cytokines (e.g. IL-4, IL-10, IL-13 and TGF-β), angiogenesis, and the induction of immune (self)-tolerance through Tregs (Wick et al., 2013). Finally, the foreign body is tolerated by the host immune system as “self”; however, the fibrotic capsule reduces the permeability of the cell chamber and often compromises oxygen supply to and/or protein secretion from encapsulated cells (Evron et al., 2018; Faleo et al., 2017; Ludwig et al., 2012) (Fig. 1B-iii). This determines the lifetime of therapeutic implants in vivo. Antifibrotic strategies to extend this window mostly involve (a) co-delivery of immunosuppressants during the entire implantation period or (b) hydrophilic treatments of the implant surface to hinder immune cells from docking on the foreign material (Vegas et al., 2016a; Veiseh et al., 2015).

Technically, every ATMP can be described as the choice of an appropriate integration technology for ectopic overexpression of one or multiple therapeutic transgenes in a corresponding host cell. The transgene product determines the eventual mechanism of action of the treatment. The location of this gene integration process defines the ATMP approach; treatments of cells ex vivo prior to implantation are designated as conventional cell therapy approaches, whereas gene integration processes that occur directly in a patient’s living tissue in vivo are classed as gene therapy (Fig. 2). Therefore, ATMPs can be sufficiently characterized
by the gene integration technology (i.e. viral vectors, non-viral polymer shells or direct electroporation of the transgenic material), the type of host cell and site of gene integration (i.e. gene therapy or cell therapy), and the delivery strategy in vivo (local or systemic) (Table S1) (Ma et al., 2019; Porteus, 2019; Sheridan, 2011).

Part 1: ATMP therapies under clinical investigation

Cell therapy approaches (ex vivo cell production)

Medicinal products containing genetically modified cells:

To create long-lasting therapeutic effects, the encoding transgenes must be stably integrated into the host cell prior to implantation or infusion (Porteus, 2019). According to current EMA regulations, this kind of medicine may belong to the class of “medicinal products containing genetically modified cells” during clinical application (EMA/CAT/GTWP/671639/2008). Currently, retroviral vectors are the predominant technology for stable transgene integration (Table S1; Table S2). Retroviruses are single-stranded RNA viruses capable of accommodating up to 8 kb of transgene cargo, which randomly integrates into the genome of the dividing host cell upon transduction (Lundstrom, 2018). Lentiviruses belong to the family of retroviruses but have the capability to infect both dividing and nondividing cells, thus providing a broader target range (Sheridan, 2011). In contrast, transposase systems such as Sleeping Beauty or PiggyBac are efficient non-viral alternatives for random gene integration with higher packaging capacities (< 20 kb) (Grabundzija et al., 2010; Holstein et al., 2018). Although random gene integration processes bear substantial safety risks (Hacein-Bey-Abina et al., 2008; Marcucci et al., 2018; McCormack and Rabbits, 2004), these technologies can achieve high transgene expression levels (Bailey and Maus, 2019). In theory, current advances
in omics and gene-editing technologies could be applied to identify and correct erroneous gene disruption resulting from the integration process in individual cell clones.

**Cellular adoptive immunotherapies.** CAR-T cell therapies illustrate successful clinical applications of retroviral vectors. This type of immunotherapy is based on the collection of a patient’s T cells via leukapheresis, followed by an *ex vivo* manufacturing process that includes T cell expansion and transduction of a chimeric antigen receptor (CAR) before reinfusion of the engineered cells into the patient (Yip and Webster, 2018). The process from cell harvest to patient administration is highly dependent on the cell source and takes approximately 2 weeks. Particularly for autologous T cells, these resource-intensive procedures account for the high cost of such therapies (Locke et al., 2017; Valton et al., 2018; Zhao et al., 2018). The chimeric antigen receptor is an artificial T-cell receptor in which the extracellular TCR domain is substituted by a single-chain variable fragment (scFv), which allows antigen-specific and HLA-independent activation of intracellular signaling (**Fig. 3A**) (Cassetta and Kitamura, 2018; Chakravarti and Wong, 2015). For example, the CAR of the FDA-approved product Kymriah (tisagenlecleucel) for the treatment of acute B cell lymphoblastic leukaemia (ALL) is composed of an scFv against the B-cell lineage antigen CD19. Anti-CD19 is fused to the native TCR signaling domain CD137 (4-1BB) and the CD3ζ costimulatory domain (**Table S1**) (Vairy et al., 2018). Upon infusion of T-cells stably expressing this lentivirus-transduced CART19 (anti-CD19-CD137-CD3ζ), the genetically modified cells autonomously migrate through the body and bind to CD19-expressing tumors, where they trigger cell killing (**Fig. 1B-i**) (Maude et al., 2018; Prasad, 2018; Schuster et al., 2019). Similarly, the CAR of Yescarta (axicabtagene ciloleucel) and Tecartus (brexucabtagene autoleucel) also contains an scFv against CD19, but the CD3ζ is fused to a different costimulatory domain (CD28) (**Table S1**) (Brentjens et al., 2013; Wang et al., 2020). Retroviral transduction of this 19-28z CAR (anti-CD19-CD28-CD3ζ) generates CAR-T cells for the treatment of adult patients with refractory large B-cell lymphoma.
and mantle cell lymphoma, respectively (Table S1) (Locke et al., 2019; Neelapu et al., 2017; Park et al., 2018; Roberts et al., 2018). All these clinically approved CAR-T products (i.e., Kymriah, Yescarta and Tecartus) employ so-called “second-generation” CAR designs, where only one costimulatory domain (CD28 or CD137) precedes CD3ζ (Kowolik et al., 2006). In many clinical trials of “second-generation” CARs, suboptimal efficiency of T-cell activation must be compensated with elevated cell doses, and this can cause severe adverse effects such as cytokine release syndrome (CRS) (Fig. 3A-i) (Grupp et al., 2013; Kochenderfer et al., 2015; Lee et al., 2015; Maude et al., 2014; Porter et al., 2011). For instance, when the target-specific scFv domain was changed to B cell maturation antigen (BCMA) for the treatment of multiple myeloma (Table S1) (Friedman et al., 2018), only 16 out of 33 patients showed a response and more than 75% of the patients developed CRS (Raje et al., 2019). Similar problems have been observed for a variety of other treatments, such as in the use of HER2-specific CARs for the treatment of breast cancer (Table S1) (Liu et al., 2017; Sun et al., 2014). “Third-generation” CARs contain multiple costimulatory domains alongside CD3ζ (Fig. 3A) (Wang et al., 2007). Nevertheless, the possible safety risks, which have actually occurred in the clinic, remain similar to those of second-generation CARs (Table S1) (Karlsson et al., 2015; Morgan et al., 2010). Interestingly, an improved third-generation CAR engineered with an intracellular IL-15Rα domain instead of CD137 (153z; scFv-CD28-IL15Rα-CD3ζ) has decreased CRS incidences in patients (Table S1) (Nair et al., 2019).

In contrast to viral transduction technologies, gene correction of cells ex vivo using designer nucleases allows precision editing of any target DNA sequence at the single base-pair level (Porteus, 2019). Designer nucleases comprise zinc-finger nucleases (ZFN), transcription activator-like effector nucleases (TALEN) or CRISPR-associated (Cas) proteins capable of targeting any genomic sequence of interest by triggering site-specific double strand breaks (DSBs). Upon co-delivery of an appropriate donor template that contains a custom-designed
DNA sequence (the therapeutic transgene), the endogenous homology-directed repair (HDR) machinery can assist in fixing the DSB and simultaneously incorporating the therapeutic transgene into the targeted locus. This process enables site-specific correction, deletion or insertion of any genetic sequence (Porteus, 2019). Such strategies were applied to knock-out specific gene products of existing “off-the-shelf” CAR-T cells, such as disruption of PD-1 using CRISPR/Cas9 to achieve resistance to immune checkpoint inhibition (Rupp et al., 2017). This strategy was also used to engineer allogeneic CAR-T cells (also known as ALLO-CAR and UCART products), either by eliminating endogenous TCR components with ZFN (Provasi et al., 2012; Torikai et al., 2012), TALEN (Qasim et al., 2017), or CRISPR/Cas9 (Ren et al., 2017), or by deleting HLA-markers with ZFN (Torikai et al., 2013) (Fig. 3A-ii). Allogenic cell therapies have higher compliance and lower manufacturing costs, since both leukapheresis and patient-specific therapy designs can be avoided. These therapies are also extremely valuable for patients for whom leukapheresis would be technically too demanding or risky (e.g. with infants or severely ill people) (Ma et al., 2019).

Similar to allogeneic CAR-T, “universal” CAR-T allows the same batches of CAR-T cells to target different tumor antigens, thereby avoiding time- and resource-intensive reengineering of new CAR constructs when targeting a different antigen (Cho et al., 2018). To achieve this goal, an antibody-coupled T cell receptor (ACTR) system was engineered by replacing the scFv-domain of a second-generation CAR with the extracellular domain of CD16 (Table S1) (Kudo et al., 2014). Since CD16 binds to the constant fragment of antibodies with high affinity, the same ACTR-expressing T cells can be used to target different tumors by using different antibodies specific for different cell surface markers (Fig. 3A-ii). The activity of universal CAR-T cells is tunable and makes the dosing of CAR-T cells much safer (Si et al., 2018). Alternatively, tandem CARs (tanCAR) feature multiple scFvs on a single CAR moiety, increasing the number of antigens that can be recognized by the same CAR-T cell (Fig. 3A-i;
Table S1) (Grada et al., 2013; Hegde et al., 2019; Qin et al., 2018; Zah et al., 2016). Apart from the engineering of antigen-specific receptors and cells, killing efficacy and specificity have been other important parameters for CAR-T development. To amplify the local cytotoxic cell response in the tumor microenvironment, CAR-T cells were engineered to secrete immunostimulatory cytokines such as IL-12 either in a constitutive (Pegram et al., 2012) or NFAT-dependent manner (Zhang et al., 2015), based on the so-called TRUCK concept (T cells redirected for universal cytokine killing) (Fig. 3A-iii; Table S1). To increase the target specificity of CAR-T cell therapies, various “logic gate” principles have been designed that allow only a pre-defined combination of input signals to activate T-cell signaling (Fig. 3A-i).

For example, multiple CAR (multiCAR) systems use two (dual-CAR; (Kloss et al., 2013; Wilkie et al., 2012)) or three (tri-CAR; (Bielamowicz et al., 2018)) different CAR molecules to program AND-type expression logics. Intracellular TCR domains (e.g. CD3ζ) and costimulatory domains (e.g. CD28 or CD137) are either expressed alone or are separately linked to different scFv-domains. Thus, activation of multiCAR occurs only when all scFvs interact with their specific antigens at the same time (Fig. 3A-i). Also, co-expression of synthetic receptors for immune checkpoint inhibitors (e.g. PD-1 or CTLA4) alongside the CAR yields inhibitory CAR (iCAR) systems following an A AND NOT B logic (Fedorov et al., 2013) (Fig. 3A-i; Table S1; Table S2).

To explore cell-specific advantages of other leukocytes in adoptive tumor targeting and killing, some CAR systems have been tested in natural killer (NK) cells (Xia et al., 2019; Xu et al., 2019b), dendritic cells (DC) (Narayanan et al., 2011) and macrophages (Hung et al., 2018; Moyes et al., 2017) (Fig. 1B-i; Table S1). As compared with T cells, the isolation and engineering of NK cells are technically more demanding. However, CAR-NK cells can offer higher tumor-killing efficacies and greater safety in vivo (Xu et al., 2019b). Monocyte-derived macrophages on the other hand, are highly mobile and can penetrate deep tissues. Thus, CAR
macrophages (CAR-MA) are an excellent option for solid tumor clearance (Moyes et al., 2017).

Importantly, cellular adoptive immunotherapies using genetically modified leukocytes are not limited to the treatment of cancer. For example, HIV-specific CARs have been clinically investigated; CAR-T cells were engineered to target infected CD4+ T-cells through broadly neutralizing antibodies (bNAds) and are intended to attenuate HIV survival by induction of T-cell-mediated T-cell killing (Ali et al., 2016; Hale et al., 2017; Liu et al., 2016a) (Fig. 3A-iv; Table S1). Also, immunosuppressive Tregs were engineered to express CARs that trigger site-specific immune tolerance to alloantigens or foreign transplants (Elinav et al., 2009; MacDonald et al., 2016; Taylor et al., 2002) (Fig. 1A-i).

**Prosthetic Blood.** While the engineering of patient-specific leukocytes has focused mainly on cancer immunotherapy, cell therapy approaches using hematopoietic stem cells (HSCs) as the host cells are promising for the treatment of various inherited blood disorders. For example, β-haemoglobinopathies are characterized by impaired blood cells resulting from inborn gene deficiencies. The gene deficiencies manifest in abnormal cell phenotypes, and the blood cells are unable to fully carry out their intended function. Since HSCs can repopulate an entire hematopoietic system, expansion and permanent genetic modification of isolated CD34+ HSCs enable mass production of gene-corrected progenitor cells *ex vivo*, which differentiate into the appropriate cell type *in vivo* upon infusion (Fig. 3B). For example, the ATMP therapy Zynteglo is based on autologous CD34+ cells transduced with a lentiviral vector harboring the βA-T87Q-globin gene, and is approved for patients with transfusion-dependent β-thalassemia (Cavazzana-Calvo et al., 2010; Negre et al., 2016) (Table S1). Similarly, Strimvelis consists of autologous CD34+ cells transduced with a retroviral vector encoding a human adenosine deaminase (ADA) transgene (Table S1). It is approved for the treatment of severe combined immunodeficiency (SCID) due to ADA shortage (ADA-SCID) and was the first commercially available ATMP based on corrective *ex vivo* cell therapy (Schimmer and Breazzano, 2016;
Gene-correction of HSCs through random integration using viral vectors to achieve ectopic overexpression of the correct gene is reminiscent of manufacturing blood “prostheses” \((i.e.\) direct replacement of a defective organ with a functional one) \((\text{Fig. 3B-i})\). In contrast, targeted gene editing (also known as “genomic surgery”) using designer nucleases allows high-fidelity knock-in and knock-out of any set of (trans)genes almost at will \((\text{Bolukbasi et al., 2016})\) \((\text{Fig. 3B-ii})\). Blood prostheses created with such “gene editing” technologies may therefore be substantially safer for patients than their virally transduced counterparts. Driven by the clinical success of Zynteglo and Strimvelis, many blood prosthesis therapies are currently under clinical trial, including treatments intended for sickle cell disease and \(\beta\)-thalassaemia \((\text{Canver et al., 2015; DeWitt et al., 2016; Traxler et al., 2016; Wu et al., 2019; Xu et al., 2019a; Ye et al., 2016})\), X-linked severe combined immunodeficiency \((\text{Schirol et al., 2017})\), Wiskott-Aldrich syndrome \((\text{Ferrua et al., 2019})\) and chronic granuloma \((\text{De Ravin et al., 2017})\) \((\text{Table S1})\). In addition, blood prosthesis therapies have played a major role in the treatment of HIV-infected patients. In the clinic, ZFN-based deletion of the CCR5 and/or CXCR4 genes in patient-specific T-cells has resulted in markedly reduced virus proliferation rates \(\text{in vivo} \) upon transfusion \((\text{DiGiusto et al., 2016; Perez et al., 2008; Tebas et al., 2014; Voit et al., 2013})\) \((\text{Table S1})\). Blood prostheses can also effectively assist CAR-T therapies. In fact, on-target off-tumor adverse effects remain a critical risk factor in CAR-mediated cell therapies. In an elegant treatment approach for myeloid leukemia, CAR-T cells targeting the lineage-specific CD33 were co-administered with gene-edited HSCs in which CD33 had been deleted with the CRISPR/Cas9 system \((\text{Table S2})\) \((\text{Kim et al., 2018b})\). Even if anti-CD33 CAR-T cells could kill healthy CD33-expressing myeloid cells (on-target, off-tumor; \text{Fig. 3A-i}), new myeloid cells would repopulate from the gene-edited HSCs that were engineered to resist elimination by the CAR-T cells.
**Solid prostheses.** Apart from the engineering of blood prostheses, gene editing can also overcome various limitations of other cell therapies. In fact, *ex vivo* modification of somatic cells can similarly create “solid” prostheses (Fig. 3C). By definition, prostheses are devices that can complement or restore defective body functions in a seamless and automated manner. While physical prostheses (e.g. artificial organs or joints) are implanted at an appropriate body site to restore mechanical functions, solid biological prostheses are based on injection of cells at body sites where specific cell-cell contacts have been missing. For example, Invossa consists of human allogeneic chondrocytes transduced with TGF-β and is approved in Korea for the treatment of knee osteoarthritis (Table S1) (Evans et al., 2018; Kim et al., 2018a).

**Somatic cell therapies (without genetic intervention):**

Traditional organ and cell transplantations were the first kinds of solid prostheses developed in biomedicine. The majority of these approaches, including stem cell therapies, do not involve (any) genetic modification. Instead, the isolated host cells are expanded *ex vivo* to allow production of sufficient cell numbers for (re)-implantation, and these cells may optionally be treated with specific chemicals and/or growth factors to trigger epigenetic changes, such as differentiation. According to EMA guidelines, cell therapies of this kind that do not intentionally change the host cell’s genomic integrity should be registered as “somatic cell therapy medicinal products” (EMA/CAT/600280/2010). For example, Alofisel (darvadstrocel) is simply based on expansion of human allogeneic adipose-derived mesenchymal stem cells (ADSC) and was approved in the EU for treatment of complex perianal fistulas in patients with Crohn’s disease; single intralesional injections of 120 million ADSCs provided clinical remission rates of >50% at 1 year follow-up (Scott, 2018) (Table S1). Mesenchymal stem cell (MSC) treatments are generally accepted as safe and have enormous therapeutic potential, but clarification of the specific molecular mechanism accounting for the proposed treatment effect remains a major challenge during clinical investigations (Galipeau and Sensebe, 2018).
general, major mechanisms of actions of MSCs include (a) tissue regeneration through self-sufficient differentiation into specific cell types, (b) immunomodulation through tissue migration and local secretion of cytokines and exosomes or (c) cell-type-specific activation through direct cell contacts (Kabat et al., 2020). Stem cells are particularly attractive, because these multipotent cells have the potential to differentiate into various cell type to aid in tissue repair (Graf and Enver, 2009). For stem-cell-based medicines, the ideal therapeutic approach would comprise complete differentiation and purification of the therapeutically active cell type \textit{ex vivo} prior to implantation or injection into the patient (Mount et al., 2015). However, the main limiting factors are the technical challenge in identifying and isolating proper stem/progenitor cells, the scarcity of robust and scalable cultivation protocols, and the shortage of quality-assurance technologies capable of characterizing differentiation efficiency (Lipsitz et al., 2017). Therefore, various treatment strategies currently under clinical testing employ a semi-rational approach, which is based on implantation of progenitor cells into the patient in the expectation that full differentiation and maturation will eventually occur \textit{in vivo}. For treatment of glaucoma and other optic neuropathies for example, implantation-ready progenitor cells can be extracted from bone marrow (Park et al., 2014b; Tsai et al., 2000), or generated \textit{ex vivo} by treatment with specific inhibitors (Kinoshita et al., 2018) (Table S1).

\textbf{Metabolic prostheses.} Cell-based treatment strategies also play a central role in the treatment of type-1 diabetes, an autoimmune disorder characterized chronic hyperglycemia due to the loss of pancreatic β-cells (Sneddon et al., 2018). Although direct transplantation of allogeneic pancreatic islets remains the gold standard in terms of therapeutic efficacy (Latres et al., 2019), this approach either critically depends on timely availability of donors or requires life-long immunosuppression (Bertuzzi et al., 2018; Ricordi et al., 2016). To overcome these issues, transplantation of allogenic islets can be achieved through CRISPR/Cas9-mediated knockout of endogenous retrovirus genomes in porcine islet cells (Niu et al., 2017) and/or encapsulation
of islets into biocompatible and semipermeable cell chambers (macroencapsulation; (Barkai et al., 2013; Ludwig et al., 2013; Pepper et al., 2015)) or alginate beads (microencapsulation; (Bochenek et al., 2018; Duvivier-Kali et al., 2001; Jacobs-Tulleneers-Thevissen et al., 2013)) that afford xenograft tolerance (Fig. 1B-iii; Table S1). Encapsulation of β-like cells produced by differentiation of human embryonic stem cells (ES; (Pagliuca et al., 2014)) or induced pluripotent stem cells ex vivo (iPSC; (Rezania et al., 2014)) also has potential for diabetes treatment (Millman and Pagliuca, 2017). Encapsulated islets or β-like cells can be seen as “metabolic prostheses”, which are capable of restoring the function of a defective organ even if the implant itself is placed at a distant location in the body (Fig. 3C). Though, stem cell-based approaches have similar types of advantages and limitations as with allogenic islet transplantation. To achieve optimal glycemic control and xenograft lifetime, it is essential that encapsulated β-like cells already show a glucose-stimulated insulin secretion (GSIS) profile similar to that of clinical-grade human islets prior to implantation. For example, based on an earlier observation that pancreatic progenitor (PP) cells could differentiate more efficiently into glucose-responsive β-like cells in vivo than in vitro (Agulnick et al., 2015), a clinical trial driven by ViaCyte Inc. used an semi-rational strategy of implanting macroencapsulated PPs inside a cell chamber that was optimized for nutrient exchange and oxygen supply (Kumagai-Braesch et al., 2013; Robert et al., 2018) (Table S1). However, differentiation in vivo might be much slower than the fibrotic reaction triggered by the implant itself, which probably accounts for the limited therapeutic efficacy, in spite of good safety results (Latres et al., 2019) (Fig. 1B-iii). In contrast, ES-derived β-like cells microencapsulated in alginate-beads 1.5 mm in diameter could restore self-sufficient glycemic control over 174 days upon implantation into mice, and this is currently the most promising preclinical study of stem cell-based diabetes treatments (Vegas et al., 2016b) (Fig. 1F; Table S2).

Safety switches:
To avoid hyperactivity of therapeutic cells *in vivo*, one alternative to cell number titration is the engineering of safety switches. Safety switches allow orthogonal control compounds to block the activity of transgenic cells on demand, while operating in parallel with the therapeutic core program (Fig. 3D). Safety switches under clinical testing include the herpes simplex virus-thymidine kinase (HSV-TK) suicide gene system (Greco et al., 2015) and the inducible caspase 9 (iCasp9) system (Straathof et al., 2005). Both systems involve transgenes that encode drug-controlled initiators of apoptosis (Fig. 3D). Constitutively expressed HSV-TK only triggers controlled cell death when ganciclovir is administered exogenously; the iCasp9 suicide switch consists of two inactive caspase 9 monomers, which are activated by dimerization in the presence of a bio-inert rapamycin analogue, AP1903. In the clinic, the iCasp9 system has been incorporated into a GD-2-specific CAR construct for treatment of neuroblastoma (Table S1) (Chen et al., 2019; Louis et al., 2011).

*Gene therapy approaches (in vivo gene delivery)*:

Non-integrative transgene delivery:

**Oncolytic DNA Viruses.** Non-integrative gene delivery are analogous to transient transfection experiments in cell culture. Transgenes are carried into the host cell to allow constant expression until the episomal vector (i.e. non-chromosomal gene carrier) is eventually “out-diluted” through cell division (Sheridan, 2011) (Fig. 2). To achieve high-level expression, non-integrating DNA viruses such as adenovirus and the related AAV system (adenovirus-associated virus) are the most widely used transgene carriers (Lundstrom, 2018; Ma et al., 2019). While adenoviral vectors allow cargo sizes of up to 8.5 kb, AAVs are generally restricted to delivery of shorter transgenes of < 5.0 kb (Wang et al., 2019). In comparison to adenoviral vectors, AAVs are safer due to their lower immunogenic and oncogenic potentials. In fact, some gene-editing-based cell therapy approaches use non-replicative adenoviral and AAV vectors to
more efficiently deliver the donor DNA required for HDR-based repair (Fig. 3B-ii) (De Ravin et al., 2016; Dever et al., 2016; Hubbard et al., 2016; MacLeod et al., 2017; Sather et al., 2015). For gene therapy approaches, non-replicative adenovirus and AAV strains are very effective to transiently deliver therapeutic transgenes into the living tissue of patients (Sheridan, 2011). For example, Gendicine, arguably the first commercial gene therapy drug that was approved for cancer treatment, is based on a replication-deficient adenovirus encoding for transgenic p53 under the control of the Rous sarcoma virus promoter (Table S1) (Guo and Song, 2018). Local injection of the purified virus into the tumor triggers tumor-specific p53 expression and apoptosis (Fig. 4A; Table S1) (Peng, 2005). Thus, Gendicine can be classified either as a conventional gene delivery approach based on ectopic transgene overexpression, or as an oncolytic virus (Ma et al., 2019). According to a strict definition, however, an oncolytic virus must have the ability not only to infect and kill, but also to specifically replicate within the tumor (Kirk and Thorne, 2009). Based on a selectively replicating adenovirus vector ONYX-015 (Kirk et al., 1998), Oncocrine (H101) was therefore designed as an oncolytic virus for the treatment of nasopharyngeal carcinoma (Table S1) (Liang, 2018). Both Gendicine and Oncocrine have been approved in China for more than a decade, but were not approved by the US FDA due to a lack of sufficient information about the two therapies (Sheridan, 2011). Because wild-type adenovirus replication lyses its host cell before transmission, engineering oncolytic adenoviruses for tumor-specific expression of the replication factor E1A is a good strategy to program tumor-specific apoptosis (Fig. 4B). This approach has been tested in many clinical trials, including for treatment of bladder cancer (Ramesh et al., 2006) and neuroendocrine tumors (Leja et al., 2011) (Table S1).

In addition to adenoviruses, herpes simplex viruses (HSV) and poxviruses (e.g. vaccinia virus) are also employed for the engineering of oncolytic viruses. Both HSV and vaccinia virus are non-integrative DNA viruses with readily designable replication-specificity, but have higher
transgene packaging capacities (> 30 kb) than adenoviruses (Lundstrom, 2018). HSV can efficiently infect almost every type of host cell upon local injection (Watanabe and Goshima, 2018). In contrast, a major advantage of oncolytic poxviruses is their ability to travel systemically through the blood and migrate self-sufficiently to a specific tumor site (Kirn and Thorne, 2009) (Fig. 4B). The oncolytic HSV Imlygic (talimogene laherparepvec), engineered for tumor-specific replication and expression of the immune stimulatory protein GM-CSF, was approved by the US FDA for the treatment of melanoma in 2006 (Raman et al., 2019) (Table S1). In the following year, JX-594 (pexastimogene devacirepvec), an oncolytic poxvirus also capable of tumor-specific GM-CSF expression (Heo et al., 2013), received orphan drug designation from the US FDA and EMA for the treatment of hepatocellular carcinoma (Table S1).

**Gene correction by overexpression.** Currently, recombinant AAVs are exclusively used for “encapsidation” to deliver DNA into specific cell types and tissues, enabling ectopic (over)expression of therapeutic transgenes (Weinmann and Grimm, 2017) (Fig. 4A). In the clinics, AAV-based gene therapy plays an important role in the correction of gene defects within particular tissues and organs (Ma et al., 2019). For instance, patients with familial lipoprotein lipase deficiency (LPLD) have elevated levels of serum triglycerides, which may cause recurrent and life-threatening pancreatitis (Bryant et al., 2013). Glybera (alipogene tiparvovec) consisted of an AAV1 vector encoding for LPL and is injected intramuscularly, leading to transient expression and subsequent secretion of the lipase in the bloodstream (Scott, 2015) (Fig. 4A; Table S1). Similarly, Luxturna (voretigene neparvovec-rzyl) is an AAV2 vector genetically engineered to express the human retinal pigment epithelial-specific protein 65 kDa (RPE65) and is approved for the treatment of mutation-associated retinal dystrophy (Ameri, 2018) (Fig. 4A; Table S1). Intravenous administration of Zolgensma (onasemnogene abeparvovec-xioi), an AAV9 vector containing a transgene encoding the human survival motor
neuron (SMN) protein, can effectively target spinal motor neurons, neuronal and glial cells of the brain (Hoy, 2019) (Fig. 4A; Table S1). Despite questionable efficiency results due to lack of an available control group, Zolgensma is currently approved for the treatment of spinal muscular atrophy (SMA) in infants (Hoy, 2019). In the not-too-distant future, AAV-based gene therapy for treatment of haemophilia is also expected to enter the market (Chapin and Monahan, 2018; Nathwani et al., 2014).

AAV and adenoviruses generally provide high transgene expression levels owing to their very high delivery efficiencies (Sheridan, 2011). However, if weak constitutive levels are needed for a specific therapeutic goal, non-viral vectors such as plasmid DNA and oligonucleotides are preferred (Ma et al., 2019). For example, direct injection or electroporation of plasmid DNA into the target tissue provides transient gene expression in a similar manner to AAV-based gene correction (Fig. 4A). Currently approved plasmid therapies include Neovasculgen (cambiogenplasmid) for the treatment of peripheral vascular disease through constitutive VEGF165 expression (Deev et al., 2018), as well as Collategene (a constitutive hepatocyte growth factor (HGF) expression vector) for the treatment of critical limb ischemia (Suda et al., 2014) (Table S1). To achieve transient transgene overexpression with non-viral vectors, mRNA encoding the desired protein or purified protein can also be directly administered into the host cell by electroporation. In contrast to ectopic overexpression of a transgene to temporarily compensate for a gene deficiency, gene correction can also be achieved by transient knock-down of specific pathologic genes using custom-designed oligonucleotides (Fig. 4A). For example, the first therapies based on RNAi (Hoy, 2018) and antisense mRNA technology (Keam, 2018; Nandakumar et al., 2018; Ottesen, 2017; Paik and Duggan, 2019; Syed, 2016) have recently entered the market.

**Integrative gene delivery in vivo:**
Gene Editing. Intuitively, targeted gene editing technologies would seem to be preferable for gene correction. Indeed, many preclinical studies are currently testing the feasibility of using ZFN (Sharma et al., 2015), mega-nucleases (Wang et al., 2018b) and CRISPR/Cas9 (Ehrke-Schulz et al., 2017; Long et al., 2016; Nelson et al., 2016; Ran et al., 2015; Tabebordbar et al., 2016; Yang et al., 2016; Yin et al., 2016), aiming to emulate the treatment efficacies of AAV-based gene correction therapies. For example, one study involving intravenous delivery of the CRISPR/Cas9 system formulated in a lipid nanoparticle carrier resulted in >80% editing of the Pcsk9 gene in mouse liver (Fig. 4A; Table S2) (Yin et al., 2017). However, since this type of gene editing results in permanent modification of the host cell genome, this editing efficiency remains too low for clinical consideration. Although optimized Cas9 variants can reduce off-target indel rates to <0.1%, even an error rate of this order is still immense in the context of the size of the human genome - and the absence of evidence in editing mistakes is not equivalent to evidence of absence (Davidovich and Cech, 2015). Therefore, even though a recent study has performed a thorough risk analysis for CRISPR/Cas9-based therapeutic genome editing while developing a rational strategy for guide RNA selection (Scott and Zhang, 2017), we believe that gene correction with non-integrative methods such as AAV, adenoviruses, plasmid DNA and oligonucleotides (Fig. 4A) remains preferable for safety reasons.

Oncolytic RNA Viruses. Apart from gene correction, integrative gene delivery in vivo is clinically acceptable if the therapeutic goal is to ultimately kill the targeted host cell. For example, Rexin-G (Mx-dnG1) is a systemically injected RNA virus approved for the treatment of metastatic cancers (Gordon and Hall, 2009). Rexin-G is a non-replicative retrovirus selectively targeting tumor-specific signature (SIG) proteins through the cryptic collagen-binding motif in the extracellular matrix, and encodes a dominant-negative mutant of human cyclin G1 (Chawla et al., 2019) (Table S1). Mechanistically, it can be defined as a systemic variant of Gendicine, which kills cancer cells by tumor-specific induction of cell cycle arrest.
instead of selective replication (**Fig. 4A**). In contrast, Toca511 is a replicative retrovirus engineered on the basis of amphotrophic murine leukemia viruses (Perez et al., 2012). Upon systemic administration, this oncolytic RNA virus selectively infects tumor cells in the brain and expresses the yeast enzyme cytosine deaminase, which converts the prodrug 5-fluorocytosine (5-FC) into a potent anticancer drug, 5-fluorouracil (5-FU) (Huang et al., 2015) (**Table S2**). Toca511 is currently in a phase 2/3 clinical trial for malignant glioma and has shown promising interim results (Lawler et al., 2017). One shortcoming of systemically delivered oncolytic viruses is the strong transgene immunogenicity triggered by these particles. In reality, clinical strains of oncolytic viruses are indeed considered “too” safe as they are efficiently cleared from the circulation by the immune system, which severely limits treatment efficacy (Willmon et al., 2009). Therefore, the current view in the field of oncolytic virotherapy is that the oncolytic virus cannot (yet) be considered as a stand-alone therapy for cancer, but can be highly efficacious when used in combination with other more established therapeutic interventions (Bell and McFadden, 2014). As an interesting alternative to circumvent the host immune barrier, oncolytic viruses can be encapsulated prior to systemic injection, either within cationic liposomes or polymers as an immuno-isolating shell (Lundstrom, 2005; Naito et al., 2007), or encoded into autologous cells to create immuno-compatible virus carriers (Willmon et al., 2009). Cell-based virus carriers are often described a “Trojan horse” strategy that uses human cells for tumor-specific migration and site-specific stimulation of virus production and secretion (Bell and McFadden, 2014; Willmon et al., 2009). Various cell types are currently being investigated as oncolytic virus carriers in (pre)clinical tests, including mesenchymal stem cells (Duebgen et al., 2014; Mader et al., 2013), neural stem cells (Tobias et al., 2013), T-cells (Cole et al., 2005; Ilett et al., 2009) and cytokine-induced killer cells (Thorne et al., 2006) (**Table S1, Table S2**). Hence, oncolytic viruses delivered by cells to their target location in vivo may be classified as a cell therapy (**Fig. 2**).
ATMP therapies have already provided impressive treatment results. The current century has witnessed almost 50 ATMP approvals, not including a variety of mysterious therapies that are not scientifically proven but have nevertheless notoriously been commercialized in some parts of the world (Yla-Herttuala, 2019). In this context, Google has recently announced a new Healthcare and Medicines policy that prohibits digital advertisements of unproven ATMPs with no clear scientific basis (Ilic et al., 2019), and this may help to minimize both the risk to patients and the likelihood of concomitant reputational damage to properly established therapies. It seems clear that genuine ATMP therapies are at the forefront of a new technological revolution in biomedicine, and the scope for engineered precision medicines in the coming decades is truly enormous (Mount et al., 2015). Of course, issues remain to be overcome. For example, the spatiotemporal control capacity of ATMP mechanisms in vivo must be dramatically increased to further enhance treatment safety and efficacy. In CAR-T cell therapies, the need for this is illustrated in classical approaches for dose-efficacy titration, which was purely based on an empirical testing of different cell numbers for injection. Such testing in patients has resulted in serious adverse effects, with overdoses of constitutively active CAR-T cells in the bloodstream causing severe cytokine release syndromes, neurotoxicity and in some cases, even death (Grupp et al., 2013; Kochenderfer et al., 2015; Lee et al., 2015; Maude et al., 2014; Morgan et al., 2010; Porter et al., 2011). In current gene therapy approaches, transgene expression strengths in vivo are typically determined by the choice of the vector system. Delivery to the target tissue and the expression level of the very same transgene vary greatly, depending upon whether a viral transduction or a naked plasmid transfection is chosen (Ma et al., 2019) (Fig. 4A). Among ATMP therapies already in the clinic (Table S1), oncolytic viruses might have made the largest
step toward achieving programmable and context-dependent therapeutic activity. Key features, including target-specificity, replication and transgene expression, have all been subjected to application-specific bioengineering, providing an excellent illustration of the huge impact that this class of precision medicines is expected to have (Chawla et al., 2019; Heo et al., 2013; Leja et al., 2011; Mader et al., 2013; Perez et al., 2012; Ramesh et al., 2006) (Fig. 4B).

**Current Research Focuses:**

**CAR-T therapy.** Synthetic biology, by focusing on the design of genetic circuits to control human cell functions with high spatiotemporal precision, is a key driver of the progress of ATMP therapies (Esensten et al., 2017; Xie and Fussenegger, 2018). In essence, synthetic biology comprises any type of genetic intervention in any biological system that aims to improve the functionality of cells, tissues or organisms. Therefore, synthetic biology can advance the development of ATMP products in almost every respect. For example, the engineering of CARs in which the scFv-domain is replaced by membrane-tethered ligands (Brown et al., 2018) or designed ankyrin repeat proteins (DARPin; (Hammill et al., 2015)) has broadened the range of possible targets for CTL-mediated killing (Fig. 3A-iv). Indeed, chimeric autoantigen receptor (CAAR)-T cells using the autoantigen desmoglein 3 (Dsg3) as an extracellular targeting domain were recently engineered to target and eliminate autoreactive B lymphocytes in pemphigus vulgaris (PV) (Ellebrecht et al., 2016). While the CAR moiety is responsible for TCR-dependent effector functions, co-expression of TCR-independent cell membrane receptors can facilitate the migration of CAR-T cells to specific sites (Chakravarti and Wong, 2015). Not surprisingly, if CAR-T cells cannot access their target cells, it is very unlikely that they can effectively control tumor growth (Tokarew et al., 2019). Thus, examples for co-receptors guiding the trafficking of CAR-T cells to their assigned destinations in vivo include natural chemokine receptors (Craddock et al., 2010; Hong et al., 2011; Moon et al.,...
and synthetic receptors activated solely by a synthetic ligand (RASSLs) engineered to be specific for the small molecule clozapine-N-oxide (CNO) (Park et al., 2014a) (Fig. 3A-iii). In the tumor microenvironment, CAR-T-cell-mediated killing inherently involves the secretion of proinflammatory cytokines, such as IL-2 and IL-10 (Roybal et al., 2016b) (Fig. 1B-i). To create customized therapeutic programs, classical TRUCK approaches co-express effector proteins to add another killing program in parallel, such as IL-12 (Pegram et al., 2012; Zhang et al., 2015), anti-cancer proteins (Boice et al., 2016) or ion channels (Eil et al., 2016) (Fig. 3A-iii; Table S2). “Converter CARs” containing an extracellular binding domain for immunosuppressive ligands (e.g. IL-4 or PD-1) fused to a TCR-activating intracellular domain were also engineered and co-expressed in CAR-T cells to redirect immune tolerance (Leen et al., 2014; Liu et al., 2016b) (Fig. 3A-iv). To achieve full control over T-cell activity, back-to-back publications from the same laboratory presented a novel CAR architecture designed on the basis of the Notch receptor (Morsut et al., 2016; Roybal et al., 2016a; Roybal et al., 2016b) (Fig. 3A). With such synthetic Notch (synNotch) receptors, T-cell activation through scFv is no longer (exclusively) synchronized with endogenous TCR-signaling, but can directly initiate transcription of any user-defined therapeutic transgene from synthetic receptor-specific promoters (Roybal et al., 2016b). Importantly, this design could substantially improve the dynamics of conditional CAR-T cell activation (Fig. 4C). For example, whereas conventional multiCAR systems require the omnipresence of different CAR parts to achieve AND gate logics (Fig. 3A-i), synNotch receptors can spatiotemporally program CAR-T cell activation by only expressing the CAR construct when specific cell contacts are matched (Morsut et al., 2016; Roybal et al., 2016a) (Fig. 4C). As an alternative to synNotch receptors, scFvs can also be fused to intracellular domains of interleukin receptors to synchronize antigen-sensing with user-defined, JAK-STAT-mediated transgene transcription (Fig. 4C) (Kojima et al., 2017). To improve the sensitivity to soluble molecules, JAK-STAT-mediated transgene expression was synchronized with various dimerization-based EpoR activation strategies (Scheller et al., 2018) (Fig. 4C).
Safety switches. In order to achieve tight control over transgene expression, toggle switches based on mutually repressive transcription units are important tools in synthetic biology (Gardner et al., 2000; Kramer et al., 2004; Muller et al., 2013). Withdrawal of one repressive module results in activation of the other module and thus, the dynamic control is superior to that achievable with inducible ON/OFF promoters (Fig. 4B) (Xie and Fussenegger, 2018). For the treatment of hepatocellular carcinoma, a toggle switch was incorporated into an oncolytic adenovirus, allowing the tumor-specific α-fetoprotein (AFP) promoter to control toggle switch expression and a specific miRNA signature to trigger activation of adenovirus replication (through E1A expression) and immunomodulation (through co-expression of IL-2, GM-CSF and anti-PD-1 scFvs) (Huang et al., 2019) (Fig. 4B). Replicating oncolytic adenoviruses containing surface modifications were also engineered for the treatment of pancreatic cancer with enhanced tumor-specificity (Yamamoto et al., 2017). To achieve maximum spatiotemporal control of self-sufficient ATMP activity in vivo, the canonical approach would be the rational choice of most appropriate combinations of tissue-specific markers to create conditionally activated transgene responses (Fig. 3A-i; Fig. 4C). In clinical settings however, patient safety would be substantially increased if the physician could use specific control signals to intervene in the therapy at any point in time and thus react to emergent situations. Current versions of such safety switches tested in the clinic are based on trigger-controlled cell death using small-molecular drugs to abort the treatment (Chen et al., 2019; Greco et al., 2015; Louis et al., 2011; Straathof et al., 2005) (Fig. 3D). However, future safety switches could use inducible transgene expression systems controlled by traceless, orthogonal and biocompatible trigger compounds, which would prevent the irreversible elimination of “expensive” ATMP products in vivo (Caliendo et al., 2019). For example, intracellular TCR signaling has been engineered to depend on drug-inducible protein dimerization in split CARs, which permit T cell activation only if an exogenous drug is present (Wu et al., 2015). Additionally, expanding
the “universal” CAR-T cell principle to accept multiple input signals renders CAR-T cell activation dependent on different combinations of exogenously applied adaptor molecules (Cho et al., 2018). In this “split, universal and programmable” (SUPRA) CAR system, the extracellular domain of CAR consists of a leucine zipper, which acts as a protein tether. Activation of SUPRA CAR depends on bi-specific adaptors simultaneously binding a target antigen through scFv and the tether through leucine zipper interactions (Cho et al., 2018) (Fig. 3A-i). By using different combinations of adaptor molecules, which are either competing for the same SUPRA CAR or simultaneously activating multiple SUPRA CARs in parallel, the same CAR-T cells can be pre-programmed to respond to different user-defined therapeutic contexts following specific antigen recognition logics (Cho et al., 2018) (Fig. 3A-i). By synchronizing CAR expression with activation of the mechanosensitive piezo channel through intracellular calcium signaling, CAR-T activity may be remote-controlled with traceless and non-invasive ultrasound waves (Pan et al., 2018) (Fig. 3D).

**Metabolic prostheses.** An ideal ATMP therapy must be safe, efficient, easy to manufacture and easy to administer (Brenner et al., 2018; Lipsitz et al., 2017). Scientifically, implantation of encapsulated stem cells-derived β-cells is considered the optimal solution for diabetes treatment (Sneddon et al., 2018) (Fig. 3C). However, the clinical success of this strategy is primarily constrained by practical issues. First, β-cell differentiation using chemical cultivation protocols requires embryonic stem cells as the source material (Pagliuca et al., 2014; Vegas et al., 2016b), which not only raises ethical and safety concerns when applied to humans, but also complicates production on a commercial scale (Rostovskaya et al., 2015). iPSCs produced from somatic tissues can also be used as source materials, but show much lower differentiation efficiency (Rezania et al., 2014). To improve the differentiation efficiency, a synthetic biology approach based on transient transfection of lineage-specific gene circuits was introduced, and afforded unprecedented production yields of iPSCs-derived β-like cells. This success was achieved by
the implementation of time- and context-dependent transcription factors, which regulate the expression of proteins involved in endogenous cell fate (Saxena et al., 2016b). However, a further hurdle facing stem-cell-based diabetes therapies is the high cost of production and quality assurance (Lipsitz et al., 2017). Therapeutically active batches of β-cells cannot be cryopreserved for re-use. In addition, technologies that can measure and standardize the quality of individual differentiation-based production runs are not yet available (Carcamo-Orive et al., 2016; Tapia and Scholer, 2016). Consequently, manufacture of stem cell-derived cell therapies is time- and resource-demanding; typically, several months are required to produce sufficient cell numbers for implantation (Rostovskaya et al., 2015). Therefore, functional mimetics of β-cells that pragmatically combine therapeutic efficacy and commercial applicability are attractive alternatives for cell-based diabetes therapy (Strzyz, 2017). For example, by using synthetic gene circuits mediating depolarization-dependent calcium entry and calcium-specific gene expression, any glycolytic human cell can be reprogrammed for glucose-dependent insulin secretion (Xie et al., 2016). These engineered cells, also known as β-cell-mimetic designer cells, have the potential to be cost-effectively produced, expanded and freeze-stored according to GMP regulations for biopharmaceutical manufacturing; these are critical features for commercialization (Fig. 3C).

By using different synthetic gene circuits encoding for other sense-and-respond therapeutic programs, metabolic prostheses can be principally engineered for self-sufficient and closed-loop control of any metabolic disorder, such as insulin resistance (Ye et al., 2017), obesity (Rossger et al., 2013b), gouty arthritis (Kemmer et al., 2010), hypertension (Rossger et al., 2013a), Grave’s disease (Saxena et al., 2016a), bacterial infections (Liu et al., 2018) or psoriasis (Schukur et al., 2015) (Fig. 3C). Furthermore, open-loop systems that allow exogenous control compounds to potentially act as safety switches have also been engineered (Bai et al., 2019; Wang et al., 2015; Yin et al., 2019). For example, one study used optogenetics to provide
communication between electronic devices and biological systems (Shao et al., 2017) (Fig. 3D). Specifically, human cells engineered for far-red-light-dependent transgene expression were controlled by an electronic microprocessor that coordinated LED illumination upon reception of specific wireless signals. This allows portable electronics, such as smartphones, to regulate gene expression, representing the ultimate level of traceless, non-invasive and long-distance remote control of cell activities.

**Therapeutic Bacteria.** Bacteria are also a powerful host cell type for the design of next-generation ATMPs. For example, engineered probiotic strains, such as *Lactococcus lactis*, can be programmed to attach to the mammalian gastrointestinal tract and trigger site-specific attenuation of *Vibrio cholerae* infections upon oral ingestion (Mao et al., 2018) (Fig. 4D). In contrast, several bacterial species, such as *E. coli* (Chowdhury et al., 2019; Geller et al., 2017; Ho et al., 2018) or *Salmonella* (Din et al., 2016; Swofford et al., 2015; Xu et al., 2014), preferentially colonize and proliferate in hypoxic and immune-privileged tumor microenvironments upon systemic administration (Fig. 4D). Therefore, the engineering of tumor-trophic bacterial strains to contain tumor-specific killing programs offers a novel and promising alternative for cancer therapies. In essence, ATMP therapies based on CAR-transgenic leukocytes (Fig. 3A), oncolytic viruses (Fig. 4B) and tumor-trophic bacteria (Fig. 4D) all rely on pre-programmed targeting of cancer cells *in vivo* through unique cell type- and species-specific properties, while encoding for similar therapeutic effects. For example, most cancer cells overexpress the cell surface marker CD47, which binds to and inactivates macrophages in the tumor microenvironment (Bailey and Maus, 2019) (Fig. 1A-ii). To overcome this survival strategy of cancer cells, the CD47 targets of macrophages can be deleted by CRISPR/Cas9-based gene editing (Ray et al., 2018). Such deletion of CD47 targets may enhance the tumor clearance capabilities of CAR-carrying macrophages (Moyes et al., 2017). Alternatively, bacteria can also be programmed for tumor-specific CD47 inhibition.
(Chowdhury et al., 2019). For instance, non-pathogenic *E. coli* were engineered to self-sufficiently invade tumors and locally mediate density-dependent control of cell lysis and drug release. During tumor-specific proliferation, these bacteria constantly produce a quorum signal AHL and an anti-CD47 nanobody. Upon exceeding a critical cell density, AHL triggers the expression of bacterial suicide genes, resulting in pulse-like induction and amplification of macrophage-mediated immune responses while attenuating CD47-mediated immune tolerance (Fig. 4D) (Chowdhury et al., 2019).

Conclusions:
In conclusion, advances in synthetic biology have the potential not only to improve existing ATMP therapies, but also to create novel therapeutic opportunities. It is not going too far to say that cell-based immunotherapies, oncolytic viruses and therapeutic bacteria might form the next three pillars of cancer therapy. Each strategy allows disease-specific programming of sophisticated sense-and-respond functions almost at will, but may differ in the ability to penetrate deep tissues due to differences in cell and particle sizes. Therefore, an ideal treatment strategy might not exist by default, as each therapy would require a rational choice of the most suitable combination of drug targets, therapeutic transgenes, vectors and administration routes (Table S2) according to disease-specific conditions. In this context, enormous progress might be achieved by using oncolytic viruses in combination with gene circuit therapies – a novel class of medicines using synthetic interconnected gene switches to compute the malignancy of individual cell populations (Liu et al., 2014; Xie et al., 2011). At present, the clinical utility of such “therapeutic biocomputers” is limited by the delivery efficiency of large-scale gene circuits into specific cells *in vivo*. Recently, a lentiviral transduction strategy has shown promising preclinical results (Nissim et al., 2017) (Fig. 4B). Novel vector systems enabling the delivery of therapeutic transgenes with designable target specificities, such as phages, are also under active development (Lemire et al., 2018).
When translating innovative synthetic-biology-inspired ATMP therapies to the clinic, the complexity of therapeutic transgenes may present the greatest uncertainty. Gene- and cell-therapies engineered to contain large amounts of foreign molecules potentially increase the level of transgene immunogenicity in the patient (Fig. 1B-ii). Therefore, selecting human-derived components when designing complicated gene circuits is advantageous, as was demonstrated in a recent report on designer cell-based therapy for diabetes and muscle atrophy (Bai et al., 2019). Apart from the therapeutic transgene, the gene delivery vectors and administration routes used by next-generation ATMP products (Table S2) have remained largely identical to those already submitted for regulatory approval and/or approved for clinical use (Table S1). Therefore, currently approved ATMP products provide an extensive pool of clinical data and experience that should be helpful to successfully translate novel therapies to the clinic. For example, designer-cell-based therapies for diabetes essentially use the same cell encapsulation techniques as used for allogenic islets and stem-cell-derived β-cells (Fig. 3C). Alginate beads have also been optimized for the encapsulation of bacteria, showcasing the possibility of preventing cell leakage when implanted into humans (Li et al., 2017). However, therapies based on encapsulated mammalian cells must overcome issues related to foreign body responses and fibrosis, which will determine the lifetime of each implantation therapy. For clinical applications, the therapeutic transgenes of designer cells can be effectively integrated into mesenchymal stem cells (MSC) using the Sleeping Beauty transposase system (Wang et al., 2018a; Xie et al., 2016; Ye et al., 2017). Both MSC-derived transplants (e.g. Alofisel) and the Sleeping Beauty technology (Kebriaei et al., 2016) are already used in clinical applications. Most recently, another designer cell-based therapy showed promising safety and efficacy results in non-human primates upon implantation of $1.5 \times 10^8$ microencapsulated cells (Yin et al., 2019), providing clues to clinically applicable cell doses (Kabat et al., 2020; Scott and Zhang, 2017; Sheridan, 2011). Thus, all the technical cornerstones of designer cell-based...
therapies have been indirectly proven safe through various independent studies, paving the way for first-in-class clinical trials in humans. In theory, the majority of synthetic biology-inspired ATMPs are already eligible for initiation of clinical trials. However, regulatory approval for registration of a clinical trial does not automatically flow from scientifically valid studies (Chari et al., 2018); for example, animal models cannot always sufficiently predict all clinically relevant information on safety and efficacy (Porteus, 2019). Thus, careful design of clinical trials is essential to address the regulatory and ethical challenges of novel ATMP therapies (Scott and Zhang, 2017). Constant (self-)education through exploration of the academic literature, compliance with current regulatory and ethical guidelines, and careful monitoring during commercialization are all imperative to anticipate potential adverse events that may be encountered by patients, in addition to post-marketing monitoring to ensure that if unexpected events do occur, they are picked up as early as possible (Kabat et al., 2020; Scott and Zhang, 2017; Sheridan, 2011).
Figure Legends:

Figure 1. Treatment strategies and molecular targets of ATMPs. (A) Endogenous (im)balances of immune tolerance exemplified by (i) autoimmune diseases and (ii) cancer progression. (B) Consequences of different therapeutic interventions for immune tolerance, including (i) cellular adoptive immunotherapies, (ii) transgenic ATMPs and (iii) treatments based on implantation of encapsulated cells. Left: Molecular mechanisms stimulating immune tolerance (avoiding immune clearance). Right: Molecular mechanisms stimulating immune clearance (suppressing immune tolerance).

Figure 2. Cell therapy and gene therapy products using ATMPs. Cell- and gene therapy approaches either use non-viral materials (naked plasmids, oligonucleotides or proteins or materials formulated in cationic polymer shells or lipid particles) or viral transgene carriers (non-integrative DNA viruses such as adenoviruses or AAV or integrative RNA viruses such as lentivirus or retrovirus) to integrate one or multiple therapeutic transgenes into host cells. In gene therapy approaches, this integration occurs directly in the patient following injection of the appropriate transgene carrier into the tissue. In cell therapy approaches, this integration occurs ex vivo in cells isolated from the patient (autologous) or derived from a donor (allogeneic). Therapeutic cells (genetically modified cells or somatic cell therapies) can be directly injected into the patient or encapsulated into a semipermeable biocompatible device to provide immune-isolation and nutrient exchange. While retroviruses and transposases trigger random transgene integration (into multiple and different genomic loci), designer nucleases such as ZFN/TALEN/Cas9 enable targeted transgene integration (into known genomic loci). For safety reasons, gene therapy approaches should prefer the use of non-integrative transgene carriers. To facilitate commercialization, therapeutically active batches of cell therapy products should be compatible with cryopreservation methods.
Figure 3. Clinically eligible cell therapy approaches. (A) Evolution of chimeric antigen receptors (CAR). Most CAR-transgenic T-cell lines (CAR-T) were produced by retro/lentiviral transduction. First-generation CARs (1G) consist of an scFv-antibody fused to the native T-cell receptor (TCR) signaling domain CD3ζ, and therefore require co-stimulatory domains such as CD28, CD137 (4-1BB) or IL15Rα on other receptors for full activation. Second-generation CARs (2G) integrate a co-stimulatory domain into the receptor architecture, while third-generation CARs comprise two co-stimulatory domains. The scFv-domain can also be replaced with antigens such as CD16 (to design universal CAR-Ts), ligands such as IL13 (to target cognate cell-surface receptors), DARPins (to target almost any protein of interest) or PD-1-receptors (to create converter CARs that transform the inhibitory effect of PD-1 into activating signals). Using the synNotch concept, scFv-binding can be synchronized with expression of any therapeutic transgene (see Fig. 4C). Current CAR-T research primarily focuses on (i) reduction of adverse effects related to on-target off-tumor toxicity, (ii) creation of allogeneic and/or universal CAR-T products to avoid patient- and/or tumor-specific re-engineering of new CAR T-cells, (iii) increasing CAR-T efficacy in terms of killing capacity and migration efficiency and (iv) broadening of the portfolio and types of possible CAR-T targets. (B) Blood replacement therapies (prosthetic blood). Defective blood cells caused by inherited genetic diseases can be “washed away” by infusions of ex-vivo-corrected CD34+ hematopoietic stem cells (HSC) that can differentiate into the appropriate cell type in vivo. (i) Currently approved gene correction strategies use retroviral vectors to restore the expression of missing genes. (ii) In the future, gene-editing technologies based on designer nucleases could directly correct genomic sequences through site-specific DNA repair. (C) Cell-based prostheses. Prostheses complement or restore defective body functions in a seamless and automated manner. Physical prostheses are implanted at the appropriate body site to restore mechanical processes. Solid prostheses are based on injection of cells (such as MSC) at body
sites where specific cell-cell contact is missing. By constantly coordinating diagnosis (detection of disease markers) with treatment (synchronized production and secretion of therapeutic proteins), encapsulated cells also provide prosthetic functions by restoring metabolic balances of nutrients and hormones. For the treatment of diabetes, impaired metabolic function of glucose-dependent insulin production can be restored with stem-cell-derived β-cells or β-cell-mimetic designer cells. Because designer cells use gene circuits to program any type of sense-and-respond behavior, this strategy can be applied in principle to any metabolic disease. (D) Safety switches in ATMP therapies and synthetic biology. Safety switches allow orthogonal control compounds to abort or resume the activity of transgenic cells at any point in time, and operate in parallel with the therapeutic core program. Early generations of safety switches are based on drug-induced apoptosis to eliminate genetically modified cells in vivo on demand. Current and future generations of safety switches are based on trigger-inducible cell activity using soluble compounds (such as antibody mixes in SUPRA CAR; see Fig. 3A) or traceless remote signals such as ultrasound and light. Optogenetics involves regulation of cell activities with light.

Figure 4. Gene therapies and future prospects for ATMPs. (A) Gene correction. Inherited genetic disorders in somatic tissues can be corrected by ectopic overexpression of the missing gene or by transient knock-down of pathologic genes through RNA interference (RNAi) or antisense mRNAs. Non-integrative overexpression is based on episomal expression from viral (adenovirus or AAV; stronger) or non-viral transgene carriers (plasmid DNA; weaker), which transiently override defective or deficient genomic expression. Integrative approaches enabling genome correction using designer nucleases are also possible but must overcome off-target editing events. (B) Oncolytic virotherapy. Adenoviruses lyse the infected host cell upon replication. Engineering oncolytic adenoviruses for tumor-specific replication (through E1A expression) enables tumor-selective re-infection and killing. Oncolytic herpes simplex viruses
(HSV) and poxviruses are DNA viruses with higher packaging capacities for therapeutic transgenes. Poxviruses and the RNA virus Toca511 are suitable for systemic administration. Lentiviruses can also be used for local delivery of oncolytic transgenes into tumors. (C) Receptor-mediated transcription. Activation of SynNotch receptors triggers proteolytic cleavage of the transmembrane domain, resulting in nuclear translocation of synthetic transcription factors and initiation of transgene expression from cognate-specific promoters. Transcription of therapeutic transgenes can also be synchronized with endogenous signaling pathways such as JAK/STAT and MAPK. Stimulation of these pathways by custom-designed cell-surface receptor actions activates synthetic promoters engineered to contain signaling-specific response elements. (D) Therapeutic bacteria. Lactobacteria can be engineered to sense infection markers in the gastrointestinal system. Upon oral ingestion, genetically engineered bacteria self-sufficiently migrate to the gut and produce specific reporter signals that can be measured in feces. Some bacterial strains such as E. coli or Salmonella preferentially colonize and proliferate in hypoxic and immune-privileged tumor microenvironments upon systemic administration. Engineering these bacteria to carry gene circuits that trigger population-density-dependent cell lysis and drug release is a promising alternative for cancer therapy.

Supplementary Material:

Table S1 - Overview of advanced therapy medicinal products (ATMPs) in the clinics.
Table S2 - Overview of advanced therapy medicinal products (ATMPs) under preclinical investigation

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Figure Legend List:

**Figure 1:** Treatment strategies and molecular targets of ATMPs.
**Figure 2:** Cell therapy and gene therapy products using ATMPs.
**Figure 3:** Clinically eligible cell therapy approaches. (A) Evolution of chimeric antigen receptors (CAR).
**Figure 4:** Gene therapies and future prospects for ATMPs.
A) Endogenous Processes
Excessive Immune Tolerance → Homeostasis → Excessive Immune Clearance

HIV-infected T-cell → T-cell Activation and Differentiation
Cytotoxic T-cell (activated) → T-cells → APC

I. Autoimmunity
v Self-antigens → Immune Attack

II. Cancer
v T-cells → Macrophages → NK-cells

B) Therapeutic Interventions
Increasing Immune Tolerance → Increasing Immune Clearance

i. Transgenic ATMPs
ii. Encapsulated Cells
iii. Adoptive Immunotherapies

Figure 1

209x296mm (300 x 300 DPI)
