



# Myeloid cell-mediated drug delivery: From nanomedicine to cell therapy

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## ARTICLE INFO

### Article history:

Received 30 January 2023

Revised 23 March 2023

Accepted 11 April 2023

Available online 15 April 2023

### Keywords:

Myeloid cells

Cell migration

Chemotaxis

Nanoparticles

Nano-engineering

Targeted delivery

Cell-carriers

Cell-engineering

## ABSTRACT

In the presence of tissue inflammation, injury, or cancer, myeloid cells are recruited to disease regions through a multi-step process involving myelopoiesis, chemotaxis, cell migration, and diapedesis. As an emerging drug delivery approach, cell-mediated drug delivery takes advantage of the cell recruitment process to enhance the active transport of therapeutic cargo to disease regions. In the past few decades, a variety of nano-engineering methods have emerged to enhance interactions of nanoparticles with cells of interest, which can be adapted for cell-mediated drug delivery. Moreover, the drug delivery field can benefit from the recent clinical success of cell-based therapies, which created cell-engineering methods to engineer circulating leukocytes as 'living drug delivery vehicles' to target diseased tissues. In this review, we first provide an overview of myeloid cell recruitment and discuss how various factors within this process may affect cell-mediated delivery. In the second part of this review article, we summarize the *status quo* of nano-engineering and cell-engineering approaches and discuss how these engineering approaches can be adapted for cell-mediated delivery. Finally, we discuss future directions of this field, pointing out key challenges in the clinical translation of cell-mediated drug delivery.

Published by Elsevier B.V.

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**Abbreviations:** EPR, Enhanced permeability and retention; HSCs, Hematopoietic stem cells; MDSCs, Myeloid-derived suppressor cells; GPCRs, G-protein-coupled receptors; CXCR4, CXC-chemokine receptor 4; CXCL12, CXC-chemokine ligand 12; SDF-1, Super Dimensional Fortress-1; CCR2, CC-chemokine receptor 2; CX<sub>3</sub>CR1, CX<sub>3</sub>C motif chemokine receptor 1; PSGL-1, P-selectin glycoprotein ligand 1; VLA-4, Very late antigen-4; VCAM-1, Vascular cell adhesion molecule 1; MAC1, Macrophage antigen 1; ITGAM, Integrin alpha M; LFA-1, Lymphocyte function-associated antigen 1; ICAM, Intracellular adhesion molecule; PECAM-1, Platelet/endothelial-cell adhesion molecule 1; CNS, Central nervous system; BBB, Blood-brain barrier; BCSFB, Blood-cerebrospinal fluid barrier; BAMS, Border-associated myeloid cells; WBCs, White blood cells; TAMs, Tumor-associated macrophages; RBCs, Red blood cells; PEG-PLL, Poly(ethylene glycol)-*b*-poly(L-lysine); PD-1, Programmed death-1; MACRO, Macrophage receptor with a collagenous structure; PLGA, Poly(lactic-co-glycolic acid); HDL, High-density lipoprotein; MSCs, Mesenchymal stromal cells; NETs, Neutrophil extracellular traps; GEMs, Genetically engineered human macrophages; GDNF, Glial cell line-derived neurotrophic factor; LNPs, Lipid Nanoparticles; AMP-CatB, AMP and cathepsin B; CAR, Chimeric antigen receptor; BiTE, Bispecific T-cell engager; PNPs, Platelet membrane-cloaked nanoparticles; MTT, Methyltransferase; EVs, Extracellular vehicles; scFv, Single-chain variable fragment.

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## 1. Introduction

Delivery of drugs to target cancer and inflammation is challenging, as systemically delivered therapeutics are limited by highly protected physiological barriers. The enhanced permeability and retention (EPR) effect is usually used as the guiding mechanism in the design of nanomedicines that target inflammation or cancer; however, a recent survey found that only 0.7 % (median) of the administered nanoparticle dose is delivered to a solid tumor [1]. Chemotaxis regulates the recruitment of circulating cells to infiltrate a disease region [2,3]. As an alternative strategy to the EPR, cell-mediated drug delivery takes advantage of chemotaxis-mediated cell trafficking, a process that offers great potential for enhancing the active transport of therapeutic cargo to the disease region. The development of approaches based on cell-mediated drug delivery has employed nanocarriers engineered either to selectively 'hitchhike' on the cell surface or to be internalized by the cells. In the past few decades, a variety of engineering methods have emerged to enhance interactions of nanoparticles with cells of interest. These methods can be adapted for cell-mediated drug delivery. Moreover, the drug delivery field can benefit from the recent clinical success of cell-based therapies (Kymriah™, Yescarta™, and Tecartus™). Knowledge developed in the cell therapy field can be utilized to engineer circulating leukocytes as 'living drug delivery vehicles' to target diseased tissues. As an important component of the immune system, myeloid cells are capable of taking up nanoparticles and efficiently homing to inflamed tissue, making them great candidates for cell-mediated drug delivery. In this review, we first provide an overview of myeloid cell ontogeny and myeloid cell trafficking in response to inflammation. We then discuss how various factors within these processes may affect cell-mediated drug delivery. In the second part of this review article, we summarize the *status quo* of nano-engineering and cell-engineering approaches and discuss how these engineering approaches can be adapted for cell-mediated drug delivery. Finally, we discuss future directions of this field, pointing out key challenges in the clinical translation of cell-mediated drug delivery. Although many of technologies and methods being discussed can be adapted for cell-based imaging and diagnosis, we limit the scope of our review to drug and gene delivery applications.

## 2. Review of myeloid cell physiology

In this section, we summarize key features of myeloid cell physiology that are critical for cell-mediated delivery. This knowledge

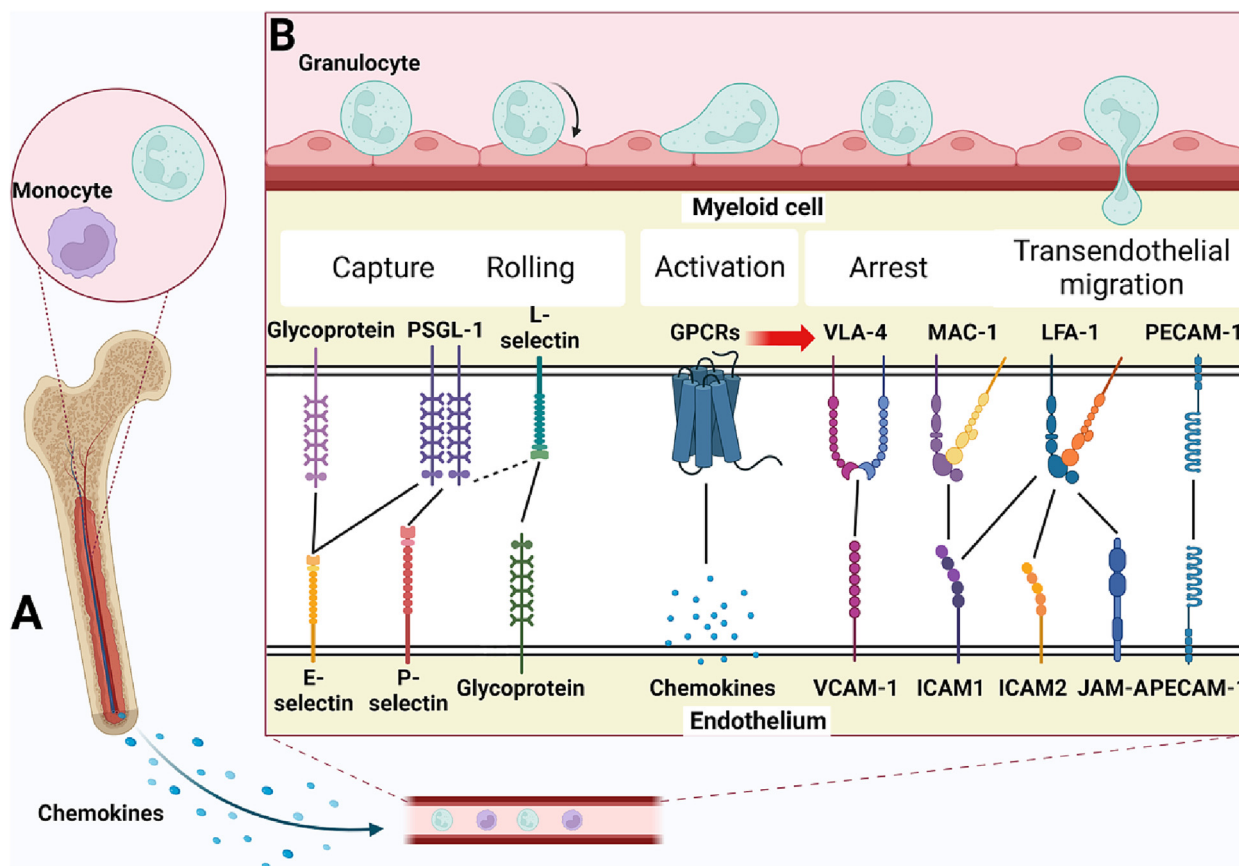
provides guidance in designing delivery platforms that utilize the trafficking of circulating cells to enhance the delivery of therapeutic molecules. We first introduce the definition of myeloid cells and how myeloid cells are generated. We then briefly review myeloid cell chemotaxis, migration, and key molecules associated with these processes.

### 2.1. Myeloid cells and myelopoiesis

Myeloid cells are a heterogeneous subgroup of leukocytes consisting of granulocytes (including neutrophils, eosinophils, and basophils), and monocytic cells (including monocytes, macrophages, and dendritic cells) [4]. In the peripheral blood, these cells represent major leukocytes whose primary function is the phagocytosis of pathogens and regulation of inflammatory progress. Myeloid cells in the blood and those that infiltrate tissues are derived from bone-marrow-derived hematopoietic stem cells (HSCs), while the tissue-resident macrophages, such as microglia in the brain, Kupffer cells in the liver, and Langerhans cells in the skin are derived from yolk sac-derived myeloid progenitors [4]. Under homeostasis, HSCs differentiate into immature myeloid cells, which then quickly differentiate into mature macrophages, dendritic cells, or granulocytes – a process termed myelopoiesis [5]. However, in pathological conditions, such as cancer, infection, and sterile inflammation, bone marrow accelerates myelopoiesis [5,6]. At the same time, the differentiation of immature myeloid cells to mature myeloid cells is blocked, which leads to the rapid expansion of a subgroup of heterogeneous immature myeloid cells with suppressive properties, known as myeloid-derived suppressor cells (MDSCs) [7].

### 2.2. Myeloid cell recruitment and chemotaxis

The recruitment of myeloid cells from the bone marrow to inflamed tissues is a chemokine-mediated process [2,3]. Chemokines control the migratory patterns of these cells through interactions with compatible chemokine receptors on the cell surface [2]. As a result of these interactions, myeloid cells egress the bone marrow and traffic via the bloodstream to the site of inflammation along chemotactic gradients – a process termed chemotaxis [3] (Fig. 1A). After arriving at the luminal side of the inflammatory site, chemokines further act on myeloid cells by activating integrins, key adhesion molecules [8]. Chemokine receptors are G-protein-coupled receptors (GPCRs). They work with their cognate chemokine(s) to regulate the specific specialized trafficking of myeloid



**Fig. 1.** Myeloid cell recruitment and diapedesis. (A) Myeloid cells egress from the bone marrow to the site of inflammation along chemotactic gradients. (B) Migration of myeloid cells from the blood vessel to the inflamed tissue is achieved by a conserved cascade of sequential steps: selectin-mediated rolling, chemokine-mediated activation, integrin-mediated arrest, and transendothelial cell migration. (Created with [BioRender.com](#)).

cells [2]. Chemokine receptors that play critical roles in mediating the trafficking and migration of myeloid cells are listed below.

#### Key chemokine receptors that mediate the trafficking and migration of myeloid cells:

**CXCR4:** In the bone marrow, homeostatic development and retention of immune cells is, to a great extent, dependent on CXC-chemokine receptor 4 (CXCR4) interactions with its complementary ligand CXC-chemokine ligand 12 (CXCL12; also known as Super Dimensional Fortress-1 (SDF-1)) [9]. As myeloid cells mature in the bone marrow, they downregulate CXCR4 expression, enabling cell egress and release into the blood [9]. After release, these cells circulate, awaiting inflammatory stimuli that would induce migration into peripheral tissues. Absence of such stimuli results in senescence and upregulation of CXCR4 expression, which directs their return to the bone marrow along CXCL12 gradients [10].

**CCR2:** CC-chemokine receptor 2 (CCR2) also regulates the egress of monocytes and MDSCs from the bone marrow via ligation with CCL2 (also known as MCP1) and CCL7 (also known as MCP3) [11,12]. CCL2 and CCR2 interactions are essential for normal inflammatory monocyte migration into peripheral tissues [13]. In addition to chemotaxis, CCR2 plays critical roles in mediating the phenotypic differentiation of myeloid cells under inflammation [14].

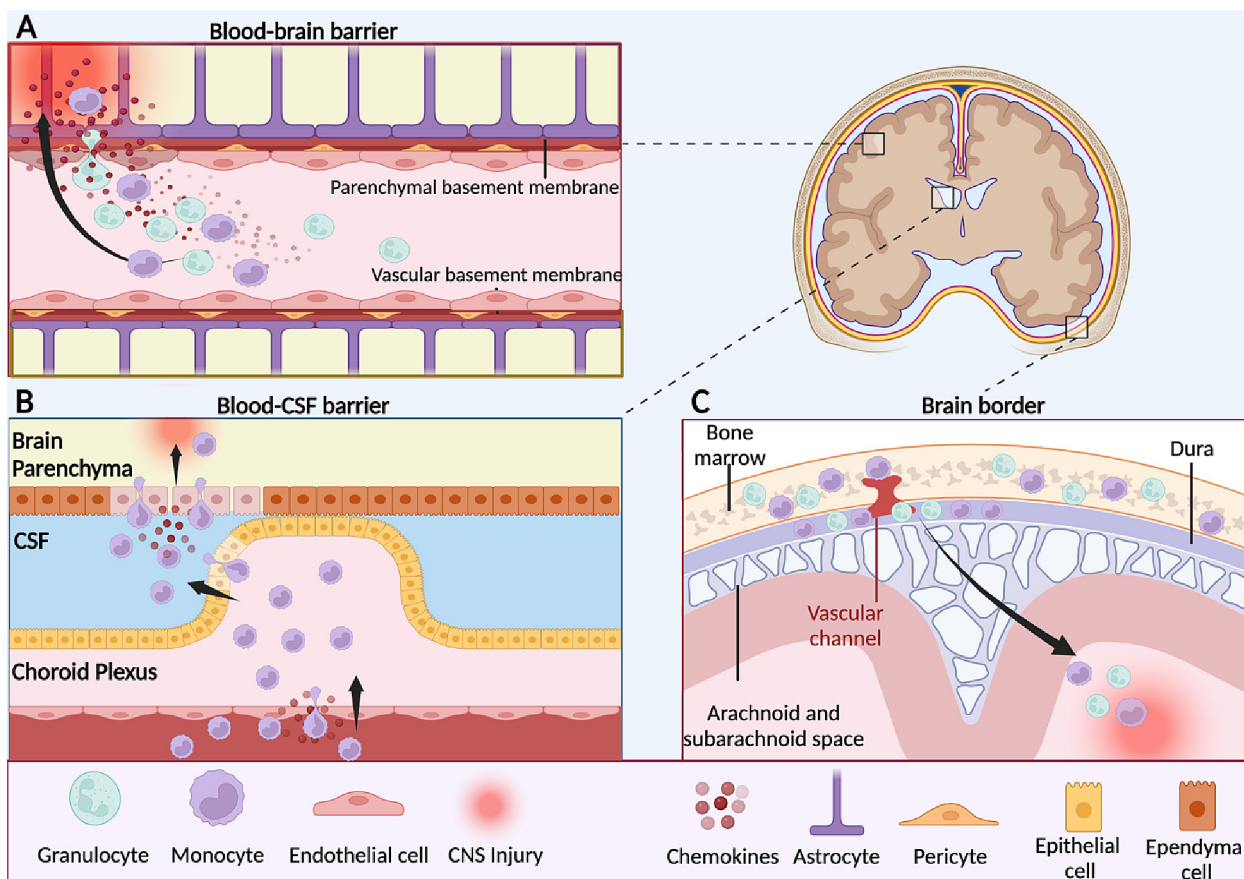
**CXCR2:** CXCR2 mediates the exit of granulocytes, such as neutrophils, from the bone marrow [15]. Binding between CXCR2 and its complementary ligands CXCL1 and CXCL2 guide granulocytes to the inflammatory site and subsequently activate these cells [16].

**CX3CR1:** The fractalkine receptor CX3C motif chemokine receptor 1 (CX3CR1) is highly expressed on anti-inflammatory or patrolling monocytes. It plays essential roles in supporting the survival of patrolling monocytes and promoting their adhesion to the vessel wall through adhesion molecules. In addition, CX3CR1 may play a role in the normal function of inflammatory monocytes [2].

#### 2.3. Myeloid cell migration and diapedesis

To infiltrate inflamed tissue, myeloid cells need to exit the circulation and migrate across the endothelium – a process referred to as diapedesis. Diapedesis is comprised of a conserved cascade of sequential steps: rolling, activation, arrest, and transendothelial cell migration, which is controlled by adhesion molecules [8] (Fig. 1B). Adhesion molecules such as selectin expressed on endothelial cells and selectin ligands expressed on myeloid cells regulate the capture of myeloid cells from the blood and subsequent rolling [17]. Next, chemokine receptors on a rolling cell become engaged by a specific chemoattractant (often a chemokine), triggering the rapid activation of myeloid cell integrins and the firm adhesion of the myeloid cell to the endothelium [8]. After firm adhesion has been established, myeloid cells transmigrate paracellularly across the endothelial cells through a gradient of junction molecules [18]. The adhesion molecules listed below include selectin ligands and integrins expressed on the surface of myeloid cells which play critical roles in mediating myeloid cell diapedesis:

**Key adhesion molecules that mediate the trafficking and migration of myeloid cells:**



**Fig. 2.** Myeloid cell migration across CNS tissue barriers. (A) Under neuroinflammation, inflamed endothelial cells shed cytokines attracting peripheral circulating myeloid cells. These peripheral myeloid cells are recruited to the site of inflammation through the weakened blood vessel lumen. (B) In response to CNS injury, monocytes transmigrate through the leaky blood vessel across the BCSFB. After leaving the choroid plexus, monocytes enter the CSF-filled ventricle and transmigrate across the ependymal layer to reach the site of injury. (C) Skull bone marrow-derived myeloid cells traffic to the dura mater through connecting channels, traverse the arachnoid and subarachnoid space, and are recruited to the site of injury. (Created with [BioRender.com](#)).

**PSGL-1:** P-selectin glycoprotein ligand 1 (PSGL-1) is a glycoprotein expressed on almost all leukocytes. It is capable of binding P-selectins and/or E-selectins (C-type lectin adhesion molecules) expressed by endothelial cells, aiding in cell adhesion and rolling [19].

**E-selectin ligands:** E-selectin ligands are glycoconjugates that contain a range of structurally diverse glycan epitopes capable of binding E-selectins. These ligands are expressed by circulating leukocytes and regulate the tethering and rolling of leukocytes at inflamed endothelium [20].

**L-selectin:** The average leukocyte will express ~ 50,000–70,000 molecules of L-selectin at the plasma membrane. The L-selectins contribute to the capture and rolling of circulating leukocytes on the endothelial surface by binding glycoproteins expressed on endothelial cells and PSGL-1 expressed on leukocytes. As circulating leukocytes migrate into the tissue, expression of L-selectin slowly turns over at the plasma membrane through a process of ectodomain shedding [21].

**VLA-4/Integrin  $\alpha 4 \beta 1$ :** Integrin  $\alpha 4 \beta 1$  (also known as very late antigen-4, VLA-4) is a transmembrane cell adhesion receptor expressed by leukocytes. Integrins remain inactive until the leukocytes are activated by chemotactic agents. For example, after the activation by SDF-1 (CXCL12), VLA-4 binds to the endothelial adhesion molecule vascular cell adhesion molecule 1 (VCAM-1), enabling firm adhesion and stimulates migration of monocytes through the endothelium [22].

**MAC-1/Integrin  $\alpha M \beta 2$ /CD11b:** Macrophage antigen 1 (MAC1; also known as integrin  $\alpha M \beta 2$ , or Integrin alpha M (ITGAM), or

CD11b) is a key adhesion molecule primarily expressed by myeloid cells. MAC1 can bind ICAM1 to facilitate cell arrest. MAC1 is a receptor for complement C3bi, mediating complement-coated particle uptake, cell-mediated cytotoxicity, phagocytosis, chemotaxis, and cellular activation [23].

**LFA-1/Integrin  $\alpha L \beta 2$ :** Lymphocyte function-associated antigen 1 (LFA-1, also known as integrin  $\alpha L \beta 2$ ) is an adhesion receptor expressed primarily on lymphocytes but also on myeloid cells. LFA-1 binds to intracellular adhesion molecule 1 and 2 (ICAM1 and ICAM2), which are expressed on endothelial cells. LFA-1 also binds to junctional adhesion molecule A (JAM-A) to facilitate the paracellular transmigration with MAC-1 [24].

**PECAM-1/CD31:** Platelet/endothelial-cell adhesion molecule 1 (PECAM-1, also known as CD31) is a transmembrane adhesion molecule expressed by endothelial cells and platelets, monocytes, neutrophils, and some types of T-cells. PECAM-1 directs myeloid cell crawling to transmigration sites through a gradient of expression on the endothelial surface [25].

#### 2.4. Tissue barriers for myeloid cell migration

Myeloid cell-mediated delivery can face several challenges depending on the tissue barriers involved. For example, central nervous system (CNS) delivery requires passage across the blood–brain barrier (BBB), which regulates the migration of myeloid cells from the systemic circulation to the brain parenchyma [26]. In addition to BBB, the migration of myeloid cells is also

regulated by the blood-cerebrospinal fluid barrier (BCSFB) [26] and the brain border (Fig. 2).

**BBB:** Peripheral circulating myeloid cell migration across the BBB (Fig. 2A) consists of the sequential steps of rolling, activation, arrest, and transmigration [26]. These processes are similar to the general processes of myeloid cell recruitment and diapedesis (Fig. 1B), which have been described in Section 2.3.

**BCSFB:** The epithelial layers of the fenestrated choroid plexus that exist between their vascularized choroidal stroma and the cerebrospinal fluid (CSF) comprise the BCSFB [26]. In response to injury, the brain parenchyma and choroid plexus generate and release chemoattractants, which recruit blood myeloid cells and aid in their migration across the BCSFB to the injured tissue (Fig. 2B) [27–29]. This pool of myeloid cells might be accessed by nanoparticles through intrathecal delivery, a delivery method already in clinical use.

**Brain border:** The brain border refers to the meninges, which comprise three membranes that surround the CNS, including a layer of dura mater, which is directly connected to the skull bone, a layer of arachnoid and subarachnoid space, and a layer of pia mater, which is connected to the brain cortex. Recent studies showed that the dura mater serves as a reservoir that hosts a unique pool of myeloid cells transiting directly from cranial bone marrow via vasculature channels rather than from the systemic circulation (Fig. 2C) [23,30]. These border-associated myeloid cells (BAMs) can migrate through meningeal barriers into parenchyma under injurious and inflammatory conditions [30].

### 3. Discussion: Biological factors that affect cell-mediated delivery

The selection of an appropriate myeloid cell subtype is critical to the success of cell-mediated delivery. Recent progress in immunobiology has identified immune cell subsets with high trafficking capability, and the mechanisms by which these cell subsets traffic to disease foci are understood [31,32]. This knowledge can be utilized by the drug delivery field to design more efficient delivery approaches. Here, we discuss a few biological factors that may affect the targeting and trafficking of myeloid cells critical to cell-mediated delivery. These factors include cell population, cell fate and lifespan, recruitment kinetics, efficiency of trafficking, accessibility by the delivery vehicles, and standard-of-care treatments.

#### 3.1. Cell population

The cell population can directly influence their delivery potential. For example, a higher cell number of circulating myeloid cells usually means a higher probability of being accessed by the delivery vehicle (i.e., nanoparticles) in the systemic circulation. At homeostasis, neutrophils comprise the most abundant immune cell population, accounting for 40–70 % of total white blood cells (WBCs); monocytes usually account for 4–11 % of total WBCs [33]; while DCs account for only 0.15–0.70 % of blood mononuclear cells [34]. Under pathological conditions, the population of immune cells can change significantly. This rapid change in the population should be considered when choosing an appropriate cell subset for targeting. For example, the population of immature myeloid cells (MDSCs) in sepsis survivors can significantly increase because of dysregulated myelopoiesis. The population of polymorphonuclear MDSCs (PMN-MDSCs, 22 % of WBCs) and monocytic MDSCs (M-MDSCs, 4.6 % of WBCs) could increase, respectively, as much as 10- or 5-fold compared to these populations in healthy donors (PMN-MDSCs: 2.1 % and M-MDSCs: 0.96 % of WBCs) [35]. The dramatic expansion of MDSCs in the blood makes them a good target for cell-mediated delivery in sepsis treatment.

#### 3.2. Cell fate and lifespan

The kinetics of myeloid cell generation, differentiation, and disappearance at both homeostasis and under inflammatory conditions can significantly influence the fate of the therapeutic cargo carried by the myeloid cells. For example, although circulating neutrophils are the major WBCs in the bloodstream, they have a short lifespan, less than 24 h [36]. If neutrophils carrying cargo do not traffic to the disease foci within this window, the therapeutic cargo will have a reduced opportunity to be delivered to the disease region. Monocytes have a longer lifespan. Tracer studies have shown that the major subset of circulating monocytes – classical monocytes (Ly6C<sup>hi</sup> in mice and CD14+/CD16- in humans) – have a lifespan of ~1–4 days [37,38]. Classical monocytes have a high capacity for tissue extravasation [38]. Having migrated to a disease region, they have the potential to differentiate into macrophages, which can persist for an even longer period [38]. Therefore, although monocytes account for a lower percentage of WBC than neutrophils, their longer lifespan and strong tissue extravasation capability can translate into an increased chance of delivering therapeutic cargo to the disease region, making monocytes a promising candidate for cell-based delivery.

It is estimated that ~10<sup>9</sup> neutrophils/kg are released and removed daily at homeostasis, and this number might increase under inflammation [39]. The removal of circulating myeloid cells will affect the fate of therapeutic cargo carried by myeloid cells. For example, most of the constant daily removal of neutrophils occurs through macrophage phagocytosis within the spleen, liver, and bone marrow [39]. This process will likely contribute to the redistribution of therapeutic cargo carried by neutrophils to these organs.

#### 3.3. Cell recruitment kinetics

Given that cell-mediated delivery relies on targeting cells recruited to the disease foci, the window of therapeutic administration needs to be well-coordinated with the kinetics of cell recruitment. The resolution of acute inflammation is a short process compared to that of chronic inflammation. As first responders, neutrophils begin to appear at a disease region 4–6 h after initiation of inflammation, peaking at 2–3 days [40]. Monocytes arrive later than neutrophils, with their population peaking at 6–7 days [40]. During this process, some monocytes can mature into macrophages and persist for a longer time at the disease region [40]. Therefore, for acute inflammation, early administration of therapeutics to target inflammation-homing cells is more likely to result in efficient drug delivery to the disease region. However, for chronic inflammation and cancer, the recruitment of myeloid cells to the disease region is a continuous process that persists as these diseases progress. The sustained myeloid cell recruitment provides a wider window for therapeutic administration. As an example, in glioblastoma, monocytes of bone-marrow origin are constantly being recruited to the tumor through CCR2 and its cognate chemokines [41,42], leading to a continuous increase in the monocyte population in the tumor stroma during the entire process of tumor development [43]. In a mouse model of glioblastoma, monocytes and monocyte-derived macrophages can account for as much as 85 % of total tumor-associated macrophages (TAMs) [41].

#### 3.4. Cell trafficking efficiency

Recent studies have identified specific subsets of myeloid cells with higher efficiency in homing to inflamed tissues than others [31,32,44]. For example, an adoptive transfer experiment in mice showed that the classic monocytes (CX3CR1<sup>lo</sup>CCR2<sup>+</sup>Gr1<sup>+</sup>) expressing high levels of CCR2 have higher efficiency in migrating into

inflamed tissue than the CX3CR1<sup>high</sup>CCR2<sup>-</sup>Gr1<sup>-</sup> subset (potentially nonclassical monocytes) [44]. In humans, CD14<sup>+</sup>CD16<sup>-</sup> monocytes share similar phenotypes and inflammation-homing potential with the mouse CX3CR1<sup>lo</sup>CCR2<sup>+</sup>Gr1<sup>+</sup> subset [44]. Therefore, delivery approaches that are based on targeting classic monocytes may lead to greater accumulation of therapeutic cargo at the site of inflammation.

Recruitment of monocytes from bone marrow via peripheral blood to inflamed tissue where they become mature macrophages and initiate repair may take days. Recent studies have identified a population of monocytes at the brain border [30,45] (Fig. 2C). Under pathological conditions such as stroke or traumatic brain injury, these cells can efficiently migrate from dura to the inflamed region, potentially through chemotaxis [30,45]. These pools of monocytes harbored in the skull marrow and dura comprise a reservoir in the vicinity of the brain that can efficiently traffic to the inflamed region. Thus, they may provide an immediate source of reparative cells that can deliver therapeutic cargo for treating neuroinflammation.

### 3.5. Cell accessibility

To efficiently deliver therapeutic cargo to a disease region through inflammation-homing myeloid cells, the myeloid cells must be loaded with the nanoparticles before they migrate to the disease region. The anatomical location of the myeloid cells determines how easily the targeted cells can be accessed by nanoparticles. For example, in glioblastoma, although microglial cells can efficiently migrate to the tumor niche during tumor development, they are hard to access by nanoparticles due to the multiple physiological barriers around the CNS and the tumor stroma, which serve as a physical barrier preventing nanoparticles from efficiently diffusing to the vicinity of microglial cells. However, circulating monocytes and macrophages from bone marrow are much easier to access by systemically administered nanoparticles. Moreover, it has recently been shown that BAMs resident at the dura mater can efficiently migrate to the site of inflammation [26] (Fig. 2C). As the blood vessels within the dura mater are fenestrated [26], this pool of BAMs is more easily accessed by nanoparticles. Thus, BAMs can be potential targets for cell-mediated delivery. Future studies will be needed for assessing which CNS delivery approaches will be suitable for enhancing nanoparticle interaction with BAMs.

### 3.6. Standard-of-care treatment

For the clinical translation of cell-mediated delivery, it is important to consider the influence of standard-of-care treatment. It is well-established that standard-of-care cancer treatments can significantly affect the population of myeloid cells and the recruitment of myeloid cells to the tumor. Specifically, chemotherapy treatment can induce neutropenia and reduce peripheral blood monocyte counts in cancer patients [46,47], potentially reducing the efficiency of myeloid cell-mediated delivery. On the other hand, radiation is known to induce the expression of chemoattractants such as CSF1, SDF-1, and CCLs, facilitating MDSC recruitment to the tumor microenvironment [48]. This effect can be utilized to enhance the efficiency of cell-mediated delivery. In this regard, a recent study showed enhanced macrophage extravasation and subsequent nanoparticle accumulation in tumor stroma after a single, low dose of radiation therapy [49]. This study demonstrated a strategy for improving cell-mediated delivery by integrating drug delivery with standard-of-care cancer treatment. Given the prevalence of cancer immunotherapy in the clinic, the effects of these treatments on myeloid cells and myeloid cell recruitment profiles need to be carefully considered in the clinical translation of cell-mediated delivery.

## 4. Delivery approaches to enhance cell-mediated delivery

Over the past 6 decades, the drug delivery field has created a variety of engineering approaches to enhance control over the distribution of drugs. Many of these existing technologies can be adopted to enhance the efficiency of cell-mediated delivery. In addition, cell therapy has achieved significant clinical success in the past few years. Much knowledge developed in the cell therapy field can be applied in the drug delivery field for developing new approaches for cell-mediated delivery. In the following sections, we selectively discuss a few studies in which nano-engineering and cell-engineering approaches are used to enhance cell-mediated delivery. These approaches can be adapted to engineer nanoparticles for myeloid cell-mediated delivery.

### 4.1. Nanoparticle surface engineering

Circulating cells have the potential to enable active transport of nanoparticle-associated drugs to diseased tissue. To enhance the interaction between nanomedicine and circulating cells, nanoparticles can be engineered for enhanced surface adsorption to, or internalization by circulating cells [50,51]. A summary of common nanoparticle surface engineering approaches is listed in Table 1. These include cellular hitchhiking, which involves nonspecific adsorption or covalent coupling/attachment; and cellular internalization, which involves phagocytosis of non-targeted nanoparticles or targeting ligand-mediated cell uptake (Fig. 3).

#### 4.1.1. Cellular hitchhiking

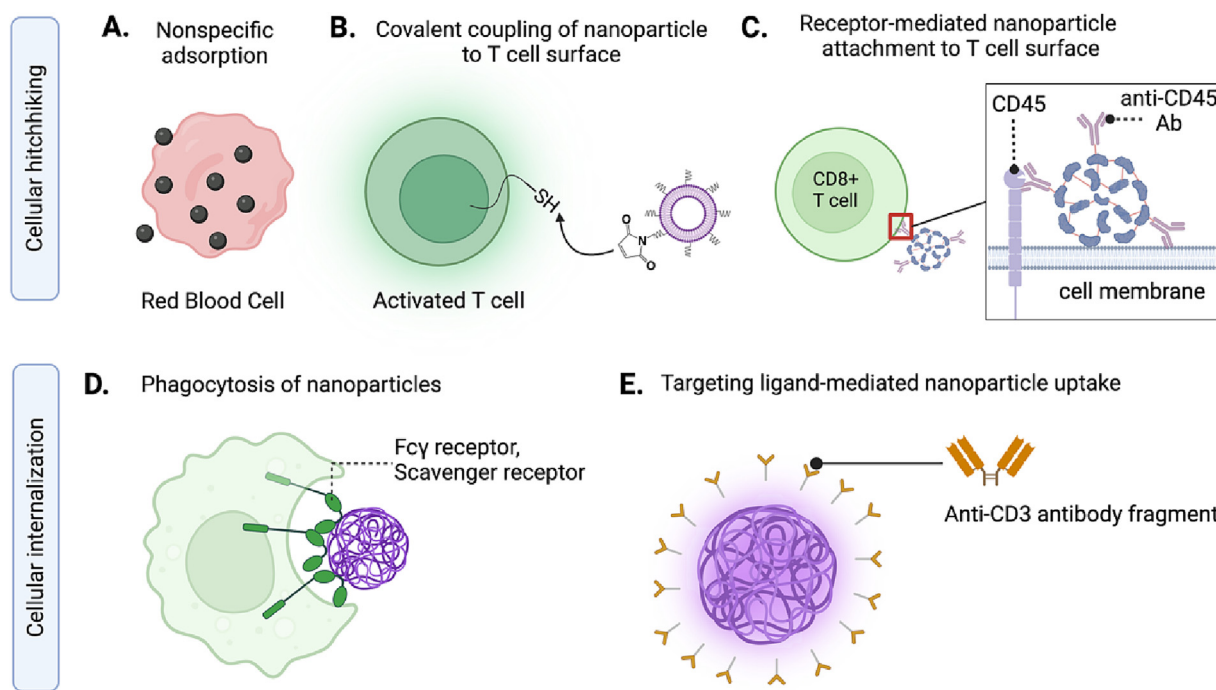
**Nonspecific adsorption.** Nanoparticle adsorption to the cell surface can be mediated by electrostatic interactions between the negatively charged membrane and positively charged nanoparticles [52]. Interactions other than electrostatic interactions, including hydrophobic, van Der Waals, and hydrogen bonding interactions may also contribute to nanoparticle attachment to the cell surface [53]. Mitragotri's group reported polymeric nanoparticles with 220 nm and 450 nm sizes bound to red blood cells (RBCs) were found to have a prolonged circulation time of up to 12 h [53] (Fig. 3A). Recent follow-up studies showed that nanocarriers adsorbed onto RBCs can achieve a ~40-fold increase of liposome delivery to the lung compared with free liposomes [54]. This approach has been validated across multiple species, including mice, rats, pigs, and *ex vivo* human lungs, making RBC hitchhiking a clinically translatable technology [54]. However, directly applying this approach for myeloid cell hitchhiking may encounter issue such as phagocytosis of nanoparticles. We will discuss in detail about this challenge and potential strategy to address this challenge in Section 5.1.

**Covalent coupling and attachment.** The nanoparticle surface can be modified with functional groups for targeting ligands to enhance their 'hitchhiking' on the cell surface through covalent coupling or receptor-mediated attachment. Motivated by the fact that activated T cells have elevated levels of cell surface free thiols relative to naïve cells [55], the Irvine group successfully anchored synthetic liposomes to the surface of activated T cells using liposomes that were surface-modified with thiol-reactive maleimide groups [56] (Fig. 3B). With this strategy, no obvious toxicity or disruption of key T cell functionalities were shown after attachment of approximately 100 nanoparticles to a T cell; such decorated T cells still showed tumor infiltration and prolonged nanoparticle retention on the cell surface [56]. A follow-up from the same group showed that the strategy did not work for 'protein nanogels' formulated from IL to 2Fc or IL-15Sa [57], as the cytokine receptors on the T cell surface can trigger the natural internalization pathways of these cytokines. However, if a small amount of anti-

**Table 1**  
Summary of nanoparticle surface engineering approaches for cell-mediated delivery.

Cellular hitchhiking	Nonspecific adsorption	Red blood cells	Carboxyl group-modified large polystyrene particles	Prolonged drug circulation [53]
			Liposomes, lysozyme dextran nanogels, PLGA-PVA nanoparticles, and albumin-nanoparticles, AAV	Target lung and brain for acute critical illness [54]
Cellular uptake/internalization	Covalent coupling or antibody-mediated attachment	T cells	Maleimide-modified liposomes or liposome-like synthetic nanoparticles	Sustained pseudo-autocrine stimulation to adoptively transferred T cells [56]
			Cytokine nanogel modified with small amount of anti-CD45 antibodies and PEG-PLL	[57]
	HSC	Platelets bearing anti-PD-1 antibodies attached to the surface of HSCs	Enhanced delivery of checkpoint inhibitor [58]	
	Phagocytosis of non-targeted nanoparticles	Neutrophils	Denatured albumin nanoparticles (Fcγ-mediated phagocytosis)	Targeted delivery across blood vessel barriers [59]
			Nanoparticles with agglutinated protein on the surface (Opsonization-mediated phagocytosis)	Acute lung inflammation detection and treatment [60]
Classical monocytes		Negatively charged PLGA polystyrene, or carboxylate microdiamonds (MARCO-mediated phagocytosis)	Therapeutic inflammatory monocyte modulation [61]	
Targeting ligand-mediated uptake	TAMs	PLGA-PEG nanoparticles	Slow-release of drugs in tumor [49,84]	
	Leukocytes	Lipoprotein-anchored LNPs, the lipoprotein can interact with Fc domain on any monoclonal antibody	<i>In situ</i> -targeting of leukocytes [64,65]	
	T cells	PbAE-PGA nanoparticles or LNP surface modified with anti-CD3 antibody fragments or anti-CD5 antibodies	<i>In situ</i> programming of T cells for disease-specific CAR expression [62,63]	

PLGA, poly(lactic-co-glycolic acid); LNP, lipid nanoparticle; PEG, poly(ethylene glycol); PLL, poly(L-lysine); PVA, poly(vinyl alcohol); PGA, polyglutamic acid; PbAE, poly(beta-amino ester); CAR, chimeric antigen receptor; AAV, adeno-associated virus; MARCO, macrophage receptor with collagenous structure.



**Fig. 3.** Schematic illustration that summarizes nanoparticle surface engineering approaches. (A) Red blood cell hitchhiking via nonspecific adsorption. (B) Maleimide-functionalized liposomes covalently coupled to activated T cell surface via thiol-maleimide conjugation. (C) Anti-CD45-modified protein nanogel for covalent attachment to T cell surface. (D) Internalization of nanoparticle via phagocytosis-associated cell surface receptors (Fcγ or scavenger receptor). (E) Enhance cellular uptake through targeted nanoparticles: Polymeric poly(β-amino ester) (PbAE) nanoparticles modified with anti-CD3 antibody fragments. (Created with BioRender.com).

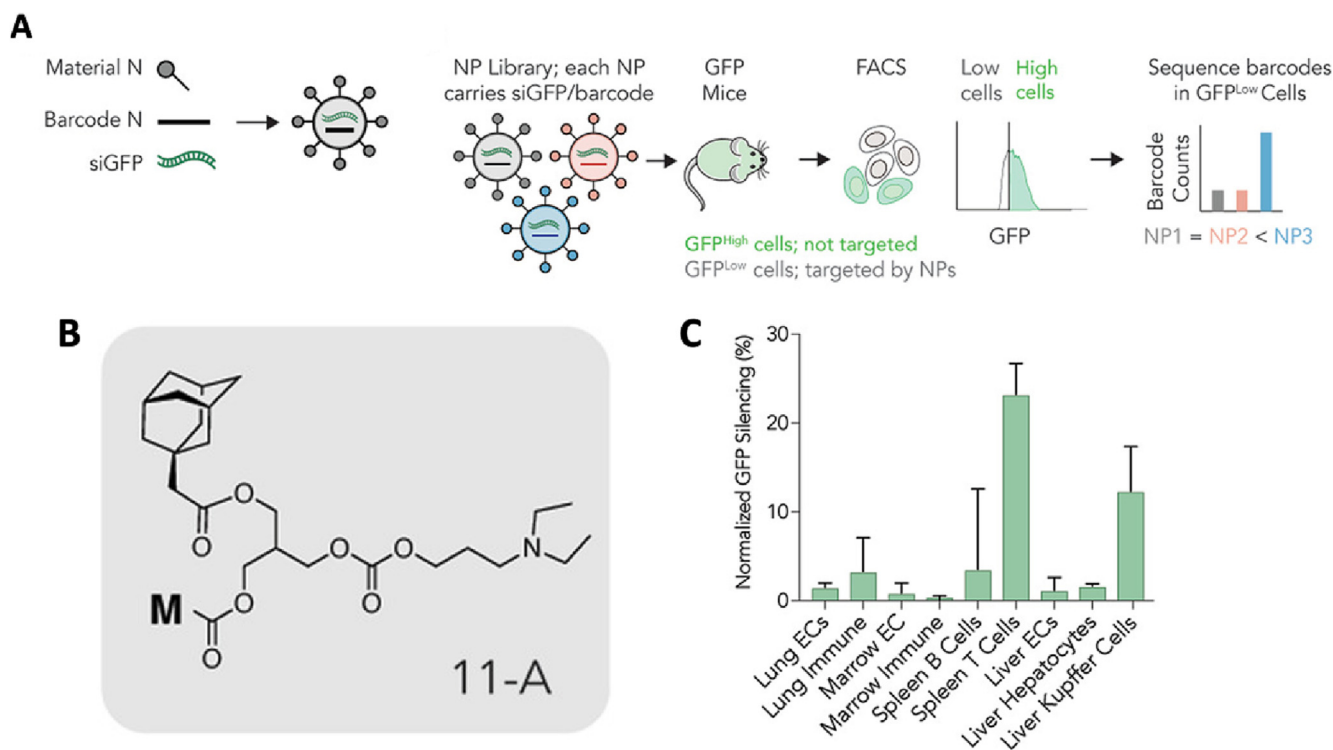
CD45 antibodies and poly(ethylene glycol)-*b*-poly(L-lysine) (PEG-PLL) were functionalized to the surface of these nanogels, the nanogels could be stably attached to the cell surface without being internalized [57] (Fig. 3C). The covalent coupling approach has also been applied to attaching blood platelets decorated with anti-programmed death-1 (PD-1) antibodies to HSCs [58].

4.1.2. Cellular uptake/internalization

**Phagocytosis of non-targeted nanoparticles.** Myeloid cells are professional antigen-presenting cells capable of phagocytosing many nanoparticles (Fig. 3D). Activated neutrophils can phagocytose

denatured albumin nanoparticles via Fcγ receptor interactions [59], providing a means for targeted drug delivery via migration of neutrophils to the site of inflammation in response to inflammatory signals [59]. When neutrophils are depleted, the transport of albumin nanoparticles across blood vessel walls is robustly abolished. It has also been shown that nanoparticles with certain surface features can trigger opsonization and formation of protein aggregates on the particle surface, leading to efficient phagocytosis by neutrophils [60].

The phagocytosis of particles can also be mediated by inflammatory monocytes through the scavenger receptor – the macro-



**Fig. 4.** Screening of DNA-barcoded nanoparticles identified specific lipid structures can enable selective transfection of LNPs in T cells. (A) Schematic illustration of the DNA-barcoding screening strategies for this constrained LNP library, where each LNP formulation contained a distinctive DNA barcode and siGFP. (B) The best performing lipid candidate contains adamantane function group in the structure. (C) siGFP LNPs formulated with lipid in B) can induce selective silencing in spleen T cells. Adapted with permission from [67] (Copyright 2019, Advanced Materials).

phage receptor with a collagenous structure (MACRO) [61]. However, it was found that uptake of biodegradable polymeric poly (lactic-co-glycolic acid) (PLGA) microparticles resulted in the sequestration of proinflammatory monocytes in the spleen followed by apoptosis of these cells [61].

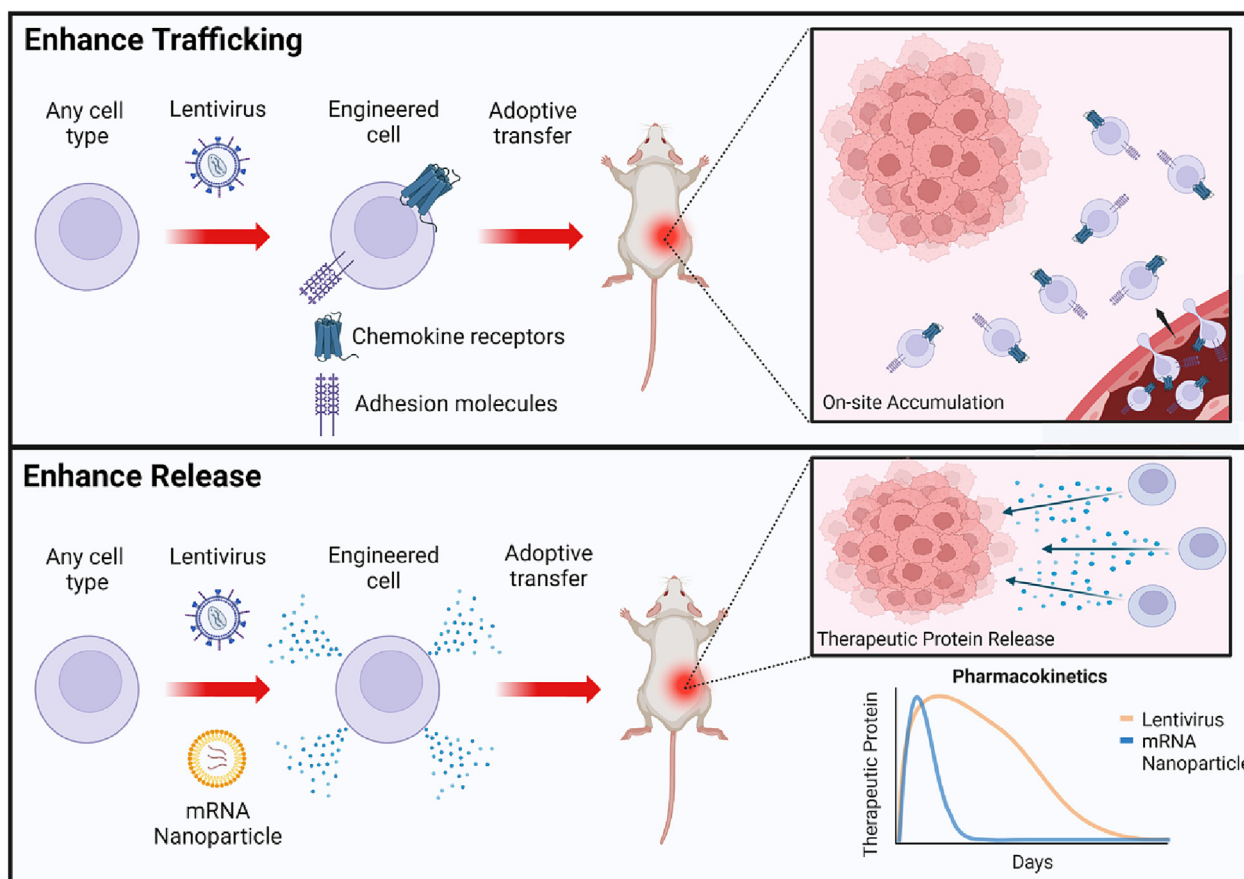
**Targeting ligand-mediated uptake.** Targeting ligands can be used to enhance nanoparticle cellular uptake, especially by non-phagocytic cells (Fig. 3E). Delivery of DNA or RNA to circulating T cells through polymer-based or lipid-based nanoparticles can be enhanced by using antibody fragments against CD3 or antibodies against CD5 as targeting ligands [62,63]. These T cell-targeting nanoparticles can significantly improve T cell targeting efficiency and can divert a large percentage of the injected dose to organs that contain T cells<sup>62</sup>. T cell-targeting nanoparticles are currently in clinical trials as alternative approaches for engineering CAR-T cells. While the chemical conjugation of antibodies to the nanoparticle surface might induce batch-to-batch variability, the Peer group has created a self-assembling modular targeted nanoparticle platform. This platform is based on a membrane-anchored lipoprotein that is incorporated into siRNA-loaded lipid nanoparticles that interact with the antibody Fc domain, enabling the construction of a theoretically unlimited repertoire of RNA-targeted carriers [64,65]. In addition to antibody-based targeting ligands, smaller-sized targeting ligands have also been used to enhance targeting efficiency. One example is the small peptide-based targeting ligand ECL1i, which allosterically binds to CCR2. A radiotracer based on ECL1i is currently in clinical trials for tracing CCR2+ inflammation-homing monocytes in pulmonary fibrosis [66].

#### 4.2. Nanoparticle library screening

An increasingly popular strategy to rationally design and identify suitable nanoparticles for selective cell-targeting is through

nanoparticle library screening. For example, the Dahlman group has reported an *in vivo* nanoparticle library screening method for siRNA delivery to T cells in the absence of targeting ligands using a high-throughput siGFP/DNA barcode-based system [67] (Fig. 4A), which allows simultaneous *in vivo* analysis of hundreds of nanoparticle formulation candidates in one animal. In this system, a specific DNA barcode was co-formulated into each nanoparticle. Successfully transfected T cells were then isolated from mice and subjected to sequencing to identify lead formulations. By combining bioinformatics, the researchers found that the conformational state of lipids can alter LNP tropism [67] (Fig. 4B, C). Recently, the Hammond group has designed another high-throughput nanoparticle screening platform called massively parallel pooled screening, which utilizes DNA barcoding, high-throughput imaging cytometry, and machine learning [68]. Briefly, using the Profiling Relative Inhibition Simultaneously in Mixtures (PRISM) method, 488 pooled, barcoded cancer cell lines were assessed for their associations with a curated nanoparticle library. The results were then analyzed via machine learning algorithms, including k-means clustering and random forest, to construct genomic nanoparticle trafficking networks and identify nanoparticle-specific biomarkers [68]. With such a sophisticated screening platform, higher predictive capacity is achievable, setting the stage for nanoparticle library screening to become a standard method for the optimization and testing of delivery materials.

The nanoparticle screening method has also been used to identify nanoparticles with monocyte-targeting selectivity. In these experiments, a library of high-density lipoprotein (HDL)-containing nanoparticles was screened using near-infrared fluorescence imaging, flow cytometry, immunofluorescence, radiolabeling, and transgenic mice [69]. They found that nanoparticle physicochemical properties (particularly size and chemical composition) were responsible for drastically different immune cell tar-



**Fig. 5.** Engineered Cells as Delivery Vehicles. Top panel depicts lentiviral engineered cells equipped with additional chemokine receptors and adhesion molecules. These modifications enhance the trafficking ability of the cells, resulting in cell accumulation at the tumor site. Bottom panel illustrates cells engineered via lentivirus or mRNA nanoparticle to enhance the sustained or transient release of therapeutic proteins at the tumor site. (Created with BioRender.com).

getting patterns. In this study, a nanoparticle formulation that selectively targets inflammatory monocytes was identified [69].

#### 4.3. Adoptive transfer of ex vivo engineered cells as delivery vehicles

The recent clinical success of cell-based therapies (Kymriah™, Yescarta™, and Tecartus™) has led to the pre-clinical development of more diverse cell-engineering approaches (Fig. 5). Additionally, a number of recently developed cell-based products have the potential to be used as cell-based delivery platforms (Table 2).

**Mesenchymal stromal cells.** Mesenchymal stromal cells (MSCs) are capable of intrinsic disease-targeting and paracrine-secretion. By removing nuclei of MSCs and modifying MSCs with chemoattractant receptors CCR2 and CXCR4, and endothelial cell-adhesion molecules PSGL-1, the Klemke lab created “cargocytes” that possess cell-like functions such as chemotactic migration toward defined chemokine signals, regulated high-affinity integrin activation, endothelial cell adhesion, and transmigration. The researchers then transfected cargocytes with mRNA encoding a therapeutic protein and showed that the cargocytes persisted for 72–96 h while continuously releasing therapeutic proteins at disease tissues [70].

**Neutrophils.** Researchers reported a neutrophil-mediated drug delivery system by pre-incubating paclitaxel-loaded liposomes with neutrophils. After surgical tumor removal, inflammatory stress occurs. The resulting upregulation of pro-inflammatory cytokines directed the systemically injected neutrophils ( $5 \times 10^6$  cells/mouse) to migrate along the chemotactic gradient and infiltrate the tumor site. Although most injected neutrophils were

detected in the liver, increased neutrophil infiltration and liposome accumulation at the brain tumor site were also observed. After arriving at the tumor site, the neutrophils were activated by inflammatory signals and released neutrophil extracellular traps (NETs), during which process the liposomes were also released [71].

**Macrophages/Monocytes.** Macrophages/monocytes are another target of interest in adoptive cell transfer. Researchers have developed genetically engineered human macrophages (GEMs) that release therapeutic proteins such as IL-12, BiTE [72,73], and glial cell line-derived neurotrophic factor (GDNF) [74]. After lentiviral transduction, GEMs stably secrete proteins as long as 80 days after transduction (Fig. 6A, B). While most systemically injected GEMs accumulated at the lung on day 1 post-injection, the GEMs did not persist in the lung (Fig. 6C, D). At day 3 post-injection, the remaining GEMs localized to the tumor where they remained until day 24 when animals were euthanized (Fig. 6C, D). Within the first 24 h, 0.99 % of intravenously injected GEMs trafficked to the tumor where they persisted for 7 (0.97 %) and 14 days (0.83 %) [72,73]. Similarly, when genetically engineered mouse macrophages were adoptively transferred into a murine model of Parkinson disease, more macrophages were accumulated at the brains of Parkinson disease mice than those of healthy mice [74]. Nanoparticles carrying mRNAs have also been used to engineer adoptively transferred macrophages. In a recent study, the Dong group transfected murine macrophages *ex vivo* using vitamin C lipid nanoparticles (VcLNP) that carry antimicrobial peptide AMP and cathepsin B (AMP-CatB)-encoding mRNA [75]. They showed that mRNA was translated in the macrophage cytoplasm, and the resulting functional

**Table 2**  
Summary of cell-based product that can be used as cell-based delivery platforms.

Cell-engineering method	Application	Ref
MSCs	Lentiviral transduction of MSC to express CCR2, CXCR4, and PSGL-1, cell enucleation through ultracentrifugation, mRNA transfection to release therapeutic protein	Enhanced inflammation-homing and therapeutic protein release [70]
Neutrophils	Incubate liposomal-formulated paclitaxel with neutrophils as novel delivery system	Targeted delivery of chemotherapeutics to the brain [71]
Monocytes/ Macrophages	Ex vivo differentiation of monocytes into macrophages, lentiviral/pDNA programming of macrophages to release protein drugs	Improve the pharmacokinetics of protein drugs [72–74]
	Ex vivo transfection of murine macrophages with LNPs carrying mRNAs that encodes for antimicrobial drugs.	Nanoparticle-enabled macrophage for the elimination of multidrug-resistant bacteria [75]
CAR-T cells	Lentiviral engineering of CAR-T cells to release proteins drugs (Bispecific antibody)	Improve the therapeutic efficacy of CAR-T and bispecific antibody [76]
B cells	CRISPR/Cas9 for replacing endogenously-encoded antibodies with antibodies targeting infectious diseases	Genetic engineering of primary human B cells to secrete pathogen-specific antibodies [77]

proteins were translocated into the lysosomes, where AMP-CatB was released, killing bacteria encapsulated in phagosomes when they fused with lysosomes [75]. Adoptive transfer of these V<sub>C</sub>-LNPs-engineered macrophages in a mouse model of sepsis significantly increased their survival.

In addition to therapeutic cargo release through genetic engineering, macrophages can also be used to load and deliver protein-based drugs [76–78] and small molecule drugs [79–81]. For example, the Batrakova group has developed a self-assembled catalase/PEI-PEG complex, termed “nanozymes,” that were efficiently taken up by macrophages within 1 h and can be slowly released in an active form for more than 24 h [76]. Packaging the redox enzyme catalase into a block ionomer complex enables drug-carrier enzyme stability by preventing macrophage-mediated enzyme degradation [77]. Following adoptive transfer, nanozyme-loaded bone marrow macrophages carry active enzyme (0.6 % of the injected dose) across the blood-brain barrier in a murine model of Parkinson’s Disease [76,77]. Once in the brain, nanozyme-loaded macrophages transport the cargo to endothelial, neuronal, and glial cells through endocytosis-independent mechanisms [76].

**CAR T Cells.** Researchers have developed a platform that incorporates both chimeric antigen receptor (CAR) and bispecific T-cell engager (BiTE) into a single engineered cell (CART.BiTE) through lentiviral transduction. In their mouse model, after a single systemic dose, CART.BiTEs were able to efficiently infiltrate a brain tumor, and the BiTEs were detected in blood and brain, indicating the BiTEs were released by CART.BiTE in vivo. However, the BiTE releasing kinetics by CART.BiTEs were not reported in this study [82].

**B Cells.** In a recent study, human and murine B cells were engineered to express antibodies targeting infections with the help of CRISPR/Cas9 technology. The engineered B cells showed sustained

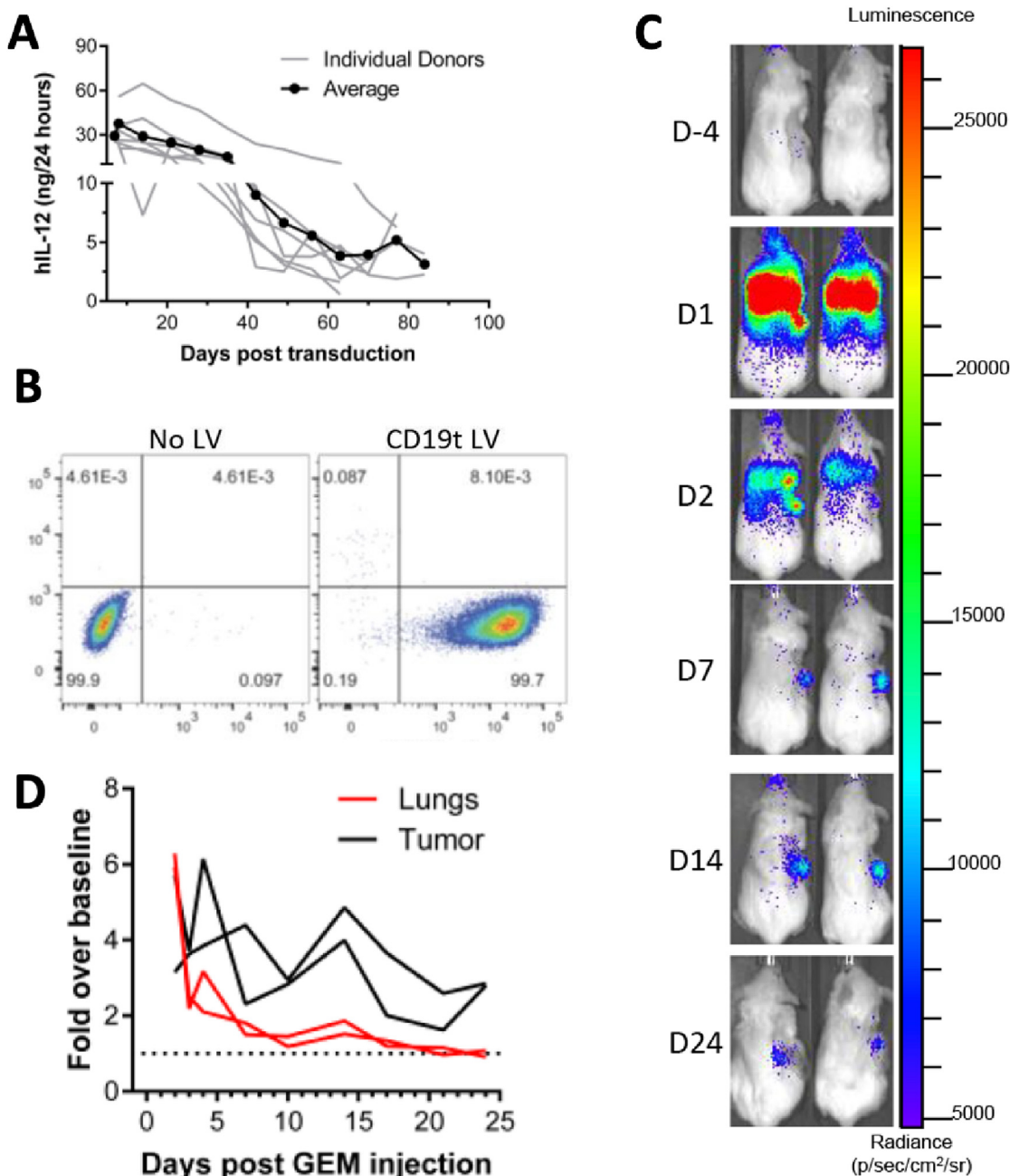
release of therapeutic antibodies over 80 days. A single injection of engineered B cells expressing RSV-specific antibodies resulted in potent protection against infection in the lymphocyte-deficient mice [83].

#### 4.4. Emerging technologies

**Immune camouflage:** an emerging platform for cell-mediated delivery is to use cell membrane-coated nanoparticles composed of a synthetically derived nanoparticle core and a surface coating of naturally derived cell membrane [84,85]. Based on this concept, the Zhang group has developed platelet membrane-cloaked nanoparticles (PNPs), which are PLGA nanoparticle cores coated in the plasma membrane of human platelets [84]. PNPs inherently mimic platelet functionalities, such as subendothelial adhesion via collagen binding, immune evasion, and selective adherence to damaged human and rodent vasculatures. An in vivo study employing a mouse cancer model showed enhanced therapeutic delivery using this cell-mimicking nanocarrier platform [84].

**Layer-by-layer coating:** Layer-by-layer (LBL) assembly is another emerging cell coating approach that utilizes electrostatic adsorption between negatively charged cell membranes and polyelectrolyte multilayers (PEMs) [86]. LBL assembly with a standard photolithography lift-off technique has been used to fabricate micron-scale patches as ‘backpacks’ for leukocytes-mediated drug delivery [87,88]. These ‘backpacks’ usually consist of three layers: a releasable region that deconstructs in noncytotoxic conditions, a payload region that encapsulates therapeutics, and a leukocyte-adhesive layer that anchors the payload to the cell membrane [89]. This strategy provides a novel method for surface functionalization of living leukocytes and was first developed for targeted delivery of therapeutics to inflamed tissues [90]. The Mitragotri group first reported a cell-based drug delivery technology using macrophages with phagocytosis-resistant backpacks [88]. Quantification analyses by time-lapse video microscopy and Methyltransferase (MTT) assay have indicated that this elongated, flat, high-aspect-ratio, and disk-shaped backpack can attach firmly to the macrophage surface and resist phagocytosis while maintaining native macrophage cellular functions [88]. A recent in vivo study published by the same group showed that phagocytosis-resistant backpack-loaded macrophages with interferon- $\gamma$  as the cytokine cargo can polarize macrophages towards proinflammatory M1 phenotypes and maintain M1 phenotypes deep within an immunosuppressive tumor [91].

**Metal-phenolic networks:** metal-phenolic networks (MPNs) are multivalent, self-assembled coatings formed by chelation between metal ions and polyphenols. The flexible structure of MNPs allow physicochemical property modifications, such as metal chelation, pH responsiveness, and mechanical and surface properties tuning [92], for versatile biomedical applications [93–95]. MPNs can also be used for single cell encapsulation and protection, which can be adapted for cell-mediated drug delivery purpose [96,97]. For example, by crosslinking the tannic acid from MPNs with the iron ion on cell surface, MNPs can form a shell-like coating on the surface of different cell types, protecting cells from unfriendly environments [98]. This shell-like coating is biodegradable under external stimuli, allowing controlled drug release at specific tissue microenvironment. One challenge associated with cellular therapy product is how to stably preserve and recover the cells. A recent study developed a self-assembling cellular coating based MNPs [99]. This coating can improve the viability and stability of the bacteria during the harsh cryo-preserving process such as stresses, including oxygen exposure and lyophilization, paving the foundation for this technology to be applied to nearly all cellular biotherapeutics [99].



**Fig. 6.** Genetically engineered human macrophages (GEMs) for targeted therapeutic protein delivery to glioblastoma. (A) GEMs are capable of sustained secretion of immunomodulator cytokine (human rIL-12) for up to 80 days, with the rIL-12 expression peaking at 8–14 days. (B) Flow cytometry quantification of GME transgene expression indicating high transfection efficiency. (C) Bioluminescence imaging shows the biodistribution of adoptively transferred GEMs. (D) Quantification of bioluminescence signal based on (C) shows initially predominant localization of GEMs to the lungs without persistence. GEMs were capable of accumulating in the tumor after 3 days and persisting up to 24 days. Adapted with permission from [72] (Copyright 2020, BMJ).

### 5. Discussion: Engineering factors that affect cell-mediated delivery

In this section, we discuss the advantage and limitations of different nano-engineering and cell-engineering approaches described above and how can they be adapted to engineer myeloid cells for cell-based delivery.

#### 5.1. Cellular uptake vs Cellular hitchhiking

Nanoparticle surface engineering is a well-studied area with various established engineering nanoparticle platforms to enhance

interactions of nanoparticles with the cells of interest either through cellular uptake or cellular hitchhiking.

**Cellular uptake.** The phagocytic nature of myeloid cells can significantly contribute to intracellular uptake of nanoparticles. For delivery strategies that rely on the cellular uptake of nanoparticles, the therapeutic cargo needs to remain intact during trafficking but readily released upon arrival at the disease site. A recent study from the Miller group elegantly elucidated the mechanism behind this progress using fluorescence live-imaging analysis [100]. The study showed that TAMs serve as the reservoirs of nanoparticles, and they slowly released and redistributed nanoparticles by directly transferring nanoparticles to neighboring tumor cells

within a range of  $<25\ \mu\text{m}$  [100]. The release of nanoparticles from cell reservoirs could also be triggered by *in vivo* signals. The highly concentrated inflammatory signals in the tumor can trigger neutrophils to release NETs [101]. This triggered-release mechanism has been utilized in a recent study of neutrophil-based delivery, in which intact chemotherapy-loaded liposomes were rapidly released from neutrophils at the tumor site along with the release of NETs [71]. In addition to the above mechanisms, intracellular therapeutic cargo can also be released through the secretion of extracellular vesicles (EVs) [102]. As the trafficking cells reach the end of their life cycle, their apoptosis may also expose the therapeutic cargo to the disease region, although this mechanism is less studied. Finally, if the nanoparticle/viral particles carry DNA or RNA, the encoded therapeutic proteins can be released extracellularly over time through protein secretion [103].

**Cellular hitchhiking.** Compared to cellular uptake, nanoparticles hitchhiking on the cell membrane are released more readily. In this case, the release of therapeutic cargo can be achieved by the direct detachment of nanoparticles from the cell membrane (for example, delivery of payloads through RBC hitchhiking can result in sustained drug release for as long as 7 days [56,104]). Alternatively, the nanomaterial (polymer-based, lipid-based, or biologics-based) can be degraded through hydrolysis [105], redox reaction [57], or dissolution [106]. One challenge particularly associated with hitchhiking on myeloid cells is to avoid phagocytosis of the nanoparticles. Certain approaches designed for cellular hitchhiking has worked for T cells, for example, maleimide-mediated covalent coupling, but has not worked for myeloid cells, due to the phagocytic nature of these cells [56]. The challenge can potentially be addressed by modifying the nanoparticle surface with anchoring antibodies such as CD47 [107], or by using an elongated, flat, high-aspect-ratio, and disk-shaped polymeric backpack, which can strongly attach to the macrophage surface and resist phagocytosis while maintaining native macrophage cellular functions [88].

## 5.2. Targeted vs non-targeted platforms

It remains a matter of debate whether targeting ligands will significantly increase the interaction of nanoparticles with the cells of interest if those cells are located within the tissue stroma. The challenge of delivery is how to enhance the transport of the nanoparticles in the tissue stroma so they can move to the vicinity of the targeted cells. This process is often limited by physical barriers such as the blood-tissue barrier and dense tissue stroma. In these cases, targeting ligands might not be useful as they do not enhance the transport of nanoparticles across these physical barriers. However, cell-mediated delivery is well-suited for targeting circulating cells in the blood, where nanoparticle transport is not limited by tissue barriers. In the blood, the transport of nanoparticles is primarily driven by the movement of the fluid, where there is a greater opportunity for the nanoparticle to reach the cells of interest. In this situation, targeting ligands can significantly enhance nanoparticle interaction with the circulating cells. This also means targeted nanoparticles may require a lower dose to achieve equal delivery efficiency compared to non-targeted nanoparticles. As the circulating cells migrate to the disease tissues, they will also direct a higher fraction of injected dose to these tissues. For example, the Stephan group reported that CD3-targeted nanoparticles transfected 11-fold more circulating T cells than did non-targeted nanoparticles. As well, CD3-targeted nanoparticles significantly increased the distribution of nanoparticles from the liver to T-cell-rich lymphoid organs such as the spleen and lymph nodes [62].

To design targeted nanoparticles as delivery platforms for cell-mediated delivery, a few important engineering factors discussed below need to be considered.

**Targeting specificity.** Cell surface receptors are usually selected as the target site when designing a targeted delivery system. To avoid toxicity induced by the off-target effect, an ideal targeting site should confer targeting specificity (i.e., the expression of the surface receptor should be limited to the cells of the target). This is often challenging for targeting a specific myeloid cell subset (for example, inflammatory monocytes), as myeloid cell surface receptors are commonly expressed across all myeloid cell subsets as well as some non-lymphoid tissues. For example, adhesion molecules such as the MAC-1 (also referred to as CD11b/CD18 or integrin  $\alpha\text{M}\beta 2$ ) is frequently selected as the target for drug delivery to monocytes/macrophages during inflammation [108]. However, MAC-1 is also expressed on the surface of many other leukocytes and tissues including spleen and kidney ([proteinatlas.org](http://proteinatlas.org)). Nanoparticles that target MAC-1 have the potential to cause off-target effects in these tissues, leading to adverse immune responses. Another frequently selected receptor for targeting TAMs and dendritic cells is the mannose receptor (CD206/MRC1) [109–111]. However, as a C-type lectin, mannose receptor expression has also been detected in other leukocytes such as dendritic cells, and non-lymphoid organs such as liver (sinusoidal endothelial cells) [112], lung (alveolar cells), and kidney (tubules) ([proteinatlas.org](http://proteinatlas.org)), leading to potential toxicity associated with off-target delivery to these tissues. Although dual-targeting delivery systems have been proposed to increase the targeting selectivity [113], this approach might face extra challenges during GMP manufacturing and in clinical translation [114].

**Functional activation/inhibition.** While targeting ligands can increase the delivery efficiency to the targeted cells, they also have the potential to induce functional activation or inhibition of the signaling pathways mediated by the targeted cell surface receptors. For cell-mediated delivery, which relies on the cell's ability to traffic to the disease region, it is critical to determine whether the targeted delivery system alters chemotaxis and migration function of the cell. For example, the Nguyen group has shown in an *in vitro* model that liposome surface modified with a CCR2-targeting single-chain variable fragment (scFv) can significantly reduce the migration efficiency of macrophages along a CCL2 gradient in a dose-dependent manner [115]. Although CCR2-targeted nanoparticles have the potential to induce migration inhibition, we would argue that targeting ligands can be used to engineer delivery system provided that the injected dose does not induce a significant inhibitory effect *in vivo*. This often requires careful evaluation. For example, ECL1i is a small peptide that selectively binds to CCR2 and inhibits CCR2-mediated chemotaxis *in vitro* [116]. However, when used as a targeting ligand for *in vivo* imaging of monocyte infiltration, the selected dose did not result in any inhibitory effects [66].

**Selection of targeting ligands.** Major types of targeting ligands include small molecule-based, aptamer-based, peptide-based, and antibody-based ligands. Small molecule-based and short peptide-based targeting ligands usually have low molecular weight, low immunogenicity, and can be manufactured at scale. However, they also have weaker interactions (binding affinity) with the targets compared to antibody-based targeting ligands [117,118]. To compensate for their lower affinity, multivalent targeting systems can be used to increase the binding avidity. Aptamers, recombinant antibodies, and antibody fragments usually have higher molecular weights. Their strong binding coefficient in the sub-nanomolar range enables efficient engagement of the nanoparticles with the targeted cells [118]. However, these molecules are expensive, are sometimes immunogenic, and may have fastidious storage condition requirements [117].

**Shortcomings of targeted nanoparticles.** Although targeted delivery platforms present obvious delivery advantages for targeting circulating cells over non-targeted delivery platforms, clinical

translation and GMP manufacturing of targeted nanoparticles may pose extra challenges compared to non-targeted nanoparticles [114]. For example, as antibody or antibody fragments are often used as the targeting ligands in the targeted platforms, translation of these platforms from mice to humans requires re-establishing humanized antibodies or antibody fragments. Moreover, as GMP manufacturing of nanomedicine requires reproducibility, the addition of surface targeting ligands adds an extra step in the complicated GMP manufacturing process and increases the challenge for limiting batch-to-batch variation.

### 5.3. Enhancing cell-mediated delivery without targeting ligands

In many cases, targeting ligands are used to enhance efficiency of delivery and selectivity for circulating target cells. However, if the targets are myeloid cells/phagocytes, then the composition of the nanoparticles can sometimes promote their internalization via phagocytosis (Fig. 5D). For example, the Brenner group has shown that unique surface properties of nanoparticles such as lysozyme-dextran nanogels, PEG-crosslinked albumin nanoparticles, and nanoparticles based on electrostatic interactions of charged proteins can trigger the opsonization and agglutination of proteins on the nanoparticle surface, resulting in efficient phagocytosis and neutrophil-mediated selective delivery to inflamed lungs. However, this phenomenon was not observed with nanoparticles such as viral capsids or bare liposomes, which cannot trigger protein agglutination on their surface [60]. In another study, microparticles derived from negatively charged polystyrene, carboxylated-microdiamonds, or biodegradable PLGA can be efficiently phagocytosed by inflammatory monocytes through the scavenger receptor MARCO [61]. These recently discovered mechanisms can be utilized to improve nanoparticle uptake by phagocytes. Since targeting ligands are not required, clinical translation of these delivery platforms will likely be easier than translation of targeted delivery platforms.

Another elegant approach to selectively targeting circulating cells without targeting ligands is through the combination of DNA-barcoding and nanoparticle screening [67]. Increasing the sample volume for *in vivo* nanoparticle screening has always been a challenge in the drug/gene delivery field [114]. DNA-barcoding technology allows the simultaneous screening of hundreds of nanoparticle formulations *in vivo*. The Dahlman group hypothesized that natural lipid trafficking pathways can promote the delivery of nanoparticles to certain immune cells. Based on this hypothesis, the group discovered that LNP formulations containing adamantane can achieve a high transfection rate of splenic T lymphocytes (Fig. 4). As HDL shows intrinsic tropism towards macrophages and monocytes [69], the DNA-barcoding technology can be used to screen HDL-modified nanoparticles for improved monocyte/macrophage targeting.

### 5.4. Adoptive transfer vs *in situ* targeting

With the recent clinical success of T cell-based cancer immunotherapies, a variety of cell therapy products are currently under active pre-clinical testing. Among these, for example, are virus-based genetic engineering approaches aimed at programming immune cells to release therapeutic proteins (Fig. 5). With these approaches, macrophages or B cells can be genetically programmed for sustained release of therapeutic cytokines or antibodies [72,83]. Adoptive transfer of these genetically engineered cells can be utilized to improve the pharmacokinetics of therapeutic proteins, especially for therapeutic cytokines or BiTEs (Fig. 6), for which clinical translation is limited by narrow therapeutic windows. Moreover, cells can be engineered to enhance their tissue trafficking capacity (Fig. 5). In a recent study, MSCs were engi-

neered to express chemokine receptor CCR2, CXCR4, and adhesion molecule PSGL1 to enhance their inflammation-homing capacity [70]. Adoptive transfer of these engineered cells enabled active transport of therapeutic cargo to the targeted sites, an achievement not obtainable with nanoparticle-based delivery platforms. Although the adoptive transfer of engineered cells is a promising approach for drug delivery, *ex vivo* cell engineering often requires a lot of time and extensive resources and is less likely to be developed as an 'off-the-shelf' therapy. Moreover, as immune cells such as myeloid cells are highly plastic, they are subjected to turning into pathological cells after interacting with the tissue microenvironment at the disease region. These limitations need to be addressed in the clinical translation of cell-based delivery platforms.

*In situ* engineering of circulating cells lies at the interface of nanomedicine and cell-based therapy. Compared to adoptive transfer of cells, it offers a quicker approach to engineering immune cells *in situ* [62,63,103,109]. As an off-the-shelf therapy, nanomedicine is less expensive to produce. However, targeting selectivity is a critical challenge for nanoparticle-based *in situ* engineering. Specifically, if the *in situ* engineering approach is used to program circulating cells to release therapeutic proteins, the accumulation of nanoparticles in off-targeted sites such as the liver, lung, and spleen might lead to increased protein secretion at these organs. The toxicity of this approach needs to be carefully evaluated.

Finally, depending on the location of disease foci, adoptive transfer and *in situ* targeting approaches might show different potential in targeted delivery to the disease tissue/organ when the delivery vehicle is administered through different delivery routes. Take CNS delivery for example, a recent study based on nonhuman primates compared the brain accumulation of adoptively transferred peripheral blood mononuclear cells (PBMCs) and monocyte-derived extracellular vehicles (EVs) delivered through different routes of administration: intraperitoneal (IP), intravenous (IV), and intrathecal (IT). The study found adoptively transferred PBMCs produced higher brain retention than EVs when delivered *i.t.*; while EVs showed superior brain accumulation than adoptively transferred PBMCs when IP and IV were chosen as the delivery routes [119].

## 6. Concluding remarks

To enhance the efficiency of cell-mediated delivery, a variety of cell types have been explored and a diversity of nano-engineering and cell-engineering approaches have been created. Although some pre-clinical success has demonstrated the potential of this new research direction [50], the translation of cell-mediated delivery platforms into the clinic will require a significant effort to integrate engineering with disease pathology. Here, we aimed to provide a summary of the biology/physiology of myeloid cell trafficking and engineering approaches related to cell-mediated drug delivery. To bridge the knowledge gaps between engineering with disease pathology, we discussed various factors that might affect myeloid cell-mediated delivery, from biological aspects (e.g., cell biology, disease pathology, and standard-of-care treatments) to engineering aspects (e.g., nano-engineering and cell-engineering approaches).

We expect to see a gradual merger of nano-engineering with cell-engineering. To move the field forward, several questions will need to be addressed:

- (1) How do we identify and target cell subsets with high inflammation-homing efficiency? As we discussed in Section 3.4., recent progress in neuro-immunology has identified monocyte/macrophage subset that can efficiently

migrate to inflamed brain tissue [30,45]. The identification of more immune cell subsets that can efficiently migrate to different organs under the context of different diseases will assist the development of more diverse cell-based delivery approaches in the future.

- (2) How will targeting ligands that interact with chemokine receptors and adhesion molecules affect the function of the cell surface receptors? As many targeting ligands have the potential to induce functional activation and inhibition of these surface receptors, it is important to use *in vitro* or *ex vivo* chemotaxis and migration assays to understand the dose-responsive effect of targeting ligands under a therapeutically relevant dose range.
- (3) Can we establish connection between nanoparticles structural properties with their intrinsic tropism towards certain immune cells? Recent studies have discovered that nanoparticle surface properties can dictate their interaction with serum complement proteins [60] and lipid structures (for LNPs) have significant influence on the natural lipid trafficking pathways [67], both of which will affect nanoparticle's intrinsic tropism towards immune cells. Towards this direction, more studies will be needed to elucidate the relation between nanoparticle's structural properties and their intrinsic tropism towards certain immune cells.
- (4) How do we quantify drug accumulation at a disease region following delivery by the trafficking cells? Both EPR effect and cell-mediated drug delivery are believed to contribute to the accumulation of therapeutic cargo within the inflamed tissue. However, there is a lack of mechanistic study to understand to which extent cell-mediated drug delivery can contribute to the drug accumulation. More sensitive quantification approaches should be developed to measure and isolate the drug accumulation contributed by both mechanisms.
- (5) What is the fate of the therapeutic cargo after the cells arrive at the disease region? Successful drug delivery requires the release of therapeutic cargo after it arrives at the disease region. More sensitive assays or quantification methods should be developed to measure the concentration of free drugs that are not associated with the delivery vehicles within the disease region.
- (6) How to control the trafficking and biodistribution of adoptively transferred immune cells? Although adoptively transferred immune cells can efficiently and actively transport to the inflamed region, a large fraction of adoptively transferred cells still localize in the off-target tissues such as lung and liver. It remains a challenge how to control the biodistribution and further enhance the trafficking efficiency of adoptively transferred immune cells.
- (7) How do we engineer immune cells with safe and controlled therapeutic drug release? Therapeutic molecules release by viral-engineered cells can lead to pro-longed uncontrollable drug release. Synthetic biology approaches to engineer cells to release cargo in response to certain extrinsic stimulus molecules can potentially address this challenge.
- (8) If cells with high plasticity are used, how do we avoid adverse effects associated with phenotypic changes? For example, myeloid cells are highly plastic and may change phenotype based on the tissue microenvironment and further contribute to the disease progression. The adverse effect associated with phenotypic change needs to be addressed if these cells are used as delivery vehicles.

To answer these questions and to facilitate the clinical translation of cell-mediated delivery strategies, an interdisciplinary effort will be required between formulation scientists, immunologists, bioengineers, and physicians.

## Data availability

Data will be made available on request.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

The authors acknowledge Ocala Royal Dames for Cancer Research Foundation, American Cancer Society, and University of Florida College of Pharmacy for their support.

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