With the rapid growth of advanced nanoengineering strategies, there are great implications for therapeutic immunostimulators formulated in nanomaterials to combat cancer. It is crucial to direct immunostimulators to the right tissue and specific immune cells at the right time, thereby orchestrating the desired, potent, and durable immune response against cancer. The flexibility of nanoformulations in size, topology, softness, and multifunctionality allows precise regulation of nano-immunological activities for enhanced therapeutic effect. To grasp the modulation of immune response, research efforts are needed to understand the interactions of immune cells at lymph organs and tumor tissues, where the nanoformulations guide the immunostimulators to function on tissue specific subsets of immune cells. In this review, recent advanced nanoformulations targeting specific subset of immune cells, such as dendritic cells (DCs), T cells, monocytes, macrophages, and natural killer (NK) cells are summarized and discussed, and clinical development of nano-paradigms for targeted cancer immunotherapy is highlighted. Here the focus is on the targeting nanoformulations that can passively or actively target certain immune cells by overcoming the physiobiological barriers, instead of directly injecting into tissues. The opportunities and remaining obstacles for the clinical translation of immune cell targeting nanoformulations in cancer therapy are also discussed.

1. Introduction

Cancer remains the main worldwide health problem, and diverse therapeutic approaches have been explored over decades. Patients can benefit from chemotherapy, radiotherapy, surgery, and newly developed immunotherapy, relying on the characteristics of tumors. Surgery, if possible, is still the most efficacious approach to clear tumors and associated lymphatics, while patients with advanced cancers need to receive chemotherapy by systemic infusion of cytotoxic anti-cancer drugs. Nevertheless, side effects associated with off-target toxicities are often the major limiting factor for the implications and therapeutic outcomes of these cancer therapies.

Nanomaterials typically in the size of 1–200 nm are revolutionizing the development of new medicines, particularly in targeted cancer therapies. Small-sized nanoformulations tend to passively target solid tumor sites driven by EPR (enhanced permeability and retention) effect, which is caused by the defective angiogenic vessels.[1] Therapeutics formulated in nanomaterial delivery platforms were initially developed to promote accumulation of chemotherapeutic agents at tumor sites and reduce unwanted systemic toxicities.[2] There are considerable chemotherapeutic nanoformulations licensed for human use, showing substantially improved therapeutic outcomes.[3] Emerging advances in nanomedicine have focused on its capability to enhance cancer immunotherapy, by precisely modulating immunosuppressive tumor microenvironment (TME).[4–7]

Beginning over 100 years ago, the concept of immune surveillance was proposed by Professor Paul Ehrlich, the winner of 1908 Nobel Prize in Physiology or Medicine. It is defined that the immune system is capable to recognise and eliminate tumor cells, thus preventing tumor progression.[8] Therapeutic vaccination
is one key strategy to empower the immune system with augmented tumor antigen specific cytotoxic T lymphocytes (CTLs) against tumor. However, the therapeutic efficacy is often compromised by suboptimal T cell priming or immunoresistance in TME. The advances in immuno-oncology have brought new insights into the area of developing novel immunotherapeutic strategies, many of which have shown remarkable success in curing patients with certain types of cancers, such as checkpoint inhibitors targeting PD-1/PD-L1 (programmed cell death-1 or its ligand), or CTLA-4 (cytotoxic T-lymphocyte antigen-4), and adoptive T cell therapies using engineered CAR-T (chimeric antigen receptor T) cells. These ground-breaking findings fueled a surge of immunotherapy under clinical or preclinical studies exploring new mono- or combined therapy. Combination immunotherapies, such as co-treatment with multiple antibodies against CTLA-4, PD-1, or PD-L1, have shown some increase in efficacy, but also elicited substantial increase in toxicity in patients. Unsurprisingly, systemic administration of these inhibitors inevitably augments the adverse effects associated with autoimmunotoxicities. Beyond that, other immuno-drugs (e.g., cytokines, receptor agonists) that modulate the functions of specific subsets of immune cells, also face the safety issue related to systemic toxicities. Thus, additional approaches that could safely and effectively drive anti-cancer immune responses remain an important unmet need.

To gain broader success of cancer immunotherapy, immuno-drug delivery nanoformulations that enable spatial and temporal regulation of immune response, have emerged in parallel with the discoveries of new immunotherapeutic agents, holding a great promise in addressing these limitations. Anti-tumor immunity is coordinated by diverse immune cells spatially and temporally across different tissues, such as T cell priming in the lymphoid organs, progenitors circulating in blood before differentiating into tissue specific phenotypes of immune cells, and effector immune cells exerting their killing functions at tumor sites. In terms of delivery function, nanoformulations can be engineered with desired properties to load, protect, and guide the therapeutic immuno-drugs to the specific subsets of immune cells at the right tissue and right time for enhanced coordination of immune response against tumor. Beyond that, accumulated evidence has also shown that nanomaterial itself can offer adjuvant effect modulating the nano-immunological activities that are determined by their chemophysical properties, such as chemical components, size and solubility, softness, deformability, topology, and chirality. These discoveries in structure-activity relationships and previously unexplored mechanisms associated with the interface of nano-immunology have been broadening and advancing the implications of highly immunogenic nanoformulations in targeted cancer therapy.

Despite that the tremendous progress in the field of nanotechnology has enhanced cancer immunotherapy, advanced nanoformulations that enable passive or active targeting delivery of systemically administrated immuno-drugs to specific subsets immune cells at peripheral, lymphatic, or tumors tissues, have not yet been timely summarized and clarified. In this review, we mainly focus on the targeting nanoengineered formulations for tissue-specific immune cell delivery of immuno-drugs, by passively and actively overcoming physical barriers post systemic administration (Figure 1a,b). We discuss specific nano-paradigms designed to target DCs, circulating monocytes, macrophages, T cells, or NK cells (Figure 1c) for enhanced cancer therapy. The associated mechanisms are emphasized by which the targeting formulations precisely regulate the anti-tumor immunity across tissues. We also briefly summarize targeting nanoformulations currently under clinical studies. We conclude this review by providing our perspectives on the potential translations and limitations of immune cell targeting nanoformulation in cancer treatment, such as safety evaluation, establishment of reliable preclinical models, and collaborative research among researchers from material science, engineering, onco-immunology, bioinformatics, and medicine. This review will provide a comprehensive and up-to-date summary of advancements in the exciting multidisciplinary field of material science and onco-immunology for enhanced cancer therapy.

2. Emerging Nanoformulations Targeting Immune Cells for Cancer Therapy

Cancer immunotherapy exploits the extraordinary capability of human immune system to fight against cancerous cells. The increasing knowledge in onco-immunology has led to innovative cancer invention approaches with several approved products for human use. Despite the rapid progress and success in this field, most cancer patients do not respond to cancer immunotherapy. It remains a major challenge to develop new modalities to enhance the response rate and therapeutic efficacy while minimize the autoimmune toxicities. The availability of new technologies in nanomaterials enables selective immunomodulation on specific immune cell types or subtypes, which plays a prominent role in tuning the interactions between innate immune cells and adaptive immunity against tumor. This approach might offer safer and promising alternative cancer treatment modalities to traditional cancer immunotherapies and therapeutic cancer vaccines. Immune cells possess phenotypic and functional heterogeneity, which are found to function differently in diverse (non)lymphoid tissues. We here will provide a brief introduction to the basic immunological functions of each subset of immune cells in cancer immunotherapy, followed by the remarkable impact of nanoformulations and insight into the key opportunities and issues.

2.1. Nanoformulations Targeting DCs

All nucleated tumor cells express major histocompatibility complex (MHC) class I molecules that can present intracellular antigen-derived peptides on cell surface, thus tumor specific CD8+ CTLs can detect and target tumors. CTLs eliminate cancer cells by producing specific cytokines (e.g., interferon (IFN)-γ) and cytotoxic enzyme molecules (e.g., granzyme). Priming CTLs in lymphoid organs by therapeutic vaccination is one preferred tool to establish efficacious anti-tumor immunity, of which DCs are vital for initiating CTLs. Targeting DCs therefore is considered as a promising strategy in cancer therapy, such as vaccination with antigens and nano-immunomodulators that can target and activate endogenous DCs in lymphoid organs. The flexibly engineered nanomaterial-based immunomodulators that target DCs enable triggering T cell anti-tumor activity rapidly and effectively.
Figure 1. Nanoengineering enhances the immunomodulation of stimulators and expansion of anti-tumor immunity, by targeting specific type of immune cells. a) Tumor-associated antigens and/or therapeutic immunostimulatory agents can be formulated in nanoparticles with specific chemophysical properties, including chemical components, particle size, solubility, softness, and topology. b) Chemophysical properties of immunotherapeutic nanoformulations determine the quality of innate and adaptive immune response, by targeting modulation of specific subsets of c) immune cells at lymphatic or tumorous tissues post systemic administration. Created with Biorender.c.

2.1.1. DCs in Skin Draining Lymph Nodes

Therapeutic vaccine nanoformulations containing small nanomaterials allow for efficient passive targeting transport of adjuvants and antigens to endogenous DCs in draining lymph nodes (dLNs) through afferent lymphatic vessels, thereby promoting presentation of antigens by activated DCs to naïve T cells and subsequently priming antigen specific anti-tumor effector T cells. Moon lab engineered ≈10 nm disc-like nanoparticles that were formed by phospholipids and apolipoprotein A1-mimetic peptides to co-deliver tumor peptide antigens and adjuvant 5'-C-phosphate-G-3' (CpG).

Following administration, this engineered nanoformulation markedly increased accumulation of delivered antigen and adjuvant molecules in dLNs compared to their free soluble form, and also prolonged the antigen presentation on DCs. Nanoformulation vaccination in mice elicited ≈30-fold greater CTLs than unpacked antigen and CpG formulation, resulting in enhanced therapeutic efficacy against B16F10 melanoma and MC38 colon cancer. Vaccines in the absence of adjuvants that provide positive stimulatory signals.

In addition to passive targeting DCs in dLNs, some nanomaterials are engineered to promote cytosolic delivery-mediated cross presentation of delivered antigens via MHC class I molecules of DCs, which is essential for increasing the magnitude of CD8+ T cell responses.

Gao and his colleagues reported ultra-pH-sensitive polymer nanoparticles with a diameter of ≈30 nm to deliver a model antigen of ovalbumin (OVA) without additional adjuvants. The designed architecture of cyclic seven-membered ring endowed this nanoparticle with potent immunogenicity on DC maturation by activating stimulator of interferon genes (STING)-type I IFN pathway. Exogenous nanoparticles are generally uptaken by DCs via endocytosis pathway and trapped in acidic endosome compartments. However, the tertiary amine group in the cyclic ring facilitated the disruption of endosomes, cytosolic delivery of exogenous OVA, and cross presentation. Following recognition of cross presented antigens, mature DCs stimulated the differentiation and proliferation of OVA-specific functional effector CTLs. Immunization with the nanoformulation, led to efficacious elimination of B16F10, MC38, and human papilloma virus (HPV) associated tumors. This
intriguing nanoformulation is projected in first human trials in 2023. The authors recently engineered a polyvalent STING nano-agonist derived from the polymer system with prolonged activation.[42] Very recently, Song and co-workers screened a library of azole molecules for a potent STING activation structure 4Blmi (as illustrated in Figure 2a), which was conjugated with polyethylenimine (PEI) mediating endocytic delivery of encapsulated antigens.[43] The designed vaccine nanoformulation (50–100 nm) elicited potent CTLs when OVA was formulated into the polymer nanoparticles (Figure 2b). This strategy also showed a
great potential in a personalized vaccine formulation, substantially regressing MC38 tumor (Figure 2c). These innovative nano-STING agonists in the format of nanoparticles with improved stability in vivo represent an attractive approach for cancer therapy in parallel with new discoveries in small molecular STING agonists.

Seder and colleagues developed a potent peptide vaccine nanoformulation in the absence of additional nanomaterial carriers. The chemically modified peptides were covalently linked with a molecule adjuvant of toll-like receptor (TLR)-7/8 agonist (Figure 3a). The conjugates were capable of self-assembling into nanoparticles (denoted as SNP-7/8a, ≈20 nm), which promoted the activation of targeted DCs in dLNs. Mice subcutaneously immunized with the nanoformulation exhibited a high magnitude of tumor specific CD8+ T cells. Along with magnitude, the functional quality and longevity of CD8+ T cells are critically important for efficient and durable anti-tumor immunity. Interestingly, the lab found that the administration route of the nanoformulation vaccine dramatically altered the targeted DC subtype, antigen presentation persistence, phenotype of functional CD8+ T cells, and therapeutic outcomes against cancer. By comparing to subcutaneous vaccination, intravenous vaccination with SNP-7/8a nanoformulation resulted in a substantially lower magnitude of neoantigen+CD8+ T cells in all tested tissues (blood (Figure 3b,c), spleen, LNs, and lungs), but higher proportion of stem-like phenotype expressing T cell factor 1 (TCF1) and PD-1 markers (Figure 3d) that demonstrated effective control on established MC38 tumor in a therapeutic model. Intravenous administration of vaccine nanoformulation targeted in splenic conventional type 1 DCs (cDC1s) and monocyte-derived DCs (mDCs) and the delivered antigens were of short-lived pharmacokinetics (peaked at 6 h and diminished at 24 h). In contrast, subcutaneous administration led to a long duration of antigens in dLN-cDC1s and prolonged DC activation up to 3 days, which probably accounted for the high number of effector CD8+ T cells. The frequency of cDC1s that are the major subset of DCs for antigen cross-presentation to CD8+ T cells, was significantly reduced post injection of SNP-7/8a nanoformulation intravenously, thus the authors proposed that monocyte-derived DCs might be the DC subset mediating stem-like CD8+ T cells, while this hypothesis needs to be validated in future evaluation. Chen and colleagues also formulated a therapeutic tumor vaccine by self-assembling dual adjuvants (CpG and short hairpin RNA) and neoantigens into nanocapsules (around 250 nm) potently activating DCs in dLNs and subsequently priming CD8+ T cell immunity. These findings from peptide-based vaccine nanoformulation provide implications in rational design of effective therapeutic vaccines for cancer treatment.

MHC class I restricted peptide-based vaccines are suitable for personalized cancer treatment, while unlikely applied in patients universally due to polymorphic human leukocyte antigen (HLA) molecules. In contrast, therapeutic messenger RNA (mRNA) cancer vaccines that can deliver whole antigen proteins are able to provide a variety of HLA epitopes. Anderson lab screened a library of ionizable lipid nanoparticles (LNPs, 100 nm in diameter) to optimize mRNA vaccination performance. Like the polymer-based nanoformulation mentioned above, the cyclic amino head groups in LNPs were found capable of activating STING-type I IFN pathway, thereby significantly promoting the immunogenicity of mRNA vaccine nanoformulation and inducing APC maturation in dLNs. This nanoformulation triggered anti-tumor immunity and demonstrated efficacious therapeutic effects in multiple mouse tumor models. The developed LNPs were protected in a filed patent for future potential clinical use.

In addition to passive targeting driven by small particle sizes less than 100 nm, active targeting strategies offer an alternative solution to augment tumor antigen specific immunity against tumor, by conjugating ligands or monoclonal antibodies (mAbs) on the surface of nanomaterials. Dolcetti and his colleague Thomas reported an oil-in-water nanoemulsion system (200 nm) conjugated with targeting mAb to Clec9a expressed by cross-presenting cDC1s. By encapsulating tumor antigens, the nanoemulsion vaccine formulation in the absence of additional adjuvants was able to target and activate cross-presenting Clec9a+DCs, thereby enhancing induction of antigen specific T cells. Immunization with the nanoemulsion vaccine formulation containing HPV E6/E7 protein or pooled B16F10 melanoma neoepitopes, showed significant suppression on the growth of HPV16 expressing TC-1 solid tumor and B16F10 tumor, respectively. Not only cross priming CD8+ T cells, but Clec9a+cDC1s are also capable of presenting glycolipid on CD1d, MHC class I like molecules, to activate invariant natural killer T (iNKT). The activated iNKT cells in turn provide helper signals to promote DC maturation and consequent antigen-specific T cell immunity. Taking consideration of this point, Dolcetti lab encapsulated E7 peptide and glycolipid ligand α-galactosylceramide (αGC) in the Clec9a-targeted nanoemulsion, to explore the feasibility of enhanced anti-tumor immunity driven by NKT cells. A single intravenous administration of the designed vaccine nanoformulation enhanced the activation of cDC1s, iNKT cells, NKT cells, and functional effector CD8+ T cells, resulting in long-term TC-1 tumor suppression in a therapeutic model. Recently, the Clec9a targeted nanoemulsion vaccine formulation containing whole OVA protein antigen showed improved immunotherapy efficacy of CAR-T cells that express transgenic T cell receptors (TCRs) specific to MHC class I or II restricted peptides. Transgenic C57BL/6-HER2 (human epidermal growth factor receptor 2) mice received the combined treatments completely regress E0771-Her2 breast cancer and MC38-HER2 tumors.

Nano vaccination formulations with a relatively large size (above 100 nm) generally are retained at the injection sites. Interestingly, Ma lab engineered deformable vaccine nanoemulsions (named DASE, Figure 4a) with a large size of ≈330 nm (Figure 4b), which were able to passively target dLNs by deforming their morphology to pass through endothelial gaps and lymphatic vessels (Figure 4c). In this vaccine formulation, albumin was applied to stabilize the squalene oil nanodrops, delivering MHC class I restricted OVA peptide SIINFEKL conjugated with a lipid chain palmitic acid (Pal-SIINFEKL). The deformable vaccine nanoformulation induced significantly enhanced SIINFEKL-specific CD8+ T cell immune response (Figure 4d), thereby promoting therapeutic effects against OVA expressing E.G7-OVA thymoma (Figure 4e), compared to albumin-based solid counterparts. Further preclinical studies on the delivery of whole tumor-associated protein antigens would pave a path to the use of deformable nanoformulation in therapeutic vaccination.
Figure 3. The administration route and dose of TLR-7/8 agonist-based vaccine nanoformulation determine the level and phenotype of neoantigen-specific CD8+ T cells. a) An illustration showing the fabrication procedure of self-assembling nanoparticle vaccines formed by MC38 neoantigen (Reps1) and TLR7/8 agonist (SNP-7/8a). b) An experimental timeline: C57BL/6 mice received SNP-7/8a vaccine (dose of 2, 8, 32 nmol) subcutaneously or intravenously on days 0 and 14. Blood samples were harvested on days 7 and 21 for the measurement of Reps1 specific CD8+ T cells. c) Reps1-tetramer+ CD44+CD8+ T cell percentages were determined by flow cytometric analysis and displayed in the representative dot plots and bar charts. d) Mice received SNP-7/8a Reps1 peptide vaccine subcutaneously or intravenously on days 0 and 14 and Reps1-tetramer+CD8+ T cells were sorted from the spleen on day 28. The sorted cells were used for single cell sequencing (scRNA seq) analysis by 10x Genomics. Monocle 3 analysis of gene expression displayed 12 distinct clusters in the uniform manifold approximation and projection (UMAP) of the sorted cells. Shown are the quantified percentages of effector and stem-like cells of total sorted cells, Pseudotime trajectory analysis, and typical gene signatures of stem-like phenotype of CD8+ T cells overlaid on the UMAP. a–d) Reproduced with permission. [50] Copyright 2020, Nature Publishing Group.
Figure 4. The deformability of vaccine nanoformulation controls its drainage to LNs, the magnitude of antigen specific CD8+ T cells and therapeutic effect against tumor. a) A scheme illustrating the pathway of deformable vaccine nanoformulation draining to the LNs by adjusting its morphology to pass through the endothelial barriers of lymphatic vessels. b) Atomic force microscopy images of DASE in a liquid solution. Scale bar: 2 μm; 100 nm (inset). c) Fluorescent images of Cy5.5 conjugated antigens in LNs harvested from mice at 24 h post administration of different vaccine nanoformulations. d) IFN-γ ELISpot images of splenocytes derived from immunized mice. e) Individual E.G7/OVA tumor growth curves of mice received different vaccine formulations as indicated. a–e) Reproduced with permission.[30] Copyright 2021, Wiley-VCH.
The effect of nanoparticle chirality on the regulation of nanoimmunological activities had been unknown until early this year. Xu and colleagues discovered that left-handed inorganic gold nanoparticles exhibited substantially stronger immune response against infectious disease [33] and cancer [58] compared to the right-hand counterparts. However, the interaction between chiral nanoparticle-formed cancer vaccine and DCs in vivo was not explored but might be interesting to fully understand the underlying mechanisms.

Several studies have reported that therapeutic outcomes in cancer treatment can be substantially improved by specifically activating other dLN-DC receptor signaling using innovative small nanomaterials [59-64] such as polymer/metal ions nanoparticles (Figure 5a,b) [62] cationic cholesterol LNPs (Figure 5c,d) [63] and pathogen-like polymer nanoparticles (Figure 5e) [64]. Harnessing the potential of targeting dLN-DCs as a deliberate strategy to trigger CTL-mediated cancer eradication is far beyond what can be achieved by the representative fascinating examples introduced above. This paradigm is expected to deliver more possibilities for advancing targeted cancer therapies.

2.1.2. DCs at Tumor Sites

As opposed to therapeutic vaccination in the periphery with exogenously derived and well-defined tumor-associated antigens (TAAs) or tumor specific antigens (TSAs), in situ vaccination (ISV) in TME sourced the endogenous antigens from the dying cancer cells, which could be presented to tumor infiltrating DCs to generate adaptive T cell immune response [36,65,66]. ISV...
approach completely avoids the challenges in some tumors where specific antigens are hard to be defined. This strategy also provides entire gamut epitopes that are unique to each individual. ISV approach was first practiced by a cancer surgeon Coley who observed that surgical site infection occasionally happened in some patients led to shrinkage of unremoned tumors, and then purposely injected bacteria into patients for cancer treatment with a fraction of responders,\(^ {67}\) though the concept of ISV was not defined or realized yet. Various ISV methods that aim to induce immunogenic cell death (ICD) or activate DCs are currently under clinical studies, such as agonists of TLR, STING and CD40 receptor. Fms-like tyrosine kinase 3 ligand (FLT3L), oncolytic viruses, and radiotherapy. Monotherapies and combined therapies of these ISV candidates under clinical consideration are introduced in detail elsewhere.\(^ {69}\) We here in this section will focus on recent advanced nanoformulations targeting DCs in TME for enhanced ISV efficacy.

Taking advantage of passive tumor accumulation and capability of delivering multiple ISV agents, our lab engineered a liposome-based nanoformulation (≈160 nm) co-encapsulating a sonosensitizer hemoporphyrin monomethyl ether (HMME) and a TLR7 agonist (R837).\(^ {68}\) Ultrasound responsive HMME enhanced sonodynamic therapy (SDT)-induced ICD, which in turn promoted DC activation in the combined immunostimulator R837, antigen presentation, and T cell priming. The enhanced ISV in combination with anti-PD-L1 mAbs significantly improved therapeutic effect against 4T1 and CT26 tumors. Recently, Wang lab reported a functional polymer-based nanoformulation (named as SPNI, with a diameter of 35 nm) consisting of semiconducting polymer for near-infrared (NIR) light-mediated photodynamic therapy (PDT) and amphilathic polymer for delivery R837 via an acid-labile linker.\(^ {69}\) Upon reaching the tumor site, R837 was released in response to acidic TME, activating DCs infiltrated in tumor. Under NIR light irradiation, SPNI induced reactive oxygen species (ROS)-mediated ICD, promoting antigen presentation on mature DCs and priming CD8+ T cells against a metastatic 4T1 tumor.

Biomimetic nanoformulations are an increasingly prevalent technology in vaccine design.\(^ {70}\) The decoration of nanoformulations with cell membrane enables targeted delivery mediated by natural ligands and immunological modulation. The cell membranes with diverse functions can be prepared from a wide range of cell types, such as red blood cells, tumor cells, and leukocytes. Coating nanoformulations with erythrocyte membranes could prolong circulation time by alleviating clearance of leukocytes. Coating nanoformulations with tumor cell membranes allow their targeting of semiconducting polymer for near-infrared (NIR) light-mediation, which has demonstrated extraordinary success in the treatment of patients with leukemia or melanoma.\(^ {82,83}\) Nevertheless, it remains of great interest to enhance T cell therapy with increased portion of responders and extended therapeutic effects to other types of solid tumors. The administration of TME-immunomodulators or cytokines is a central approach under preclinical and clinical studies for enhanced T cell therapy, while these strategies usually show substantial toxicity in patients due to off-target effects. Rationally engineered nanoparticles that are conjugated with or encapsulated immunodrugs enable active targeting to the surface of T cells and subsequent modulation in a controlled manner. Schmid et al. reported PLGA polymer decorated with polylethylene glycol (PEG) and anti-CD8a antibodies, capable of targeting CD8+ T cells that were circulating in mouse blood, or resident in lymphoid organs and tumors.\(^ {84}\) To target a phenotype of exhausted CD8+ T cells in tumor, anti-PD-1 antibody was conjugated on the surface of PLGA/PEG nanoparticles. The delivery of a transforming growth factor-beta receptor

**2.2. Nanoformulations Targeting T Cells**

Therapeutic cancer vaccine effectiveness is often compromised by T cell exhaustion that can be driven by the suboptimal priming of CTLs (such as persistent antigenic stimulation, lack help signals from DCs and CD4+ T cells)\(^ {79,80}\) or the existence of inhibitory signals from tumor or stromal cells.\(^ {81}\) Exhausted T cells exhibit a dysfunctional state, losing their capability of producing cytokines and cytotoxic enzymes against tumor cells. Functionally impaired T cells are heterogeneous, with increased expression of inhibitory receptors of PD-1, lymphocyte activation gene 3 (LAG3), T cell immunoglobulin and mucin domain 3 (TIM-3), eomesodermin (EOMES), CTLA-4, and/or CD244. Immunotherapies by a blockade of the inhibitory receptor signaling are an effective strategy in cancer treatment, though only a small fraction of patients (≈20%) can benefit from this approach.\(^ {82}\)

Adoptive transfer of tumor-specific T cells that are engineered or expanded ex vivo is an alternative curative therapeutic option, which has demonstrated extraordinary success in the treatment of patients with leukemia or melanoma.\(^ {82,83}\) Nevertheless, it remains of great interest to enhance T cell therapy with increased portion of responders and extended therapeutic effects to other types of solid tumors. The administration of TME-immunomodulators or cytokines is a central approach under preclinical and clinical studies for enhanced T cell therapy, while these strategies usually show substantial toxicity in patients due to off-target effects. Rationally engineered nanoparticles that are conjugated with or encapsulated immunodrugs enable active targeting to the surface of T cells and subsequent modulation in a controlled manner. Schmid et al. reported PLGA polymer decorated with polylethylene glycol (PEG) and anti-CD8a antibodies, capable of targeting CD8+ T cells that were circulating in mouse blood, or resident in lymphoid organs and tumors.\(^ {84}\) To target a phenotype of exhausted CD8+ T cells in tumor, anti-PD-1 antibody was conjugated on the surface of PLGA/PEG nanoparticles. The delivery of a transforming growth factor-beta receptor
1 (TGFβ1) inhibitor (SD208) that obstructed TGFβ signaling mediated immunosuppression, enabled restoring the function of effector CD8+ T cells against MC38 tumor, while free drugs showed limited therapeutic effect. This targeting strategy was also able to deliver a TLR7/8 agonist (R848) of innate immunity, increasing the response of MC38 and B16F10 tumors to anti-PD-1 mAbs. In contrast, the combination of free R848 and anti-PD-1 mAbs had no effect. Sustained release of R848 and specific delivery enabled significantly improved infiltration of CD8+ T cells, accounting for the superior therapeutic effect in cancer treatment. Inspired by the fact that primed T cells possess a higher level of reductive thiols on the cell surface than T cells with a naive phenotype (Figure 6a), the same lab reported reduction-responsive protein nanogels (80–130 nm) that were formed by cross-linking the protein molecules (interleukin (IL)155a, IL-2Fc, bovine serum albumin (BSA) or IgG) using NHS-SS-NHS (Figure 6b). The disulfide groups allowed responsive release of cargo proteins in the presence of reducing agents of glutathione (GSH) (Figure 6c,d) or activated T cells stimulated with anti-CD3/CD28 antibodies. Surface conjugation of nanogels with anti-CD45 specific antibodies prolonged the retention of nanogels specifically on the surface of naive T cells up to 7 days, preventing fast cell internalization of carried IL155a. Systemic administration of IL155a nanogels together with adoptive transferred mouse T cells or human CAR-T cells substantially improved therapeutic effect against B16F10 tumor (Figure 6f) or U-87 MG human glioblastoma (Figure 6g–i) in vivo, compared to systemic administration of free IL155a. Chen lab designed a multifunctional nanof ormulation (≈145 nm), which comprised 1) magnetic iron oxide nanoparticles for tumor accumulation under an external magnetic field; 2) fucoidan with inherent therapeutic effect and immunostimulatory functions; 3) aldehyde-dextran for conjugation of anti-PD-L1 mAbs (checkpoint inhibition) and anti-CD3/CD28 mAbs (T cell activation). Post intravenous administration, this multiple functional nanof ormulation augmented effector T cell mediated anti-tumor and anti-metastatic capabilities in 4T1 and CT-26 tumor models.

Agonistic OX-40 antibodies currently under clinical studies are effective in stimulating T cell activation and proliferation for enhanced anti-tumor activity, while the corresponding stimulatory receptor OX-40 molecules can be downregulated on T cells in TME. To promote OX-40 stimulatory signaling, Dong lab screened a library of phospholipid nanoparticles to optimize the delivery efficacy of OX-40 mRNA in vitro and in vivo, thereby increasing OX-40 receptor expression levels on T cells in TME. This T cell targeting strategy significantly enhanced therapeutic effect of anti-OX-40 antibodies in A20 and B16F10 tumor models, particularly in combined treatments with anti-CTLA-4 and anti-PD-1 mAbs. Stephan and colleagues designed a stable and effective polymer-based nanof ormulation with a size of ≈155 nm to achieve T cell targeting delivery of a plasmid DNA encoding tumor specific CARs in vivo, instead of engineering CAR-T cells ex vivo. Specifically, the biodegradable poly (β-amino ester) (PbAE) polymer nanof ormulation was functionalized with 1) anti-CD3 antibodies for targeting circulating lymphocytes; 2) microtubule-associated and nuclear localization signal peptides for targeting the cell nuclear via microtubule transport machinery. The multifunctional nanof ormulation enabled selective targeting delivery of DNA molecules to T cells persistently expressing leukemia-specific 194-1BBz CAR, leading to potent regression of Flp-ALLO1 leukemia.

Different from exogenous synthetic nanocarriers, endogenous exosomes are natural membrane vesicles secreted by cells, showing low immunogenicity and reduced clearance possibility by circulating immune cells (such as monocytes and macrophages). Zhang lab engineered an interesting exosome-based nanof ormulation named SMART-Exos (synthetic multivalent antibodies retargeted exosomes, Figure 7a), which were derived from genetically edited HEK293 cells expressing anti EGFR and anti-CD3 mAbs to link T cells and EGFR-expressing MDA-MB-468 human breast cancer cells (Figure 7b). Following intravenous administration, αCD3/αEGFR SMART-Exos were able to target (by αCD3) and then direct (by αEGFR) the T cells of transplanted human blood mononuclear cells (PBMCs) to MDA-MB-468 breast tumor in immunodeficient NSG mice, eliciting potent anti-tumor immunity (Figure 7c). Recently, Azab and colleagues developed decorated LNPs with multiple mAbs against T cell receptors and tumor antigens, enhancing the engagement between T cell and tumor cells (Figure 8a). The designed T cell multiple specific nanoenagers exhibited substantially increased half-life and T cell accumulation at the tumor site (bone borrow, Figure 8b), compared to iso- and bispecific-nanoenagers. The superior anti-tumor activity of the multiple specific nanoenagers was demonstrated in an aggressive multiple myeloma mouse model (Figure 8c).

Nanof ormulations enabled responsive production of immunomodulators under external stimuli (e.g., lights and ultrasound), thereby offering a strategy to enhance anti-tumor efficacy of adoptive T cells infiltrated in TME in a controlled manner. Recently, Kwong laboratory utilized PEG-coated gold nanorods (13 × 47 nm) to passively target tumor infiltrating engineered T cells. Under NIR light irradiation at tumor site, gold nanorods mediated thermal control on the expression of IL155a or NKG2D stimulatory receptors by engineered adoptive αCD19 CAR T or Pmel-1 specific T cells, significantly improving therapeutic outcomes in multiple tumor models (K562, MDA-MB-468, and B16F10). The localized photothermal control strategy would enable targeted delivery of other immuno-drugs to augment T cell potency against tumor and reduce off-target toxicity. However, the penetration depth of NIR light is limited (a few centimeters), which greatly hinders the applicability of these strategies in clinic. Therefore, a plasmonic transducer is most likely required in practical applications. Alternative heating modalities, such as ultrasound, will address the limitations and have showed a great potential in thermal control of T cell gene expression. This concept provides localized modulation of adoptive T cells by external stimuli for improved efficacy in the treatment of solid malignancies.

Regulatory T cells (Tregs) are a crucial subset of CD4+ T cells expressing FOXP3, which mediate immune tolerance by suppressing immune response against self-antigens as well as tumor antigens. In the context of TME a relatively high ratio of Tregs to effector T cells suppresses anti-tumor immunity, promoting tumor progression. Accumulating evidence has demonstrated that cancer immunotherapy with therapeutics depleting Tregs can effectively enhance anti-tumor activity. Nevertheless, depletion of Tregs is also able to elicit deleterious autoimmunity at non-tumor tissues. Kim and co-workers developed
Figure 6. Adjuvant drugs formulated in protein nanogels substantially improve therapeutic effect of T cell therapy, by specifically activating T cells in a TCR-signaling responsive manner. a) A scheme illustrating the formation of protein nanogels (NG) and its triggered release of adjuvant drugs by the reduction activity of primed CD8^+ T cells. b) A TEM image of IL-15Sa-NG and the hydrodynamic sizes of IL-15Sa-NG, IL-2Fc-NG, BSA-NG, and IgG-NG measured by dynamic light scattering. Scale bar: 50 nm. c) The release profiles of IL-15Sa in the forms of redox-responsive or non-degradable NG. d) Free or released cytokines measured by a matrix assisted laser desorption/ionization (MALDI) mass spectrometry. e) The average B16F10 tumor growth curves and f) survival rates of mice administrated intravenously with different formulations as indicated. g) Experimental timeline: NSG mice were inoculated subcutaneously with luciferase-U-87 MG cells on day 0, followed by intravenous transfer of adoptive human T cells that contained CAR-T cells targeting epidermal growth factor receptor (EGFR) on day 7. Mice received different treatments as indicated. U-87 MG tumors in mice received with different treatments were detected by bioluminescence in vivo imaging at h) different time points and i) the survival curves are shown. a–i) Reproduced with permission.[85] Copyright 2018, Nature Publishing Group.
neuropilin-1 (Nrp1) decorated PLGA/lipid hybrid nanoparticles for targeted delivery of anti-CTLA-4 mAbs to Nrp1 receptor expressing intratumoral Tregs. The engineered nanoformulation elevated the frequency of tumor infiltrating CD8+ T cells by blocking Tregs-mediated immunosuppression, thereby enhancing tumor regression.

T-cell-based therapy has transformative potential as alternative modalities in cancer treatment. The scientific breakthroughs have led to three approved products in the clinic. There is a large pipeline of investigative T cell therapies with hundreds of clinical trials underway. Despite the advancements of T cell therapies, innovative strategies are necessary to fulfill their potential in cancer treatment, such as 1) promoting T cell activation and proliferation in vivo; 2) preventing T cell exhaustion and off-tumor target effects; 3) redirecting efficient T cells tracking to the tumor site. Nanomaterial-based targeting formulations exemplified above have shown their unique capabilities to meet these optimization criteria. Beyond targeting and modulating T cells in vivo, nanoformulations carrying immuno-stimulators or cytokines might simultaneously stimulate other immune cells, and restore their functions impaired by the immunosuppressive tumor environment, such as DCs, macrophages, NK cells, and monocytes. The unique chemicals or metal ions released from the nanoformulations might also tune the crosstalk between immune cells and stromal cells via activating unexplored signaling pathways. In all, nanoformulations targeting T cells open the possibility of ultimately expanding the T cell therapies to the treatment of solid tumors.

2.3. Nanoformulations Targeting Tumor Associated Macrophages and Monocytes

Along with exhausted CD8+ T cells, tumor associated macrophages (TAMs) are predominant immunosuppressive cell types in TME, which play a critical role in tumor progression to malignancy. The frequency of TAMs is often correlated with the resistance to cancer immunotherapies. Therefore, TAMs are an appealing population to target. For example, the use of antibodies to deplete a specific subtype of TAMs, or target a scavenger receptor on TAMs in vivo was considered as a promising strategy in promoting cancer regression. TAMs are...
highly heterogeneous with diverse functions among different tumor types, such as promoting tumor migration and invasion, suppressing effector T cell-mediated anti-tumor immunity, and stimulating angiogenesis.\(^{[101]}\) The inherent plasticity of TAMs fosters the polarization from an M2-like to an M1-like phenotype. In most types of tumors, immuno-suppressive M2-like cells prevail, thus administration of immuno-stimulatory molecules in TME can reeducate M2-like cells to M1-like cells, promoting the therapeutic efficacy of cancer immunotherapies. For instance, IL-12, agonists of TLRs and CD40, and IFN-γ were found capable of reprogramming TAMs from M2 to M1 phenotype.\(^{[102–104]}\) The use of nanoformulations enabled in vivo targeting delivery of immuno-drugs to M2 phenotype of TAMs with improved phenotypic conversion toward M1 against cancer.

Weissleder lab reported a \(\beta\)-cyclodextrin-based nanoformulation to encapsulate and deliver R848 molecules to TAMs in vivo, driving M2 to M1 phenotype alternation.\(^{[105]}\) Mice intravenously administered with the nanoformulation showed significant regression of MC38 and B16F10 tumors. The combination with anti-PD-1 mAbs demonstrated improved immunotherapy outcomes. Of note, R848 delivered by nanoformulations that were discussed above in the section of targeting DCs, was found capable of promoting the maturation of tumor infiltrating DCs, thus it is possible that these R848-nanoformulations passively targeting to TME might modulate both DCs and TAMs. Instead of delivering immuno-stimulators, Stephan and colleagues developed cationic PbAE polymer nanoparticles (\(\approx 100\) nm) to deliver anionic mRNA encoding interferon regulatory factor 5 (IRF5) and IKKβ, which were transcription factors regulating M1 polarization in TME.\(^{[131]}\) IRF5/IKKβ mRNA-PbAE nanoformulation demonstrated effective genetic reprogramming macrophages toward M1-like cells in vivo. Intraportal administration of IRF5/IKKβ mRNA-PbAE nanoformulation increased T cell infiltration into solid ID8 ovarian cancer, thereby substantially reducing tumor growth. Following intravenous injection, IRF5/IKKβ mRNA-PbAE nanoformulation enabled control on lung metastases in B16F10 model. This approach also improved therapeutic efficacy of radiotherapy in the treatment of glioma. Importantly, repeated intravenous injections of the nanoformulation were found well tolerated without obvious systemic toxicities. This safe and advanced technology is projected to be evaluated in patients with ovarian cancer, as illustrated in Figure 9.

PI3-kinase \(\gamma\) (PI3Ky) signaling obstructs the activation of nuclear factor kappa B (NF-\(\kappa\)B) activation via Akt and mammalian target of rapamycin (mTor), which promotes suppressive immune environment during tumor growth. Selective blocking macrophage P13Ky signaling pathway was discovered as a potent strategy in regulating TAM polarization for cancer immunotherapy.\(^{[106]}\) To achieve long-term and systemic modulation of immunosuppressive microenvironment, Song et al. recently designed an albumin nanoparticle containing P13Ky inhibitor (IPI-549) and paclitaxel (PTX), which remodeled the TAM in both tumor-dLNs and tumor sites via the repolarization of M2

Figure 8. Multiple specific T cell nano-engagers increase the half-life of therapeutic mAbs and prevent tumor escape caused by antigen loss, conferring improved cancer immunotherapy. a) Illustrated are engineered different T cell nanoengagers as indicated and bispecific T cell nanoengagers-mediated tumor killing. b) The biodistributions of adoptive human T cells and c) multiple myeloma progression curves in NSG mice received with different nanoengagers as indicated in (a). a–c) Reproduced with permission.\(^{[94]}\) Copyright 2021, Nature Publishing Group.
Figure 9. Cationic PbAE polymer nanoformulation delivers anionic mRNA encoding transcription factors (IRF5 and IKKβ) to ovarian tumor, specifically regulating M1 polarization. Illustrated is the design of planned first clinical trial, in which ovarian cancer patients will receive repeated intraperitoneal infusions of the developed mRNA nanoformulation programing TAMs for therapeutic evaluation. Reproduced with permission.[31] Copyright 2019, Nature Publishing Group.

to M1 phenotype, thereby regressing metastatic breast cancer in murine models.[107]

“Marker of self” CD47 ubiquitously expressing on all cells interacts with signal regulatory protein alpha (SIRPα) on macrophages and DCs,[108,109] avoiding the destruction by macrophage-mediated phagocytosis. SIRPα-CD47 inhibitory checkpoint plays a crucial role in protecting healthy cells, while tumor cells can significantly upregulate their CD47 expression compared to normal cells (two to six times) to escape from the recognition and destruction by macrophages. Therefore, block SIRPα-CD47 inhibitory signal has become a promising strategy under clinical trials to treat patients with advanced malignancies and lymphoma.[110] Administration of anti-CD47 mAbs that block CD47-SIRPα axis led to an increased frequency of M1-like TAMs against tumor.[111] In triple negative breast cancer (TNBC), increased TAMs were found promoting tumor growth (Figure 10a), thus Zhang and colleagues engineered a liposome-based nanoformulation (≈120 nm, Figure 10b,c) to target and modulate TAMs for enhanced treatment of breast cancer.[112] Specifically, anti-CD47 mAbs were conjugated on the surface of liposomes through a matrix metalloprotease 2 (MMP2) responsive phospholipid (Figure 10d), and the anti-cancer small drug PTX was encapsulated into the phospholipid bilayers. Following intravenous administration, the nanoformulation exhibited increased accumulation at the CD47 expressing tumor site than liposomes without anti-CD47 mAbs. The responsive release of anti-CD47 mAbs from the nanoformulation induced significantly enhanced polarization of M2 to M1 phenotype TAMs, with the aid of PTX-induced ICD. The nanoformulation-mediated-TAM reprogramming in turn promoted effector T cell infiltration in tumor, thereby exhibiting superior anti-tumor activity than free anti-mAbs or PTX in a therapeutic MDA-MB-231 breast cancer model (Figure 10e).

Monocytes circulating in blood are progenitors to TAMs and monocyte derived DCs. In response to the contexts of tissues and diseases, such as inflammatory environment in cancer, monocytes can be recruited to tissues and differentiate into terminal effector cells, such as M2-like cells in tumor.[113] Inspired by this fact, Hyeon and colleagues developed a new nanoformulation targeting circulating immune cells in blood for enhanced cancer therapy.[114] As illustrated in Figure 11a, anti-CD11b mAbs conjugated with trans-cyclooctene (Anti-CD11b-TCO) were intravenously injected in mice bearing 4T1 tumor, to target CD11b expressing monocytes circulating in blood. At 24 h post administration, mesoporous silica nanoparticles (MSNs) modified with tetrazines (MSNs-Tz, around 50 nm, Figure 11b) were injected to tag CD11b+ cells through the biorthogonal click reaction between TCO and Tz. Compared to passive targeting approach, the monocyte-mediated delivery allowed deeper penetration of MSNs in tumor (Figure 11c), resulting in enhanced therapeutic efficacy of MSNs-carried chemodrug doxorubicin against 4T1 tumor (Figure 11d). It would be interesting to further explore whether the penetration depth of therapeutic nanoformulation affects the recruitment or differentiation of macrophages.

In a recently highlighted preprint[115,116] that has not been peer-reviewed yet, Krummel and colleagues revealed the co-dependency of TAMs and dysfunctional T cells in promoting tumor immune evasion. Dysfunctional T cells and TAMs engaged through persistent antigen specific synaptic interactions.
exhausted T cells. Synchronously, the factors produced by the dysfunctional T cells lead to the recruitment of extra monocytes, differentiating into TAMs. These findings suggest the need for new therapies that target both TAM and exhausted T cells to interrupt the positive feedback circuit, reversing immunosuppression and promoting anti-tumor immunity. In addition, the authors used the cutting edge technique of ZipSeq (single cell spatial transcriptomics),\textsuperscript{[117]} to “label” the cells from the outer to inner area of tumor prior to scRNA sequencing. It was found that TAMs and CD8+ T cells sitting in the inner region of tumor present with more impaired function on antigen presentation and exhausted status, compared to those in outer region. Therefore, new targeting nanoformulations that can penetrate the hypoxic tumor core would improve the therapeutic outcomes of the
immunodrugs targeting both TAMs and exhausted T cells. Tumor infiltrating immune cells are temporally, spatially, and functionally heterogeneous. With the advances in new technologies, such as CITE-seq (cellular indexing of transcriptomes and epitopes by sequencing) and spatial transcriptomics, biologists and immunologists can uncover the mechanisms of tumor immunosuppression heterogeneity, offering new potential targets for cancer therapies. The combination of advanced nanoformulation technologies that enable specific site targeting would aid the development of new immunotherapeutics for better cancer treatment.

2.4. Nanoformulations Targeting NK Cells

NK cells that are innate cytotoxic lymphocytes can rapidly recognize and kill tumor cells, which are regulated by the joint signals from stimulatory and inhibitory receptors. In contrast to CTLs, prior antigen exposure is not required for NK cell natural cytotoxicity. NK cells express stimulatory receptors, such as NKG2D and NKP44L, being able to recognize the altered proteins expressed by cancer cells. Like CTLs, activated NK cells can directly eliminate cancer cells by the release of cytotoxic enzymes and cytokines. Tumor cells often have downregulated...
MHC class I molecules, to reduce the recognition and attack by antigen specific CTLs, while NK cells can exert their antigen unspecific cytolytic function on the cells with absent expression of MHC class I molecules that, in fact, suppress the cytotoxic activity of NK cells through the interaction with inhibitor KIRs (killer cell immunoglobulin-like receptors), thereby minimizing the undesired destruction on healthy cells.[118] NK cells are a unique subset among immune cells with powerful anti-tumor efficacy, thus NK-cell-based cancer therapies are extensively explored under clinical trials.[119]

Antibody-dependent cellular cytotoxicity (ADCC) is a crucial mechanism underlying NK cell therapy against antibody-opsonized cancer cells, triggered by the interaction between the CD16 receptor expressed on NK cells and Fc fragment of antibodies. For example, following administration of anti-GD2 mAbs that target the molecules highly expressed by neuroblastoma cells, allogenic NK cells were transferred into children with neuroblastoma, resulting in enhanced NK cells mediated ADCC therapeutic effect with 40% responders.[120] To achieve spatial control of ADCC-based therapeutics, Lu and colleagues engineered responsive nanoformulations for controlled release of encapsulated IgG antibodies in acidic tumor environment, thereby augmenting CD16-Fc signaling mediated NK cell immunotherapy.[121] As illustrated in Figure 12a, BSA nanocapsules were first prepared by coating with a layer of crosslinked polymer. PBA(phenylboronic acid) and IgG were conjugated on the surface of BSA nanocapsules, followed by coating with PMPC-b-PAm/Pm/Glu. The resultant nanoformulation showed a small size of ≈50 nm (Figure 12c). The polymer layer of PMPC-b-PAm/Glu displayed responsive release of inner core particles in the presence of elevated proton or sialic acid (SA). These properties enabled nBSA-PBA-IgG core particles precisely released at acidic TME following passive accumulation, and then bound with SA overexpressed on the surface of tumor cells (Figure 12b). Tumor infiltrating NK cells (Figure 12f) were activated by interacting with Fc fragment of IgG tagged on tumor cells, resulting in enhanced ADCC-mediated regression of 4T1 and B16F10 tumor (Figure 12d,e).

To potentiate NK cell mediated anti-tumor activity, therapeutic antibodies have been recently engineered and produced with single-chain multifunctional fragments that target NK cell receptors together with tumor antigen, termed bispecific or trispecific killer engagers (abbreviated as BiKEs or TriKEs).[122] Instead of engineering antibodies, Wang and colleagues designed biocompatible PEG-PgL nanoparticles functionalized with trispecific killer engagers, consisting of mAbs targeting activation of NK-cell receptor CD16 and 4-1BB, and human tumor antigen EGFR (around 100 nm in diameter, Figure 13a,b).[123] Additionally, a therapeutic anti-cancer drug of epirubicin (EPI) can be encapsulated into the nanoenagers for combined therapy (Figure 13a,b). Mechanically, the nano-TriKEs allowed spatiotemporal regulation on the engagement of NK cells and tumor cells by specifically targeting EGFR-overexpressing tumor cells and potentiating NK-cell activation via CD16 and 4-1BB receptors. The activated NK cells secreted cytotoxic cytokines and enzymes to kill the engaged cancer cells (Figure 13a). The therapeutic effect of the nanoenagers was evaluated in multiple tumor models, including EGFR expressing mouse breast cancer A431, human breast cancer MB468 xenograft models, and human colorectal adenocarcinoma EGFP+/HT-29 and lymphoma model EGFP-Raji dual xenograft models in T cell-deficient mice (Figure 13c). The results revealed tumor specificity and superior killing potency to free antibodies (Figure 13c). The combination of nano-TriKEs and EPI demonstrated significantly improved cancer therapy. It would be interesting to compare the anti-tumor efficacy and safety of the nano-TriKEs with therapeutic TriKEs antibodies. Furthermore, the therapeutic effect of this platform in humanized mouse model would provide additional preclinical evidence for its clinical translation in future.

There are several studies of nanoformulations targeting other types of immune cells ex vivo, such as neutrophils,[10,124–126] before infusion back to animals for potent cancer immunotherapy, which is out of our scope on in vivo targeting nanoformulations. Thus, the details of these studies are not introduced here. The summary of exemplified nanoformulations for targeted cancer immunotherapy is listed in Table 1. Inorganic nanoformulations, such as silica nanoparticles, can be precisely engineered with various sizes, porosity, shape, and topology. Most inorganic candidates are relatively stable under physiological conditions, while potential toxicity caused by mental ions formulated in organic nanoparticles is the key limiting factor for their clinical development.[127] In contrast, organic nanoformulations, particularly lipid and polymeric nanoparticles, offer advantages in fabrication simplicity, biocompatibility, and easy modulation of biological properties.[128] For these reasons, the advanced and promising candidates under clinical trials in cancer patients are often organic nanoformulations, which are described in next section.

3. Clinical Development of Nanoformulations in Targeted Cancer Immunotherapy

The immuno-drugs in a manner of nanoformulations have demonstrated encouraging preclinical therapeutic outcomes that are not achieved by free immuno-molecules in solution, supporting their success moving from the bench to clinical trials (Table 2). For example, poly-ICLC, an agonist for TLR3 containing double-stranded RNA (ds-RNA) protected by polylysine, is a promising candidate at advanced clinical development. In 2011, Sékaly and colleagues reported a small-scale human study on the innate immune response to poly-ICLC.[129] Following subcutaneous administration, poly-ICLC showed upregulated genes associated with innate cell pathways, particularly type I IFN and inflammasome signaling, similar to those induced by microbials. Given the potent adjuvance and DC targeting capability, poly-ICLC alone, conjugation with peptide/protein antigens, or combination with immune checkpoint inhibitors (ICIs), has been studied in phase 1/2 clinical trials to assess its safety and efficacy in patients with colon cancer, triple-negative breast cancer, ovarian, or glioblastoma (Table 2). The preliminarily promising results demonstrated tolerability and anti-tumor activity.[130] ISCOMs (immune-stimulating complexes) that consist of saponin QuilA adjuvant, phospholipid, and cholesterol are cage-like nanoformulation adjuvants with a diameter of 40–60 nm, [131] which can carry peptide/protein antigens and target APCs in lymphatic system. ISCOMs were first described in 1984,[132] representing another type of well-known adjuvants under clinical trials.[133] For example, the combination of ISCOMs and tumor
Figure 12. Multifunctional protein/polymer nanoformulation allows precise release of IgG antibodies at TME, targeting tumor surface antigen and enhancing ADCC-mediated NK therapy. Illustrated are a) the fabrication procedure of immunomodulating nanoparticles (IMN) and b) the therapeutic mechanism of IMN in targeted cancer therapy. c) A TEM image (inset) and hydrodynamic size of IMN determined by dynamic light scattering (DLS). d) C57Bl/6 mice bearing established B16F10 or e) SA-depleted B16F10 tumor with a size of 50 mm³ received different treatments as indicated. Shown are average tumor growth curves and survival rate curves. f) CD54⁺ NK and CD69⁺ NK cell percentages of pre-gated NK1.1+ cells in B16F10 tumor samples derived from mice received treatments as indicated. a–f) Reproduced with permission. [121] Copyright 2019, Wiley-VCH.
antigen New York esophageal squamous cell carcinoma-1 (NY-ESO-1) protein\textsuperscript{,134} was found safe and highly immunological with high-titer antigen specific antibody responses and a broad range of T cell immunity in participants with resected NY-ESO-1 positive melanoma in a phase I clinical trial\textsuperscript{,135}. The patients intramuscularly vaccinated with ISCOMs/NY-ESO-1 appeared improved relapse-free survival compared to those received placebo or NY-ESO-1 antigen alone\textsuperscript{,136}. In a phase 2 clinical trial, the vaccine did not impact survival endpoints of patients with fully resected melanoma\textsuperscript{,137} of which the downregulation of antigen and/or HLA class I molecules might lead to the immune escape, though vaccination induced strong antigen specific immunity.

Further development of ISCOMs+NY-ESO-1 cancer vaccine was terminated.

AS15 is a liposomal nanoformulation adjuvant comprised of monophosphoryl lipid A (MPL), QS-21, and CpG 7909. A phase 2 clinical study (NCT00086866) revealed that AS15 combined with melanoma antigen family A, 3 (MAGE-A3) tumor antigen induced strong clinical activity (robust MAGE-A3 specific antibody and T cell immune response) in patients with melanoma\textsuperscript{,138} thus phase 3 studies of MAGE-A3+AS15 therapeutic vaccines were initiated in patients with surgically resected melanoma (NCT00796445) and non-small-cell lung cancer (NSCLC) (NCT00480025). The clinical outcomes revealed that
Table 1. The most recent advanced nanoformulations targeting immune cells for cancer therapy.

| Nanomaterials                        | Size   | Targeting subsets of immune cells | Targeting strategies | Immuno-cargo molecules                                                                 | Tumor model          | Mechanistic insights                                                                                     | Ref. |
|-------------------------------------|--------|------------------------------------|----------------------|----------------------------------------------------------------------------------------|----------------------|----------------------------------------------------------------------------------------------------------|------|
| Lipid/peptide nanodiscs             | 10 nm  | dLN-DCs                            | Small size           | Antigen (SIINFEKL; Adpgk; 27+M30+TRP2) + CpG                                           | MC38, B 16F10        | Cytosolic pathway mediated cross presentation for enhanced CTLs against tumor                               | [37] |
| PC7A polymer nanoparticles          | 30 nm  | LN-resident CD8+ DC                | Small size           | Antigen OVA; HPV E7 peptide                                                            | B16-OVA, TC-1        | STING pathway activation and cross presentation for induction of potent CTLs against tumor.              | [40] |
| Azole/PEI                          | 50–100 nm | dLN-DCs                          | Small size           | OVA; tumor cell lysis                                                                  | B16-OVA; MC38        | Same as above                                                                                               | [43] |
| Peptide/TLR7/8a micelles           | 20 nm  | dLN-cDC1s (S.C.); monocyte-derived DCs (I.V.) | Small size           | Antigen (7 MC38 neoantigen; HPV E6/E7; self-antigen Trp1)+TLR7/8a adjuvant              | MC38, TC-1, B 16F10 | I.V. administration targeted monocyte-derived DCs for induction of stem-like CD8+ T cells for potent anti-tumor activity. | [49, 50] |
| CpG/shRNA nanoparticles             | 250 nm | dLN-DCs                            | Small size           | Antigen (SIINFEKL;Adpgk)+Adjuvant (CpG+shRNA)                                          | MC38                 | Dual adjuvants delivered antigen and activated dLN-DCs concurrently, triggering potent anti-tumor T cell immunity. | [51] |
| Ionizable lipid nanoparticles       | 100 nm | dLN-DCs                            | Small size           | mRNA encoding tumor antigens (OVA and E7)                                              | B16-OVA, TC-1        | Heterocyclic lipids promoted mRNA transfection and STING activation for enhanced anti-tumor immunity.   | [53] |
| Nanoemulsion                       | 200 nm | Splenic Clec9a+cDC1s               | Anti-Clec9a mAb      | HPV E7/E7 protein, B 16F10 neoepitopes                                                  | TC-1, B16F10         | Active targeting delivery of tumor antigens to cross presenting DCs for high magnitude of T cell immunity against tumor. | [54] |
| Polymer/Mn2+                        | 150 nm | LN-DCs                             | Small size           | OVA+Nod1 agonist                                                                      | B16-OVA              | Nanoformulation modulated DC activating by cross presentation of antigens and Nod1 receptor signaling against tumor. | [62] |
| Cationic cholesterol lipid nanoparticles | 70 nm | LN-DCs                             | Small size           | OVA+CpG                                                                              |                      | Nanoformulation activated DC via TLR signaling promoted cross presentation capability for enhanced T cell priming and anti-tumor activity. | [63] |
| Mannan/PLA/PEI polymer nanoparticles | 100 nm | LN-DCs                             | Small size           | Antigen (OVA, MC38 tumor lysis+adjuvants (TLR4 agonist of mannan+CpG)                  | B 16-OVA; MC38       | Dual adjuvants activated dLN-DCs concurrently, triggering potent anti-tumor T cell immunity.               | [64] |
| Albumin-stabilized nanoemulsion     | 330 nm | LN-DCs                             | Deformability        | Antigen (SIINFEKL)                                                                    | E.G7/OVA             | The deformable nanoemulsion enabled drainage to LNs for potent T cell priming against tumor.                | [30] |
| Liposome                            | 160 nm | Tumor-DCs                          | EPR effect           | R837 (TLR7 agonist) + sonosensitizer (HMME)                                            | 4T1, CT26            | R837 together with SDT-induced ICD promoted DC activation and tumor antigen presentation for enhanced therapeutic effect. | [68] |
### Table 1. (Continued).

| Nanomaterials                          | Size  | Targeting subsets of immune cells | Targeting strategies | Immuno-cargo molecules | Tumor model | Mechanistic insights                                                                                                                                                                                                 | Ref. |
|----------------------------------------|-------|-----------------------------------|----------------------|------------------------|-------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| Cell membrane/PLGA                    | 160 nm| Tumor-Clec9a+CDC1s                | CBP-12 peptide targeting Clec9a | 2′,3′-cGAMP (STING agonist) | TC-1, B16F10 | STING pathway-mediated cDC1 activation in tumor for antigen cross presentation, priming polyfunctional CD8+ T cells against tumor.                                                                                         | [74] |
| PLGA/PEG nanoparticles                 | 270 nm| (PD-1+) CD8+ T cells              | Anti-CD8a, anti-PD-1 mAbs | TGFβR1 inhibitor (SD-208), TLR7/8 agonist (R848) | MC38, B16F10 | Sustained release of SD-208 efficiently obstructed TGFβ signaling-mediated immunosuppression, restoring the function of effector CD8+ T cells against tumor; Delivered R848 restored innate immunity.                                    | [84] |
| Protein self-linked nanogels           | 80–130 nm | Primed T cells                  | Anti-CD45 mAbs and reducing agents | IL-15 super agonist (IL-15Sa) | B16F10, U-87 | IL-15Sa precise release to primed T cells substantially augmented T cell expansion against tumor.                                                                                                                     | [85] |
| Fucoidan/dextran/iron oxide nanoparticles | 145 nm | PD1+ CD8 T cells                | External magnetic field | Anti-CD3, anti-CD28, anti-PD-L1 mAbs, fucoidan | 4T1, CT-26 | Precise co-delivery of anti-PD-L1/T cell activator mAbs enhanced tumor infiltrating T cells and reduced Tregs, for improved anti-tumor effect.                                                                             | [86] |
| Phospholipid nanoparticles            | 50 nm | Tumor CD8+ T cells               | EPR effect            | OX40 mRNA               | B16F10, A20 | Increased expression of OX-40 receptor on T cells enhanced anti-OX-40 Abs-mediated T cell activation and proliferation to combat tumors.                                                                                     | [88] |
| PbAE polymer nanoparticles            | 155 nm | CD3+ T cells                    | Anti-CD3 mAbs+nuclear transport peptide | Plasmid DNA antigen | Eu-ALL01 | Increased expression of leukemia-specific 194-18Bz CAR enhanced T cell-mediated regression of leukemia.                                                                                                               | [89] |
| Exosome nanoparticles                 | 100 nm | CD3+ T cells                    | Anti-CD3 mAbs         | Anti-CD3, anti-EGFR mAbs | MDA-MB-468 | Linking CD3+ adoptive T cells and EGFR+ tumor cells promoted T cell anti-tumor immunity.                                                                                                                                 | [93] |
| Lipid nanoparticles                   | Not provided | CD3+ T cells                  | Anti-CD3 mAbs         | Anti-CD3, BCMA, CS1,CD38 mAbs | BCWM.1; MM.1S | Multiple nanoengagers increased half-time of mAbs and tumor accumulation and prevented tumor escape.                                                                                                                  | [94] |
| PEG/Gold nanorods                     | 13 × 47 nm | CART cells, TCR engineered T cells in tumor | ERP effect            | -                      | K562, MDA-MB-468, B16F10 | Thermal control production of IL-15Sa or NGK2D stimulatory receptors by engineered adoptive αCD19 CART T or Pmel-1 specific T cells against tumors.                                                                 | [95] |
| Nanomaterials                  | Size  | Targeting subsets of immune cells | Targeting strategies | Immuno-cargo molecules | Tumor model | Mechanistic insights                                                                 | Ref.  |
|-------------------------------|-------|----------------------------------|----------------------|------------------------|-------------|---------------------------------------------------------------------------------------|-------|
| β-cyclodextrin nanoparticles  | 30 nm | TAMs                             | EPR effect           | R848                   | MC38, B16F10 | Altering suppressive M2-like phenotype to an anti-tumor M1 phenotype promoted anti-tumor activity. | [105] |
| Albumin                      | 143 nm| TAMs                             | EPR effect           | PI3Kγ inhibitor (IPI-549) | 4T1 and MMTV-PyMT spontaneous breast cancer | Remodulating macrophages in both tumor sites and lymph nodes, polarizing M2 to M1, and increasing T cell immunity for long term tumor remission | [107] |
| PbAE polymer                 | 100 nm| TAMs                             | EPR effect           | IRF5/IKK/mRNA          | B16F10, ID8, glioma | Genetically reprogramming M2 with increased IRF5/IKK expression regulated M1 polarization for enhanced T cell tumor infiltration. | [31]  |
| Liposome                     | 120 nm| TAMs                             | Anti-CD47 mAbs       | Anti-CD47 mAbs+PTX     | MDA-MB-231 | Responsive release of anti-CD47 mAbs promoted polarization of M2 to M1 phenotype for enhanced cancer treatment in combination with PTX. | [112] |
| Tz-silica nanoparticles      | 50 nm | CD11b+ monocytes                 | Click chemistry interaction | Doxorubicin           | 4T1        | Monocyte-mediated delivery allowed deep tumor penetration for enhanced therapeutic efficacy of drugs. | [114] |
| Protein/polymer nanoparticles| 50 nm | NK cells                         | IgG                  | IgG                    | B16F10, 4T1 | Fc-CD16 interaction enhanced NK cell-mediated tumor regression.                      | [121] |
| PEG-PLGA                     | 100 nm| NK cells                         | Anti-EGFR antibody, anti-CD16, anti-4-1BB mAbs | Anti-EGFR antibody, anti-CD16, anti-4-1BB mAbs | B16F10, A431, MB468, HT29 | This multifunctional nanoparticle potentially activated NK cells against tumor | [123] |
Table 2. Summary of clinical studies of nanoformulations targeting immune cells for cancer therapy.

| Nanomaterials          | Targeting subsets of immune cells | Administration approach | Immuno-cargo molecules | Models | Product name | Status                                                                 | Stage (Clinicaltrials.gov identifier) |
|------------------------|----------------------------------|-------------------------|------------------------|--------|--------------|----------------------------------------------------------------------|----------------------------------------|
| Polylysine/carboxymethylcellulose | DCs                              | i.m.                    | Polynosinic-polycytidylic acid (Poly I:C) | Multiple cancers | Poly ILC | Completed/recruiting/terminated/completed/completed | Phase 1/2 (NCT00374049/NCT02834052/NCT03721679/NCT00986609/NCT02166905/NCT02078648) |
| Liposome               | APCs                             | i.m.                    | Adjuvant (saponin Quil A) and antigen (NY-ESO-1) | Melanoma | NY-ESO-1 IS-COMATRIX | Completed/competed                                                      | Phase 2 (NCT00199901/NCT00518206) |
| Liposome               | APCs                             | i.m.                    | Adjuvants (MPL + QS-21 + CpG) and tumor antigens | Multiple cancers | AS15 | Completed/completed/completed/terminated/completed/completed/completed/completed | Phase 1/2/3 (NCT00952692/NCT1498172/NCT2364492/NCT1435356/NCT0148928/NCT1835878/NCT00358526/NCT00286866/NCT00796445/NCT01480025) |
| Liposome               | dLN-APCs                         | s.c.                    | TAA and MPL             | Multiple cancers | L-BLP25 | Terminated/completed/completed/completed/completed/completed/completed/completed | Phase 1/2/3 (NCT1423760/NCT1462513/NCT094548/NCT1496131/NCT1735387/NCT1507103/NCT0157196/NCT0960115/NCT1015443/NCT0157209/NCT0828009/NCT0040918/NCT0925548/NCT02049151) |

(Continued)
| Nanomaterials                        | Targeting subsets of immune cells | Administration approach | Immuno-cargo molecules                                                                 | Models                  | Product name      | Status                        | Stage (Clinicaltrials.gov identifier) |
|------------------------------------|-----------------------------------|-------------------------|----------------------------------------------------------------------------------------|-------------------------|-------------------|-------------------------------|--------------------------------------|
| Cholesteryl pullulan nanogels      | APCs                              | s.c.                    | HER-2/NY-ESO-1                                                                           | Multiple cancers        | CHP-HER2; CHP-NY-ESO-1 | Completed                     | Phase-1 (NCT00291473)                |
| Liposome (DPX)                     | APCs                              | s.c.                    | Antigens (TAA peptides) and adjuvants (polynucleotide and Montanide ISA51 VG)           | Multiple cancers        | DPX-0907          | Completed                     | Phase-1 (NCT01095848)                |
| Liposome (DPX)                     | APCs                              | s.c.                    | As above                                                                               | Multiple cancers        | DPX-Survivac      | Recruiting/completed/active, not recruiting/recruiting/completed/active, not recruiting/recruiting/completed/active, not recruiting/recruiting/recruiting | Phase-1/2 (NCT04895761/ NCT01416038/ NCT0275250/ NCT03029403/ NCT03332576/ NCT03836352/ NCT03349450/ NCT05243524/ NCT04920617) |
| Liposome (DPX)                     | APCs                              | s.c.                    | E7                                                                                     | HPV related head and neck, cervical or anal cancer | DPX-E7 vaccine       | Active, not recruiting       | Phase-1/2 (NCT02865135)              |
| VLP                                | APCs                              | s.c.                    | Antigen (Melan-A peptides) and adjuvant (CpG-A)                                        | Melanoma                | MeIQbG10          | Completed/completed/completed/completed/completed/completed/completed/completed | Phase-1/2 (NCT00306566/ NCT00306514/ NCT00306533/ NCT00651783/ NCT00324623) |
| Liposome                           | APCs                              | s.c.                    | Antigen (MUC1) and adjuvant (PET lipid A)                                              | Solid tumors            | ONT-10            | Completed/completed/completed/completed/completed/completed/completed/completed | Phase-1 (NCT0156789/ NCT0178964/ NCT0270372) |
| DMS-5000/liposome                  | DCs                               | i.v.                    | Antigens (Gp100, melan A/MART-1, tyrosinase) and adjuvants (IFN-γ)                      | Melanoma                | Lipovaxin-MM       | Completed                     | Phase-1 (NCT01052142)                |

(Continued)
Table 2. (Continued).

| Nanomaterials | Targeting subsets of immune cells | Administration approach | Immuno-cargo molecules | Models | Product name | Status | Stage (Clinicaltrials.gov identifier) |
|---------------|----------------------------------|--------------------------|------------------------|--------|--------------|--------|--------------------------------------|
| Liposome      | APCs                             | s.c.                     | Tumor-derived antigen and IL-2 | Lymphoma | -            | Completed | Phase-1 (NCT00020462) |
| Liposome      | Peripheral immune cells          | i.v.                     | Plasmid DNA complex     | Leukemia | JVS-100      | Completed | Phase-1 (NCT00860522) |
| Liposome      | APCs                             | s.c.                     | Extract of a person’s cancer cells and IL-2 | Leukemia | Oncoquest-CLL | Active, not recruiting | Phase-1 (NCT01976520) |
| Liposome      | T cells                          | s.c.                     | IL-2                   | Melanoma | -            | Completed | Phase-2 (NCT00004104) |
| Heat-shock protein | APCs               | s.c.                     | Autologous tumor antigens | Multiple tumors | gp96 | Unknown/completed/unknown | Phase-1/2 (NCT02317471/ NCT02122822/ NCT02133079) |
| Lipid         | APCs                             | s.c.                     | Antigens (peptides) and adjuvants (CpG-7909) | Multiple solid tumors | ELI-002 2P | Recruiting | Phase-1 (NCT0483017) |
| PLGA          | iNKT and APCs                    | i.v.                     | NY-ESO-1 and iNKT activator threitolceramide-6 | Advanced solid tumor | PRECIOUS-01 | Recruiting | Phase-1 (NCT04751786) |
| PEI           | dLN-APCs                         | i.m.                     | DNA encoding neuroblastoma-associated antigen fused with potato virus X coat protein | Neuroblastoma | DNA-PEI polyplex | Recruiting | Early Phase-1 (NCT04049864) |

(Continued)
| Nanomaterials | Targeting subsets of immune cells | Administration approach | Immuno-cargo molecules | Models | Product name | Status | Stage (Clinicaltrials.gov identifier) |
|--------------|-----------------------------------|-------------------------|------------------------|--------|--------------|--------|-------------------------------------|
| IL-15 superagonist complex nanogel | CD8+ T cells and NK cells | i.v./i.p./s.c. | IL-15 N72D + rmlIL-15 Fc | Hematological malignancies/Solid tumors | ALT-803 | Completed/active, not recruiting/withdrawn | Phase-1/2/3 (NCT01727076/ NCT03563157/ NCT03647423/ NCT03586869/ NCT03387058/ NCT03329248/ NCT02890758/ NCT02523469/ NCT02559674/ NCT02384954/ NCT01853897/ NCT03127081/ NCT02093593/ NCT03054909/ NCT03565551/ NCT10946789/ NCT03052116/ NCT02138734/ NCT03082125) |
| Lipoplex | Splenic cDCs, pDCs, and macrophages | i.v. | mRNA encoding TSAs | Melanoma Stage III/IV; unresectable melanoma | BNT111 | Recruiting/active, not recruiting | Phase-1/2 (NCT04526899/ NCT02410733) |
| Lipoplex | As above | i.v. | As above | Metastatic castration-resistant prostate cancer | BNT112 | Recruiting | Phase-1/2 (NCT04382898) |
| Lipoplex | As above | i.v. | mRNA encoding HPV16 E6/E7 | HPV16+ head and neck squamous cell carcinoma | BNT113 | Recruiting | Phase-2 (NCT04534205) |
| Lipoplex | As above | i.v. | mRNA encoding TAA As | Triple-negative breast cancer | BNT114 | Active, not recruiting | Phase-1 (NCT02316457) |
| Lipoplex | As above | i.v. | As above | Ovarian cancer | BNT115 | Active, not recruiting | Phase-1 (NCT04530946) |
| Lipoplex | As above | i.v. | As above | Non-small-cell lung cancer | BNT116 | Not yet recruiting | Phase-1 (NCT05142189) |

(Continued)
Table 2. (Continued).

| Nanomaterials                  | Targeting subsets of immune cells | Administration approach | Immuno-cargo molecules                                                                 | Models                                                                 | Product name | Status                                                                 | Stage (Clinicaltrials.gov identifier) |
|-------------------------------|-----------------------------------|--------------------------|----------------------------------------------------------------------------------------|------------------------------------------------------------------------|--------------|------------------------------------------------------------------------|----------------------------------------|
| DOTAP Liposome                | As above                          | i.v.                     | Total tumor mRNA and pp65 full length LAMP mRNA                                         | Pediatric high-grade glioma/glioblastoma                              | RNA-LP       | Recruiting                                                             | Phase-1 (NCT04573140)                  |
| Lipoplex                      | As above                          | i.v.                     | mRNA encoding personalized cancer vaccine                                               | Multiple resected/advanced cancers                                    | BNT122       | Recruiting/active, not recruiting/active, not recruiting/withdrawn/active, not recruiting | Phase-1/2 (NCT04486378/NCT04161755/NCT03815085/NCT04267237/NCT03489962) |
| Lipoplex                      | CAR-T cells                       | i.v.                     | mRNA encoding CD3xCLDN6 bispecific antibodies                                           | Solid tumor                                                           | BNT211       | Recruiting                                                             | Phase-1/2 (NCT04503278)                |
| LNP                           | NK cells                          | i.v.                     | mRNA encoding anti-Claudin 18.2 antibodies                                              | Solid tumor                                                           | BNT141       | Recruiting                                                             | Phase-1/2 (NCT04683939)                |
| Cationic polymer/lipid formulation | Tumor-T cells, tumor cells       | i.v.                     | mRNA encoding CD3xCLDN6 bispecific antibodies                                           | Solid tumor                                                           | BNT142       | Recruiting                                                             | Phase-1/2 (NCT03526230)                |
| LNP                           | T cells                           | i.v.                     | mRNA encoding optimized IL-2                                                           | Solid tumor                                                           | BNT151       | Recruiting                                                             | Phase-1/2 (NCT04455620)                |
| LNP                           | As above                          | i.v.                     | mRNA encoding IL-2 and mRNA encoding IL-7                                              | Solid tumor                                                           | BNT152 and BNT153 | Recruiting                                                             | Phase-1 (NCT04710043)                  |
| LNP                           | dLN-APCs                          | i.m.                     | mRNA encoding personalized cancer vaccine                                               | Multiple cancers                                                      | mRNA-4650    | Terminated                                                             | Phase-1/2 (NCT03480152)                |
| LNP                           | As above                          | i.m.                     | As above                                                                                | Solid tumor/melanoma                                                  | mRNA-4157    | Recruiting/active, not recruiting                                      | Phase-1/2 (NCT03313778/NCT03489788)    |
| Lipopolyplex                  | As above                          | s.c.                     | As above                                                                                | Multiple advanced digestive system cancers                            | -            | Unknown                                                                | Not Applicable (NCT03468244)           |
| LNP                           | As above                          | i.m.                     | mRNA encoding KRAS mutated proteins                                                    | Multiple KRAS mutant advanced/metastatic cancers                     | mRNA-5671    | Active, not recruiting                                                 | Phase-1 (NCT03948763)                  |
MAGE-A3+AS15 vaccine alone did not show therapeutic efficacy in these cancer patients and the development of the therapeutic vaccine for use in these malignancies has been stopped.\(^{[139,140]}\) A phase 2 study of MAGE-A3+AS15 vaccine in patients who experienced surgical removal of bladder cancer was also terminated (NCT01435356). In parallel, the combination therapy of MAGE-A3+AS15 vaccine and IL-2/ICIs was evaluated in clinical development. A phase 2 clinical study (NCT01266603) proved the safety profile of the combination therapy similar to that of high dose IL-2 monotherapy and showed that vaccination provided strong anti-tumor activity in melanoma patients.\(^{[141]}\) The clinical immune monitoring data suggested a potential treatment strategy by combining MAGE-A3+AS15 vaccine and ICIs. Another completed phase 1 study investigated the Bacillus Calmette-Guerin (BCG) modulation of MAGE-A3+AS15 vaccine in the patients with bladder cancer (NCT01498172, Table 2).

Beyond MAGE-A3 tumor antigen, AS15 has been under clinical development in combination with other types of cancer antigens for the treatment of patients with other malignancies, such as combination with HER2\(^{+}\) protein antigen for treating HER2\(^+\) metastatic breast cancer (phase 1, NCT0058526;\(^{[142]}\) phase 2, NCT01140738;\(^{[143]}\) phase 1, NCT00952692;\(^{[144]}\) phase 2, NCT01853878) and melanoma (phase 1/2, NCT01149343\(^{[146]}\)). Wilms’ tumor 1 (WT1) antigen for treating WT1\(^+\) breast cancer (phase 2, NCT01220128, terminated), P501 antigen for treating prostate cancer (phase 1, NCT00148928), and MAG-Tn3 for HER2- breast cancer (phase 1, NCT02364492\(^{[147]}\)). Despite the observed acceptable safety profile and immunogenicity, GSK decided to terminate the clinical development of cancer vaccines consisting of recombinant proteins and AS15, considering the negative results of the AS15 adjuvant platform in two phase 3 clinical trials of MAGE-A3 therapeutics.

Liposomal nanoformulation L-BLP25, cancer associated mucin 1 (MUC1) vaccine, is another well investigated therapeutic candidate in phase 1/2/3 clinical trials (Table 2), of which the clinical results demonstrated that L-BLP25 was well tolerated and capable of inducing T cell immune response in patients with NSCLC.\(^{[148]}\) In a phase 2b clinical study, patients vaccinated subcutaneously with L-BLP25 appeared a potential benefit in survival time compared to those only received best supportive care.\(^{[149]}\) Given these promising clinical outcomes, a phase 3 trial was initiated to investigate whether L-BLP25 could improve the survival time of patients after chemoradiotherapy, while the preliminary clinical results did not show significant difference between patients received vaccination and placebo.\(^{[150]}\) The clinical development of this product has been stopped.

A nanogel formed by cholesteryl pullulan (named CHP, 25 nm in diameter\(^{[151]}\)) was well examined in protein antigen delivery, and the resulting CHP-antigen vaccine formulations were capable of targeting APCs and inducing antigen specific T cell immunity against murine tumor.\(^{[152]}\) A protein cancer vaccine of CHP-HER2 was assessed in a clinical study in patients with HER2\(^+\) cancers, showing clinical response with HER2-specific T cell immunity.\(^{[153]}\) Vaccination of patients with CHP-NY-ESO-1 vaccine also induced antigen specific T cell response\(^{[154]}\). Instead of carrying recombinant protein antigens, DPX-0907 is a strongly immunogenic liposomal vaccine formulation (120 nm in diameter) delivering multiple HLA restricted peptides and polynucleotide-based adjuvants. DPX-0907 was capable of inducing multifunctional effector T cell immune response against antigens in patients with breast, ovarian, or prostate cancer (phase 1, NCT01095848\(^{[155]}\)). The vaccine formulation of DPX- Survivac using surviving HLA peptides also showed induction of robust polyfunctional T cell immunity in ovarian cancer patients (phase 1, NCT01416038\(^{[156]}\)). These promising clinical activities lead to the further clinical evaluation of this peptide cancer vaccine platform as monotherapy or combination therapy with cyclophosphamide/radiation/IDO1 inhibitor in patients with HER2- breast cancer (phase 1, NCT04895761), ovarian, peritoneal carcinoma, or fallopian tube cancer (phase 1/2, NCT02785250; NCT03029403; phase 1, NCT0332576; phase 2, NCT05243524), hepatocellular carcinoma, NSCLC, bladder cancer (phase 2, NCT03836352), large B cell lymphoma (phase 2, NCT03349450 and NCT04920617). DPX-E7 vaccine currently is under phase1/2 clinical trial (NCT02865135) to evaluate its safety profile and therapeutic efficacy in patients with HPV16 associated malignancies (head and neck, cervical or anal cancer).

MelQbG10 is another type of peptide-based vaccine,\(^{[157]}\) consisting of 1) highly immunogenic virus-like particles (VLP, 30 nm in diameter) formed by shell proteins derived from bacteriophage Qbeta, and immunostimulatory G10, unmodified A-type CpG, and 2) peptides derived from antigen Melan-A/MART-1 (melanoma antigen recognized by T cells 1) that is expressed in advanced melanoma.\(^{[158]}\) The first phase 1/2 clinical trial of MelQbG10 revealed that immunization with MelQbG10 was well tolerated and capable of inducing CDB\(^+\) T cell immunity in patients with melanoma.\(^{[159]}\) In combination of additional adjuvants, MelQbG10 vaccine increased memory and effector phenotypic T cell response in patients with melanoma (phase 2, NCT00651703\(^{[157]}\)). The combination therapy of MelQbG10 and chemotherapy (cyclophosphamide, fludarabine phosphate) was evaluated in melanoma patients recruited under a phase 1 study (NCT03024623). Several other types of liposome, heat shock protein, lipid, PLGA or PEI-based vaccine, or immune-stimulator nanoformulations formed by different components were evaluated in phase1/2 clinical trials (such as ONT-10, Lipovaxin-MM,\(^{[160]}\) JVRS-100, ELI-002 2P and PRECIOUS-01\(^{[161]}\)), which are summarized in Table 2.

The recombinant IL-15 superagonist complex nanogel that targets NK and T cells as introduced in the section above, named ALT-803, has been evaluated under phase 1/2/3 clinical trials (as listed in Table 2) to assess the safety and efficacy for treating patients with advanced solid tumors (such as NSCLC, melanoma, renal carcinoma, colon cancer, and breast cancer), or leukemia. Phase 1 clinical studies revealed that ALT-803 was well tolerated, increased expansion of NK cells (NCT01727076\(^{[162]}\)) and CD8\(^+\) T cells (NCT01855897\(^{[163]}\)), and promoted anti-tumor activity (NCT02523469,\(^{[164]}\) NCT02138734\(^{[165]}\)) in cancer patients. Recent clinical results from a phase 2/3 trial (NCT03022825) of ALT-803 combined with BCG showed exceeded clinical response, safety, and efficacy, compared to available options for patients with bladder cancer.\(^{[166]}\) These encouraging clinical outcomes support further development of ALT-803 product with numerous clinical trials underway, particularly in the combination with...
chemotherapy, NK cell therapy, cancer vaccines, radiation therapy, and/or ICIs. The preferred nanoformulations that are currently active under clinical studies are LNP-mRNA technology, of which lipoplexes enables targeted delivery of therapeutic mRNA cancer vaccines to APCs in lymph organs, and subsequent proliferation of abroad T cell immunity in preclinical and clinical settings. Sahin and colleagues revealed that the surface charge of lipoplexes determined their biodistribution post intravenous administration in mice. Near neutral lipoplexes were found precisely and effectively targeting splenic CD11c+ cDCs, plasmacytoid DCs (DCs), and macrophages, which promoted encoded antigen expression in APCs and triggered highly potent innate and adaptive immunity against aggressive tumors in mice. This mRNA vaccine approach has been extensively evaluated under phase 1 clinical trials in cancer patients, such as the products BNT111, BNT112, and BNT113 encoding TSAs, BNT114, BNT115, and BNT16 encoding TAAs, and BNT122 encoding personalized cancer vaccines (Table 2). The clinical study results of BNT111 (NCT02410733) recapitulated the observations in mice. The translation of mRNA encoding melanoma antigen was found in DCs resident at lymphoid organs, inducing strong polyclonal functional CD4+ and CD8+ T cells against antigens in patients with advanced melanoma. BNT111 monotherapy, or combination therapy with anti-PD-1 mAbs demonstrated tumor regression in patients. Another lipoplex-mRNA product named BNT211 encodes antigen claudin-6 (CLDN6) that is a tetraspan membrane protein integral to tight junction formulation and widely expressed in tumors, which empowered proliferation of adoptive CLDN6-CAR-T cells to regress large CLDN6+ solid tumors in mouse models. The preliminary clinical data showed encouraging safety profiles and expansion of CLDN6-CAR-T cells in patients with CLDN6+ tumors. Along with these therapeutic mRNA cancer vaccines, BioNTech’s product pipelines on clinical program include LNP-mRNA products encoding antigen specific therapeutic mAbs (BNT141 and BNT142) or cytokines (BNT151, BNT152, and BNT153), which are currently being assessed in patients with solid tumors. In parallel, Moderna is investigating the applicability of mRNA immune medicines for the treatment of cancer patients, such as mRNA-4650 and mRNA-4157 therapeutic mRNA vaccines. The unique amine-lipid structure endowed Moderna’s nanoformulation with enhanced endosomal escape, excellent safety profiles, and rapid clearance. Moderna mainly focuses on the development of lipid-delivered personalized cancer mRNA vaccines. Identification and selection of appropriate immunogenic neoantigens are critically important for the effectiveness of cancer mRNA vaccines. Moderna has developed different pipelines to identify neoantigens that can be recognized by intratumor T cells, such as discovering driver mutations by exome sequencing of tumor samples in the development of product mRNA-4650. Moderna also has TAA encoded mRNA vaccine products under clinical development, such as mRNA-5671 for the treatment of patients with Kirsten rat sarcoma viral oncogene homolog (KRAS) mutant advanced cancers (NCT03948763, Table 2). The recent remarkable success of mRNA-lipid in the deployment of COVID-19 mRNA vaccines is fostering the research interest and clinical development of mRNA therapeutics delivered by nanoformulations in cancer immunotherapy. The efficiency of nanoformulations targeting immune cells relies on the biochemical features of nanoformulations, administration routes, and subsets of immune cells. For example, near neutral lipid-based mRNA nanoformulations were found efficiently targeting splenic APCs post intravenous administration, demonstrating potent cancer immunity in preclinical and clinical studies. Following intravenous administration, the reduction responsive nanogel was found efficiently targeting activated T cells for elimination of established tumors, which supported its movement from bench to clinical trials.

4. Conclusions and Future Directions

The use of nanomaterials for targeted chemotherapy has been approved in clinical practice, which enables selective tumor accumulation of systemically administrated cytotoxic chemotherapeutics. Modulation of the nanomaterial constructs, such as small sizes and surface decoration with targeting ligands, improves tumor-specific targeting and consequently reduces off-target adverse effects. Recent research interest in nanomaterial delivery platforms has been shifting rapidly toward cancer immunotherapy for improved implementation of therapeutic immuno-drugs. Current summary reports mainly focus on the targeting strategies (both local and systemic administration) for the modulation of tumor specific immune cells, structural cells, and physiology features in TME. Local administration offers an easy targeting approach, but not practical in the case of hard-to-reach cancer sites. Besides, establishing successful anti-tumor immunity requires coordination of innate and adaptive immune system across tissues, involving lymphoid organs, blood stream, or tumor tissues. In view of these two points, this review highlights recent advances in systemically administrated immuno-drug nanoformulations (Figure 14) that are capable to target specific subsets of immune cells located in lymphoid organs, bloodstream, and tumor sites for enhanced cancer immunotherapy. The directing strategies of nanoformulations consist of passive targeting (Figure 14a) driven by size exclusive transport or EPR effect and active targeting (Figure 14b) driven by the conjugated specific ligands, such as mAbs, peptides, and small molecules. The exemplified nanoformulations were engineered to direct immuno-drugs to specific subsets of DCs, T cells, NK cells, TAMs, or monocytes, to promote their functional activity or restore impaired function, thereby enhancing anti-tumor immunity (Figure 14c). The associated mechanistic insights into the regulation of anti-tumor immunity are summarized in Table 1. Immune cell-targeting nanoformulations represent an attractive strategy for cancer therapy, potentiating the efficacy and decreasing off-target toxicity. Some intriguing candidates are currently under clinical studies (Table 2). Despite the advantages and rapid progress, there are still several challenges and obstacles to be addressed for advancing the clinical translation of these nanoformulations in cancer treatment.

1) Differences in species-specific immune system: The choice of preclinical settings in the evaluation of therapeutic nanoparticle platforms is critically important for their translation from animals to humans. For instance, mouse xenograft tumor models are simple to implement, while do not closely replicate the human oncology. In addition, the species-specific immune
systems are different in mice and humans,[178,179] thus the anti-tumor immune response in mice hardly reflects those in humans. Patient-derived xenograft (PDX) model is biologically more relevant to human settings, but the immunodeficient animals require to be humanized with human immune system, such as engraftment of peripheral blood mononuclear cells.[177,180] Humanized mouse models serve as a preclinical bridge, improving the understanding of the immune functions of novel immuno-drug nanoformulations in adoptive T cell therapies as well as therapeutic neoantigen vaccines. Despite some success, humanized mouse models remain constrained by low engraftment rate, long-term duration, varieties in MHC antigens, and suboptimal development of lymphoid organs. The genetically engineered mouse models that enable spontaneous development of cancer are closely relevant to the features of TME in humans.[177] For example, HPV transgenic mice resemble closely the immune signatures of the HPV associated squamous cell carcinoma,[181,182] which is an ideal mouse model to investigate the modulation of nanoformulations on tumor infiltrating immune cells. It requires interdisciplinary team engaged in a close and intense collaboration, across the scientists in material science and onco-immunology and clinicians. Thus, whether the outstanding immuno-therapeutic efficacy and safety achieved in mice models can predict the performance in human clinical trials remains to be extensively evaluated in the future.

2) Feasibility of scale up production: Cost-effective and feasible large-scale manufacturing of these nanoformulations are major concerns that prevent their clinical translation. Usually, new delivery systems that utilize Food and Drug Administration (FDA)-approved materials (i.e., lipid, polymer) are more likely to move from the lab toward the clinic than those unapproved ones.[183,184] As yet, of all the submissions to FDA for drug products using nanoplatforms, liposome was reported to be the most prevalent category, mainly owing to the simplicity in manufacturing at large scale.[185] Many FDA-approved nanoformulation modulated immunotherapy has already been applied in clinic for cancer treatment (Table 2), most of which are lipid-based formulations. For example, the technology of using lipids to deliver mRNA to DCs previously reported in Nature[167] is being tested in clinical trials in patients with melanoma (NCT02410733). However, in the context of nanoplatforms for T-cell targeted therapy, many studies involved complex design process. With the formulation complexity increasing, manufacturing costs and the regulatory procedures to prove safety and effectiveness increase as well.[186] Thus, in future studies, researchers in this field should keep the ultimate goal of the nanomedicine-“widespread clinical application” in mind, when designing the nanomaterial solutions.

3) Difficulties in precise targeting to tissue-specific subsets of immune cells: It remains a challenge to completely resolve off-target immune toxicities while maintaining sustained anti-tumor effects in patients receiving immuno-drugs. Nanoparticles should be engineered to precisely target and modulate tissue-specific immune cells, reducing adverse effects. The accumulated evidence on ligands revealed that distinct subsets of immune cells can express the same
bionarkers. For instance, M1- and M2- phenotypes of TAMs display shared mannose/galactose receptors,\(^\text{[187]}\) Similar to T cells, NK cells express several immune checkpoint molecules on their surface (e.g., TIM-3, CTLA-4, and PD-1),\(^\text{[188–190]}\) along with some intracellular signaling components (i.e., CDk8, Cbl-b).\(^\text{[191]}\) Thus, nanoformulations targeting these shared components can stimulate undesired immune cell responses in cancer treatment, which might inevitably increase unwanted side effects. To advance nanoformulations targeting specific receptors, receptor humanized mouse models are designed to evaluate the efficacy and safety.\(^\text{[195]}\) Also, cancer patients often receive substantial conventional therapies (e.g., radiotherapy and chemotherapy) prior to immunotherapy. Thus, the influence of radio/chemo-therapeutics should be explored on the biomarkers expressed on patient immune cells for the selection of accurate immune cell-targeted therapies.

4) Exploration of new mechanisms at the interface of nanoformulations and immune system: The rapid development of cutting-edge biotechnologies, such as transcriptomics, scRNA sequencing, and CITE-seq that enables gaining information of surface proteins using available antibody-bound oligos in RNA sequencing, have advanced our understanding on the cross-talk between structure cells and immune cells either at normal\(^\text{[192]}\) or cancerous tissues.\(^\text{[193]}\) The integration of multiple high dimensional multilomics analysis approaches enables identification of new subtypes of disease or tissue specific immune cells and their functions on immune regulation as well as receptor-ligand networks, deepening our knowledge of immune modulation mechanisms across different organs. The applications of these technologies would uncover novel interaction mechanisms occurring at the interface of nanoformulations and tissue resident immune cells at the injection sites, bloodstream, lymphoid organs, and tumor sites. The observations at mRNA levels are not always correlated well with functional protein levels, thus discoveries derived from these high dimensional sequencing data should be validated at protein levels using biomolecular tools and (Symphony) multichannel flow cytometry analysis. The newly defined mechanisms associated with nano-immunological activities will provide new guidelines for engineering the next generation of nanoformulations.

5) Comprehensive safety evaluation: The potential toxicity of these fascinating nanoparticles is a burning matter, restraining their clinical translation. Aside from nanotoxicity\(^\text{[194]}\) associated with low degradation, DNA damage, or metal poisoning on the central nervous system, nano-immunoformulations could also potentially cause a diverse range of chronic and irreversible immune-related adverse events.\(^\text{[18]}\) Upon entering biological systems, nanoformulations tend to form a corona by interaction with proteins or lipids in the biological fluids.\(^\text{[195]}\) Protein corona was discovered responsible for recognition by innate immune cells and triggering adaptive immune response.\(^\text{[195]}\) For example, APC can recognize and uptake nanoparticles with a corona formed by serum components via the lipoprotein receptors. The interactions between nanoformulations and biological molecules, tissues, immune cells, and intracellular biomolecules, determine the fate and performance of nanoformulations in vivo. The study of corona-nanoformulations is helpful for the prediction of immunological response of nanoformulations, therefore diverse methods have been developed to characterize the nanocomplexes. Cryo-transmission electron microscopy can visualize the network of corona on the surface of nanoparticles. \(^\text{[196]}\) Chemical analysis methods can reveal the compositional profiling of corona in combination with mass spectrometry.\(^\text{[197]}\) Affinity chromatography is able to quantitatively measure the total protein in corona adsorbed on organic nanoparticles. \(^\text{[198]}\) Recently, a biolayer interferometry-based fishing strategy was developed for in situ analysis of corona protein adsorption and dissociation processes.\(^\text{[199]}\) In the context of tracking the distribution of nanoformulations in the immune system, in vivo imaging and inductively coupled plasma analysis in combination with fluorescence-activated cell sorting technology offer quantitative approaches to analyze the accumulation of nanoformulations in specific subsets of immune cells in lymphoid organs. The activation status of immune cells induced by nanoformulations can be revealed by flow cytometry analysis. The advanced knowledge in the interactions between nanoformulation and proteins or immune cells provides insights into nano-protein complexes mediated immune response and potential adverse immunotoxicity. For these reasons, the biological safety of engineered nano-immune formulations should be systematically evaluated from different aspects, though current preliminary data did not show obvious acute toxicities in preclinical and clinical studies.

Current emerging nanoeengineering technologies have shown great potential in amplifying the potency and reducing adverse effects of immunotherapeutic substances. Some investigative nanof ormulation-based targeted cancer therapies are currently under clinical studies. Accumulated evidence exists suggesting that the physiochemical features of nanomaterials along with the immuno-drugs determine the modulation of immune cells, initiating and shaping anti-tumor immunity. However, the associated mechanisms remain not fully understood. The advances in highly dimensional biological technologies offer a high possibility to discover unexplored mechanisms, broadening and deepening the knowledge in this field. Close collaborations among nano-engineers, onco-immunologists, and bioinformaticians are encouraged to foster the translation of nanof ormulations, benefitting cancer patients in the future.

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**Conflict of Interest**

The authors declare no conflict of interest.
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