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To cite this article: Duy Hieu Truong, Thi Thu Phuong Tran, Han Thuy Nguyen, Cao Dai Phung, Tung Thanh Pham, Chul Soon Yong, Jong Oh Kim & Tuan Hiep Tran (2018): Modulating T-cell-based cancer immunotherapy via particulate systems, Journal of Drug Targeting, DOI: 10.1080/1061186X.2018.1474360

To link to this article: https://doi.org/10.1080/1061186X.2018.1474360

Accepted author version posted online: 09 May 2018.
Published online: 03 Jun 2018.

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ABSTRACT

Immunotherapy holds tremendous promise for improving cancer treatment in which an appropriate stimulator may naturally trigger the immune system to control cancer. Up-to-date, adoptive T-cell therapy has received two new FDA approvals that provide great hope for some cancer patient groups. Nevertheless, expense and safety-related issues require further study to obtain insight into targets for efficient immunotherapy. The development of material science was largely responsible for providing a promising horizon to strengthen immunoengineering. In this review, we focus on T-cell characteristics in the context of the immune system against cancer and discuss several approaches of exploiting engineered particles to manipulate the responses of T cells and the tumour microenvironment.

Introduction

In addition to surgery, radiotherapy and chemotherapy, immunotherapy has become one of the pillars of current cancer treatment [1–3], particularly in treating patients with advanced or refractory cancers as seen by the approval of the US Food and Drug Administration (FDA) of sipuleucel-T (Provenge®, Dendreon) for advanced prostate cancer [4], and ipilimumab (Yervoy®, Bristol-Myers Squibb) for metastatic melanoma [5]. Due to general advances in science, particularly molecular biology, immunotherapy possesses several advantages over traditional cancer treatment regimens, including high specificity and excellent safety profiles with limited or even no undesirable effects. The key difference between cancer immunotherapy and the other three above-mentioned approaches is its exploitation of patients’ own immune system to combat and destroy cancer cells through activation and expansion of cancer-specific T lymphocytes (Figure 1). To reach its destination, anticancer-specific immunity requires three steps [6,7]. First, antigen presenting cells (APCs), such as dendritic cells (DCs) and macrophages, capture and process tumour-associated antigens (TAAs) into antigenic peptides which are then combined with human leukocyte antigen (HLA) molecules and presented on the cell surface for recognition by T-cell receptors (TCRs) (signal 1). Second, the costimulatory molecules B7 on APCs must bind to CD28 on T cells to activate T cells (signal 2) [8]. Both signal 1 and signal 2 are needed to guarantee full and optimal activation of T cells without causing T-cell tolerance. Sometimes, an extra signal (signal 3), which involves cytokines, such as interleukin (IL)-2, can play a critical role in shaping the immune response [8,9]. Finally, activated cancer-specific T lymphocytes move to tumour sites and eradicate cancer cells after recognising TAAs [10].

Acknowledging their crucial roles in cancer immuno-surveillance and eradication, several therapies have been developed to produce, manipulate and/or enhance cancer-specific T lymphocytes over the past three decades [11,12]. The first option is adoptive T-cell therapy (ACT) which is the most powerful approach in cancer immunotherapy [13]. This approach involves the isolation of tumour-specific T cells, their expansion ex vivo to obtain sufficiently high number, and infusion back into the patient. These T cells can be isolated from resected tumours (also known as tumour-infiltrating lymphocytes or TILs) [14–16] or genetically modified by transfection with TCRs or chimeric antigen receptors (CARs) [17–20]. Very recently, the FDA approved two CAR-based gene therapy products, namely tisagenlecleucel (Kymriah®, Novartis) for refractory/relapsed B-cell acute lymphoblastic leukaemia (ALL) [21,22] and axicabtagene ciloleucel (Yescarta®, Kite) for refractory/relapsed large B-cell lymphoma [23,24], suggesting a very promising way to develop future ACT therapies.

The use of immune checkpoint inhibitors, which affect the intrinsic regulatory mechanisms of T lymphocytes, has also been taken into consideration [25–27]. These antibodies can bind to immune checkpoint receptors, such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), programmed death 1 (PD-1) and its major ligand PD-L1, thereby blocking the transmission of inhibitory signals to T lymphocytes. Several promising clinical trials have been performed with numerous FDA-approved drugs that are available on the market such as nivolumab and pembrolizumab [4,5,28–31]. Additionally, combinations of immune checkpoint blockade with ACT or other therapies such as chemotherapy and granulocyte-macrophage colony-stimulating factor (GM-CSF) have demonstrated superior outcomes compared to those mentioned alone [12,32–34].

Beyond the intrinsic aforementioned regulatory pathway, the tumour microenvironment (TME) is also an interesting target that has drawn attention for developing cancer immunotherapy [35–37]. Normally, regulatory T lymphocytes (Tregs), myeloid-derived suppressor cells (MDSCs), transforming growth factor (TGF)-β, and other mediators are upregulated in the TME and inhibit cancer-specific T-lymphocyte functions resulting in immunosuppression and poor...
immunotherapeutic efficacy by various mechanisms (Figure 2) [38–44]. For example, Tregs can suppress APCs via CTLA-4, consume IL-2, release inhibitory cytokines (IL-10, TGF-β and IL-35), express granzyme/perforin and degrade adenosine triphosphate (ATP). MSDCs are a highly diverse population of immature myeloid cells that can produce arginase 1 and release reactive oxygen species, nitric oxide and immune-suppressive cytokines [45,46]. TGF-β is upregulated in various types of cancer, such as melanoma, breast, stomach and liver cancers, and plays a key immunosuppressive signal in promoting tumour evasion [47,48]. TGF-β can suppress the activation and attenuate the cytotoxicity of cytotoxic T lymphocytes (CTLs), DCs and macrophages, while inducing the production of Tregs [49–52]. Some potent TGF-β inhibitors have been developed but have thus far demonstrated poor pharmacokinetics, low tumour penetration capacity and high toxicity [53–55]. These above issues highlight the urgent need for exploring efficient strategies to successfully regain control of the TME for effective cancer immunotherapy.

Along with the dramatic advances in the field of cancer immunology, we have also witnessed impressive progress in fabricating delivery systems with improved pharmacokinetics and cell/tissue targeting to increase specificity and efficacy and decrease toxicity, potentially overcoming some of the limitations of current radiotherapy and chemotherapy [57–62]. Hence, delivery systems targeting immune molecules and cells are supposed to support the development of cancer immunotherapy of which the principle is mentioned above. Moreover, these particulate carrier systems can be used for labelling and tracking immune cells by different imaging techniques to better understand the cellular mechanisms of tumour development and metastasis as well as to assess the efficacy of some treatment regimes, thereby, guiding the engineering of next-generation cancer immunotherapy and vaccines.

In this review, we aim to summarise the advances in designing micro- and nano-scale synthetic particles for modulating T-cell-based cancer immunotherapy. The synthetic particles include those modulating single or multiple targets or those being used to label T cells for disease diagnosis and/or therapeutics. In the context of synthetic particles for modulating T cells for treatment, we discuss various approaches, including: (1) artificial APCs, (2) T cells as promising delivery carriers, (3) particulate systems that modulate checkpoint inhibition, (4) particulate systems that modulate the TME and (5) combination therapies. The T cell-targeted nanoparticles (NPs) for various imaging techniques are mentioned in the last section to fulfil the broad picture which is illustrated in Scheme 1.

**Synthetic particles for modulating T cells**

*Artificial antigen presenting cells (aAPCs)*

Natural APCs, especially DCs, are required for the activation of T lymphocytes, the use of which in cancer treatment has demonstrated favourable clinical outcome and minor undesirable effects [6,63]. Nonetheless, their preparation for clinical use poses some serious challenges. Autologous DCs must be isolated from peripheral blood, *ex vivo* stimulated to generate sufficient amounts and then transfused back into patients. This is a time-consuming and expensive process that may also result in the generation of DCs of variable quality [64,65]. Additionally, it is difficult to standardise the treatment protocol when using patient-derived autologous DCs [10,64]. To overcome these drawbacks, aAPCs have been generated as ‘off-the-shelf’ alternatives to induce the production of tumour-specific CTLs both *ex vivo* and *in vivo*. These alternatives include magnetic beads, latex microspheres, carbon nanotubes (CNTs) and others as summarised in Table 1.

Magnetic beads and latex microspheres are powerful platforms for developing a cellular or cell-free aAPCs owing to their ability to simultaneously present antigens and co-stimulatory signals, leading to the efficient induction and expansion of antigen-specific T cells *ex vivo* to the same level as peptide-pulsed DCs [66–68]. Durai et al. [68] conjugated HLA-A2 Ig and an anti-CD28 antibody onto superparamagnetic beads which were then loaded with melanoma-associated antigen recognised by T cells (MART-1) peptide. Melanoma-associated antigen specific CTLs from peripheral blood T lymphocytes were rapidly expanded and demonstrated potent antitumor effector functions *in vitro*. Noninvasive bioluminescence imaging (BLI) demonstrated that the biodistribution of the aAPCs in the body in the beads preferentially localised to the tumour site in HLA-A2̂ melanoma tumour-bearing mice three days after transfer. Following injection into mice, MART-1 specific aAPC-induced CTLs
significantly reduced the tumour size and weight in the tumour prevention model and established model. Shen et al. [69] co-immobilised HLA-A2/peptide tetramers and an anti-CD28 antibody onto the surface of 5 μm-sulphated polystyrene microspheres. Following three rounds of immunisation into naïve HLA-A2/Kb transgenic mice, the tumour antigen-specific CTLs in the spleen were expanded five-to-six-fold. The cytolytic activity of splenocytes upon administration of MART1-aAPCs and co-incubation of MART-1 peptide on melanoma cell line 501 was apparently higher than that on breast cancer cell line MDA-MB-231, suggesting that the recognition of endogenously expressed tumour antigen on the surface of human target cells and cytolysis of these cells by aAPCs were antigen-specific. Later, the same authors [70] coupled H-2Kb/TRP2 tetramers, anti-CD28 and anti-4-1BB antibodies onto the surface of sulphated polystyrene latex beads. After two intravenous (iv) and one subcutaneous (sc) injections, aAPCs demonstrated the higher levels of CD69, CD25, IFN-γ and higher cytolytic activity of splenocytes against B16 cells in both naïve and antigen-primed mice models compared to those of two controls, anti-CD28/4-1BB/His bead and anti-His bead. Moreover, administration of aAPCs significantly inhibited tumour growth and markedly delayed the tumour progression with an increase in mean survival time on mice challenged with B16 melanoma cells, but not with S180 fibrosarcoma cells.

Using another approach, Lu et al. [71] attached H-2Kb-Ig/pTRP2 dimeric complexes (as signal 1), and a combination of anti-CD28 antibody, 4-1BB ligand (4-1BBL) and CD83 molecules (as signal 2) to latex beads. The expanded allo-restricted CTLs demonstrated...
antigen-specific cytolysis against RMA-S lymphoma cells pulsed with the peptide pTRP2 and H-2Kb+ melanoma cells expressing TRP2 which could be inhibited by anti-H-2Kb monoclonal antibody Y3. The pTRP2-specific allo-restricted CTLs markedly reduced the pulmonary metastasis and greatly improved the survival of mice (>60 days vs. <25 days in untreated or control groups) in established tumours.

Carbon nanotubes, which possess very high surface area-to-volume ratio, have been of great interest of Fadel et al. In one early study, they adsorbed anti-CD3 on single walled carbon nanotube (SWNT) bundles [72]. Results showed these materials markedly enhanced the activation of T cells compared to the equivalent amount of anti-CD3 in solution and other high-surface-area materials studied (i.e. activated carbon, polystyrene and C60 NPs). After chemical treatment with HNO3 or HNO3/LiBH4, modified SWNTs possessed higher surface areas compared to untreated SWNTs that could facilitate higher capacity for anti-body adsorption and T cell activation. This phenomenon was explained in their later study by using functionalised SWNT bundles adsorbed with anti-CD3 (αCD3) and anti-CD28 (αCD28) [73]. They found that large antibody stimuli (5–6 μm in size) were clustered on the surface of functionalised as opposed to untreated nanotubes that would probably increase the dwell time of antibodies around antigens and, thereby, effective half-life of this receptor ligand complex.

Perica et al. [75] synthesised nanoscale quantum dot (QD) aAPCs of approximately 30 nm in size and iron-dextran aAPCs of 50–100 nm in size by binding chimeric MHC-Ig dimer (as signal 1) and chimeric B7.1-Ig or an anti-CD28 antibody (as signal 2) to the surfaces of streptavidin-coated QDs and dextran-coated iron oxide NPs, respectively. These nanoscale aAPCs induced antigen-specific T cell expansion in vitro, in which the optimal effect occurred in the presence of both signal 1 and signal 2. Upon a single sc injection of QD aAPCs into the mouse melanoma model, tumour growth was markedly inhibited in mice treated with T cells and cognate QD aAPCs. The in vivo experiment was also designed to observe the impact of the administration route and T cells with/without pulsing by aAPCs. After treatment with iron-dextran aAPCs, all three mice groups treated with one sc and one subsequent intravenous injection (sc/iv), two continuous sc injections (sc/sc) or two continuous iv injections (iv/iv) exhibited less tumour growth compared to non-cognate aAPCs receiving iv/sc as the control. However, a significant reduction of tumour growth over the entire treatment course compared to the control group was only observed in the sc/iv and sc/sc treated groups, but not in the iv/iv treated group. This observation suggested that the sc route of administration of nanoscale aAPCs was significant to the antitumor efficacy which might have resulted from retention in the local lymph nodes. Based on the paramagnetic properties of iron-dextran NPs, the same authors hypothesised that application of an

![Scheme 1](image-url)
Table 1. Synthetic particles for modulating T cells.

| Type | Materials | Active agents | Characteristics | References |
|------|-----------|---------------|-----------------|------------|
| 1. Artificial antigen presenting cells (aAPCs) | Polymer-based particles | Polystyrene | HLA-A2/peptide tetramers; anti-CD28 | Expansion of tumour-specific CTLs | [69] |
| | | Polystyrene | H-2K b/TRP2 tetramers; anti-CD28; anti-4-1BB | Inhibition of tumour growth and improvement of survival | [70] |
| | | Polystyrene | H-2K b- Ig/pTRP2 dimeric complexes; anti-CD28; 4-1BB ligand; CD83 | Reduction of pulmonary metastasis and improvement of survival of tumour-bearing mice | [71] |
| | Inorganic particles | Single walled carbon nanotube (SWNT) | Anti-CD3; anti-CD28 | Increase of T cell activation | [72,73] |
| | | Quantum dot | Chimeric MHC-Ig dimer; chimeric B7.1-Ig | Expansion of antigen-specific CTLs in vitro and inhibition of tumour growth | [75] |
| | | Dextran-coated iron oxide nanoparticles | Chimeric MHC-Ig dimer; anti-CD28 | Inhibition of tumour growth | [75] |
| | | | MHC-Ig dimer; anti-CD28 | Decrease of tumour size and improvement of survival under magnetic field | [76] |
| | Hybrid particles | Polystyrene; iron oxide nanoparticles | MART-1; HLA-A2 Ig; anti-CD28 | Reduction of tumour size and weight | [68] |
| | | Carbon nanotube; PLGA | MHC-l; sCD28; IL-2; magnetite | Induction of CD8+ T cells in vitro and inhibition of tumour growth | [74] |
| | | Polymerised alginate; synthetic collagen-mimetic peptide | IL-15 superagonist; anti-CD3/CD28/CD137 | Complete tumour eradication and improvement of survival in mice | [77] |
| 2. T cells as delivery carrier for nanoparticles | Lipid-based particles | Liposomes | IL-15 superagonist; IL-21 | Tumour eradication and improvement of survival in mice bearing established B16 melanomas | [79] |
| | | Liposomes | NSC-87877 | Enhanced accumulation of T cells in prostate tumours, inhibition of tumour growth and improvement of survival | [80] |
| | | Nanocapsules | Topoisomerase I poison SN-38 | Eradication of lymphoma cells in vitro, inhibition of tumour growth and improvement of survival | [81] |
| | Inorganic particles | Gold colloid nanoparticles | – | Increase of migration and accumulation of GNP at tumour sites in vivo | [82] |
| 3. Particulate systems that modulate checkpoint inhibition | Polymer-based particles | Dextran | Anti-PD-1; glucose oxidase | Synergistic antitumor effect when combined anti-PD-1 and glucose oxidase | [87] |
| | | Alginate hydrogel | RMP1-14; celecoxib | Synergistic antitumor effect when combined anti-PD-1 and celecoxib | [88] |
| | | Folic acid-functionalised polyethylenimine | PD-L1 siRNA | Efficient PD-L1 knockdown | [102] |
| | | PEG-PLA; cationic lipid | CTA-4-siRNA | Down-regulation of CTL-4 expression | [103] |
| | Inorganic particles | Gold nanoparticles | Anti-PD-L1 | Similar anti-tumour efficacy with standard-of-care treatment | [91] |
| | Lipid-based particles | Liposomal cerasome | Anti-PD-L1; gadoterate meglumine; IRDye800CW | Preferential accumulation at 4T1 breast tumour and CT26 colon tumour sites | [92] |
| 4. Particulate systems that modulate the tumour microenvironment | Hybrid particles | Liposomal polymeric gels | TGF-β inhibitor (SBS05124); IL-2 | Inhibition of solid tumour growth or lung metastases and improvement of survival | [105] |
| | | Liposome–protamine–hyaluronic acid nanoparticles | Trp 2 peptide; CpG; TGF-β siRNA | Down-regulation of TGF-β expression and inhibition of tumour growth | [106] |
| | | Very small size proteoliposomes (VSSPs) | – | Reprogram of MDSCs to a more anti-tumour phenotype | [119] |
external magnetic field could enhance the binding of these nano-scale aAPCs to TCRs, thereby, boosting T cell activation [76]. As expected, magnetic application nearly doubled the TCR cluster size and enhanced T cell proliferation in vitro and after adoptive transfer in vivo. Magnetic-enhanced aAPC-activated T cells significantly inhibited B16 melanoma growth and increased survival compared to the untreated control, T cells alone and aAPC-stimulated T cells without a magnetic field.

Polymerised alginate and a synthetic collagen-mimetic peptide were used to create macroporous scaffolds that contained an IL-15 superagonist (IL-15/IL15Rα) [77]. In incompletely resected 4T1 breast tumour model, iv injection of T cells showed quite similar effects in preventing metastatic relapse with controls resulting from the accumulation of T cells in the spleen and liver, while direct intracavitary administration demonstrated a little advantage, but did not prevent recurrence. In contrast, scaffold-delivered T cells

**Table 1.** Continued

| Type               | Materials                                | Active agents                                      | Characteristics                                      | References |
|--------------------|------------------------------------------|----------------------------------------------------|-----------------------------------------------------|------------|
| Lipid-based particles | PEGylated lipid nanocapsules             | Lauroyl-modified form of gemcitabine 6-Thioguanine | Efficacy enhancement of OVA-targeting ACT            | [114]      |
| Polymer-based particles | Copolymer of polyethylene glycol (PEG) and polypropylene sulphide | Depletion of suppressive MDSCs and enhancement of ACT efficacy in melanoma cells | | [115]      |
|                    | Poly(amideamine) (PAMAM) dendrimers; 3,4,5,6-tetrahydrophthalic anhydride (THPA) | All-trans-retinoic acid (ATRA)                      | Inhibition of HepG2 proliferation and enhancement of differentiation of HepG2 into more mature forms | [125]      |

**Figure 3.** Design of CNPs. Schematics describing the combination of multivalent antigen presentation, paracrine delivery of cytokine, and T-cell enrichment capabilities into CNPs. (a) Schematic showing bundled CNTs binding neutravidin to present biotinylated T-cell stimuli and PLGA nanoparticles encapsulating magnetite and IL-2. (b) Schematic highlighting three properties of the engineered CNP platform: multivalent antigen presentation, paracrine release of IL-2 and magnetic separation of CNPs from T cells. (c) Work flow diagram depicting the T-cell stimulation process and cell separation using CNPs. OT-1 CD8+ T cells were purified from splenocytes, and incubated with CNPs for three days. Activated T cells were then separated from CNPs and injected peritumourally into B6 mice, which had previously been inoculated with the B16 tumour for 10 days. Reprinted with permission from [74].
exhibited 167-fold higher proliferation at the resection site, substantial infiltration into peritumoural tissue and 54-fold higher accumulation in tumour-draining lymph nodes compared to directly injected, IL-15/IL15Rα-prestimulated T cells, leading to complete tumour eradication and higher survival in all mice. The efficacy of the material-deployed T cells was also observed on advanced-stage unresectable tumours using a model of stage 3 ovarian carcinoma suggesting that the biomaterial-based devices might provide an approach to bypass immunosuppressive barriers created by tumours and minimise side effects introduced by ACT procedure.

**T cells as promising delivery carriers**

More than one decade ago, Steinfeld et al. [78] encapsulated doxorubicin-coated magnetic NPs into T lymphocytes to investigate their feasibility as drug delivery systems to autonomously target tumour sites. Unfortunately, more than 60% of the cells died owing to the cytotoxicity of doxorubicin released after approximately 15 h. Despite these challenges, the potential of this idea urged other groups to develop their own T-cell systems as delivery platforms for nanoparticulate cargos (Table 1) because of many advantages associated with using T cells to transport therapeutic drugs such as their high specificity, magnification of CTL activity, and induction of long-term immune responses [78].

Irvine’s group [79] proposed a synthesis principle by conjugating maleimide-functionalised liposomes and liposome-like synthetic NPs to the free thiol groups on the surfaces of T cells and then attaching thiol-terminated polyethylene glycol (PEG) to the remaining maleimide groups of the complex (Figure 4(a)). They found that the conjugation process did not compromise key cell functions such as T cell recognition or killing of ovalbumin (OVA) peptide-pulsed target cells and in vivo tissue homing of T cells. Furthermore, encapsulation of both an IL-15 superagonist (IL-15Sa) and IL-21 into these NPs strongly amplified the proliferation of melanoma-specific pmel-1 T cells, leading to the eradication of tumours and improved survival in mice bearing established B16 melanomas (Figure 4(b,c)).

Using the above conjugation principle, the same authors [80] synthesised liposome-decorated T cells as delivery vehicles for NSC-87877 – a dual inhibitor of key phosphatases Shp1 and Shp2 which downregulate T-cell activation in the synapse at the T-cell/APC contact zone. T cells carrying Shp inhibitor-loaded liposomes augmented the accumulation of T cells in prostate tumours by 5.7-fold compared to T cells accompanied by the same dose of the unencapsulated soluble drug. In addition, liposome-decorated T cells significantly decreased the tumour burden by 5.2-fold and increased the survival rate compared to untreated groups after T cell transfer, as opposed to T cells alone or a combination of T cells and the systemically administered drug. Next, to overcome the limitations caused by poor pharmacokinetics of a potent topoisomerase I poison SN-38, this group [81] loaded this compound into nanocapsules and then attached to T cell surfaces. The functionalised T cells efficiently killed lymphoma cells in vitro without being affected by the cytotoxicity of SN-38. After adoptive transfer into tumour-bearing mice, these T cells effectively delivered SN-38 to lymphoid organs reaching a level of 90-fold higher in lymph nodes compared to that of the free drug at 10-fold higher doses intravenously administered. More importantly, animals receiving functionalised T cells showed remarkable 60-fold reduction in the tumour burden on day 16 and improved life span without risks of increased adverse effects.

Kennedy et al. [82] used human T cells as delivery systems for gold colloid NPs (40–45 nm) to treat cancer. The internalisation of gold nanoparticles (GNPs) exerted no adverse effect on T cell viability or function. GNP-loaded T cells enhanced the migration and accumulation of GNPs to tumour sites in vivo by fourfold compared to PEGylated GNPs, while still remaining the normal biodistribution of T cells. These results paved the way for future explorations of anti-cancer therapy of GNP-T-cell systems by modifying T cells to maximise their immunotherapy and biodistribution advantages, which can then be combined with the imaging and photothermal therapy (PTT) advantages of GNPs.
Particulate systems that modulate checkpoint inhibition

Many clinical trials have demonstrated durable objective responses in patients with advanced melanoma, lung cancer or urothelial carcinoma during treatment with anti-CTLA-4, anti- PD-1 and anti-PD-L1 monoclonal antibodies [28–30,83,84]. Unfortunately, only a small fraction of patients benefited from these therapies, and significant immune-related toxicities still need to be reduced for better patient compliance [85,86]. Accordingly, controlled delivery systems have been developed to improve the therapeutic efficacy by enhancing the accumulation of monoclonal antibodies in tumour tissues and to reduce off-target toxicities (Table 1).

Wang et al. [87] reported the integration of self-degradable hyaluronic acid and pH-sensitive dextran NPs that co-loaded anti-PD-1 and glucose oxidase to fabricate a self-dissociated microneedle patch (Figure 5). The microneedle exhibited the sustained release of anti-PD-1 owing to the dissociation of dextran NPs in the acidic environment that was generated from the conversion of glucose to gluconic acid. In the B16-F10 mouse model of melanoma, there were significantly higher immune responses upon a single administration of a microneedle patch in comparison with those administered via a microneedle without glucose oxidase or intratumoural administration of anti-PD-1 alone at the same dose. Moreover, co-delivery of anti-PD-1 and anti-CTLA-4 in the microneedle patch showed significant synergistic effects in melanoma treatment.

The anti-PD-1 monoclonal antibody (RMP1-14, Bioxcell) and celecoxib were co-encapsulated into an alginate hydrogel system [88]. In B16-F10 melanoma and 4T1 metastatic breast cancer models, this hydrogel system maintained high drug concentrations in peripheral circulation and within tumour regions, leading to enhanced antitumor effects of RMP1-14, celecoxib and both components. These synergistic antitumor activities were expectedly obtained from the increase of CD4^+ interferon (IFN)-γ^+ and CD8^+ IFN-γ^+ T cells together with the dampened immunosuppressive environment in the tumour and weakened angiogenic and inflammatory responses.

Theranostics, which can combine the advantages of therapy and diagnostics in one application, is a novel promising platform in personalised cancer treatment [89,90]. To develop a non-invasive tool for the early prediction of therapeutic responses, Meir et al. [91] combined computed tomography (CT) with PEGylated GNPs conjugated to anti-PD-L1 (αPDL1-GNPs). Owing to the enhanced uptake of αPDL1-GNPs into the tumour, the CT signal reached the maximum intensity as soon as 48 h post-injection, which showed linear correlation with tumour growth at day 8 and quantitative T cell infiltration as measured at day 11 (end of experiment). Moreover, these theranostic NPs demonstrated similar antitumor efficacy as standard-of-care treatment at five times higher doses, which might therefore reduce their toxicity. Du et al. [92] recently reported a dual-mode imaging technique by combining magnetic resonance imaging (MRI) and near-infra-red fluorescence. They encapsulated paclitaxel into PD-L1-targeted nanohybrid liposomal cerasome NPs, which were labelled with an MRI contrast agent (gadoterate meglumine) and an infra-red dye (IRDye800CW), and intravenously injected these labelled NPs into mice with 4T1 breast tumours and CT26 colon tumours. The relevant signal intensities were enhanced in both animal models which resulted from the preferential accumulation of NPs at tumour sites. Furthermore, bioluminescence images showed that these PD-L1-targeted NPs were the most effective treatment against tumours and metastases compared to non-targeted NPs, PD-L1 and paclitaxel, without obvious toxicity.

The fast growth of gene technology led to the emergence of ribonucleic acid (RNA) interference resulting from the discovery of small interfering RNAs (siRNAs) that can inhibit specific genes [93,94]. However, its potential use in disease treatment is hampered by lack of efficient delivery strategies [93,95]. Especially, the escape of siRNAs from endosome into cytosol remains a major challenge for their delivery to target sites because of the possibility of their enzymatic degradation in lysosomes [96,97]. Bearing that in mind, many systems have been researched to overcome that hurdle by exploiting the acidic endosome environment and enhancing endosomal escape through the formation of ion pairs, ‘proton sponge effect’, destabilisation of endosomal membrane,

Figure 5. Schematic of the microneedle patch-assisted delivery of anti-PD-1 (aPD1) for the skin cancer treatment. (a) Schematic of the aPD1 delivered by a microneedle patch loaded with physiologically self-dissociated NPs. With GOx/CAT enzymatic system immobilised inside the NPs by double-emulsion method, the enzyme-mediated conversion of blood glucose to gluconic acid promotes the sustained dissociation of NPs, subsequently leading to the release of aPD1. (b) The blockade of PD-1 by aPD1 to activate the immune system to destroy skin cancer cells. GOx: glucose oxidase; CAT: catalase. Reprinted with permission from [87]. Copyright (2016) American Chemical Society.
and epithelial ovarian cancer cells, which overexpress PD-L1 and folate receptors, folic acid-functionalised polyethylenimine polymers loading PD-L1 siRNA were synthesised [102]. The folic acid-functionalised cationic polymers complexed with the negatively charged siRNA which in turn enhanced siRNA uptake, resulted in efficient PD-L1 knockdown and sensitised ovarian cancer cells to T cell killing in vitro. In another approach, CTA-4-siRNA was encapsulated into NPs constructed from poly(ethylene glycol)-block-poly(ol-l-lactide) (PEG-PLA) and N,N-bis(2-hydroxyethyl)-N-methyl-N-(2-cholesteryoxyxcarbonyl-aminooxy) ammonium bromide (BHEM-Chol) [103]. These NPs effectively delivered CTA-4-siRNA to T cells, down-regulated CTL-4 expression, and enhanced the activation and proliferation of T cells in vitro. Upon systemic delivery of NPs, while anti-CD4+ and CD8+ T cells increased, the ratio of Tregs decreased. Most importantly, these NPs significantly delayed tumour growth and prolonged survival in B16 melanoma-bearing mice.

**Particulate systems that modulate the tumour microenvironment**

The TME is a complex area in both structural and functional terms in relation to immune cells, extracellular matrix, blood and lymphatic vasculature [104]. Furthermore, both soluble mediators, such as TGF-β and indoleamine 2,3-dioxygenase (IDO), and cellular mediators, including MDSCs and Tregs, are upregulated in this area. These complexities and abnormalities of TME play a crucial role in tumour progression and metastasis that can prevent most conventional therapies from being successful [35]. Herein, we discussed several TME-targeting strategies using nanoparticulate systems to address the aforementioned mediators (Table 1).

**Transforming growth factor-β**

TGF-β is a crucial factor in the TME that promotes tumour growth and metastasis; therefore, systemic administration of TGF-β inhibitors can possibly manipulate the TME. However, minimising the toxicity of TGF-β inhibitors is required before further therapeutic efficacy can be assessed. Park et al. [105] fabricated a hybrid platform called nanoscale liposomal polymeric gels that co-delivered a TGF-β inhibitor (SB505124) and IL-2. This core–shell system demonstrated the sustained release of two encapsulated agents to TME. Following intratumoural and systemic administration, this hybrid system significantly suppressed tumour growth of solid tumours or lung metastases and increased the survival rate of tumour-bearing mice. These synergistic effects involved the activation of both innate and adaptive arms of the immune system as a result of the enhanced activity of natural killer cells and of intratumoural activated CD8+ T cells.

To boost the efficacy of lipid-calcium-phosphate nanoparticulate vaccine that co-encapsulated a tumour antigen (Trp 2 peptide) and an adjuvant (CpG oligonucleotide) on advanced tumours, Xu et al. [106] deliberately silenced TGF-β in tumour cells by using liposome–protamine–hyaluronic acid NP-delivered TGF-β siRNA. This combined therapy down-regulated TGF-β in the TME by approximately 50%, increased levels of tumour infiltrating CD8+ T cells and decreased level of regulatory T cells that resulted in lowered tumour growth and promoted efficacy of the therapeutic vaccine.

**Myeloid-derived suppressor cells**

Modulation and attenuation of MDSCs – a population of myeloid cells that suppresses the immune system – should be taken into consideration to modulate and attenuate. Current drug therapies that target and modulate MDSCs, e.g. sunitinib, gemcitabine and 5-fluourouracil, are expensive and show inconsistent efficacy and safety outcomes [107,108]. Hence, it is clinically significant to develop affordable, less toxic and higher efficacious alternative strategies comprising decreases/elimination of MDSCs in the tumour, functional inhibition of MDSCs, and promotion of the differentiation of MDSCs into mature APCs resulting in enhanced local T cell functions.

For direct elimination of MDSCs, interleukin-4 receptor α (IL-4Rα) is a target of interest. In addition to the overexpression on the surface of a variety of solid tumours such as head and neck squamous cell carcinoma, glioblastoma, and renal cell carcinoma [109], IL-4Rα is also overexpressed by MDSCs and the blockade by receptor binding molecules or antibodies is thought to effectively induce apoptosis of MDSCs [110]. On various murine xenograft models, Kawakami et al. observed that the use of recombinant IL-4 cytokotins significantly delayed the tumour progression of IL-4Rα-expressing head and neck, breast, prostate cancers [109,111,112]. A clinical study showed positive clinical responses following the intratumoural administration of an IL-4 cytokitin into patients with high-grade gliomas [113]. These results suggested that the recombinant IL-4 cytokotins could directly eliminate IL-4Rα overexpressing cells and by decorating NPs that contain IL-4 cytokotins with MDSC-targeting molecules (e.g. IL-4Rα-binding aptamers), we might develop NP systems with improved specificity and minimal off-target toxicities. This targeting strategy can also be applied to other cytoktoy drug-loaded NPs as well. However, no studies have been reported using this strategy so far. An innovative MDSC targeting was proposed by encapsulation of a lauroyl-modified form of gemcitabine (GemC12) into PEGylated lipid nanocapsules (LNCs) [114]. These produrg-loaded LNCs showed efficient depletion of the monocytic MDSC subset. In comparison with free gemcitabine hydrochloride, there was a decrease in the percentage of spleen and tumour-infiltrating monocytic-MDSCs and improved efficacy following sc injection of GemC12-loaded LNCs into melanoma and lymphoma-bearing mice owing to the preferential uptake of LNCs by monocytic cells rather than by other immune cells. Remarkably, even administered at very low dose, GemC12-loaded LNCs relieved tumour-associated immunosuppression and significantly enhanced the efficacy of OVA-targeting ACT. Earlier, Jeanbart et al. [115] formulated 25-nm polymeric micelles encapsulating 6-thioguaine using a conjugation reaction between 6-thioguaine and a block copolymer of PEG and polypropylene sulphide. These micelles depleted monocytic MDSCs in the spleen and tumour as well as granulocytic MDSCs in the draining lymph nodes after two days of injection into two tumour models, namely E.G7-OVA thymoma and B16-F10 melanoma, in vivo. Following a single injection, 6-thioguaine-delivered micelles decreased circulating monocytic and granulocytic MDSCs in both tumour models up to a seven-day period. Moreover, the combination of these micelles and OVA-specific CD8+ T cells markedly enhanced the efficacy of ACT in OVA-expressing melanoma cells possibly because of the previous depletion of suppressive MDSCs by these micelles.

Regarding indirect killing of MDSCs, the use of immunological adjuvants seems to be a feasible approach. Immunological adjuvants, such as CpG oligonucleotides and very small size proteoliposomes (VSSPs), have demonstrated positive effects on regulating MDSCs in animal models [116–118]. In tumour-bearing mice, iv injections of VSSPs NPs significantly reduced the immunosuppressive characteristics of splenic MDSCs, successfully reprogrammed MDSCs to a more anti-tumour phenotype and potently reduced their capacity to suppress the activation of CTLs [119].

To convert MDSCs into mature APCs, all-trans-retinoic acid (ATRA), which may promote the differentation of immature
myeloid cells in vitro and in vivo resulting in enhancement of cancer vaccines’ efficacy, might be a potential agent [120,121]. To effectively target tumour-induced MDSCs, plasma levels of highly hydrophobic ATRA must be higher than 150 ng/ml necessitating a means to improve its bioavailability [122]. When loaded into liposomes and polymeric micelles, these systems have showed higher plasma concentrations following iv injection than oral or iv free ATRA administration [123,124]. Another study using pH-sensitive NPs prepared from poly(amidoamine) (PAMAM) dendrimers and 3,4,5,6-tetrahydrophthalic anhydride (THPA) for ATRA delivery showed that these NPs enhanced both the proliferative inhibition of HepG2 cell line and the differentiation of this cell line into more mature forms compared to free ATRA at higher concentration and longer treatment period [125]. All together, these results implied the potential utilisation of ATRA as an MDSC-targeting agent in designing NPs for the treatment of solid tumours and haematologic cancers.

**Synthetic particles for modulating multiple targets**

**Combination with photodynamic therapy (PDT)**

Photodynamic therapy is composed of three basic components, namely photosensitisers, light and oxygen [126,127]. Upon administration of the photosensitisier into the body, a light source at a specific wavelength is applied to induce a photochemical reaction and generate the highly cytotoxic singlet oxygen (\(^{1}O_2\)). The antitumor effects of PDT are proposed based on three interrelated mechanisms including direct elimination of tumour cells, damage to the tumour vasculature and activation of immune responses [126,127].

To test the hypothesis that effective PDT can sensitise tumours to immune checkpoint blockade therapy, Lin’s group [128] fabricated immunogenic nanoscale coordination polymer core–shell NPs with the photosensisitiser pyropheophorbide–lipid conjugate (pyrolipid) in the shell and a coordination of Zn and pyrophosphate in the core, namely, NCP@pyrolipid (Figure 6(a)). They found...
that these NPs ablated tumour cells and induced the release of TAAs, which promoted the activation and proliferation of effector T cells. Upon combination with anti-PD-L1 therapy, these NPs showed synergistic effects in the eradication of light-irradiated primary tumours and the regression of non-irradiated distant tumours. In another study, this group [129] encapsulated an IDO inhibitor into the highly porous structure of chlorin-based nanoscale metal-organic framework (nMOF) to form IDO inhibitors (TBC-Hf) (Figure 6(b)). Consistent with the previous study, PDT treatment also demonstrated a strong abscopal effect when combined with IDO inhibitor checkpoint blockade.

Recently, Xu et al. [130] prepared upconversion nanoparticles (UCNPs) co-encapsulating chlorin e6, which is a photosensitiser, and imiquimod (R837), which is a Toll-like receptor 7 agonist. These authors observed the excellent efficacy of UCNPs-based PDT when combined with anti-CTLA-4 to remove local tumours, inhibit distant tumours and create a long-lasting immune response against tumour rechallenge. Taken together, these results confirmed that the NP-mediated PDT both killed tumour cells and sensitised checkpoint blockade therapy through a strong tumour-specific immune response (Table 2).

**Combination with photothermal therapy**

Photothermal therapy involves the use of a photo-absorbing agent, which can convert near-infra-red laser into heat to thermally ablate cancer cells [131]. Upon near-infra-red laser irradiation, PDT can induce tumour cell death, thereby releasing TAAs into the surrounding microenvironment to prime antitumor immune responses. This phenomenon has led to the development of nanoparticulate systems that might synergise the effects of PDT and immunotherapy (Table 2).

An attempt at combining anti-CTLA-4 checkpoint blockade with PDT was performed by Chen et al. [132] using adjuvant PLGA NPs that encapsulated an indocyanine (ICG) – a photothermal agent – and imiquimod (R837) (Figure 6(c)). Upon irradiation with a near-infra-red laser, the formed PLGA-ICG-R837 NPs photothermally ablated primary tumours and caused the release of TAAs which resulted in vaccine-like immune responses in the presence of the adjuvant (R837). In metastatic models using breast cancer (4T1) and colorectal cancer (CT26) cells, the NP-based PDT plus anti-CTLA-4 blockade therapy almost inhibited tumour growth owing to the great reduction in the percentage of Tregs caused by CTLA-4 blockade therapy, which in turn markedly increased both the CD8+ T effector/Treg ratio and CD4+ T effector/Treg ratios. Furthermore, the combination therapy also showed long-term immune-memory effects to protect mice against tumour rechallenge 40 days after removal of the primary tumour. Using PEGylated SWNTs, which possess the strong optical absorption and high photon-to-thermal energy conversion efficiency in the near-infra-red region [133], as photothermal agents, Wang et al. [134] also found a significant enhancement of antitumor effect of SWNT-based PDT when combined with anti-CTLA-4 therapy in a lung metastasis model.

Later, Cano-Mejia et al. [135] investigated the combination of anti-CTLA-4 checkpoint blockade immunotherapy and Prussian blue nanoparticle (PBNP)-based PDT to treat neuroblastoma, which is a common, difficult-to-treat cancer in children. The pH-dependent stability of PBNPs rendered them appropriate for intratumoural administration. PBNP-based PDT slowed tumour growth, increased the infiltration of lymphocytes and T cells to the tumour area in vivo and provoked an antitumor immune response that synergised the antitumor effects of anti-CTLA-4 immunotherapy. The combination therapy showed complete tumour regression and long-term survival in higher percentage of mice compared to anti-CTLA-4 alone, PBNPs alone, PDT alone, and the untreated control, and it also protected previously PDT-treated mice against neuroblastoma rechallenge as a result of immunity developed against these tumours.

In another field, GPNs have been extensively studied for PDT and combined phototherapy owing to their biocompatibility, strong absorption capability, efficient heat conversion, high photostability and controllable surface chemistry [136,137]. Bear et al. [138] synthesised hollow gold nanoshells and utilised these gold nanoshell-enabled PDT to treat mouse B16-F10 tumour models. Unexpectedly, they found that PDT-induced immune response was not sufficient to delay the growth of poorly immunogenic B16-F10 tumours, but accelerated the growth of distant lung metastases that resulted from the substantial increase in the production of MDSCs in the distal TME. Interestingly, the combination of PDT and ACT demonstrated prevention of primary tumour recurrence post-ablation, significant inhibition of contralateral tumour growth, and abrogation of PDT-induced metastatic tumour growth. Recently, PEGylated gold nanostar-mediated PDT was combined with anti-PD-L1 immunotherapy in a new two-pronged modality, called synergetic immuno photothermal nano therapy (SYMPHONY) [139]. The authors found that SYMPHONY treatment completely eradicated primary tumours and distant tumours and improved overall survival in mice injected with MB49 bladder cancer cells. Also, this combined therapy induced long-term cancer vaccine effect upon rechallenge against MB49 cancer cells. Immune cell phenotyping analysis revealed that while MDSCs were markedly down-regulated, the expressions of PD-1 on CD4 and CD8 T cells in spleens were increased in the SYMPHONY-treated group providing the proof of concept for its synergistic effect. Taken together, these above results implied powerful synergistic effects when combining checkpoint blockade with PDT to eradicate tumours.

### Table 2. Combination of checkpoint blockade and phototherapy.

| Type                          | Materials                     | Immune target | Photosensitiser/photothermal agent | References |
|-------------------------------|-------------------------------|---------------|-----------------------------------|------------|
| 1. Combination with photodynamic therapy (PDT) |                               |               |                                   |            |
| Hybrid particles              | Zn pyrophosphate (ZnP); lipidsa (DOPA, DOPC, DSPE-PEGc2k, cholesterol) | PD-1          | Pyropheophorbide–lipid conjugate (pyrolipid) | [128]      |
|                               | Nanoscale metal-organic framework (nMOF) | IDO           | S,10,15,20-Tetra(p-benzoato)chlorin (H2TBC) | [129]      |
| Metal-based particles         | Upconversion nanoparticles (UCNPs) | CTLA-4        | Chlorin e6                        | [130]      |
| 2. Combination with photothermal therapy (PTT) |                               |               |                                   |            |
| Polymer-based particles       | PLGA                          | CTLA-4        | Indocyanine green (ICG)           | [132]      |
| Inorganic particles           | PEGylated SWNTs               |               |                                   | [133]      |
|                              | Prussian blue nanoparticle (PBNP) |               | –                                 | [134]      |
|                              | Hollow gold nanoshells        |               | ACT                               | [137]      |
|                              | PEGylated gold nanostar       | PD-1          | –                                 | [138]      |

aDOPA: 1,2-dioleoyl-sn-glycerol-3-phosphate sodium salt; DOPC: 1,2-dioleoyl-sn-glycero-3-phosphocholine; DSPE-PEGc2k, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-(ammmopolyethylene glycol)2000.
Other combinations
Mortensen et al. [140] aimed to combine ACT with boron neutron capture therapy by transfecting T cells with functionalised boron carbine NPs. The NPs were mostly internalised into cells to reach a concentration of 0.13 pg boron/cell and kept in the cell population for up to eight days by transferring them into daughter cells during several division cycles, which might suggest an effective way to kill neighbouring cells upon neutron irradiation in cancer treatment using ACT.

Kuai et al. [141] reported the formulation of a new vaccine by decorating synthetic high-density lipoprotein (sHDL) nanodiscs with cysteine-modified antigen peptides and cholesterol-modified CpG. This vaccine showed more sustained antigen presentation and significantly enhanced the delivery of antigen/adjuvant to lymph nodes. Although the formulation markedly elicited neointergen-specific CTLs, this monotherapy failed to reject the neoantigen-expressing tumours owing to the upregulation of PD-1 and PD-L1 in the TME. Remarkably, the combination therapy of nanodiscs and anti-PD1/anti-CTLA-4 impressively eradicated established MC-38 and B16-F10 tumours in approximately 90% of mice.

T cell-targeted nanoparticles for imaging
Regarding the crucial roles of immune cells, such as T lymphocytes, in immunotherapy, several non-invasive cellular tracking methods have been developed to track the migration and better understand the fate of these immune cells and their therapeutic potential in cancer treatment, including fluorescence imaging, MRI, BLI, nuclear imaging (i.e. positron emission tomography (PET), single-photon emission computed tomography (SPECT)), and confocal photothermal microscopy [142–146]. Generally, the labelling procedures, either direct or indirect, should have high selectivity and specificity, a suitable safety profile, appropriate pharmacokinetics, good stability in vivo, and economical effectiveness [147]. Direct labelling is a simple and widely used method in which T cells are isolated from the subject, ex vivo expanded, labelled with NPs as imaging probes in vitro, and then injected into the subject and tracked by suitable imaging modalities as mentioned above. Indirect labelling, on the other hand, is more complicated and requires transfecting T cells with an enzyme/receptor/fluorescent protein-encoded reporter gene by using nonviral or viral agents, such as polymers, liposomes and transfection regents. The translated products then activate the imaging probes, thereby, allowing visualisation of the labelled cells. The advantages and disadvantages of these two labelling methods have been summarised elsewhere [143,148].

Optical fluorescence and bioluminescence imaging
For optical fluorescence imaging, T cells can be labelled by fluorophores or QDs by a direct labelling strategy or fluorescent proteins...
by an indirect labelling strategy. Some studies have used QDs to map sentinel lymph nodes and image tumour targeting and development; however, the lack of toxicity evidence has rendered QDs less popular for in vivo use [149–151].

To specifically target ACT T cells in vivo for repeated stimulation or tracking purposes, maleimide-functionalised PEGylated liposomes were conjugated to targeting ligands such as IL-2-Fc or anti-Thy1.1 F(ab’)_2 [152]. Both targeted stealth liposomes demonstrated specific and strong binding to pmel-1 T cells in vitro and in vivo following a single injection. Remarkably, liposomes conjugated with IL-2-Fc repeatedly boosted the proliferation of transferred pmel-1 T cells in a lung metastasis mouse model, as seen by BLI (Figure 7). The results may pave the way for the future delivery of imaging contrast agents or adjuvant drugs to T cells to track adoptive T cells or enhance the efficacy of ACT in vivo.

**Magnetic resonance imaging**

MRI is a widely used imaging technique in clinical studies and has four classes of contrast agents, including paramagnetic gadolinium complexes, superparamagnetic iron oxide (SPIO) NPs, ^19^F-containing probes and chemical exchange saturation transfer (CEST) probes; however, the application of gadolinium complexes in T cell tracking is rarely reported [153].

Using an HIV Tat peptide, Kircher et al. [154] produced highly derivatised cross-linked oxide nanoparticles (CLIO-HD) for ex vivo lymphocyte labelling. CLIO-HD-labelled T cells could be detected in vitro up to 120 h and in vivo in the tumour for a maximum of 48 h with detection thresholds as low as <2 cells/voxel and <3 cells/voxel, respectively. High-resolution MR images demonstrated three-dimensional heterogeneity of T cell recruitment to B16-OVA tumours in vivo in which labelled T cells accumulated in different areas inside the tumours at each serial administration.

**Nuclear imaging techniques**

PET and SPECT are high sensitive imaging techniques that make them clinically translatable for disease diagnosis. To track T cells in vivo by PET, Bhatnagar et al. [155] ex vivo radio-labelled genetically modified CAR T cells with a complex of GNPs and ^64^Cu^2+^ (denoted as GNP–^64^Cu/PEG2000). The labelled T cells were then intravenously injected into mice and imaged by µPET/CT and BLI. Co-localisation of the signals from the transverse, coronal and sagittal planes of the mouse by the PET and BLI methods supported the ability to track T cells in vivo by µPET/CT scanner. Additionally, in contrast to the preferential accumulation in the spleen and liver of the cell-free GNP–^64^Cu/PEG2000, labelled T cells mostly delivered the complex to the lung suggesting their role as delivery carriers for NPs without sequestration of the reticuloendothelial system (RES) via enhanced permeability and retention effect [82,156]. Using a similar approach, this research group formed a dual-modality complex (super paramagnetic iron oxide NPs – Copper-64; SPION–^64^Cu) for ex vivo labelling non-phagocytic primary T cells [157] (Figure 8(a,b)). Almost all cells were labelled within 10 min as confirmed by flow cytometry and iron content analysis with minimal cytotoxic effects. Notably, SPION-loaded T cells retained their tumour targeting activity in a B-cell lymphoma tumour model in vitro.

Exploiting the FDA-approved and relatively long half-life of ^11^In-oxine as a cell tracker, Pittet et al. [158] reported a rapid,
non-invasive and longitudinal imaging technique to precisely track *ex vivo* labelled CTL localisation in mice by combining SPECT and X-ray CT. Additionally, this combined technique also revealed the association between CTL accumulation and tumour growth or rejection.

GNPs have commonly been used as contrast agents for CT imaging [159–162]. Upon covalent conjugation of 20 nm-GNPs with glucose, Meir et al. [163] used these coated GNPs to *ex vivo* label melanoma-specific TCR-transduced (targeted) T cells. Following injection into mice bearing human melanoma xenografts, GNP-labelled targeted T cells showed migration and accumulation into tumour sites using CT imaging (Figure 8(c)), and significant tumour regression was observed compared to GNP-labelled non-transduced (non-targeted) T cells or GNPs alone. Moreover, the validation step showed excellent correlation between CT and fluorescent imaging of dual-labelled T cells, which encapsulated GNPs and green fluorescent protein, confirming the reliability of the GNP-based CT imaging technique.

Conclusions and future perspectives

Herein, we summarised several particulate systems that modulate T-cell-based cancer treatment through the use of aAPCs, modulation of immune checkpoint blockade and the TME, use of T lymphocytes to deliver drugs and use of imaging agents for tracking T cells and monitoring therapeutic responses. The particulate systems can be used to modulate single targets or multiple targets in combination therapies for therapeutic improvement. Our review demonstrates promising results when combing immunotherapy with other therapies, such as PTT or PDT.

Although immunotherapy for cancer treatment has experienced dramatical advances with the recent FDA approval of two novel gene therapy products, e.g. tisagenlecleucel (Kymriah®Novartis) and axicabtagene ciloleucel (Yescarta®Kite) [24], these therapies still face many challenges. First, these therapies are still too expensive, with costs of US$475,000 and US$373,000, respectively. Second, these therapies require state-of-the-art facilities and highly skilled personnel, which returns to the matter of expense, and they also require great effort and time, especially in developing countries where cancer is rapidly rising due to the air and water pollution. Third, they have serious problems regarding their safety profiles, especially cytokine release syndrome (CRS), which can lead to a high fever and flu-like symptoms due to a systemic response to the activation and proliferation of CAR-T cells, and neurologic toxicity. These adverse events can be fatal and life-threatening, making these products available under a restricted programme, called the risk evaluation and mitigation strategy (REMS). Fortunately, FDA also approved tocilizumab (Actemra®, Genentech Inc., South San Francisco, CA) to minimise the severity of CRS accompanied with CAR T-cell therapy [21]. Therefore, although the idea of using genetically modified T cells dates back more than two decades, much work remains to further improve CAR T-cell therapy.

Since TAAs are also expressed in some normal tissues at low levels, which may lead to immune intolerance, there is an urgent need to search for more tumour-specific targets. Due to the development of genomic sequencing and bioinformatics, another class of tumour antigens called tumour-specific neoantigens, which are mutation-based peptide sequences found only in tumour cells and that are entirely absent from normal cells, has arisen as an ideal target for cancer immunotherapy. Numerous studies have shown a positive correlation between neoantigen-reactive T cells and tumour regressions, especially in checkpoint blockade therapy [164–167]. These results suggest that direct transfer of neoantigen-specific T cells into patients would be a good therapeutic approach. In addition, personalisation of T cell-mediated cancer immunotherapy may avoid the difficulty of identifying public antigens that are exclusively expressed on tumour cells; hence, targeting neoantigens may be safe and effective [168]. The opportunity seems to just be opening for certain groups of patients who can afford this therapy, but it is still valuable in the long term for cancer treatment.

To boost the immune response and avoid tumour resistance, combination therapies that modulate multiple targets are preferable over single therapies. Immunotherapy can combine with chemotherapy, radiotherapy or with each other to overcome limitations regarding the response rates and duration of therapy. The combination approach is a promising approach that shows synergistic effects on tumour growth and overall survival in preclinical studies. The rationale for these combinations is that chemotherapy or radiotherapy can trigger the release of TAAs from dead cells, which consequently leads to cross-presentation to T cells and enhancement of immunotherapy. Nevertheless, the immune-related effects of these agents still raise concerns that require further investigations. Another major challenge is to identify the optimal dose and schedule of combination therapies, which requires early phase clinical trials in patients.

Lastly, concern regarding what particles can induce T cell besides manipulating immune activities still require many more investigations. The long-term toxicity and immunogenicity of NPs have been reported elsewhere without a comprehensive understanding of the molecular and cellular mechanisms, especially inorganic material-based particles [169,170].

Disclosure statement

No potential conflict of interest was reported by the authors.

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