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In Vivo T Cell-Targeting Nanoparticle Drug Delivery Systems: Considerations for Rational Design

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ABSTRACT

T cells play an important role in immunity and repair and are implicated in diseases, including blood cancers, viral infections, and inflammation, making them attractive targets for the treatment and prevention of diseases. Over recent years, the advent of nanomedicine has shown an increase in studies that use nanoparticles as carriers to deliver therapeutic cargo to T cells for *ex vivo* and *in vivo* applications. Nanoparticle-based delivery has several advantages, including the ability to load and protect a variety of drugs, control drug release, improve drug pharmacokinetics and biodistribution, and site- or cell-specific targeting. However, the delivery of nanoparticles to T cells remains a major technological challenge, which is primarily due to the nonphagocytic nature of T cells. In this review, we discuss the physiological barriers to effective T cell targeting and describe the different approaches used to deliver cargo-loaded nanoparticles to T cells for the treatment of disease such as T cell lymphoma and human immunodeficiency virus (HIV). In particular, engineering strategies aiming to improve nanoparticle internalization by T cells, including ligand-based targeting, will be highlighted. These nanoparticle engineering approaches are expected to inspire the development of effective nanomaterials that can target or manipulate the function of T cells for the treatment of T cell-related diseases.

KEYWORDS nanomedicine, lymphocyte, cancer, infection, immunotherapy, receptor-mediated endocytosis, stealth, endosomal escape, tissue-resident, immune evasion

VOCABULARY SECTION

T cell differentiation: A process in which an immature (naïve) T cell changes into a more specialized T cell subtype after antigenic stimulation. Various stages of differentiation are

characterized by the expression of particular clusters of differentiation molecules on the cell surface.

Immunotherapy: A type of biological treatment that boosts or suppresses (parts of) the immune system to better respond to infection or disease.

Receptor-mediated endocytosis: The process of internalizing cargo, such as protein, metabolite, virus, nanoparticle, by cells upon binding of a surface receptor. This process is also referred to as clathrin-mediated endocytosis.

Stealth: A property ascribed to particles that exhibit reduced immune recognition, typically because of a surface coating that prevents binding of specific proteins that mark foreign materials for immune clearance (opsonization). Stealth particles exhibit low (*i.e.*, nonspecific) cellular association and improved bioavailability *in vivo.*

Protein corona: A protein layer formed on a particle surface through molecular adsorption of proteins after introduction of the particles in a biological medium. The protein corona plays an important role in the surface properties of a particle and subsequent bio–nano interactions.

Biocompatibility: A property of a (nano)material that characterizes its ability to perform its desired function in living tissues without eliciting undesirable side effects such as immune activation, toxicity, or other injury.

The human immune system consists of specialized cells, tissues, and organs that together provide a defense mechanism against a wide variety of pathogens or injury.[1] T cells play an essential role in the adaptive arm of the immune system and can be found throughout the body. T cell precursors originate from the bone marrow and migrate to the thymus where they mature before entering the periphery. During maturation, all T cells develop signatory T cell markers including a T cell

receptor, coreceptor signaling domain CD3, and the lesser-known costimulatory receptor CD2.[2] In addition, T cells will differentiate into either of the two main subtypes: CD4 helper T cells or CD8 cytotoxic T cells.[1,2] Naïve CD4 and CD8 T cells patrol the bloodstream and lymphoid organs, such as the lymph nodes, spleen, or mucosa-associated lymphoid tissues (Figure 1a), until they encounter their specific antigen. CD4 T cells recognize antigens presented on major histocompatibility complex class II (MHC-II) by antigen-presenting cells such as dendritic cells[3] (Figure 1b). Upon activation, CD4 T cells can further differentiate into pro- or anti-inflammatory helper T cell subtypes (Th1/Th17 and Th2/regulatory T cells, respectively), which are characterized by the secretion of different cytokine signatures.[1] These cytokines modulate the behavior of other immune cells to thereby amplify or dampen the immune response. Furthermore, these cytokines can attract other immune cells, including more T cells, to home to the site of inflammation.[4] In contrast, CD8 T cells recognize antigens presented by infected or malignant cells *via* MHC-I and upon activation excrete cytotoxic granules (granzymes, perforin, interferon-γ (IFN-γ), and other cytokines) to the target cell[5] (Figure 1b). Once activated, both proliferating CD4 and CD8 effector T cells infiltrate various non-lymphoid tissues during an inflammatory response and they can continue to reside as tissue-resident memory cells (Figure 1a). Together, over the course of an inflammatory response, many T cell subtypes arise, with each subtype exhibiting a specific function and receptor expression pattern and being individually implicated in disease.[1]

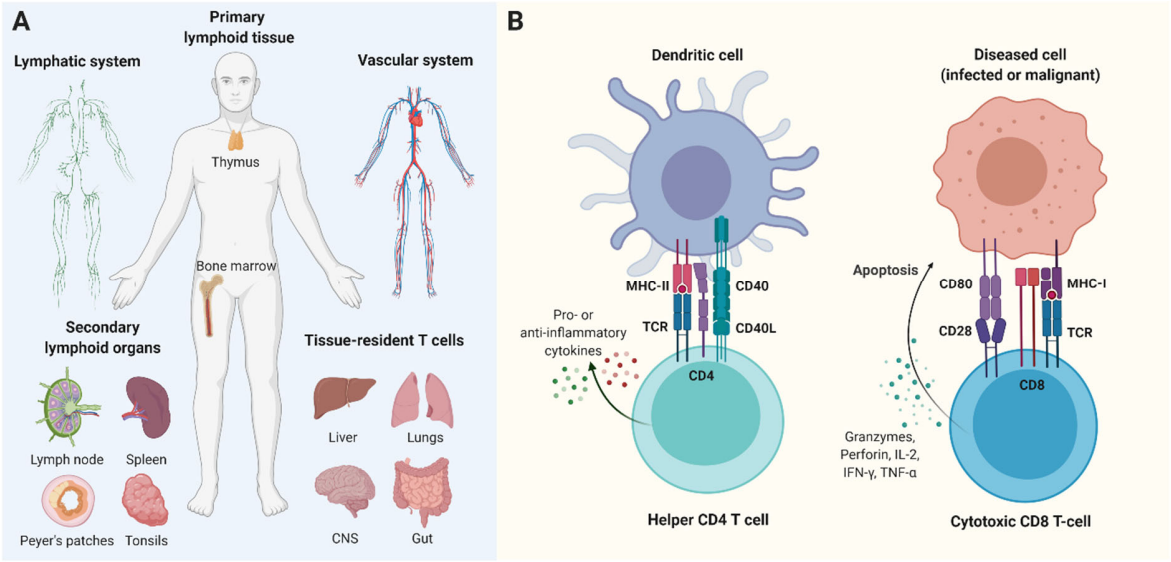

Figure 1. T cell origin, distribution, and function. (A) T cells are derived from primary lymphoid tissues and patrol the lymphatic system, vascular system, and secondary lymphoid organs until they encounter their cognate antigen. Additionally, T cells can enter non-lymphoid tissues to facilitate an inflammatory response or reside in these tissues as tissue-resident memory cells. (B) T cells are divided into two major categories. CD4 T cells, or helper T cells, recognize antigens presented by MHC-II on antigen-presenting cells, such as dendritic cells, and regulate other immune cells. CD8 T cells, or cytotoxic T cells, recognize antigens presented by MHC-I on diseased or infected cells and release toxins to induce apoptosis of target cells. CD, cluster of differentiation; CNS, central nervous system; IFN, interferon; IL, interleukin; MHC, major histocompatibility complex; TCR, T cell receptor; TNF, tumour necrosis factor. Created with BioRender.com.

T cells are of significant interest for the development of a range of immunotherapies.[6] These therapies can aim to deplete specific pro- or anti-inflammatory subsets of T cells,[7–9] skew the differentiation of naïve T cells into one of these subsets,[10–12] or use T cells as drug carriers to the site of disease.[13–15] Furthermore, immune checkpoint blockade has gained significant interest as a

strategy to boost T cell responses to diseased or infected cells.[16] Advances in gene transfer and genome-editing technologies have led to the development of chimeric antigen receptor (CAR)-T cell therapy, in which T cells are genetically engineered *ex vivo.* Following reinfusion (adoptive transfer), these cells can seek and infiltrate the tumor microenvironment or target infected or auto-antibody-producing cells.[6,17–19] Moreover, T cells are direct subject of pathology in the form of T cell lymphocytic leukemia, T cell lymphoma, and human immunodeficiency virus (HIV) infection.[20–23]

In each of these medical indications, significant therapeutic challenges exist, in which regard targeted delivery of therapeutic agents to T cells could have clinical relevance and potential (Figure 2). In brief, the solutions that delivery vehicles can provide are twofold. Firstly, targeted drug formulations can enhance the therapeutic index, *i.e.*, by increasing drug delivery to target cells or tissues while reducing off-target toxicity. This strategy could benefit chemotherapeutic interventions in cancers, including T cell leukemia and lymphoma, which suffer from the common side effects of chemotherapy as healthy rapidly dividing cells are eradicated in addition to the cancer cells.[14,24] Similarly, strategies that are aimed at achieving HIV remission off antiretroviral treatment are hindered by off-target effects of the therapeutic agents currently studied.[25] As such, targeted delivery to CD4 T cells, the main HIV reservoir, or an even more specific population of HIV-infected cells can potentially contribute to finding a cure. Reducing off-target toxicity can also progress gene silencing and immunotherapeutic strategies, *e.g.*, efforts to silence cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), an immune checkpoint inhibitor, *via* targeted delivery of small-interfering ribonucleic acid (siRNA) against CTLA-4 to restore antitumor functions.[26,27] Furthermore, the reduced off-target effect can potentially warrant the use of more potent therapeutics such as diphtheria toxin or ricin A. Targeted delivery of these pro-apoptotic drugs has

been suggested in the context of depleting HIV-infected CD4 T cells or depleting suppressive regulatory T cell subsets for immunotherapeutic purposes.[9,28] Current strategies for *in vivo* drug targeting to T cells include the use of immunotoxins, that is, targeting moieties directly conjugated to the drug or toxin. Denileukin diftitox (Ontak), a bacteria-derived IL-2/diphtheria toxin fusion protein, was one of the earliest T-cell-targeting drugs approved by the US Food and Drug Administration (FDA) in 1999 but has been discontinued since 2014 because of safety issues.[29] Generally, immunotoxins suffer from poor solubility, limited stability, and rapid clearance, which has prompted efforts in using delivery vehicles to address these challenges.[30] Secondly, the use of a delivery vehicle can overcome the need for adoptive transfer of *ex vivo* engineered or drug-loaded T cells. *In vivo* targeting of T cell subsets can greatly reduce the cost of cellular hitchhiking-based drug delivery systems by avoiding the need to individually prepare patients' cells *ex vivo*.[13] The same hurdles hamper widespread applications of CAR-T cell therapy, which currently relies on the costly *ex vivo* genetic engineering of each individual patient's cells prior to reinfusion.[31,32] Moreover, reinfusion of the expanded engineered CAR-T cell can be accompanied with adverse events such as cytokine release syndrome, resulting in multiple organ failure and neurological toxicity (reviewed therein[33]), which potentially can be circumvented by CD8 T cell specific *in vivo* delivery. Furthermore, *in vivo* delivery of gene editing machinery such as the clustered regularly interspaced short palindromic repeats (CRISPR) toolbox to T cells presents potential for therapy and immunomodulation. Although *in vivo* expression of the Cas9 protein has the potential downside of being immunogenic,[34] this approach has nevertheless been suggested as a strategy to eradicate latent HIV or knock out the immune checkpoint receptor programmed cell death protein 1 (PD-1) in T cells, among others.[22,23,35,36]

Current research strategies for *in vivo* drug delivery include viral and nonviral methods; however, both methods suffer from low drug delivery efficiencies.[37,38] Two adeno-associated viral vector-based therapeutics, voretigene neparvovec (Luxturna) and onasemnogene abeparvovec (Zolgensma), have been approved by the FDA. However, the cost associated with large-scale vector production is limiting similar therapeutics being used in the clinic.[39] Further limitations of viral vector-based delivery methods are associated with limited choice of cargo, potential oncogenicity,[40] and immunogenicity.[41] Therefore, the development of safer and more effective drug delivery methods is warranted. The use of engineered nanoparticles as delivery platforms has been suggested as a potential approach to T cell reprogramming and delivery of therapeutics to T cells in general. Nanomedicines have already demonstrated significant innovations in cancer medicine by offering highly versatile drug carriers.[24,42] In addition, nanoparticle-based vaccines were recently developed and approved by the FDA as a coronavirus disease 2019 (COVID-19) vaccine.[43,44] However, T cells do not take up nanoparticles as readily as cancer cells because of their small cellular size, high nucleus-to-cytoplasm ratio, nonphagocytic nature, and low rates of endocytosis. In addition, it has been suggested that slow acidification of endosomes in primary T cells reduces transfection efficiency of vectors that rely on pH-dependent release of cargo.[45] Cell exposure to a foreign nanomaterial is likely to induce an immune response or affect immune cell functions depending on the material and cell type.[46] Furthermore, T cell activation has been associated with various cellular changes, including altered expression and spatial reorganization of surface receptors,[47,48] all of which may have significant implications in nanoparticle–cell interactions. This has created major challenges for materials scientists and biologists aiming to develop T cell-targeted nanomedicine.

Therefore, the overall aim of this review is to provide scientists in the physical and life sciences with an understanding of the biological barriers to T cell delivery and the nanoparticle engineering strategies available. Additionally, we provide an overview of T cell-targeted nanoparticles explored to date and detail considerations for future efforts in the rational design of T cell-targeted nanoparticle systems.

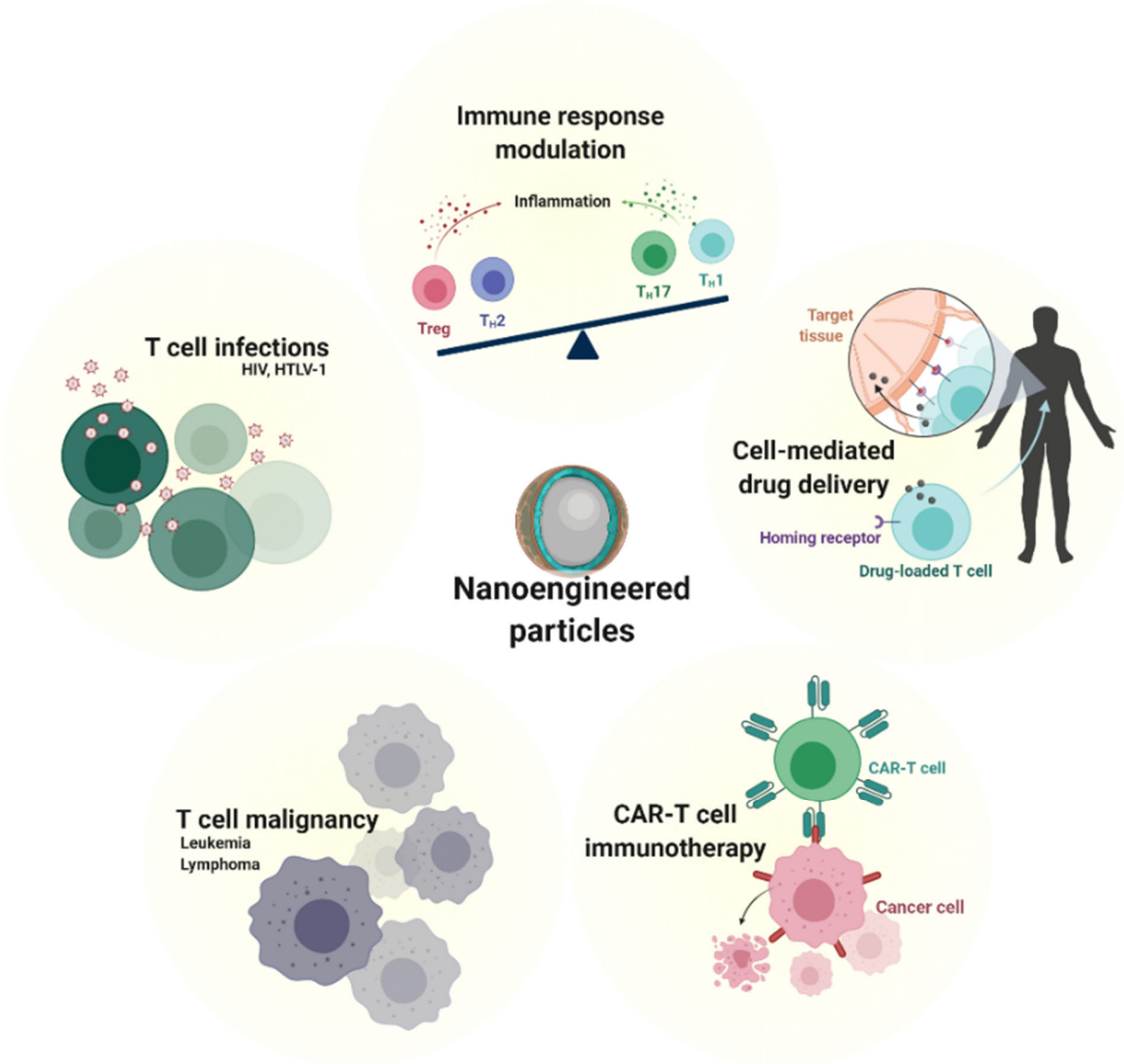

Figure 2. Possible applications of T cell-targeted nanoparticles. Targeting T cells can serve to modulate immune responses by influencing the balance between pro- and anti-inflammatory T cell subsets. The inherent homing capacity of T cells to the site of disease can be exploited for T cell-

mediated drug delivery to specific target tissues. CAR-T cell therapy is a promising immunotherapy for cancers and nanoparticles can be used to deliver the CAR-encoding genetic material to T cells *in vivo*. Furthermore, T cells can be the subject of disease themselves and T cell-targeted nanoparticles can be used to treat T cell leukemias, lymphomas, or chronic viral infections. CAR, chimeric antigen receptor; HIV, human immunodeficiency virus; HTLV-1, human T-cell lymphotropic virus type 1; $T_H x$, helper T cell subtype *x*; Treg, regulatory T cell. Created with BioRender.com.

NANOPARTICLE DESIGN: EXPLORING THE OPTIONS

A plethora of nanoparticles are being developed for therapeutic delivery, diagnostic, and imaging applications.[29,49] Based on their chemical composition, nanoparticle systems can be divided into several broad categories: polymer-based nanoparticles, lipid-based nanoparticles, and inorganic nanoparticles. Each system carries its benefits and limitations in terms of drug loading, stability, biocompatibility, and biodegradability. An overview of nanoparticle subclasses used for T cell delivery is presented in Table 1. The physicochemical properties of these nanoparticles, including size and surface charge, are important parameters known to influence their interaction with cells and downstream delivery efficacy.[50]

Influence of Drug Type on the Choice of Nanomaterial. The main types of therapeutics delivered to T cells are small hydrophobic molecules, proteins, and nucleic acids. Although most nanoparticle systems are highly versatile, not all types of therapeutics can be loaded onto or into every type of nanoparticle. As such, the type of therapeutic cargo is a major determinant in the choice of nanomaterial (Figure 3).

Many chemotherapeutic agents, as well as some of the therapeutics studied to eradicate latent HIV are small hydrophobic molecules. For these types of drugs, lipid-based materials and oil-

based nanoemulsions allow drug loading by complexing with lipids or encapsulation in an oily core, respectively.[51–53] Amphiphilic polymeric materials, including poly(lactic-*co*-glycolic acid) (PLGA)-based nanoparticles, also allow encapsulation of hydrophobic drugs *via* hydrophobic interactions with the water-insoluble portion of the polymer during nanoparticle assembly.[54] In addition, small drug molecules have been conjugated to the surface of gold and other inorganic nanoparticles.[55]

Proteins such as diphtheria toxin, ricin A, and the Cas9 protein of the CRISPR toolbox can be loaded into various types of nanoparticles by physical entrapment or chemical conjugation. For instance, using a water-in-oil-in-water double emulsion technique, proteins can be entrapped in hydrophobic polymer-based nanoparticles such as PLGA.[54] Proteins can also be entrapped in liposomes,[51] mesoporous silica nanoparticles,[56] $CaCO_3$ particles,[57] and metal–phenolic capsules,[58] or incorporated into the caveosphere outer membrane *via* fusion to caveolin-1.[59] Additionally, the thiol (–SH) groups present in most proteins can be exploited to covalently bind protein therapeutics to the surface of gold nanoparticles.[60,61] In general, however, forming stable protein nanoformulations is challenging because of the potential occurrence of protein degradation.[54,62] To overcome this barrier, a strategy was proposed recently, in which a variety of nanomaterials can be coated with protein–phenolic network assemblies that retain the structure and function of the protein.[62]

For certain immunotherapeutic strategies, as well as strategies to engineer CAR-T cells or perform CRISPR-mediated genome editing in T cells *in vivo*, the therapeutic agents are based on nucleic acids, including siRNA, messenger RNA, and plasmid DNA. Nucleic acids are innately hydrophilic and negatively charged. Two widely studied cationic polymers for nucleic acid delivery are poly-L-lysine and polyethyleneimine (PEI).[63,64] However, their therapeutic application

is limited because of low transfection efficiency *in vivo* and high cytotoxicity (for PEI). The recent generation of cationic polymers that has been developed includes chitosan, poly-β-amino esters (PBAE),[65,66] and glycogen nanoparticles.[67] The attachment of nucleic acids on solid nanoparticles has also been studied *via* electrostatic interactions or covalent attachment using nucleic acids with added functional end groups; the solid nanoparticles include inorganic nanoparticles such as gold, silica, and iron oxide.[68]

Influence of the Nanoparticle Material on Surface Engineering Options. In addition to the inherent physicochemical properties of nanoparticles, the nanoparticle surface can be modified to tether or conjugate ligands, including targeting molecules, drugs, detection probes, biotin, and stealth macromolecules.[69] Surface modification of nanoparticles can be achieved by using one or more chemical methods to either form a noncovalent interaction or covalent bonds between the nanoparticle surface and ligand (reviewed therein[69,70]). Noncovalent interactions include electrostatic, hydrophobic, van der Waals forces, hydrogen bonding, and ligand exchange,[71,72] whereas covalent attachment involves chemical conjugation between the nanoparticle surface and functional end groups on the drug or ligand, with or without the aid of a cross-linker. The choice of the modification strategy is influenced by the functional groups available on the nanoparticles and ligands, and whether the reaction conditions can maintain the stability of the nanoparticles and the function of the conjugated drug or ligand (Figure 3).

In summary, the choice of nanoparticle material is often dictated by the type of cargo to be delivered *i.e.*, the physicochemical properties of the drug, and in turn, the chosen material dictates subsequent surface modifications (Figure 3). The ability to scale up nanoparticle production at reasonable cost plays a key role in determining the suitability of the nanoparticles for clinical application.

Table 1. Overview of nanoparticle subclasses used for T cell delivery based on material type

Nanoparticle	Subclass	Description	Reference
Polymer-based	Polyethyleneimine	Polyethyleneimine is a cationic polymer with well-described endosomal escape capabilities. However, its therapeutic application is limited owing to low transfection efficiencies and high cytotoxicity.	[64]
	Polyesters (*e.g.*, polylactic acid, poly(lactic-*co*-glycolic acid))	Polyesters are FDA-approved hydrophobic polymers and biodegradable *via* Krebs cycle resulting in minimal toxicity. They require surface modification to be soluble in water, can form micelles to encapsulate hydrophobic drugs or capsules to carry hydrophilic molecules, and suffer from high burst release of the loaded drug.	[54,73]
	Natural polymers (*e.g.*, chitosan, alginate, hyaluronan)	Natural polymers are biocompatible and biodegradable. Cationic chitosan forms complexes with negatively charged cargo and drug release is pH-dependent. The presence of reactive groups allows conjugation with ligands.	[74–76]
	Dendrimers (*e.g.*, polyamidoamine)	Dendrimers are repetitively branched molecules or oligonucleotides that can adopt a spherical morphology. Drug loading is achieved through physical encapsulation in internal cavities or conjugation to the terminal functional groups.	[77]

Lipid-based	Liposomes	Liposomes consist of an aqueous core surrounded by one (unilamellar) or more (multilamellar) lipid bilayers that may contain stabilizing components such as cholesterol and ceramide. They can load hydrophobic drugs within the bilayer or the aqueous core, can fuse with target cell membranes, and are FDA-approved.	51
	Solid lipid nanoparticles	Solid lipid nanoparticles are composed of a solidified lipid matrix as the core with a surface coating of surfactant or phospholipid. Both hydrophobic and hydrophilic drugs can be dissolved or dispersed within the core. Solid lipid nanoparticles display improved cell uptake compared to liposomes but they can suffer from poor drug loading capacity and leakage.	52
	Emulsions	Emulsions consist of an oil core stabilized by fatty acids, triglycerides, or polymer shells. They typically suffer from poor drug loading and stability, and synthesis reproducibility.	53

	Caveospheres	Caveospheres are formed by the expression of human caveolin-1 in *Escherichia coli* (*E. coli*). Functionalization of caveospheres can be achieved by encoding fusion proteins containing caveolin-1. Drug loading is complicated by the dependency on natural processes within *E. coli*. Caveospheres are potentially immunogenic owing to the presence of bacterial components.	[59,78]
Inorganic	Gold	Gold nanoparticles are small (typically 1–100 nm), inert, and nontoxic. They can be conjugated to a variety of drug classes, including peptides, proteins, nucleic acids, and chemotherapeutic agents, *via* biodegradable linkers. However, sub-10 nm gold nanoparticles have a short *in vivo* half-life due to renal clearance.	[60,61]
	Iron oxide	Iron oxide nanoparticles are small (typically 1–100 nm) spherical nanoparticles with superparamagnetic properties. They are mainly used for magnetic resonance imaging or magnetic fluid hyperthermia-based therapy but can be coated with therapeutic compounds for drug delivery or theranostic applications.	[55,79]

THE BIO–NANO INTERFACE: IMPROVING NANOPARTICLE FATE AND NANOPARTICLE–T CELL INTERACTIONS

As most nanoparticles are prepared from exogenous materials (*i.e.*, originating from outside the organism), they face multiple effective clearance mechanisms that have evolved in the human host.

Firstly, upon entering the bloodstream, synthetic nanoparticles will attract and adsorb proteins and other plasma components to form the so-called protein corona (or biomolecular corona).[80,81] The presence of immunoglobulins and other opsonins in this protein corona makes these nanoparticles prone to sequestration by phagocytes in the mononuclear phagocyte system (MPS; also called the reticuloendothelial system) including Kupffer cells of the liver.[82,83] In addition, nanoparticles with a diameter of less than ~6 nm are efficiently excreted *via* the kidneys.[84,85] Often less than 5% of the administered nanoparticles reach their target tissue.[82,86]

The physicochemical properties of a nanoparticle, including size, shape, composition, and surface properties, can influence how it interacts at the bio–nano interface.[50] As such, rational nanoparticle design is essential to control the fate of nanoparticles in the complex *in vivo* environment. There has been limited work on the effect of physicochemical properties on nanoparticle–T-cell interactions and nanoparticle uptake by T cells.[87] We can, however, learn from what is known about bio–nano interactions in the various organs and tissues where T cell targeting for therapeutic purposes is desired. This section describes four core aspects of T cell targeting and discusses strategies to improve uptake by T cells *in vivo*, by reducing nonspecific interactions and improving targeting to T cells specifically.

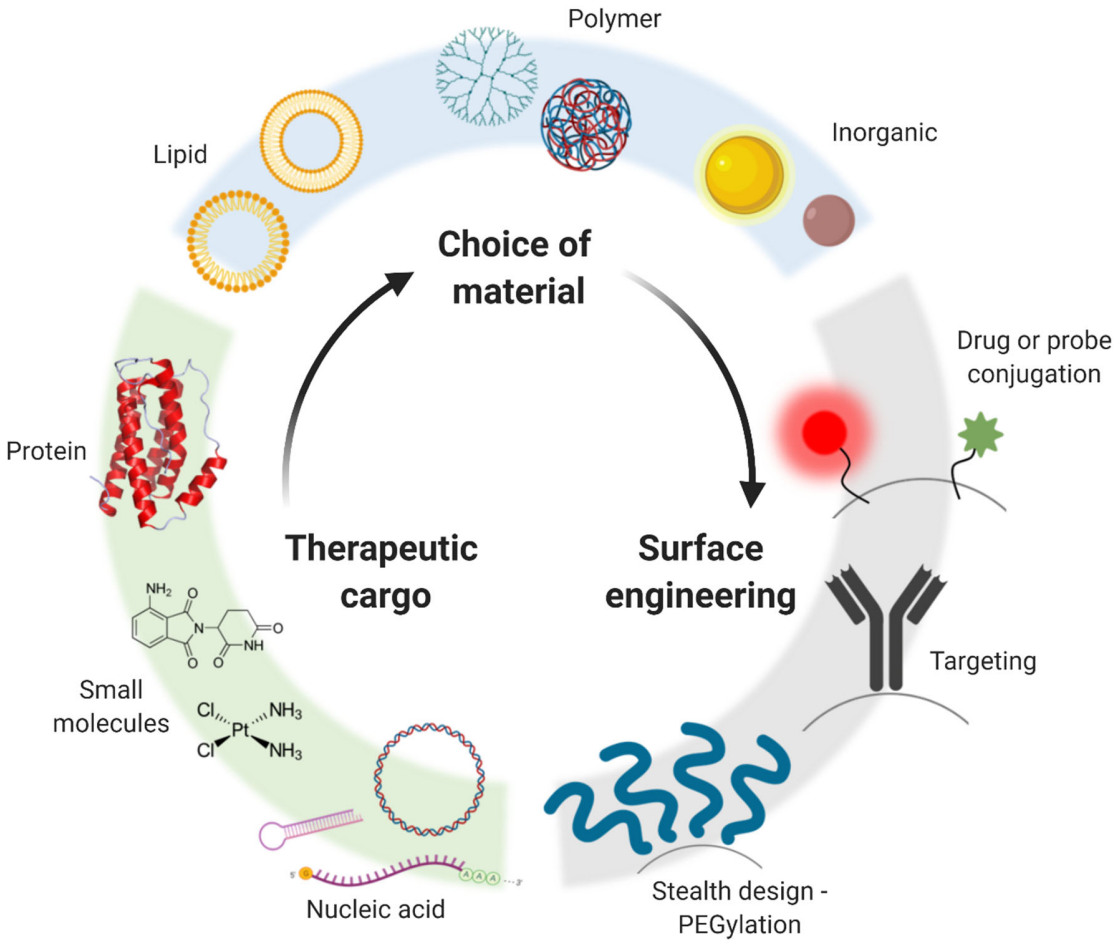

Figure 3. The therapeutic cargo informs the choice of the nanoparticle material that in turn influences the surface modification approach employed. Different classes of drugs, including proteins, small molecule drugs, and nucleic acids, can be delivered using nanoparticles. Not all nanoparticle types are equally suitable for the delivery of each of these drug classes. The possibilities for surface engineering are subsequently dependent on the material chosen, the presence of functional groups in both the nanoparticle and the ligand (*i.e.*, drug, probe, targeting moiety, or stealth molecules). Created with BioRender.com.

Bio–Nano Interactions in Whole Blood: The Importance of Stealth and Protein Corona. Although only an estimated 2–3% of all T cells are found in the blood,[1] circulating T cells are an

important target for nanoparticle-based drug delivery. In the case of T cell leukemia or HIV infection, circulating T cells are the direct subject of disease,[20,88] where for CAR-T therapy or other immunotherapies circulating T cells could simply provide the most accessible T cell pool to target.[32] Even when circulating T cells are not the intended target T cell subset, many therapeutics are dependent on the bloodstream for circulation throughout the body.

Studying the behavior of nanoparticles in this physiological compartment has been achieved *in vitro* by incubating nanoparticles with whole blood and assessing nanoparticle association with various blood cell populations.[89,90] Although only a few such studies have specifically examined differential association with T cells, the available data suggest that in the absence of targeting strategies, T cells demonstrate considerably lower nanoparticle association compared with other immune cells.[59,91–93] Controlling interactions between nanoparticles and off-target blood cells through surface engineering strategies is thus essential to achieve efficient nanoparticle-based drug delivery to T cells.

Avoiding immune recognition and clearance by off-target cells is commonly referred to as "stealth".[94,95] The design of stealth nanoparticles is a major field of ongoing investigation. Conjugation with the hydrophilic and neutrally charged polyethylene glycol (PEG), referred to as PEGylation, is well known to significantly increase blood circulation time, and multiple PEGylated nanoformulations are commercially and clinically available.[29] However, as PEG is an exogenous compound, administration of PEGylated formulations can induce an immunoglobulin M (IgM) response by the spleen, which results in accelerated blood clearance (referred to as the "ABC phenomenon") upon repeated dosing of PEGylated nanomedicine.[96,97] Other non-exogenous stealth strategies have also been explored including the use of proline–alanine–serine (PAS) (PASylation)[98,99] and CD47, a marker of "self" over-expressed on red blood cell membranes

to prolong blood circulation.[100] Further research is warranted to progress these compounds into nanoformulations approved for clinical use.

While the above-described strategies aim to generate low-fouling (low-binding) nanoparticles with minimal formation of a protein corona, it has been suggested that the protein corona can be controlled and exploited rather than being considered a hindrance to achieving the desired bio–nano response.[101] For instance, the recruitment of certain types of proteins from blood (*e.g.*, albumin, apolipoprotein E (ApoE)) on the nanoparticle surface can help improve the biodistribution profile of nanoparticles, overcome biological barriers, and direct them to targeted regions and tissues.[102] In this regard, the concept of forming a personalized protein corona using a patient's specific serum composition is being explored.[103]

Targeting Tissue-Resident T Cells. When T cells in the bloodstream are not the intended targets, further biological barriers and tissue-specific clearance mechanisms can provide additional challenges to efficient nanoparticle delivery. As discussed above, T cells circulate in the lymphatic and vascular systems and reside in secondary lymphoid tissues and other organs including the liver, lungs, brain, and gut (Figure 1a). Within these tissues, they perform specialized roles and functions in immunity and disease that may be of specific interest for therapeutic interventions. The following sections discuss nanoparticle delivery to these target sites.

Delivery to lymph nodes can be particularly relevant in the context of T cell lymphoma and HIV, where the lymph nodes form major reservoirs of malignant and latently infected T cells, respectively.[104] Additionally, the lymph nodes are of interest for immunomodulatory interventions that directly act on naïve T cells to regulate their activation and differentiation upon antigen recognition.[105] Delivery to the lymph node paracortex, where T cells mainly reside, can be achieved *via* intramuscular injection and subsequent uptake by the lymphatic system or *via* blood

circulation and subsequent migration through the walls of high endothelial venules.[105] For the former strategy, a nanoparticle size range of 10–100 nm is considered to be an ideal size range for efficient internalization of nanoparticles by the lymphatics.[105] Extravasation can be enhanced *via* targeting of the peripheral node addressin on the capillary wall, thereby mimicking the natural homing process of T cells into the lymph node.[106] PEGylation of polymer nanoparticles has also been shown to enhance lymph node targeting *in vivo.*[107]

The liver is an immunologically complex organ that is subjected to cancers, inflammatory diseases, such as liver fibrosis, and viral or parasitic infections such as hepatitis B virus and malaria infection.[108] Liver tissue-resident T cells have been suggested as targets for immunotherapeutic strategies, for example targeting liver fibrosis.[109] While a majority of nanoparticles localize in the liver following intravenous injection, most are internalized by Kupffer cells (macrophages), limiting access to liver-resident T cells.[110] Strategies to prevent uptake by Kupffer cells include presaturation of Kupffer cells by nontherapeutic particles[111] and transient depletion of Kupffer cells,[112] although this might be challenging to achieve clinically.

Tissue-resident T cells in the lungs can be involved in the defense against respiratory pathogens and lung cancers. Alternatively, they can play a role in airway inflammation in response to allergens and acute cellular rejection following lung transplantation.[113] In the lungs, mucus presents a physical barrier to the delivery of nanoparticles *via* inhalation in addition to the cellular barrier posed by the alveolar macrophages that roam the pulmonary tissue.[102,114] To enter the alveolar region where most T cells reside, nanoparticles can be injected systematically or inhaled in liquid droplets or powders.[115] Our recent studies showed that the deposition of metal–phenolic capsules could be tuned precisely to the alveolar region *versus* the bronchi by controlling the

aerodynamic diameter of the capsules.[116] The physicochemical properties of nanoparticles that affect their fate in the lungs are reviewed by Liu *et al.*[117]

Targeting T cells within the central nervous system (CNS) is of interest for immunotherapy against brain tumors or to reduce pathogenesis of several neurodegenerative orders to which T cells have been linked.[118,119] However, nanoparticle delivery to the CNS is complicated by the blood–brain barrier (BBB), which prevents access to many biomolecules and nanoparticles.[120] The strategies used to overcome or bypass the BBB to facilitate nanomaterial delivery to the brain are discussed therein.[120] In addition to manipulating the BBB, *e.g.*, by focused ultrasound or microwaves to make it susceptible to drug extravasation, the surface modification of nanoparticles with cationic and amphiphilic components or receptor targeting ligands has been investigated to exploit the transport mechanisms across the BBB.[120] To bypass the BBB completely, nanoparticles have been administered *via* alternative routes to enable access to the CNS, including intratympanic or inner ear delivery,[121] intranasal delivery,[122,123] or direct tumor delivery through minimally invasive surgery.[124]

The gut presents an important drug target in strategies to eradicate HIV, as it is considered a major reservoir of HIV-infected T cells.[125] Gut-residing T cells are furthermore known to play a role in inflammatory bowel disease pathogenesis, the antitumor response against gastrointestinal cancers and maintaining tolerance against gut microbiota and food antigens.[126] Targeting T cells in the mucosal intestinal tissue can be attempted through oral administration; however, the wide variation in pH, the presence of mucus, and the inherent physiological functions of the gastrointestinal tract complicate efficient delivery.[127] Various nanoparticle assemblies that seek to address these challenges have been investigated for oral delivery.[127] For example, to achieve targeting to the T cell-rich small intestine epithelium[128] and gut-associated lymphatic tissue,

nanoparticles must withstand the acidic conditions in the stomach. This tolerance can be achieved by using materials that are insensitive to acidic pH (*e.g.*, polyanionic polymers and enteric polymers). In addition, the nanoparticles must overcome mucus when targeting the underlying cells in the intestine. Surface modification of nanoparticles with either PEG[129] or mucus-degrading enzymes[130,131] has been shown to improve mucus penetration, although nanoparticle size, charge, and composition also play a role.[132] Surface modification of nanoparticles to increase interaction with target cells or increase residence time in the small intestine using various ligands, including lectins, Claudin 4, Fc regions of antibodies, and vitamin B12, has been described and are summarized therein.[127] Lipid-based nanocarriers have been attractive for targeting the intestinal lymphatic system because of their role in the oral adsorption of dietary lipids.[133–135] Lopinavir, a hydrophobic antiretroviral drug, formulated in solid lipid nanoparticles showed almost a 5-fold higher intestinal lymphatic transport compared with lopinavir suspension.[136]

In summary, targeting T cells present in different sites in the body presents a major technological challenge—specifically, realizing an optimized nanoparticle system capable of reaching multiple T cell target sites *via* one route of administration is challenging. Alternatively, targeting T cells one site at a time is likely more feasible and the fate of the nanoparticle rests on the ability of the nanoparticle to overcome or exploit the physical and biological barriers present at each target site, which can be facilitated by precise nanoparticle engineering.

Targeting Nanoparticles through Ligand–Receptor Interactions. Advantages of targeted nanomedicines are the increased specificity of drug delivery and concurrent reduction of off-target effects and toxicity. Multiple targeting molecules or ligands have been explored as surface decorations of nanomedicine including antibodies or antibody fragments, proteins, and aptamers.[137,138]

The choice for a target receptor for nanoparticle-mediated drug delivery is influenced by (i) the level of specificity of that receptor to the intended target population and (ii) the receptor density on the cell surface.[137] In T cell targeting studies, the most commonly chosen receptors to date are general T cell markers *i.e.*, CD3, CD4, CD7, and CD8,[14,19,139–142] likely because of their high expression levels and (mostly) exclusivity to T cells. However, as has been observed with immune checkpoint inhibitors, such general targeting to all T cell subsets can result in dose-limiting toxicities.[16] As such, if possible, markers of specific cell subsets or markers overexpressed by malignancies are chosen *e.g.*, PD-1, CD25/IL-2 receptor, or protein tyrosine kinase 7 (PTK7).[143,144] An alternative targeting strategy could be to use antibodies that target a foreign antigen expressed on the diseased target cells. For example, in HIV infection, HIV envelope-specific antibodies such as bispecific T cell-engaging (BiTE) antibodies or dual affinity retargeting proteins (DARTs) that attract killer cells have been explored as an antiviral strategy.[145,146] Attaching such antigen-specific antibodies to nanoparticles has the potential to achieve highly targeted drug delivery to HIV envelope-expressing cells. However, a singular surface biomarker of disease is not always available or known.[147] In addition, it is important to note that when antibodies are used as the targeting ligands on nanoparticles, receptor targeting *via* antibody binding *in vivo* can induce receptor signaling, potentially triggering cellular responses. For example, CD3 stimulation leads to immunosuppression and T cell anergy,[148] a desirable effect in an organ transplant setting but highly non-desirable otherwise.

Of note, targeting and stealth are highly interrelated concepts and efficient targeting is unlikely to occur in the absence of stealth. However, stealth strategies can compromise targeting abilities and *vice versa*. Therefore, a balance between stealth and targeting is required to create biologically effective nanoparticles.[94]

Nanoparticle Internalization and Endosomal Escape. Upon reaching the target tissue and T cell subset, most nanoparticle systems require internalization for drug release.[149] The main routes of internalization are phagocytosis, micropinocytosis, and receptor-mediated endocytosis (RME). The mechanism of internalization depends on the size of the nanoparticle that is to be internalized[150] (Figure 4). The availability of these endocytic pathways differs between cell types, and phagocytosis in particular is restricted to certain cell types (professional phagocytes), such as macrophages and other antigen-presenting cells, as well as a limited number of non-professional phagocytes (fibroblasts, epithelial, and endothelial cells).[82,151]

Nanoparticle-mediated drug delivery to T cells is complicated by the nonphagocytic nature of T cells. Nanoparticle internalization into these cells is thus limited to macropinocytosis, RME, and a few other, less relevant pathways (Figure 4). Nanoparticle uptake through macropinocytosis is nonspecific and the vesicles formed during macropinocytosis are heterogeneous in size but generally 0.2–1 μm.[150,152,153] While professional phagocytes undergo constitutive micropinocytosis,[152] this endocytic pathway seems restricted to activated T cells[154] and is generally considered as inefficient. In contrast, RME is a highly specific mechanism of uptake that is induced by receptor binding.[155] Classic examples of receptors known to internalize through RME include the transferrin receptor[156,157] and chemokine receptors.[158] The efficiency of RME is strictly dependent on the internalization kinetics of the target receptor, the activation state of the cell, and the size of the particle to be taken up.[85] Studies on receptor internalization kinetics have been reported,[159,160] and chemokine receptors are generally considered to be rapidly recycling.[158] However, comparative studies in T cells are lacking. For RME, which is mediated by clathrin-coated vesicles, an optimum nanoparticle diameter of ~50 nm has been suggested and the

efficiency of endocytosis is known to decrease with increasing particle size.[85,120,155,161–163] Altogether, these restrictions pose significant challenges to nanoparticle delivery to T cells.

Once a nanoparticle is internalized, controlling its intracellular trafficking and subsequent drug release poses a further challenge.[149] Entrapment in acidic endosomal or lysosomal compartments can result in drug degradation and recycling to the cell membrane (exocytosis), leading to a loss of therapeutic efficacy. Several strategies for endosomal escape have been reported and nanomedicines have been designed accordingly: (i) cationic polymers and lipids can interact with the negatively charged endosomal membrane and cause membrane flipping and destabilization ("flip-flop" mechanism); (ii) polymers containing protonatable functional groups can induce endosomal swelling and rupture ("protein-sponge effect"); (iii) cationic lipid carriers can induce membrane fusion; and (iv) pore-forming peptides can self-assemble into drug-releasing pores in the endosomal membrane.[149,164] Studies in this context often approach nanoparticle endosomal escape from a nanoscience perspective and may disregard cell-specific effects. However, it has recently been suggested that T cells may present additional barriers to endosomal escape compared to the commonly used HeLa cells in the form of slower endosomal acidification, enhanced autophagy, and the presence of immune-sensing proteins.[165]

Of note, nanoparticle internalization is not required for all drug delivery systems. Other strategies for drug delivery include fusion of liposomal formulations with the cell membrane and extracellular release of hydrophobic drugs in the microenvironment of the target cell.[14,166–170] The latter strategy, although highly promising, is limited to small molecule drugs that can permeate the cell membrane without any vectors and may be less effective for nucleic acid or protein delivery.

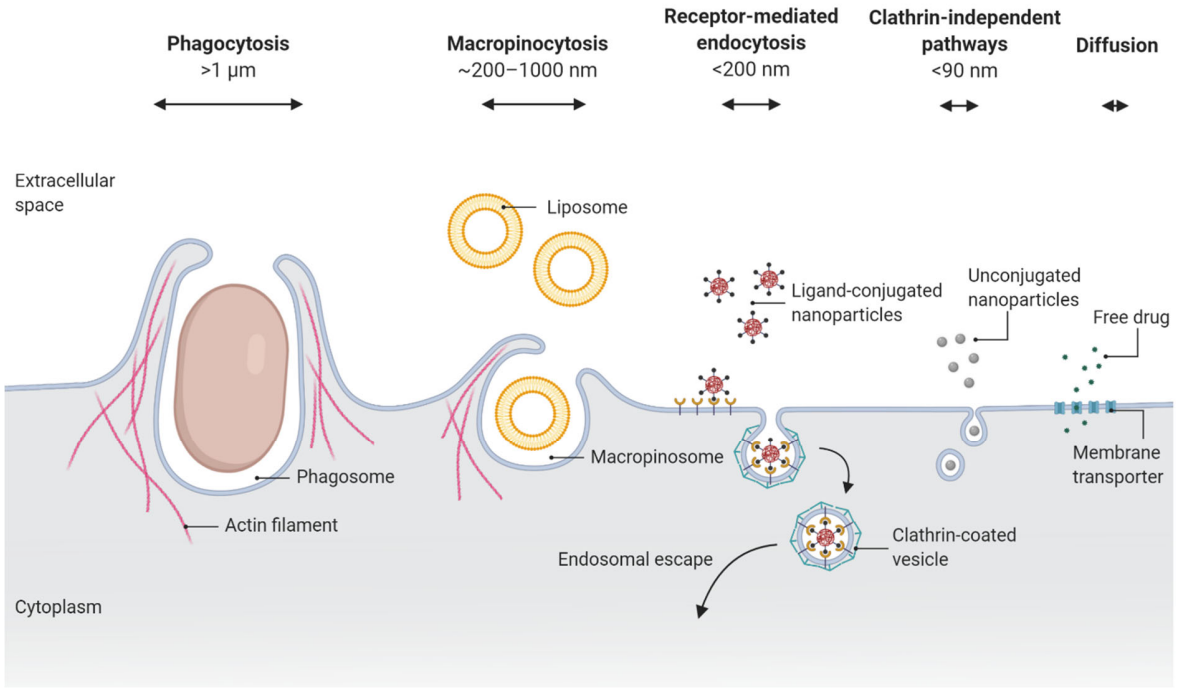

Figure 4. Routes of nanoparticle endocytosis. Four main routes of endocytosis can be distinguished depending on the size of the nanoparticle being taken up. Phagocytosis: phagocytes can engulf large particles of >1 µm but this process is restricted to certain cell types. Macropinocytosis: macropinosomes can vary in diameter but the efficiency of nanoparticle uptake decreases with increasing particle size. RME and clathrin-independent pathways are the main routes of uptake for smaller particles of <200 nm. In contrast, small-molecule drugs can diffuse through the plasma membrane or internalize through membrane transporters. RME is induced by receptor binding of ligand-conjugated nanoparticles. Upon internalization into a clathrin-coated vesicle, the nanoparticle needs to escape endosomal compartments to release its cargo into the cytoplasm. Created with BioRender.com.

NANOPARTICLE-MEDIATED DRUG DELIVERY TO T CELLS: SUCCESSES TO DATE

Despite the importance of T cells in various diseases, there are relatively few studies exploring direct T cell-targeted drug delivery using nanoparticles. Most reports focus on drug delivery to the T cell surface, thus not requiring nanoparticle internalization.[14,167–169,171] Although this is a valuable approach for drugs that can be delivered to the cell surface, in this review, we focus on strategies for nanoparticle-mediated intracellular drug delivery to T cells, noting that most reported studies originate from the field of oncology[19,26,140,144,172–177] and HIV.[178–183] For a detailed listing of the studies described in this section, see Table 2.

Material Characterization. In the T cell field, efforts have revolved around the delivery of a plethora of different therapeutic compounds ranging from siRNA and plasmid DNA to peptides and small hydrophobic drugs. Hydrophobic polymer- and lipid-based nanoparticles are most commonly used and these have been exploited for the delivery of both nucleic acids[19,139,178] and hydrophobic drugs.[173,174,179,184] Gold nanoparticles have been used for the delivery of nucleic acids,[61] however, to our knowledge, there are no reports of this strategy in a T cell context. Specifically, gold nanoparticles have only been used for intracellular delivery of small hydrophobic drugs to T cells *in vitro*.[140,175,176] It is challenging to derive guidance from these few reports in terms of the optimal nanomaterial for delivery to T cells. Studies have indicated that the conjugation ability of the nanoparticle system to a targeting molecule is a major rationale for the choice of material.[19,59,175,176] Irvine and coworkers initially performed studies on liposomal delivery of hydrophobic drugs but subsequently used gold-based nanoparticles because of their higher drug loading capacities.[140,174,185] The ease of synthesis was also provided as a rationale for the use of gold nanoparticles.[175,176]

The average nanoparticle size used for intracellular delivery to T cells is around 100 nm (range from 14.9 to 354 nm), with gold nanoparticles being significantly smaller than the lipid-based or

polymeric nanoparticles used (Table 2). The surface charge of the nanoparticles used is mostly negative, although not all studies report a ζ-potential measurement (Table 2). Further studies are needed to assess whether a negative nanoparticle surface charge effectively increases drug delivery to T cells.

Only a selection of these studies discuss the mechanism of drug release from the nanomaterial. Most of the studies report a sustained drug release over time,[170,173,174,177,183] which in one study is shown to be further triggered after nanoparticle internalization.[173] Drug release from gold nanoparticles has been described to be pH-dependent[175,176] or triggered by endosomal cleavage of the nanoparticle shell.[140]

Table 2. Overview of T cell-targeted nanomedicines for intracellular drug delivery

Nanoparticle	Size (nm)	ζ-potential (mV)	Cargo	Targeting ligand	Application	*In vivo*[a]	Reference
Polymeric							
Chitosan	305–354	15.2–17.5	siRNA	Anti-CD7	Fundamental, RNAi	×	[141]
PLGA	200	−23	no cargo	cIBR peptide targeting LFA-1	T-ALL treatment	×	[172]
PLGA–lipid–PEG	~110	~ −25	Imatinib	tLyp1 peptide targeting Nrp1	Cancer immunotherapy	✓	[173]
PBAE–PGA	155 ± 40	−7.8 ± 2.1	Plasmid DNA	Anti-CD3e	CAR T cell therapy	✓	[19]
Carbosilane	43.8	not reported	Doxorubicin	Aptamer targeting PTK7	T-ALL treatment	×	[144]
PEI–melittin	~70–200	~ –2 to 7	siRNA	Transferrin	Pulmonary delivery; asthma	✓	[186,187]
Lipid-Based							
Caveospheres	40	not reported	no cargo	Anti-CCR5	Fundamental, targeted delivery	×	[59]
Liposomes	129 ± 5	−10	siRNA	Anti-CD4	Fundamental, RNAi	✓	[139]
Liposomes	173 ± 13	not reported	no cargo	Anti-CD90 or anti-IL-2R	Fundamental, targeted delivery	✓	[185]
Liposomes	83 ± 11	not reported	TGFβ inhibitor	Anti-CD90 or anti-CD45	Cancer immunotherapy	✓	[174]
Liposomes	141 ± 25	−9.2	siRNA	Anti-LFA-1	RNAi for anti-HIV prophylaxis	✓	[178]
Liposomes	219	not reported	Bryostatin-2, nelfinavir	Anti-CD4	HIV cure	×	[179]

PEG–liposomes	195.4 ± 3	−41.1	Methotrexate	Peptide targeting CD40L	Autoimmune diseases	✓	[184]
Inorganic							
Gold	~23	not reported	TGFβ inhibitor	Anti-CD8	Cancer immunotherapy	✓	[140]
Gold	14.9 ± 0.5	−32.4 ± 1.6	Daunorubicin	Aptamers targeting PTK7 and nucleolin	T-ALL treatment	✖	[175,176]

[a]Where applicable, *in vivo* studies have been performed in mice. CAR, chimeric antigen receptor; cIBR, cyclo(1,12)-Pen-PRGGSVLVTGC-OH; Fundamental, fundamental research (*i.e.*, no clinical application specified); LFA-1, lymphocyte function-associated antigen-1; Nrp1, neuropilin 1; PBAE, poly-β-aminoester; PEG, polyethylene glycol; PEI, polyethyleneimine; PGA, polyglutamic acid; PLGA, poly(lactic-*co*-glycolic acid); PTK7, protein tyrosine kinase 7; RNAi, RNA interference; T-ALL, T cell acute lymphoblastic leukemia; TGFβ, Transforming growth factor beta.

Nanoparticle Targeting. The rationale behind the use of antibodies for nanoparticle targeting is twofold: (i) targeted nanoparticles increase the delivered drug dose to target cells through enhanced nanoparticle–cell interactions and (ii) the off-target effects of the therapeutic cargo are reduced. Several studies have consistently shown that conjugation of nanoparticles with a targeting ligand increases the nanoparticle–cell association with target cells in a monoculture.[19,59,139–141,172,178,179,184,185] *In vitro* analyses of selective nanoparticle association have shown an enhanced association with target cells *versus* nontarget cells in a mixed cell culture.[59,140,179] To date, studies that specifically assess selective nanoparticle internalization, as opposed to association, by target T cells are lacking.

In vivo data generally showed increased colocalization of antibody-conjugated nanoparticles with their target tissue or target cell.[19,139,140,173,174,178,185] In a study using polymeric nanoparticles consisting of PBAE and polyglutamic acid, targeting resulted in a different biodistribution in mice with CD3-targeted nanoparticles accumulating in lymphoid organs,[19] whereas nontargeted nanoparticles accumulated in the liver (Figure 5A and B). A separate study showed that in the absence of antibody conjugation, PEGylation of poly(methacrylic acid)-based nanoparticles improved targeting to lymph nodes, including the T cell-rich paracortex[107] (Figure 5C and D).

In some of the studies listed in Table 2, the choice of the targeting ligand was based specifically on the efficiency of the receptor to induce RME.[19,59,141,174,185–187] Based on these studies, CD3,[19] CD7,[141] CCR5,[59] transferrin receptor,[186,187] CD90,[174,185] and IL-2R[185] would make good candidates for inducing nanoparticle internalization in T cells (Figure 6A). However, transferrin receptor expression is not limited to T cells, thus potentially limiting its targeting specificity.[186,187] CD4 and CD45 are shown to be non-internalizing surface receptors,[59,174] although Ramishetti *et al.* achieved intracellular siRNA delivery *via* CD4-targeted lipid nanoparticles.[139] The authors showed that

following nanoparticle association and/or internalization, the surface expression of the targeted CD4 receptor was reduced, possibly due to receptor sequestering[139] (Figure 6B). Some of these findings were confirmed in a study that sought to identify a surface anchor, rather than a cycling receptor, and found that CD45 internalized more slowly than CD2, CD8, CD11α, or CD90.[169]

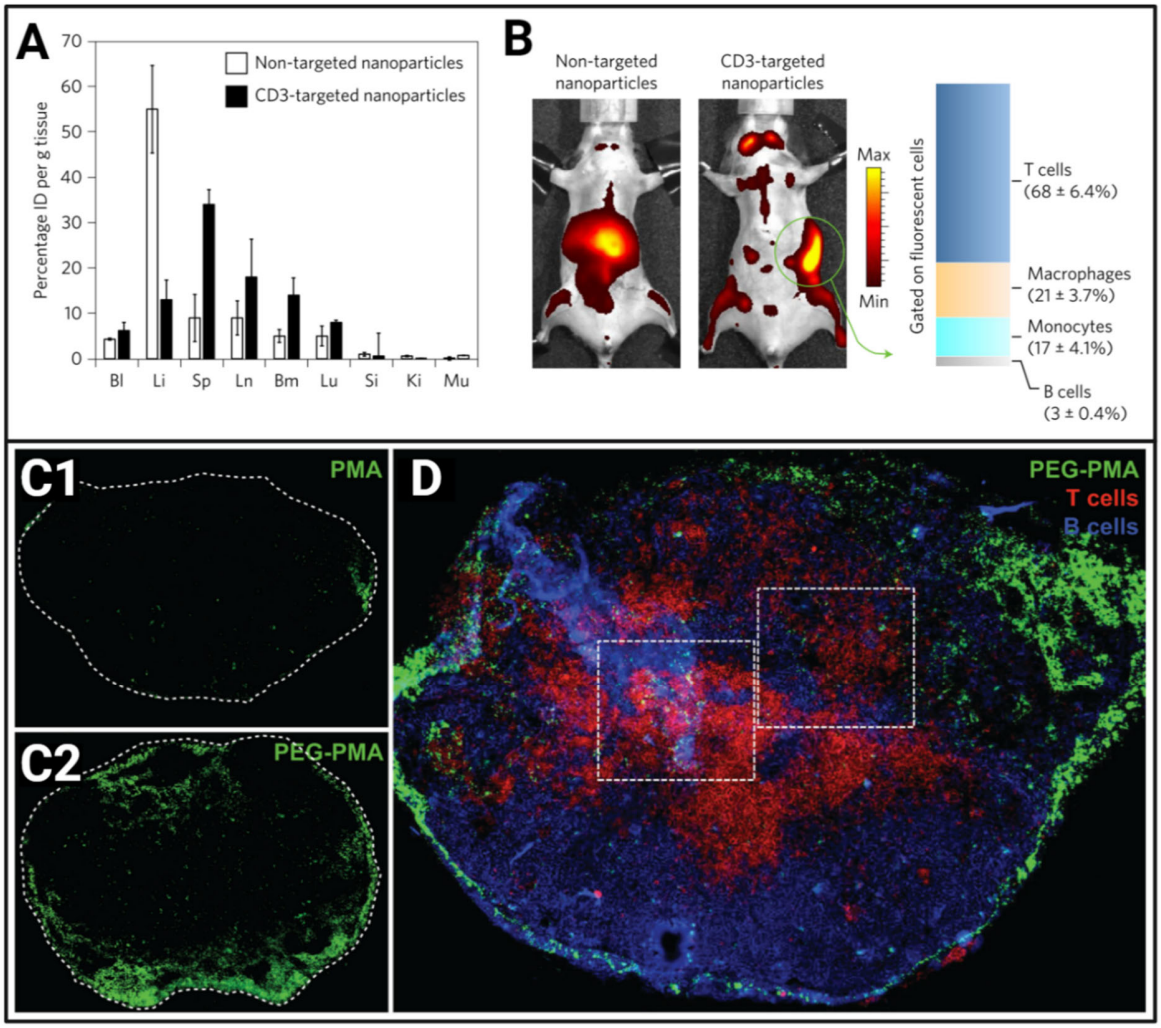

Figure 5. Nanoparticle surface engineering through targeting and stealth modifications alters the biodistribution profile of the nanoparticles. (A, B) CD3 conjugation enhances nanoparticle accumulation in tissues associated with T cell residence: biodistribution of fluorescent T cell targeted or nontargeted nanoparticles 4 h after tail-vein injection (A) and bioimaging of nanoparticle distribution in mice (B). In (A), data are expressed as injected dose (ID) per gram of

tissue. Bl, blood; Li, liver; Sp, spleen; Ln, lymph node; Bm, bone marrow; Lu, lung; Si, small intestine; Ki, kidney; Mu, muscle. Data are from 10 mice per treatment condition pooled from 2 independent experiments. Each bar represents the mean percentage of ID per gram tissue ± standard error of the mean. In (B), one representative mouse from each cohort (n = 10) is shown. The bar graph reflects percentages of splenocytes positive for fluorescent nanoparticles in mice treated with CD3-targeted nanoparticles, as measured by flow cytometry. Adapted with permission from reference 19. Copyright 2017 Springer Nature. (C, D) PEGylation improves the number of poly(methacrylic acid) (PMA) nanoparticles found in lymph nodes and in the deeper paracortex where T cells reside: fluorescence microscopy of lymph nodes of mice injected with PMA (C1) or PEG–PMA (C2) nanoparticles and fluorescence microscopy of lymph nodes of mice injected with PEG–PMA nanoparticles (D). In (C), the dashed white line represents the periphery of the lymph node. In (D), counterstaining was performed for T and B cell zones. Adapted with permission from reference 107. Copyright 2016 WILEY-VCH Verlag GmbH & Co. KGaA.

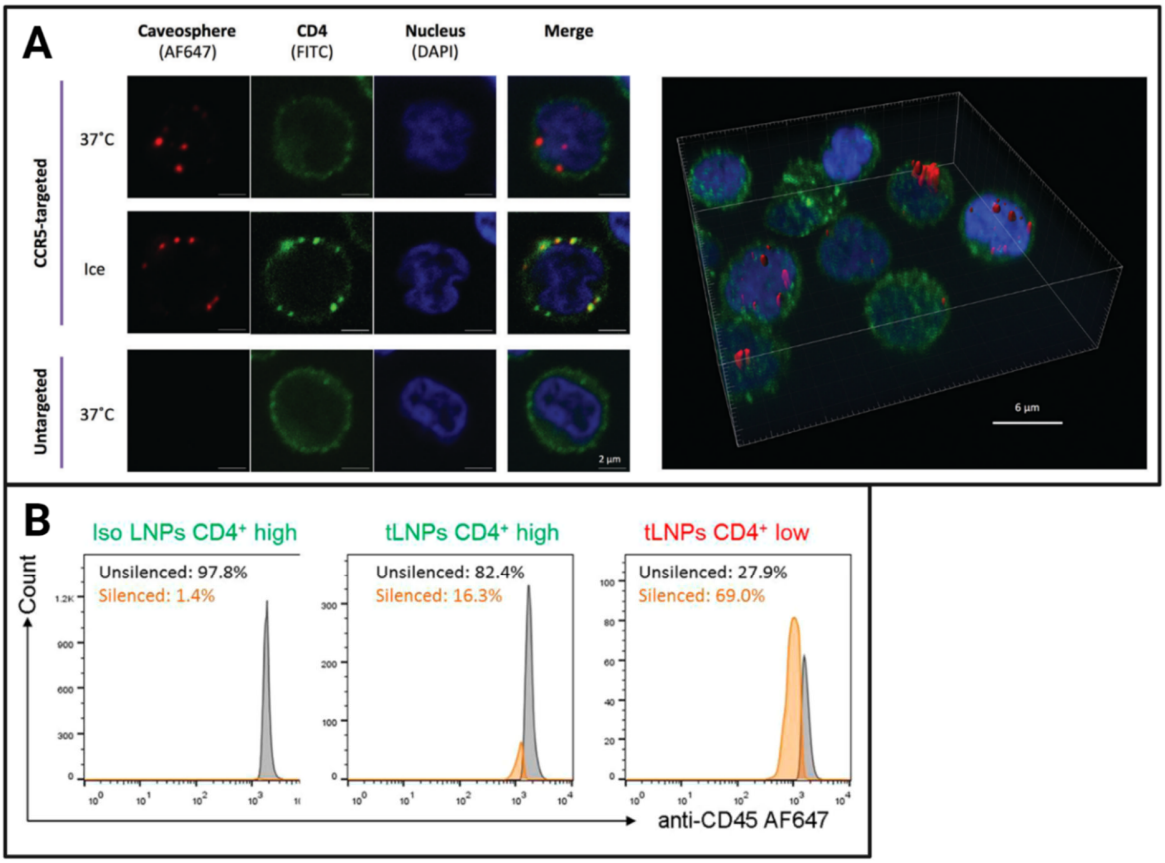

Figure 6. Nanoparticle targeting to certain receptors expressed on the T cell surface can enhance nanoparticle internalization through receptor-mediated endocytosis. (A) Internalization of CCR5-targeted caveospheres into primary CD4 T cells is temperature- and receptor-mediated: (Left) cross-sectional images of representative cells and (Right) representative field of view showing caveospheres internalized by primary CD4 T cells from 2 independent experiments (>100 cells per condition). AF647, Alexa Fluor 647; DAPI, 4′,6-diamidino-2-phenylindole; FITC, fluorescein. Adapted with permission from reference 59. Copyright 2016 The Royal Society of Chemistry. (B) CD4-targeted lipid nanoparticle (tLNP) internalization by mouse splenocytes followed by functional silencing compared to nontargeted LNP (isotype, Iso LNP). Nanoparticle internalization was associated with reduced CD4 receptor levels on the cell surface, suggesting receptor sequestering due to receptor-mediated endocytosis. An hour post-

administration of tLNPs or isoLNPs loaded with siCD45, splenocytes were collected and labeled with anti-CD4 PE. CD4+ T cells of isoLNPs, $CD4^{high}$ (green), and $CD4^{low}$ (red) cells of tLNPs were separated using BD FACSAriaIII cell sorter. Sorted cells were cultured *in vitro* for 3 days, stained with anti-CD45 AF647, and analyzed by flow cytometry. Adapted with permission from reference 139. Copyright 2015 American Chemical Society.

Rational Design of T Cell-Targeting Nanoparticles. Based on the literature published to date, the following features are suggested to be important for targeting T cells *in vivo* (Figure 7).

(i) For efficient T cell entry, nanoparticles should ideally be <200 nm in diameter to be compatible with RME. An optimum size of ~50 nm is suggested.[85,155] However, nanoparticles should remain larger than ~10 nm to avoid renal clearance *via* glomerular filtration.[79,85]

(ii) Nanoparticles should preferably interact with specific receptors on the T cell surface that, upon ligation, induce their own endocytosis (RME) through clathrin-coated vesicles, as this is the most efficient route of uptake for T cells. Therefore, for T cell targeting, nanoparticles should be conjugatable to antibodies or other RME ligands.[154,155] The intracellular signaling that arises from this receptor ligation should be nontoxic to the cells.

(iii) For compounds that are unable to cross a cellular membrane (lipid bilayer)—*i.e.*, large compounds and hydrophilic compounds—nanoparticles are required to induce endosomal escape and subsequent cytosolic delivery to prevent endosomal entrapment, recycling to the plasma membrane, or transport to lysosomes (with potential degradation).[149]

(iv) Nanoparticles should be immune evasive *i.e.*, avoid engulfment (phagocytosis) by macrophage-like cells in both the blood as well as MPS that is triggered by nanoparticle opsonization. This also generates a further restriction in the ideal nanoparticle size to <100 nm, which promotes escape from MPS scavenging.[137,188,189]

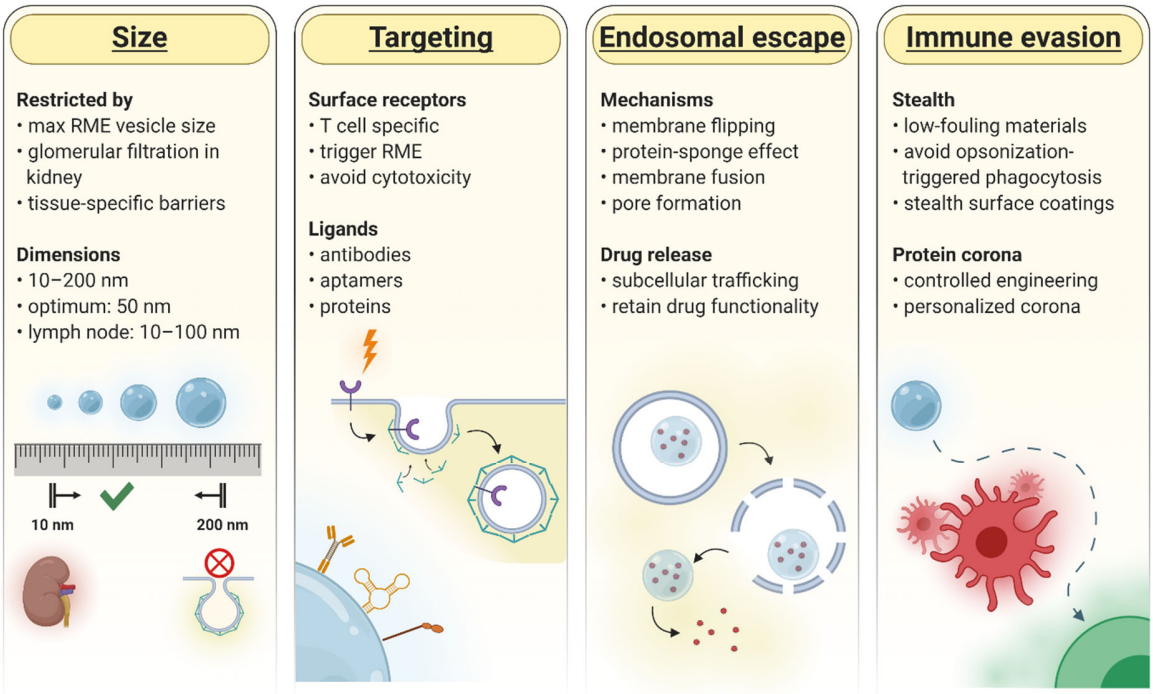

Figure 7. Important features for future rational design of T cell-targeting nanoparticles. *In vivo* drug delivery to T cells using nanoparticles remains a technological challenge. The nature of T cells, their *in vivo* biodistribution, and complex cellular environment pose barriers that require specific and rational material engineering solutions to address those challenges. RME, receptor-mediated endocytosis. Created with BioRender.com.

In summary, the use of nanomedicine for *in vivo* targeting of T cells is a promising but still nascent field and studies to date are mostly proof of concept. More fundamental, comparative studies of nanoparticle internalization kinetics into T cells are needed. These will validate and potentially expand the recommendations illustrated in Figure 7, which are likely to advance translation of *in vitro* T cell-targeting nanoparticles to clinically approved formulations.

FUTURE PERSPECTIVES

The key challenges in *in vivo* nanoparticle-mediated drug delivery are to: (i) achieve drug targeting to the cell population or anatomical site of interest; (ii) achieve high internalization

efficiency and intracellular drug release once the target cell has been reached; and (iii) avoid nonspecific clearance of nanoparticles by off-target cells. Although these challenges are universal across all fields of nanomedicine, there are several challenges when designing drug delivery systems aiming to target T cells.

First, the existence of many T cell subsets, each with different roles and functions in disease, necessitates an understanding of the surface markers that identify the particular T cell subset of interest. In contrast to cancer nanomedicine, where specific biomarkers are often well characterized, selecting a target receptor for T cell-directed nanomedicine is likely to be more complex. In some fields, such as the targeting of HIV-infected cells in a person on antiretroviral therapy, a well-characterized and reliable biomarker is still lacking.

Second, an understanding of the nanoparticle physicochemical properties that induce optimal interactions between nanoparticles and T cells is needed. Previous studies provide good starting points but additional fundamental studies, including those screening various nanoparticle classes and their surface characteristics, are expected to provide a better understanding of the interactions of nanomedicines with T cells. Furthermore, given the nonphagocytic nature of T cells, the focus may need to shift toward actively inducing nanoparticle uptake through RME. This has not been a major focus in the field, as most nanoparticle systems currently being developed target phagocytic or cancerous cells that are more receptive to nanoparticle uptake. We propose that the concept of induced nanoparticle uptake into T cells through RME should be further investigated and results from these studies should form the basis of future rational design.

Third, even with an increased understanding of strategies for induced endocytosis, designing stealth nanoparticles to increase blood circulation times will be essential to maximize targeting to T cells, including tissue-resident T cells. Progress made in the field of low-fouling surface coatings

as well as an increased understanding of protein corona formation and engineering can be exploited where applicable.

Efforts in each of these areas are likely to significantly boost the upcoming field of T cell-targeted nanomedicine. Nanoscience research is increasingly driven by clinical needs, and clear biological mandates will better position future research.[190] Regulatory approval of nanomedicine remains complex owing to the multifaceted design of nanomedicines, which spans more than one regulatory domain.[191,192] Clear demonstration of the safety of the used nanomaterial will be paramount to the approval of nanomedicines and thus to the future use of nanoparticles for *in vivo* T cell targeting.[19] Nevertheless, the recent accelerated approval of nanoparticle-based COVID-19 vaccines and the precedent set by T cell-targeted immunotoxins provide promise for approval of future T cell-targeted nanomedicine.

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Table of Contents graphic