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Combining nanomedicine and immune checkpoint therapy for cancer immunotherapy

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Abstract

Cancer immunotherapy has emerged as a pillar of the cancer therapy armamentarium. Immune checkpoint therapy (ICT) is a mainstay of modern immunotherapy. Although ICT monotherapy has demonstrated remarkable clinical efficacy in some patients, the majority do not respond to treatment. In addition, many patients eventually develop resistance to ICT, disease recurrence, and toxicity from off-target effects. Combination therapy is a keystone strategy to overcome the limitations of monotherapy. With integration of ICT and any therapy that induces tumor cell lysis and release of tumor-associated antigens (TAAs), ICT is expected to strengthen the coordinated innate and adaptive immune responses to TAA release and promote systemic, cellular anti-tumor immunity. Nanomedicine is well-poised to facilitate combination ICT. Nanoparticles with delivery and/or immunomodulation capacities have been successfully combined with ICT in preclinical applications. Delivery nanoparticles protect and control the targeted release of their cargo. Inherently immunomodulatory nanoparticles can facilitate immunogenic cell death, modification of the tumor microenvironment, immune cell mimicry and modulation, and/or *in situ* vaccination. Nanoparticles are frequently multi-functional, combining multiple treatment

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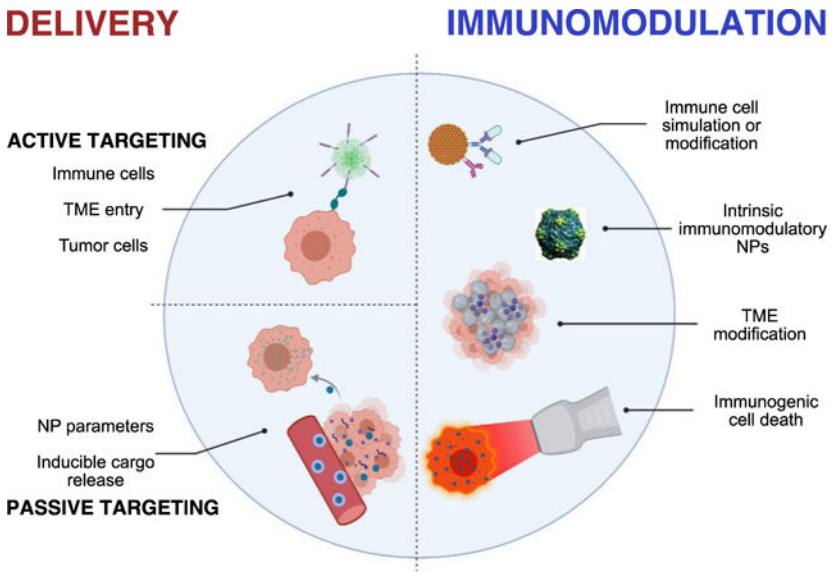
strategies into a single platform with ICT. Nanomedicine and ICT combinations have great potential to yield novel, powerful treatments for patients with cancer.

Graphical Abstract



Nanoparticles with targeted delivery and immunomodulation capabilities promote novel immune checkpoint therapy combination therapeutic strategies.

Introduction

As understanding of the complex interactions between cancers and the immune system has advanced, cancer immunotherapy has increasingly gained traction as a promising treatment strategy. The immune system plays a critical role in tumor surveillance and rejection. Many cancers are adept at suppressing anti-tumor functions of the immune system and evading immune responses (D. S. Chen & Mellman, 2013; Finn, 2008). Immunotherapy modulates the activity of the immune system to promote its anti-tumor functions and overcome immunosuppression (Alexander, 2016). Dr. William Coley's studies of intratumoral injections of bacterial toxin, later known as "Coley's toxin", provided early evidence in support of this approach to cancer therapy.

Coley's toxin produced promising therapeutic results, including complete tumor regression in multiple patients (Nauts et al., 2007). This material from pathogens acted as a "danger" signal at the site of the tumor to stimulate local engagement of the innate arm of the immune system. The local innate immune response can subsequently prime the adaptive arm of the immune system against the tumor. The primed adaptive immune response can effect selective, systemic, and durable anti-tumoral immunity (Marabelle et al., 2017; Singh & Overwijk, 2015). More recently, the presence of tumor-infiltrating lymphocytes (TILs) has been correlated with improved clinical prognosis (Galon et al., 2006; Imai et al., 2000; Kawata et al., 1992). Early evidence of the potential of the endogenous immune system to recognize and eradicate tumor cells drove the development of immunotherapy treatments to

potentiate and augment this effect. As a result, the field of immunotherapy has boomed with diverse immunotherapeutic strategies to modulate the dynamic and complex activities of the immune system that underlie cancer immunity.

Cancer immunity is a cyclic process of interactions between the cancer and the immune system. Ideally, it leads to an effective anti-tumor response without developing autoimmunity or immunosuppression. Tumor lysis can be induced by cytotoxic immune cells or by cancer therapies, including radiation, chemotherapy, or minimally invasive, imaging-guided interventions, such as ablation, chemoembolization or radioembolization. With tumor lysis, damaged tumor cells release tumor-associated antigens (TAAs) into the tumor microenvironment (TME), inducing local inflammation and an innate immune response. Infiltrating antigen presenting cells (APCs) process and transport TAAs to draining lymph nodes. Cross-presentation of antigens to T cells leads to priming the adaptive arm to launch systemic, cellular anti-tumor immunity, even at distant metastatic sites, known as the ‘abscopal effect’ (Marabelle et al., 2017; Singh & Overwijk, 2015).

Dysfunction of the “cancer-immunity cycle” impedes the effective eradication of tumors by the immune system. Many aggressive tumors possess this feature (D. S. Chen & Mellman, 2013). Tumors may contribute to cancer-immunity dysfunction through suppression and evasion of immunosurveillance. Tumor cells commandeer signaling pathways that regulate activation and effector functions of many types of immune cells. They also appropriate pathways that promote recruitment and proliferation of immunosuppressive cells, such as tumor-associated macrophages and regulatory T cells (Tregs), within the TME (D. S. Chen & Mellman, 2013; Finn, 2008). Checkpoints within this cycle determine whether the immune system will generate immunity against or tolerance towards the tumor. The therapeutic strategy of targeting these immune checkpoints through immune checkpoint therapy (ICT) has resulted in dramatic, durable clinical responses, including complete remissions in some cases. Thus, ICT has become a mainstay of modern immunotherapy.

Immune checkpoint inhibitors (ICIs) have emerged as the principal class of ICT in the clinical setting. ICIs target checkpoint molecules to disinhibit effector T cell activity. As monotherapies, ICIs have produced remarkable clinical outcomes for melanoma, non-small cell lung cancer, and renal cell carcinoma. Ten to 35% of patients demonstrated therapeutic responses and some survived 10 years from treatment (Hellmann et al., 2016; Topalian et al., 2015). While these outcomes are remarkable, ICT carries risks of autoimmune disease-like side effects and most patients with advanced or metastatic disease do not respond to existing ICI treatments. Even those who do respond initially tend to recur with resistant disease. Tumor heterogeneity, ranging from variations in cell populations within a tumor and among metastases in a single patient to differences in tumors among patients with the same diagnosis, plays a notable part in the limited responses, recurrence, and resistance (Emens et al., 2017). Differences among patients, such as metabolism and immune response, further contribute to the diversity of patient outcomes.

Effective combination strategies generally target distinct mechanisms in summative or synergistic ways to overcome the complexity and variability of cancer genotypes and phenotypes (Grzywa et al., 2017; L. Li et al., 2019; Palmer & Sorger, 2017). Cultivation of

novel ICT-based combination therapy strategies can substantially improve patient outcomes while limiting side effects. Combination therapy may aim to enhance the function of ICT, or to synergize with ICT through components that independently modulate immune system function and tumor-immune interactions. Nanoparticles are well-equipped to address these challenges through their capacity for targeted drug delivery and their immunomodulatory properties to sensitize tumors to ICT. Here, we succinctly review ICT and its limitations, explore relevant aspects of nanoparticle design for combinatorial immunotherapy, and discuss approaches to combining nanoparticles and ICT for cancer immunotherapy, including outcomes of preclinical studies.

Immune Checkpoint Therapy

First-generation ICT

ICT promotes potent anti-tumor immune responses by altering immune checkpoint signaling pathways. The main targets of clinically available ICIs are two immune checkpoint proteins on the surface of activated T cells, cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) or programmed cell death-1 (PD-1), and the ligand for PD-1, PD-L1, which is found on a variety of cell types. The detailed mechanisms of these signaling pathways and the treatment outcomes of their inhibition have been extensively reviewed elsewhere (Alexander, 2016; Pardoll, 2012; Ribas & Wolchok, 2018; Topalian et al., 2015; Wei et al., 2018). It is critical, however, to note the non-overlapping and complementary way anti-PD-1/PD-L1 and anti-CTLA-4 monoclonal antibodies (mAbs) block inhibition of effector T cell activity.

When bound to its ligands PD-L1 and PD-L2, the PD-1 receptor inhibits the activity of effector and memory T cells. After activation, T cells may express PD-1 receptors as a negative feedback mechanism to limit their activity. Exposure to inflammatory cytokines can induce expression of PD-L1 on somatic cells, including tumor cells, stromal cells, and immune cells within the TME. Thus, tumor cells and other TME cells expressing PD-L1 can suppress TIL activity (Figure 1). Inhibition of PD-1 and/or PD-L1 signaling alleviates suppression of activated T cells. This allows effector T cells to carry out their anti-tumor functions within the TME and to coordinate enhanced anti-tumor activities among other immune cells (Pardoll, 2012; Ribas & Wolchok, 2018; Topalian et al., 2015). Several formulations of anti-PD-1 and anti-PD-L1 mAbs have been increasingly incorporated into first-line therapy in the clinical setting. Anti-PD-1/PD-L1 mAbs have been FDA-approved for a range of cancers, including metastatic melanoma, non-small cell lung cancer (NSCLC), Merkel cell carcinoma, hepatocellular carcinoma (HCC), renal cell carcinoma (RCC), and urothelial carcinoma (Antonia et al., 2018; Brahmer et al., 2015; Gallacher et al., 2019; Hargadon et al., 2018; Ribas et al., 2016; Ribas & Wolchok, 2018; Robert et al., 2015).

Anti-CTLA-4 mAbs target a different immune checkpoint pathway, but also have demonstrated effectiveness in clinical application. Both $CD4^+$ (helper) and $CD8^+$ (cytotoxic) T cells initiate surface expression of CTLA-4 soon after stimulation by antigen presentation on type I or II major histocompatibility complex molecules (MHC-I and MHC-II). CTLA-4 blocks the co-stimulatory signal required for subsequent T cell amplification and proliferation, thereby providing a negative feedback mechanism to limit the immune response. CTLA-4 interferes with an early, critical step in priming an adaptive immune

response (see Figure 1). CTLA-4 also promotes the immunosuppressive functions of Tregs within the TME. In contrast to PD-1/PD-L1, the actions of CTLA-4 occur on a more systemic scale within secondary lymphoid organs, as well as the TME. CTLA-4 inhibition alleviates the suppression of T cell proliferation in response to APC cross-presentation and priming and promotes Treg depletion within tumors. Since 2011, anti-CTLA-4 mAbs have been FDA-approved for use as adjuvant therapy against melanoma and are the subject of clinical trials for other cancers, including HCC (Alexander, 2016; Hargadon et al., 2018; Pardoll, 2012; Ribas & Wolchok, 2018; Topalian et al., 2015).

Early clinical investigations of ICI centered on monotherapeutic strategies, which yielded marked improvement in response rates and survival. CTLA-4-targeted ICI monotherapy in patients with advanced-stage disease, or who had undergone prior treatment, demonstrated the possibility of dramatic, durable responses against melanoma with ICI. Long-term survival was observed in 21% of these patients (Schadendorf et al., 2015). CTLA-4 ICI yielded milder efficacy in other tumor types, such as NSCLC and RCC (Hellmann et al., 2016; Topalian et al., 2015). Responses to PD-1/PD-L1 ICI single-agent treatment were generally improved over CTLA-4 ICI; however, this varied with cancer type. Patients with stage III or IV melanoma had response rates of 35–40% (Larkin, Lao, et al., 2015; Ribas et al., 2016); although in patients with the rare desmoplastic-type melanoma and in patients with Hodgkin lymphoma, response rates could exceed 70%. In patients with advanced stage disease, including NSCLC, head and neck cancer, and urothelial cancer, low response rates of 20% or less were observed with anti-PD-1 mAb monotherapy (Bellmunt et al., 2017; Ferris et al., 2016; Garon et al., 2015; Ribas & Wolchok, 2018). Further, long-term follow-up of initial ICI responders revealed that as many as 33% of patients developed tumor recurrence and ICI resistance (Ribas & Wolchok, 2018). While CTLA-4 and PD-1/PD-L1 ICI monotherapy set a new standard in cancer therapy, the dramatic, durable responses occurred in the minority of patients. These therapies are also limited by their autoimmune disease-like side effects, including colitis, inflammatory hepatitis, and dermatitis. CTLA-4 ICIs are associated with more pronounced side effects because of their system-wide actions. Limited responses and serious potential side effects render ICI monotherapy insufficient to achieve durable tumor suppression and eradication in most patients.

Outcomes have further improved in recent phase II and III clinical trials of combined CTLA-4 and PD-1/PD-L1 ICI therapeutic strategies. This combination takes advantage of the distinctive and complementary mechanisms of action of CTLA-4 and PD-1/PD-L1 ICI. In stage III and IV melanoma patients, response and 5-year survival rates improved to 58% and 52%, respectively, with combined anti-PD-1 mAb anti-CTLA-4 mAb treatment (Larkin et al., 2019). Combination ICI therapy also improved outcomes in non-melanoma cancers, including RCC and NSCLC. This improvement over standard treatment or ICI monotherapy with combined CTLA-4 and PD-1/PD-L1 ICI therapy; however, was more modest for some cancers than that observed in melanoma. Combined anti-PD-1 mAb anti-CTLA-4 mAb ICI in patients with stage IV or recurrent NSCLC demonstrated a response rate of 36% compared to 30% with standard chemotherapy. Clinical investigations of combined demonstrated that the combination of PD-1/PD-L1 and CTLA-4 inhibitors leads to greater response rates and longer survival than either monotherapy; nonetheless, toxicity remains a challenge (Khair et al., 2019; Larkin, Chiarion-Sileni, et al., 2015; Postow et al., 2015).

Despite the potential for dramatic clinical responses to ICIs, there remains room for further improvement in patient outcomes with immunotherapeutic treatment. Even with combined ICI therapeutic strategies, in some cancers only a minority of patients demonstrate clinical responses to these therapies and, for those who do respond, the effects may not be durable. This response may depend on the nature of the interaction between the specific tumor and the immune system. Tumors that are relatively immunogenic and exhibit extensive TIL presence on histologic examination (i.e. “hot” tumors) tend to respond more robustly to ICIs. Conversely, “cold” tumors, which have very little or no TIL presence, generally respond poorly to ICI treatment. An important strategy to address this limitation is one that promotes conversion of a “cold” tumor into a “hot” tumor (Galon et al., 2006; Galon & Bruni, 2019). Generation of anti-tumor immune responses, where they have not yet occurred, depends on treatments that modify other immune system functions, in addition to TIL activation and Treg suppression. These functions include adaptive immune system priming, innate immune system activity, and tumor infiltration by TILs. Anti-PD-1 mAb and anti-CTLA-4 mAb ICI established that powerful anti-tumor immune responses could be elicited through disinhibition of tumor-targeting T cells. Further progress is being fueled by the expansion of immunotherapy strategies to target other aspects of T cell function, as well as other immune system components and functions.

Next-generation ICT

The next generation of ICT promises to overcome the limitations of the mAbs that comprise first-generation ICIs. Next generation ICT employs other classes of molecules with different co-signaling targets that modulate T cell and other immune cell functions.

Novel T cell co-inhibitory signals under investigation include lymphocyte-activation gene 3 (LAG-3), T cell immunoglobulin and mucin-domain containing molecule 3 (TIM-3), T cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT), and V-domain Ig-containing Suppressor of T-cell Activation (VISTA) (see Figure 1). LAG-3 is expressed on a variety of lymphocytes and dendritic cells (DCs). Ligands of LAG-3 include MHC-II and lectins secreted into the TME or expressed on tumor cells. LAG-3 ligand binding leads to inhibition of effector T cell anti-tumor functions and DC activation. In several types of cancer, LAG-3 is upregulated on activated $CD4^+$ and $CD8^+$ T cells, natural killer (NK) cells, and Tregs. TIM-3 regulates effector T cell exhaustion and Treg function in the TME. It is co-upregulated with PD-1 in Tregs and exhausted $CD8^+$ TILs. TIGIT is highly expressed on lymphocytes, notably NK cells, Tregs, $CD4^+$ and $CD8^+$ T cells. TIGIT signaling leads to depressed NK cell and $CD8^+$ cytotoxicity, DC co-stimulation capabilities, and modulation of Treg functions (Burugu et al., 2018; Tundo et al., 2019; Wei et al., 2018). VISTA is expressed on T cells and APCs. When expressed on T cells, it may serve as a receptor to an unknown ligand. VISTA signaling decreases production of IFN-γ and TNFα cytokines, which results in reduced T-cell proliferation and increased Treg production (Burugu et al., 2018; Wei et al., 2018). In several tumor types, PD-1, TIM-3, and TIGIT are all co-expressed in severely exhausted $CD8^+$ TILs. Hence, combination ICT strategies to inhibit these immune checkpoint pathways together could restore anti-tumor functions to exhausted TILs more effectively than blocking a single pathway (Tundo et al., 2019; Wei et al., 2018).

ICT, targeting co-stimulatory molecules expressed by T cells and other immune cell types, can also promote anti-tumor immunity. 4–1BB and OX40 are co-stimulatory molecules that demonstrate potential therapeutic efficacy (see Figure 1). 4–1BB is transiently expressed on the surface of activated T cells, mature DCs, and NK cells. Its ligand is displayed on APCs. Binding of the ligand leads to enhanced activation, proliferation, and survival of cells expressing 4–1BB (Etxeberria et al., 2020). OX40 is expressed on the surface of activated T cells, NK cells, and Tregs, and its ligand is also expressed on activated APCs. Stimulation of OX40 on T cells promotes their survival. OX40 also dampens Treg cell function and prevents conversion of naïve T cells into Tregs (Aspeslagh et al., 2016; Wei et al., 2018). Clinical trials are currently investigating these novel ICT strategies as monotherapy or in combination with anti-CTLA-4 mAbs or anti-PD-1/PD-L1 mAbs, as well as in combination with established cancer treatments including radiation, chemotherapy, ablation, chemoembolization and radioembolization (Burris et al., 2017; Hargadon et al., 2018; Hellmann et al., 2016; Khair et al., 2019; Longo et al., 2019; Meng et al., 2017; Qin et al., 2019; Tundo et al., 2019; Yap et al., 2019).

Current mAb-dependent ICT has been subject to the limitations inherent to antibodies. Although delivery or display of antibodies on nanoparticles is feasible (as shown by several examples described below), the conjugation of these high molecular weight biologics can be cumbersome and expensive. These limitations have fueled the rapid development of a variety of other classes of small molecule or peptide-based agents as ICIs (Table 1). As compared to mAbs, small molecule-based ICT has the potential for improved stability, enhanced membrane permeability, and wider options for routes of administration, including enteral routes. Several small molecule ICIs targeting PD-1/PD-L1, CTLA-4, LAG-3, and VISTA have been described (Sasikumar & Ramachandra, 2020). For example, oxadiazole/thiadiazole derivatives CA-170 and CA-327 (structures not publicly disclosed) antagonize both PD-1/PD-L1 and VISTA or PD-1/PD-L1 and TIM-3 signaling pathways, respectively. CA-170 is currently in a phase II clinical trial as an oral treatment for solid tumors and Hodgkin lymphoma (NCT02812875) (Curis, Inc., 2020).

ICI peptides have been developed to target the PD-1/PD-L1 pathway. For example, AUNP-12 is a competitive inhibitor of PD-1 consisting of a branched peptide (main sequence, SNTSESFKFRVTQLAPKAQIKE and side branch, SNTSESF) designed to mimic the endogenous PD-1 receptor (Sasikumar et al., 2019; Sasikumar & Ramachandra, 2020). Nonmimetic peptides with binding affinity for PD-L1 are also being developed. The 12 amino acid D-peptide, ^{D}PPA-1 (NYSKPTDRQYHF), impedes the interaction of PD-1 and PD-L1. The D-peptide configuration renders the peptide more resistant to proteolysis (Chang et al., 2015). In preclinical studies, both AUNP-12 and ^{D}PPA-1 have demonstrated *in vivo* efficacy as PD-1/PD-L1 peptide inhibitors (Chang et al., 2015; Sasikumar et al., 2019). Another example is a macrocyclic peptide, BMS-986189 (structure not publicly disclosed), which is in a phase I clinical trial (NCT02739373) (Bristol-Myers Squibb, 2018).

Next generation ICT allows modification of a wider array of immune cells through novel pathways. Prior clinical trials and experience have shown that combinations of ICIs targeting different checkpoints are more effective than monotherapy (Khair et al., 2019; Larkin, Chiarion-Sileni, et al., 2015; Postow et al., 2015). The development of diverse, novel

immune checkpoint targets and small molecule ICIs provides more ICT combination possibilities with the potential to customize treatments for different cancer types and different patient populations. Combination treatment strategies also afford opportunities in dosing and administration of the component therapies to reduce toxicity and side effects in vulnerable patients (Mokhtari et al., 2017).

Combination immunotherapy is a critical element in the cancer immunotherapy progress. Combination therapy may be directed toward improvement of the intrinsic function of ICT. Taking a broader approach, however, combination therapy also may be developed to synergize with ICT by modifying tumor-immune interactions to make tumors more vulnerable to anti-tumor immune responses. The ultimate goal is induction of a robust immune response to TAA released by conventional cancer treatments such as radiation therapy (Asna et al., 2018), chemotherapy (Da Silva et al., 2016), ablation, or embolization (Longo et al., 2019). Integration of immunotherapy with any therapy that induces tumor cell lysis and release of TAAs is expected to be synergistic. ICT employed in this regimen can strengthen the coordinated innate and adaptive immune responses to TAA release to induce systemic, cellular anti-tumor immunity (see Figure 1 for an overview of the cancer immunity cycle). Nanoparticles are emerging as a highly promising adjunct to combination immunotherapy. With their great diversity, flexibility, and capacity for multi-functionality, nanoparticles can be designed to promote cooperative and coordinated therapeutic effects through co-delivery and targeting of multiple therapeutic agents, and even through immunomodulation. Nanoparticles are poised to take on these roles in combination therapy strategies and potentially improve therapeutic outcomes with ICT.

Nanoparticles for targeted delivery in combination with ICT

Nanomedicine is increasingly joining the cancer immunotherapy revolution. Nanoparticle technologies encompass a vast diversity of shapes, sizes, and materials, including metal nanoparticles, polymeric nanoparticles (of synthetic and biological origin), lipid nanoparticles, and viral vectors or non-infectious plant viral nanoparticles (Figure 2). With unique and tunable physiochemical properties, nanoparticles can surmount many of the limitations of ICT alone. Several nanotechnologies are FDA-approved for the delivery of drugs and contrast agents (Chariou et al., 2020; Kim et al., 2010; Shi & Lammers, 2019; Tran et al., 2017); however, the combination of nanoparticles and ICT for cancer immunotherapies are being explored primarily in the preclinical setting.

Nanoparticle-mediated ICT drug delivery can provide protection, localization, and controlled release of the ICT cargo. Thus, it can enhance the efficacy of ICT by increasing the amount of ICT reaching the target site, while reducing toxicity by limiting activity at non-target sites (Riley et al., 2019; Zang et al., 2017). Nanoparticles also can co-deliver multiple cargos to facilitate combination therapy (Q. Chen et al., 2016; K. Cheng et al., 2018; Duan et al., 2019; Feng et al., 2018; Kosmides et al., 2017; Lang et al., 2019; G. Li et al., 2019; Mi et al., 2018). Additionally, nanoparticles can serve as immune modulators by mimicking or enhancing immune cell functions (Cervera-Carrascon et al., 2018; Chao et al., 2019; Chiang et al., 2018; Engeland et al., 2014; Gandhapudi et al., 2019; Liu et al., 2017).

This nanoparticle-mediated immune modulation can be employed in concert with ICT as part of multifaceted, combinatorial treatment strategies.

Nanoparticle design can be tuned to optimize features of ICT or other therapy that can influence their function, including *in vivo* stability, solubility, circulation half-life, and accumulation in tumors or other target sites, such as lymphoid tissues for anti-CTLA-4 ICI. These features are influenced by the size, surface charge, shape, material composition, and surface chemistry of the nanoparticles (Sindhwani et al., 2020; Wilhelm et al., 2016). Efficient nanoparticle-mediated delivery of ICT after systemic administration may be accomplished through passive and active targeting strategies. In this discussion, passive targeting strategies refer to those exploiting the physiological or pathophysiological features of the target, which often reflect the surrounding milieu (Au et al., 2020; K. Cheng et al., 2018; Kosmides et al., 2017; Lang et al., 2019; S.-Y. Li et al., 2016; Nikpoor et al., 2017). Active targeting approaches take advantage of specific molecular signatures associated with a target, often through ligand-binding interactions (Au et al., 2020; K. Cheng et al., 2018; Du et al., 2017; Kosmides et al., 2017; G. Li et al., 2019; S.-Y. Li et al., 2016; Mi et al., 2018).

Passive targeting for nanoparticle-facilitated ICT delivery

The pattern of passive accumulation of nanoparticles depends on their size and surface charge. Nanoparticles smaller than 100 nm in diameter are more likely to extravasate through abnormal tumor vessels, while even smaller nanoparticles are better able to penetrate the dense extracellular matrix of the TME (Hubbell & Langer, 2013; Wilhelm et al., 2016). Nanoparticles larger than 200 nm in diameter demonstrate greater tendency to be cleared by the reticuloendothelial system (RES) (Blanco et al., 2015). Very small nanoparticles, such as those less than 5.5 nm, can be cleared by the renal system (Wilhelm et al., 2016). Slightly negative or neutral surface charges promote longer persistence of nanoparticles within circulation. More positively charged nanoparticles have a higher rate of nonspecific uptake by cells. This property may be an advantage or a hindrance depending on the cell type encountered by the nanoparticles. The RES tends to clear nanoparticles with strong positive or negative surface charges (Figure 3) (Hubbell & Langer, 2013; Wilhelm et al., 2016). Structure-function properties such as these guide the design of targeted drug delivery systems.

Several nanoparticles designed to deliver anti-PD-1/PD-L1 mAbs or antagonistic small molecules within the TME incorporate these parameters (Au et al., 2020; K. Cheng et al., 2018; Kosmides et al., 2017; Lang et al., 2019; S.-Y. Li et al., 2016; Nikpoor et al., 2017). Polyethylene-glycol-(PEG) coated and non-coated liposomes were examined as anti-CTLA-4 mAb carriers. Both carrier nanoparticles loaded with anti-CTLA-4 mAbs were approximately 140 nm in diameter, but the non-PEGylated liposome carried a stronger negative charge than the PEGylated liposome. Compared to free anti-CTLA-4 mAbs and CTLA-4-non-PEGylated liposomes, the CTLA-4-PEGylated liposomes exhibited 3-fold greater intratumoral accumulation. Splenic accumulation of CTLA-4-non-PEGylated liposomes was almost 40 times greater than that of free anti-CTLA-4 mAbs and 17.5 times greater than that of CTLA4-PEGylated liposomes. In the C26 colon carcinoma tumor model,

however, the CTLA-4-PEGylated liposomes demonstrated only modest improvements in slowing tumor growth and prolonging survival as compared to free CTLA-4. Interestingly, there were no significant differences in levels of $CD4^+$, $CD8^+$, or Tregs in the tumor or draining lymph nodes. Although tumor homing was achieved, the limited improvement in therapeutic benefit may be attributed due to the highly stable liposome formulations. Only 10% of the antibody payload was released within the TME, whether PEGylated or non-PEGylated liposomes were used (Nikpoor et al., 2017). These nanoparticles improved accumulation of the ICT in the target site and stability in circulation, but optimization of ICT function also requires release to facilitate access of the ICT to its target site of action. Improved liposome designs and integration of controlled-release mechanisms through internal triggers (pH, enzymes) or external triggers (including light, heat, and ultrasound) can overcome these challenges (Zylberberg & Matosevic, 2016).

In another example, a 140-nm polymer nanoparticle formulation, composed of a poly(ethylene glycol)-block-poly(D,L-lactide) copolymer and a cationic lipid, was administered systemically to deliver CTLA-4 small interfering RNA (siRNA) to impede CTLA-4 production in $CD4^+$ and $CD8^+$ T cells of the tumor, spleen, lymph nodes, and blood (S.-Y. Li et al., 2016). The nanoparticle enabled broad distribution to the target organs while still facilitating accumulation within the TME. It resulted in more dramatic increases in levels of $CD4^+$ and $CD8^+$ TILs and better therapeutic efficacy than observed with the CTLA4-PEGylated liposomes. While most nanoparticles delivering ICT were designed with neutral or weakly negative zeta potentials to enhance biocompatibility (Au et al., 2020; K. Cheng et al., 2018; Kosmides et al., 2017; Lang et al., 2019), the nanoparticles delivering siRNA exhibited a weakly positive surface charge (G. Li et al., 2019; S.-Y. Li et al., 2016). This positive surface charge may have facilitated interactions of the nanoparticles with target T cells, albeit at the risk of increased clearance, possibly by the RES.

That the copolymer-cationic lipid nanoparticle-delivered siRNA CTLA-4 therapy was more efficacious than the liposome nanoparticle-delivered anti-CTLA-4 mAb strategy may reflect differences in formulation chemistry and drug release, as well as the distinct mechanisms of action of siRNA versus mAbs. It is also possible that positive-charged nanoparticles may have been better able to interact with target cells than the negative-charged liposomes. The siRNA may have been more efficiently released from the copolymer-cationic lipid nanoparticles following uptake by target cells. In contrast, the anti-CTLA-4 mAbs may have been trapped inside the stable liposome nanoparticles in the TME. These examples illustrate how the activity of nanoparticle-based cancer immunotherapy depends on a multitude of design parameters, each of which must be carefully optimized to yield efficacious formulations.

Strategic design of the material composition of nanoparticles can also achieve selective, controlled release within a target site. Some nanoparticles capitalize on common characteristics of the TME or tumor cells, such as presence of matrix metalloproteinases (MMPs) and an acidic pH, as release triggers for ICT and other drug cargoes (K. Cheng et al., 2018; Feng et al., 2018; Lang et al., 2019; F. Zhou et al., 2019). Some nanoparticles execute co-delivery strategies, with a single nanoparticle carrying ICT and a cytotoxic chemotherapeutic (K. Cheng et al., 2018; Lang et al., 2019). Lang et al. designed a

spherical nanodevice with two concentric layers. The outer layer consisted of an MMP-sensitive copolymer encapsulating a PD-1/PD-L1 inhibitory small molecule, HY19991, an anti-cancer stem cell agent, thioridazine, and pH-sensitive micelles containing the cytotoxic chemotherapeutic drug paclitaxel. The presence of MMPs led to degradation of the outer layer and release of the HY19991, thioridazine, and paclitaxel-containing micelles in the TME. When endocytosed by tumor cells, the micelles dissembled in the acidic endo/lysosomes, thereby mediating intracellular release of the cytotoxic paclitaxel and triggering cancer cell death. Treatment with this nanodevice in MCF-7 breast tumor-bearing mice led to 83% survival longer than 60 days. This effect reflects a substantial improvement over treatment with unencapsulated HY19991 and paclitaxel-containing micelles, which had no survivors at 55 days (Lang et al., 2019). This chemo-immunotherapy combination strategy aims to concentrate chemotherapy within tumor cells to induce cell death and subsequent release of TAAs in parallel with delivery of an immunostimulatory agent or ICT within the TME. This strategy primes anti-tumor immunity in concert with chemotherapy-induced debulking and inhibition of cancer stem cells. Such multilayered and multi-staged, targeted nanoparticle strategies could optimize spatial and temporal control of delivery to achieve powerful synergy among these effects.

Cheng et al. employed a single-layered nanoparticle containing amphiphilic peptides to co-deliver two different immunotherapeutics within the TME: A PD-1/PD-L1 peptide ICI, ^{D}PPA-1, and an IDO inhibitor, NLG919 (K. Cheng et al., 2018). Indoleamine 2,3 dioxygenase (IDO), which is primarily produced by tumor cells and myeloid-derived suppressor cells, represents another mechanism of immunosuppression in the TME. Increased IDO activity may deplete tryptophan and other byproducts of tryptophan catabolism in the TME, thereby promoting anergy and apoptosis of T cells and differentiation of Tregs. Inhibitors of IDO have demonstrated efficacy in the preclinical setting (Labadie et al., 2019; Muller et al., 2019), while its performance in clinical trials has yielded inconsistent results (Burris et al., 2017; Incyte Corporation, 2020; Long et al., 2019). In this combination immunotherapy design, the hydrophobic domain of the amphiphilic peptides contained a target site for MMPs. The peptides of the nanoparticle formed a tight shell, with the hydrophobic components oriented centrally, surrounding the cargo at a neutral pH. Within the weakly acidic TME, the nanoparticle swelled, which allowed MMPs to access the inner hydrophobic domains and disrupt the nanoparticle structure, co-releasing the ^{D}PPA-1 and NLG919. Nanoparticle-facilitated delivery of ^{D}PPA-1 or NLG919 significantly improved efficacy over administration of either therapeutic independently without nanoparticle-facilitated delivery. Nanoparticle-facilitated co-delivery of ^{D}PPA-1 and NLG919 also significantly improved survival and tumor growth suppression in tumor-bearing mice compared to nanoparticle-facilitated delivery of either therapeutic alone (K. Cheng et al., 2018).

Nanoparticles can passively accumulate in a target site by engineering their size, surface charge, or even multi-layered inducible features that exploit physiological or pathophysiological characteristics of the target tissue or organ. Nanoparticle materials can also be programmed to generate spatiotemporally controlled release, particularly for combinations of therapies with different targets. Chemo-immunotherapy strategies, for example, employ two different classes of cancer therapies with distinctive mechanisms and

sites of action. Through sequential release techniques, each co-administered therapy could be separately delivered to its target site (K. Cheng et al., 2018; Lang et al., 2019; F. Zhou et al., 2019). Many of these passive targeting strategies are foundational in nanoengineering and underscore active targeting strategies that further optimize delivery efficiency.

Active targeting for nanoparticle-facilitated ICT delivery

Nanoparticles can be engineered to display active targeting moieties, such as ligands, to actively promote target site deposition. For intratumoral accumulation, nanoparticle carriers may be decorated with ligands to direct them to immune cells, tumor cells, the tumor vasculature and other sites of TME entry, or the surrounding extracellular matrix of the TME (see Figure 3) (Ruoslahti et al., 2010; Sindhwani et al., 2020; Wilhelm et al., 2016). Several reports have employed such active targeting strategies to enhance nanoparticle-facilitated delivery of ICT (Au et al., 2020; K. Cheng et al., 2018; Du et al., 2017; Kosmides et al., 2017; G. Li et al., 2019; S.-Y. Li et al., 2016; Mi et al., 2018).

For example, Li et al. displayed an active targeting peptide Lin TT1 on their self-assembling micelles (G. Li et al., 2019). Lin TT1 engaged in low-affinity binding of p32 cell surface receptors on tumor vessel endothelial cells, tumor cells, and tumor-associated macrophages and triggered macropintocytosis-mediated translocation of nanoparticles across these cells. In this manner, Lin TT1 actively facilitated uptake from systemic circulation and tumor tissue penetration of its conjugated nanoparticle (G. Li et al., 2019; Sharma et al., 2017). Therefore, Lin TT1-mediated targeting enabled nanoparticle extravasation while enhancing intratumoral penetration and distribution of the nanoparticle and its payload; specifically, Lin TT-1-targeted nanoparticles were used co-deliver siRNA for PD-L1 and an IDO inhibitor. Intravenous administration of the targeted nanoparticles enhanced tumor delivery of the therapeutic payloads by approximately five-fold as compared to intravenous administration of free therapeutics. This nanoparticle-facilitated co-delivery led to prolonged tumor growth suppression and increased intratumoral levels of $CD8^+$ TILs and interferon-γ in the 4T1 murine breast tumor model compared to nanoparticle-facilitated delivery of PD-1 siRNA or IDO inhibitor alone (G. Li et al., 2019).

Nanoparticles have been used to deliver ICT, as described above. But ICT can also be used to target the nanoparticles to tumors, because the receptors can be overexpressed in the TME. A doxorubicin-loaded liposomal nanoparticle displaying anti-PD-1 mAbs enhanced accumulation of nanoparticles within 4T1 tumors and extended median survival by approximately 40 days more than those treated with control nanoparticles displaying IgG or with free doxorubin (Du et al., 2017).

Active drug delivery strategies have also been devised to target immune cells, including T cells. Treg suppression was achieved through combined therapy of free anti-CTLA-4 mAbs with active nanoparticle delivery of imatinib, a tyrosine kinase inhibitor that promotes apoptosis of Tregs and impedes their immunosuppressive functions. Imatinib was loaded into a polymer poly(lactic-co-glycolic) acid (PLGA) core. This core was then coated in a mixture of PEG-distearoyl-phosphatidylethanolamine (PEG-DSPE) and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine. A targeting peptide that binds the Neuropilin-1 receptor on Tregs, tLyp, was conjugated to the PEG-DSPE. The tLyp was displayed on the surface of

the nanoparticle and helped target delivery of the imatinib cargo to Tregs. In combination with intravenous administration of free anti-CTLA-4 mAbs, the nanoparticles significantly reduced Tregs and increased $CD8^+$ CTLs within the B16 melanoma tumor model. This treatment regimen also delayed tumor progression compared to anti-CTLA-4 mAbs alone, as evidenced by nearly a 3.5-fold difference in tumor volume (Ou et al., 2018).

Active targeting of nanoparticles could be further enhanced by utilizing multiple targeting moieties in a single nanoparticle design. Chiang et al. produced nanoparticles loaded with anti-PD-1 mAb decorated with anti-CD3 antibodies on their surface. The nanoparticles bound to the CD3 T cell surface marker, which facilitated anti-PD-1 mAb delivery directly to T cells. The nanoparticles were also ferromagnetic, which facilitated further targeting when an external magnetic field was applied to the tumor. This approach enhanced tumor accumulation of the anti-PD-1 mAb payload and improved efficacy over nanoparticles with only anti-CD3 targeting (Chiang et al., 2018). In another nanoparticle design strategy, nanoparticles decorated with both anti-PD-1 (antagonistic) and anti-OX40 (agonistic) mAbs co-delivered these ICTs simultaneously to enhance delivery through synergistic pathways. This improved targeting and delivery of these therapies to T cells than co-administration of free anti-PD-1 and anti-OX40 mAbs (Mi et al., 2018).

A multivalent active targeting strategy not only drives the nanoparticles to a target site but also can attract key immune cells to the site. A chemo-immunotherapy strategy used nanoparticles loaded with the cytotoxic chemotherapeutic, epirubicin, to target tumor cells and NK cells, while also attracting NK cells to the TME (Au et al., 2020). The nanoparticles displayed a tumor-targeting component, an anti-epidermal growth factor receptor (EGFR) antibody, and two NK cell-activating components, anti-CD16 and anti-4–1BB antibodies. These nanoparticles combine chemotherapy with ICT directed at the co-stimulatory 4–1BB molecule. The NK cell-stimulating antibodies facilitated recruitment and activation of NK cells, but depended upon the EGFR-directed recruitment of NK cells to the tumor, which enhanced cell death of EGFR-expressing tumors. The release of the epirubicin within the tumor further promoted cell death. In an EGFR-overexpressing murine tumor model, A431, trivalent targeting of epirubicin-loaded nanoparticles demonstrated the greatest efficacy, with delayed tumor progression and 50% survival at 75 days when no other treatment groups had survivors. This multifunctional and multivalent targeting nanoparticle strategy facilitated simultaneous co-localization of NK cells, tumor cells, and the cytotoxic chemotherapy (Au et al., 2020). Nanoparticles with multiple targeting mechanisms can increase targeting specificity or bring two different targets into spatial proximity to improve therapeutic efficacy.

Active targeting strategies facilitate nanoparticle-mediated targeted delivery by directing the nanoparticle and its cargo to a specific site of action, be it a physical location (Du et al., 2017; G. Li et al., 2019) or a cell type (Au et al., 2020; Chiang et al., 2018; Mi et al., 2018; Ou et al., 2018), while decreasing off-target deposition. This approach can improve the specificity of a therapeutic effect, often more so than through passive accumulation of the nanoparticle without the active targeting moieties. As with passive targeting, these nanoparticles can also permit spatiotemporally controlled cargo release to further support targeting. Thus, combining targeting delivery nanoparticles with ICT improves efficacy and

helps to reduce possible toxicity, addressing some of the key challenges of ICT in the clinical setting.

Nanoparticles for immunomodulation in combination with ICT

While nanoparticles have been effectively employed in drug delivery and targeting to enhance immunotherapeutic efficacy, another combination strategy utilizes nanoparticles to modulate anti-tumor immune responses to augment the efficacy of ICIs. Most combination strategies employ one or more immunomodulation approaches, including induction of immunogenic cell death (ICD), modification of the TME, modification or mimicry of immune cell function, and/or *in situ* vaccination (see Figure 3). These approaches were typically combined with systemically-administered free ICI mAbs (Cano-Mejia et al., 2017; Cao et al., 2020; Cervera-Carrascon et al., 2018; Chao et al., 2019; Q. Chen et al., 2016; Duan et al., 2016, 2019; Engeland et al., 2014; Feng et al., 2018; Gandhapudi et al., 2019; Ganesh et al., 2018; Lebel et al., 2016a; Liu et al., 2017; Min et al., 2017; Wang & Steinmetz, 2020; Woller et al., 2015), a few approaches employed nanoparticles with both ICT delivery and intrinsic immunomodulation functionalities (Chiang et al., 2018; Engeland et al., 2014; Gandhapudi et al., 2019). Of note, most immunomodulatory nanoparticles depended upon local administration of nanoparticles within or near the target site, employing intratumoral injections of nanoparticles (Cano-Mejia et al., 2017; Cervera-Carrascon et al., 2018; Chao et al., 2019; Q. Chen et al., 2016; Engeland et al., 2014; Gandhapudi et al., 2019; Lebel et al., 2016a; Liu et al., 2017; Saha et al., 2017; Wang & Steinmetz, 2020; Woller et al., 2015). These strategies may offer the benefit of enhancing anti-tumor immune responses in a manner that is complementary to the ICT-mediated disinhibition or activation of effector and memory T cells.

The immune system can be prompted to recognize tumor cells and mount an anti-tumor response when tumor cells undergo ICD. This form of cell death attracts and activates APCs to take up antigen from dying tumor cells. Signaling cascades within the dying tumor cells lead to surface expression of signals, including calreticulin, that enhance phagocytosis of the cells and lead to priming of an adaptive anti-tumor response (Galluzzi et al., 2017). ICD can be triggered through radiation, thermal ablation, embolization, and some types of chemotherapy (Fahmueller et al., 2013; Galluzzi et al., 2017; Yu et al., 2014). Nanoparticles can be utilized to deliver such agents (e.g. for chemotherapy or photodynamic therapy (PDT)) or serve as sensitizing agents to induce photothermal therapy (PTT) (e.g. Prussian blue nanoparticles) or magnetic hyperthermia (MHT) (e.g. iron oxide nanoparticles) (Cano-Mejia et al., 2017; Cao et al., 2020; Chao et al., 2019; Q. Chen et al., 2016; Chiang et al., 2018; Duan et al., 2016; Hoopes et al., 2017; Wang et al., 2014; Xu et al., 2017). Light of a specific wavelength absorbed by a sensitizer material can be converted into reactive oxygen species (ROS) for PDT or into thermal energy for PTT (Q. Chen et al., 2016; Duan et al., 2016). MHT uses alternating magnetic fields to generate heat within a magnetic sensitizer material (Chao et al., 2019). ROS and high temperatures can both trigger ICD in tumor cells, subsequently inducing an anti-tumor immune response. Several studies demonstrated substantially improved long-term survival and delayed tumor progression with combination strategies including ICD induced through localized hyperthermia or ROS generation (Cano-

Mejia et al., 2017; Chao et al., 2019; Q. Chen et al., 2016; Duan et al., 2019). As with immune response-priming tumor lysis therapies, ICD is expected to synergize with ICT.

Chen et al. created nanoparticles comprised of the photosensitizer indocyanine green and the toll-like receptor (TLR) 7 agonist R837, co-encapsulated within polymer poly(lactic-co-glycolic) acid (PLGA). The combination therapy was used to treat one of bilateral tumors using CT26 colon and 4T1 breast tumor models or to treat a metastatic 4T1 tumor model. Nanoparticles were administered intratumorally and excited by external laser to induce PDT resulting in ICD. The encapsulated TLR7 agonist served as an additional immunostimulatory signal to enhance the local inflammatory response. When combined with anti-CTLA-4 mAbs, this approach also suppressed the growth of the untreated CT26 and 4T1 tumors through an abscopal effect, with improvement in survival (70% of animals alive at day 70 as compared to approximately day 34 for animals treated with surgery + anti-CTLA-4 or PDT alone) (Q. Chen et al., 2016). In a similar strategy, MHT was used with iron-based nanoparticles (FeNPs) and PLGA conjugated to R837. Again, a bilateral CT26 tumor model was used. The systemically administered FeNPs could be directed to the tumor using an external magnetic field. When combined with anti-CTLA-4 mAb, this treatment resulted in 100% survival and sustained tumor regression of treated and untreated tumors at 60 days (Chao et al., 2019).

Another ICD-based approach employed a nanoparticle delivering a combination of oxaliplatin, which can induce ROS and ICD, and an anti-malarial drug dihydroartemisinin, which also produces ROS. Systemic administration of the free oxaliplatin and dihydroartemisinin with free anti-PD-L1 mAb was highly toxic. Introduction of a multi-layered delivery nanoparticle, containing the oxaliplatin and dihydroartemisinin, improved selective tumor accumulation of these drugs and reduced toxicity. The nanoparticle-delivered dual-pronged ROS approach with ICT achieved complete eradication of CT26 tumors in all animals, which was not achieved with free anti-PD-L1 mAb monotherapy or combined free oxaliplatin and dihydroartemisinin (Duan et al., 2019).

Nanoparticles can also shift the TME toward an immunostimulatory state by directly altering the conditions of the environment or the activities of the cells within it. One of the critical features of the TME is hypoxia, which arises from the rapid growth of tumor cells amid insufficient blood supply. Hypoxia causes dysfunction of immune cells, especially T cells. Tumor-specific CTLs are unable to carry out their cytotoxic function in the hypoxic TME. One study aimed to alleviate this hypoxia in combination with anti-PD-L1 mAbs ICI through nanoparticles loaded with catalase and the cytotoxic chemotherapeutic, doxorubicin (Zou et al., 2018). The catalase oxidized hydrogen peroxide to oxygen. These particles were multifunctional, as the doxorubicin has been reported to induce ICD, and the nanoparticles were coated in a membrane that mimicked that of the model tumor cells, B16F10 melanoma. When combination treatment with anti-PD-L1 mAbs and nanoparticles containing only doxorubicin was compared to combination treatment with nanoparticles containing both catalase and doxorubicin, the presence of catalase dramatically improved tumor growth suppression and increased intratumoral $CD8^+$ TILs and interferon-γ levels (Zou et al., 2018). Nanoparticle-mediated TME modification to alleviate hypoxia can thus act synergistically to enhance the efficacy of ICI and chemotherapy.

TMEs frequently contain low levels of the cytokines and chemokines that would facilitate CTL trafficking to and infiltration of tumors, such as CXCL10 and CXCL11. These chemotactic signaling deficits impede the targeting and infiltration of tumors by anti-tumor effector T cells. In response, Liu et al. used a multifunctional nanoparticle based on an oncolytic vaccinia virus modified to express CXCL11 (Liu et al., 2017). This modified vaccinia, in combination with anti-PD-L1 therapy, administered *in situ* produced substantial increases in survival: median survival at approximately 50 days vs. approximately 30 days for anti-PD-L1 alone vs. 22 days for vaccinia virotherapy alone. This effect was associated with increases in activated $CD8^+$ CTLs within tumors and reductions in Tregs and exhausted $CD8^+$ T cells. PD-L1 expression was also increased on immune and tumor cells in contrast with reduced PD-L1 expression with anti-PD-L1 treatment alone. The vaccinia virotherapy provided multiple anti-tumor effects that may be synergistic with ICI therapy. The direct tumor cytotoxicity by the oncolytic virus and immunogenicity of the vaccinia viral particles within the tumor can trigger an innate immune response that could prime an adaptive immune response. Further, increased expression of CXCL11 within the tumor likely facilitates intratumoral trafficking and infiltration of CTLs generated in the anti-tumor immune response. Greater expression of PD-L1 on immune cells enhances the effect of anti-PD-L1 therapy on these cells. When coupled with ICI therapy, the modified vaccinia virotherapy addressed multiple immunosuppressive mechanisms to facilitate effector T cells in performing their cytotoxic functions within the TME (Liu et al., 2017).

Nanoparticles have also been developed to directly modify the action of immune cells or simulate their functions to overcome the immunosuppressive TME. Modified oncolytic viruses have been engineered to induce expression of immune cell-activating cytokines in the TME (Cervera-Carrascon et al., 2018; Saha et al., 2017). A notable example is Herpesvirus-based talimogene laherparepvec (T-VEC), which obtained FDA approval in 2015 (Bommareddy et al., 2018; Russell et al., 2019). T-VEC was engineered to selectively infect tumor cells and encode granulocyte-stimulating factor for expression. In another example, Saha et al. engineered an oncolytic herpes simplex virus to express IL-12, a pro-inflammatory cytokine, for the treatment of model glioblastomas. In this study, the combined treatment with both anti-PD-L1 and anti-CTLA-4 mAbs increased anti-tumor M1 phenotype macrophages and reduced the numbers of Tregs (Saha et al., 2017, 2018). Oncolytic viruses have also been engineered to express siRNAs against CTLA-4 and PD-L1 (Engeland et al., 2014).

Nanoparticles can also be designed to perform or simulate some of the functions of DCs and other immune cells that are impaired by the TME (Hickey et al., 2017; Saxena & Bhardwaj, 2018). Antigen-capturing nanoparticles (AC-NPs) bind to a diverse range of TAAs and are subsequently taken up by APCs. AC-NPs facilitate antigen presentation and adaptive immune response priming. Here, peptide antigens were loaded onto AC-NPs *ex vivo* or *in vivo* within irradiated tumors. In a bilateral B16F10 melanoma model, one tumor was irradiated and injected with the nanoparticles in combination with systemic anti-PD-1 therapy. AC-NPs were observed within DCs and macrophages in tumor-draining lymph nodes. This combined therapy suppressed the growth of the untreated tumor and induced complete responses in 20% of animals at 80 days compared to no surviving animals after 35 days in animals treated only with radiation and anti-PD-1 mAbs. Higher $CD8^+$ and $CD4^+$ T

cell and lower Treg levels were observed in the untreated tumors of the animals that received the combination of radiation, AC-NPs, and anti-PD-1 mAbs, as compared to those treated only with radiation and anti-PD-1 mAbs (Min et al., 2017).

Artificial APCs (aAPCs) have shown promise as a means of increasing activation of the adaptive immune response. aAPCs fulfill the endogenous APC roles of presenting antigens and co-stimulatory signals to effector T cells to activate them. aAPCs generally display an antigen-MHC-I complex and co-stimulatory molecules on their surface. They may also release cytokines to further promote the activation and proliferation of the effector T cells interacting with the aAPC. aAPCs are not subject to the limitations of endogenous APCs; they are unaffected by TME immunosuppression, and they constantly present antigen. One report used intravenously administered aAPCs composed from PLGA and containing IL-2 and anti-CTLA-4 mAb to activate anti-tumor T cells in a mouse model of melanoma. The aAPCs were externally coated with a dimer of melanoma antigen ($TRP2_{180–188}$) bound to a mouse MHC-I ($H\text{-}2K^b$:Ig fusion protein) and anti-CD28. This treatment elicited enhanced efficacy and specificity against the melanoma. Mice treated with these aAPCs also demonstrated increases in anti-$TRP2_{180–188}$ $CD8^+$ CTLs in the blood, spleen, and tumor (Zhang et al., 2017). These studies combined ICT with nanoparticles designed to promote a targeted, adaptive anti-tumor immune response through mimicry of antigen-capture or antigen presenting functions.

Nanoparticles with the ability to directly modify immune cell function and alter the TME through innate immune system activation can serve as adjuvants when coupled with TAAs for the purpose of cancer vaccines. Local administration can activate the innate immune system response *in situ* and elicit cell-mediated anti-tumor responses (Fiering, 2017; Sheen & Fiering, 2019). By this mechanism, the profile of cytokines and innate immune cells within the TME shift toward a more immunostimulatory state (Hashiguchi et al., 2010; Lee et al., 2017; Murray et al., 2018; Wang et al., 2019), and infiltrating APCs sample a diverse array of TAAs, customizing the vaccination to the specific tumor (Marabelle et al., 2017; Singh & Overwijk, 2015). In doing so, APCs and other mediators of innate immunity prime the adaptive immune system, leading to systemic anti-tumoral immunity, which includes the expansion of populations of circulating effector B and T cells targeted against local, regional, and distant disease.

This immunotherapeutic strategy may employ nanoparticles to deliver classical adjuvants (Chao et al., 2019; Q. Chen et al., 2016; Xu et al., 2017) or use nanoparticles comprised of intrinsically immunogenic materials or structures. Some cationic lipids can activate an innate immune response through TLR-dependent or -independent mechanisms. They may also facilitate cellular uptake of protein antigens to enhance cross-presentation of antigens for adaptive immune response priming. In one report, intratumoral administration of a cationic lipid enantiomer, R-1,2-dioleoyl-3-trimethyl-ammonium-propane (R-DOTAP), with peptide antigens was combined with systemic anti-PD-L1 mAb. R-DOTAP also activated TLR7 and TLR9 and enhanced dendritic cell activation and cross-presentation (Gandhapudi et al., 2019). Some carbon nanotubes also have inherent adjuvant properties (Dumortier, 2013; Fadel & Fahmy, 2014; Palomäki et al., 2011), and they can act as photosensitizers for PTT (Wang et al., 2014). As mentioned previously in the active

targeting section, Chiang et al. created magnetic- and antibody-targeted nanoparticles made of fucoidan-dextran-based iron oxide (Chiang et al., 2018). Fucoidan is an anionic, sulfated polysaccharide from Fucus vesiculosus, a type of brown seaweed. Fucoidans can interact with TLRs (Makarenkova et al., 2012) and enhance phagocyte and NK cell activities and activate antigen-specific effector T cells. Notably, this multifunctional design also permitted combination of immunomodulation and delivery strategies within a single nanoparticle platform (Jin et al., 2014).

As with synthetic nanoparticles, viruses and virus-like particles (VLPs) can be modified to carry or display cargoes of interest (chemotherapy, siRNA, genes encoding cytokines, etc.) and virus-based therapies have been combined with ICI (Cervera-Carrascon et al., 2018; Engeland et al., 2014; Lebel et al., 2016a; Liu et al., 2017; Wang & Steinmetz, 2020; Woller et al., 2015). Examples of oncolytic virus therapy were discussed above; here we want to draw attention to the plant viruses and bacteriophages that are noninfectious to humans and present an emerging class of nanomaterials. A number of plant virus and bacteriophage-based nanoparticles are in preclinical development (Shoeb & Hefferon, 2019; Wen & Steinmetz, 2016) and several approaches are in clinical testing (Mohsen et al., 2020). In some approaches, VLPs have been utilized to package and protect TLR agonists, such as CpG oligodeoxynucleotides (ODNs), which are TLR9 agonists. *In situ* vaccination using Qβ bacteriophage VLPs loaded with CpG ODNs demonstrated *in vivo* efficacy in preclinical murine tumor models of lymphoma and head and neck cancer. Encapsulation by Qβ VLPs protects the CpG ODNs from premature degradation and enhances their uptake by APCs. Of note, efficacy of this strategy depended on priming the immune system to generate anti-Qβ Abs with repeated injections of Qβ alone prior to tumor challenge and *in situ* vaccination (Y. Cheng et al., 2020; Lemke-Miltner et al., 2020). A similar *in situ* vaccination approach employed a plant VLP based on the cowpea chlorotic mottle virus (CCMV) to encapsulate CpG ODNs. The CpG ODN-loaded CCMV-VLPs enhanced *ex vivo* tumor-associated macrophage uptake of CpG ODNs. *In vivo*, intratumoral treatment with the CCMV-VLPs containing CpG ODNs augmented tumor-associated macrophage phagocytic activity. CCMV alone exerts minimal modulatory effect on immune system function; thus, immunostimulation via CpG ODN is critical to generate an anti-tumor immune response (Cai et al., 2020). Some plant viruses and VLPs have potent, intrinsic immunostimulatory properties and can be applied in intratumoral *in situ* vaccination strategies as a single agent or in combination. For example, intratumoral administration of VLPs from papaya mosaic virus (PapMV) has been shown to activate innate immune responses and prime subsequent adaptive anti-tumor responses through TLR7 signaling, likely mediated by the RNA encapsulated into the VLPs (Lebel et al., 2016b). PapMV *in situ* vaccination combined with systemic anti-PD-1 therapy suppressed B16F10 melanoma growth and increased activated $CD8^+$ CTLs (Carignan et al., 2018).

Another *in situ* vaccination approach uses the cowpea mosaic virus (CPMV) and its VLPs. CPMV has been demonstrated as a highly potent adjuvant to prime anti-tumor immunity with demonstrated potent efficacy leading to tumor regression and overall survival in a wide variety of animal tumor models, including melanoma, ovarian cancer, breast cancer, colon cancer, and glioma (Cai et al., 2019; Kerstetter-Fogle et al., 2019; Lizotte et al., 2016; Murray et al., 2018; Patel et al., 2018; Shukla et al., 2019; Wang et al., 2019; Wang

& Steinmetz, 2019). This efficacy has also been replicated in canine patients (Hoopes et al., 2018). CPMV *in situ* vaccination primes innate immune cell activation, which leads to adaptive immune system-mediated, anti-tumor responses. These responses included increased tumor infiltration by $CD4^+$ and $CD8^+$ effector T cells and memory T cells (Wang et al., 2019). Following CPMV *in situ* vaccination, expression of PD-1 and OX40 was differentially increased in TILs in models of ovarian carcinoma, colon carcinoma, and melanoma. Upregulation of a checkpoint protein sensitizes the tumor to a specific ICT. For B16F10 melanomas and CT26 tumors, OX40 expression was increased on more T cell types and to a greater degree than PD-1. Combination therapy with CPMV *in situ* vaccination and intratumoral administration of anti-PD-1 mAb or agonistic anti-OX40 mAb ICT showed dramatic differences in efficacy against B16F10 and CT26 tumors. CPMV with anti-OX40 mAbs delayed tumor progression, prolonged survival, and increased TILs. The CPMV and anti-PD-1 mAb combination was no more efficacious than CPMV monotherapy. On the other hand, both OX40 and PD-1 expression were increased on TILs in the ID8-Defb29/Vegf-A-luc ovarian cancer model. Correspondingly, both combination therapy regimens, CPMV and anti-OX40 mAbs or CPMV and anti-PD-1 mAbs, substantially increased therapeutic efficacy over CPMV alone (Wang & Steinmetz, 2020).

These numerous and diverse examples demonstrate that nanomedicine synergizes effectively with ICT (Figure 4). Nanoparticles play roles in stimulating and modulating immunity (e.g., their use as adjuvants for in situ vaccination) but they also allow the targeting of ICT agents and/or the co-delivery of synergistic active ingredients. Multiple immunomodulatory strategies produce or support an anti-tumor immune response by inducing ICD within the tumor, altering the TME to an immunostimulatory state, or vaccinating against *in situ* TAAs. Many of these approaches aim to produce an adaptive immune response mediated by effector T cells. In some cases, nanoparticles may alter the expression patterns or activities of the TILs in ways that can sensitize the tumor to treatment with a specific ICT. Further, multifunctional design allows for combination of immunomodulation and delivery strategies within a single nanoparticle.

Conclusions

ICT has ushered in a new paradigm of cancer treatment, centered on immunotherapy. Despite clinical successes of ICT, substantial opportunities remain for improving treatment response, reducing recurrence, and minimizing toxicity profiles. Combination strategies employing nanoparticles with ICT have revealed immense potential for overcoming the limitations of ICT. Nanoparticles may serve as targeted delivery systems for ICT alone or in combination with other therapies. They can also serve as de facto immunotherapeutic agents that synergize with ICT. Finally, nanoparticles offer the opportunity to combine multiple treatment and localization strategies into one platform. While these combination therapies have been explored exclusively in preclinical studies, the widespread, separate clinical use of ICT and nanoparticles indicate the future potential for clinical translation and integration of combinatorial nanoparticle and ICT strategies into established cancer treatment paradigms. Combinatorial nanoparticle and ICT strategies are well-positioned to synergize with tumor ablation, embolization, radiation, and chemotherapy and advance cancer therapy toward the goals of maximized therapeutic efficacy and minimized toxicity.

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Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

References

Alexander W (2016). The Checkpoint Immunotherapy Revolution. Pharmacy and Therapeutics, 41(3), 185–191. [PubMed: 26957887]

Antonia SJ, Villegas A, Daniel D, Vicente D, Murakami S, Hui R, Kurata T, Chiappori A, Lee KH, de Wit M, Cho BC, Bourhaba M, Quantin X, Tokito T, Mekhail T, Planchard D, Kim Y-C, Karapetis CS, Hiret S, … PACIFIC Investigators. (2018). Overall Survival with Durvalumab after Chemoradiotherapy in Stage III NSCLC. The New England Journal of Medicine, 379(24), 2342–2350. 10.1056/NEJMoa1809697 [PubMed: 30280658]

Asna N, Livoff A, Batash R, Debbi R, Schaffer P, Rivkind T, & Schaffer M (2018). Radiation therapy and immunotherapy—A potential combination in cancer treatment. Current Oncology, 25(5), e454–e460. 10.3747/co.25.4002 [PubMed: 30464697]

Aspeslagh S, Postel-Vinay S, Rusakiewicz S, Soria J-C, Zitvogel L, & Marabelle A (2016). Rationale for anti-OX40 cancer immunotherapy. European Journal of Cancer, 52, 50–66. 10.1016/j.ejca.2015.08.021 [PubMed: 26645943]

Au KM, Park SI, & Wang AZ (2020). Trispecific natural killer cell nanoengagers for targeted chemoimmunotherapy. Science Advances, 6(27), eaba8564. 10.1126/sciadv.aba8564 [PubMed: 32923587]

Bellmunt J, de Wit R, Vaughn DJ, Fradet Y, Lee J-L, Fong L, Vogelzang NJ, Climent MA, Petrylak DP, Choueiri TK, Necchi A, Gerritsen W, Gurney H, Quinn DI, Culine S, Sternberg CN, Mai Y, Poehlein CH, Perini RF, & Bajorin DF (2017). Pembrolizumab as Second-Line Therapy for Advanced Urothelial Carcinoma. New England Journal of Medicine, 376(11), 1015–1026. 10.1056/NEJMoa1613683

Blanco E, Shen H, & Ferrari M (2015). Principles of nanoparticle design for overcoming biological barriers to drug delivery. Nature Biotechnology, 33(9), 941–951. 10.1038/nbt.3330

Bommareddy PK, Shettigar M, & Kaufman HL (2018). Integrating oncolytic viruses in combination cancer immunotherapy. Nature Reviews Immunology, 18(8), 498–513. 10.1038/s41577-018-0014-6

Brahmer J, Reckamp KL, Baas P, Crinò L, Eberhardt WEE, Poddubskaya E, Antonia S, Pluzanski A, Vokes EE, Holgado E, Waterhouse D, Ready N, Gainor J, Arén Frontera O, Havel L, Steins M, Garassino MC, Aerts JG, Domine M, … Spigel DR (2015). Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer. The New England Journal of Medicine, 373(2), 123–135. 10.1056/NEJMoa1504627 [PubMed: 26028407]

Squibb Bristol-Myers. (2018). Randomized, Double-Blinded, Placebo-Controlled, Single Ascending Dose Study to Evaluate the Pharmacokinetics, Safety, Tolerability and Pharmacodynamics of BMS-986189 in Healthy Subjects (Clinical Trial Registration No. NCT02739373). clinicaltrials.gov. https://clinicaltrials.gov/ct2/show/NCT02739373

Burris HA, Gordon MS, Hellmann MD, LoRusso P, Emens LA, Hodi FS, Lieu CH, Infante JR, Tsai FY-C, Eder JP, Cleary JM, Jelovac D, Tsuhako AL, Mueller L, Lin R, Morrissey K, Mahrus S, Morley R, Pirzkall A, & Davis SL (2017). A phase Ib dose escalation study of combined inhibition of IDO1 (GDC-0919) and PD-L1 (atezolizumab) in patients (pts) with locally advanced or metastatic solid tumors. Journal of Clinical Oncology, 35(15_suppl), 105–105. 10.1200/JCO.2017.35.15_suppl.105

Burugu S, Dancsok AR, & Nielsen TO (2018). Emerging targets in cancer immunotherapy. Seminars in Cancer Biology, 52, 39–52. 10.1016/j.semcancer.2017.10.001

Cai H, Shukla S, & Steinmetz NF (2020). The Antitumor Efficacy of CpG Oligonucleotides is Improved by Encapsulation in Plant Virus-Like Particles. Advanced Functional Materials, 30(15), 1908743. 10.1002/adfm.201908743 [PubMed: 34366757]

Cai H, Shukla S, Wang C, Masarapu H, & Steinmetz NF (2019). Heterologous Prime-Boost Enhances the Antitumor Immune Response Elicited by Plant-Virus-Based Cancer Vaccine. Journal of the American Chemical Society, 141(16), 6509–6518. 10.1021/jacs.9b01523 [PubMed: 30995022]

Cano-Mejia J, Burga RA, Sweeney EE, Fisher JP, Bollard CM, Sandler AD, Cruz CRY, & Fernandes R (2017). Prussian blue nanoparticle-based photothermal therapy combined with checkpoint inhibition for photothermal immunotherapy of neuroblastoma. Nanomedicine: Nanotechnology, Biology and Medicine, 13(2), 771–781. 10.1016/j.nano.2016.10.015

Cao Q, Wang W, Zhou M, Huang Q, Wen X, Zhao J, Shi S, Geng K, Li F, Hatakeyama H, Xu C, Piwnica-Worms D, Peng W, Zhou D, Sood AK, & Li C (2020). Induction of antitumor immunity in mice by the combination of nanoparticle-based photothermolysis and anti-PD-1 checkpoint inhibition. Nanomedicine: Nanotechnology, Biology and Medicine, 25, 102169. 10.1016/j.nano.2020.102169

Carignan D, Herblot S, Laliberté-Gagné M-È, Bolduc M, Duval M, Savard P, & Leclerc D (2018). Activation of innate immunity in primary human cells using a plant virus derived nanoparticle TLR7/8 agonist. Nanomedicine: Nanotechnology, Biology and Medicine, 14(7), 2317–2327. 10.1016/j.nano.2017.10.015

Cervera-Carrascon V, Siurala M, Santos JM, Havunen R, Tähtinen S, Karell P, Sorsa S, Kanerva A, & Hemminki A (2018). TNFa and IL-2 armed adenoviruses enable complete responses by anti-PD-1 checkpoint blockade. OncoImmunology, 7(5), e1412902. 10.1080/2162402X.2017.1412902 [PubMed: 29721366]

Chang H-N, Liu B-Y, Qi Y-K, Zhou Y, Chen Y-P, Pan K-M, Li W-W, Zhou X-M, Ma W-W, Fu C-Y, Qi Y-M, Liu L, & Gao Y-F (2015). Blocking of the PD-1/PD-L1 Interaction by a D-Peptide Antagonist for Cancer Immunotherapy. Angewandte Chemie International Edition, 54(40), 11760–11764. 10.1002/anie.201506225 [PubMed: 26259671]

Chao Y, Chen G, Liang C, Xu J, Dong Z, Han X, Wang C, & Liu Z (2019). Iron Nanoparticles for Low-Power Local Magnetic Hyperthermia in Combination with Immune Checkpoint Blockade for Systemic Antitumor Therapy. Nano Letters, 19(7), 4287–4296. 10.1021/acs.nanolett.9b00579 [PubMed: 31132270]

Chariou PL, Ortega-Rivera OA, & Steinmetz NF (2020). Nanocarriers for the Delivery of Medical, Veterinary, and Agricultural Active Ingredients. ACS Nano, 14(3), 2678–2701. 10.1021/acsnano.0c00173 [PubMed: 32125825]

Chen DS, & Mellman I (2013). Oncology Meets Immunology: The Cancer-Immunity Cycle. Immunity, 39(1), 1–10. 10.1016/j.immuni.2013.07.012 [PubMed: 23890059]

Chen Q, Xu L, Liang C, Wang C, Peng R, & Liu Z (2016). Photothermal therapy with immune-adjuvant nanoparticles together with checkpoint blockade for effective cancer immunotherapy. Nature Communications, 7(1), 1–13. 10.1038/ncomms13193

Cheng K, Ding Y, Zhao Y, Ye S, Zhao X, Zhang Y, Ji T, Wu H, Wang B, Anderson GJ, Ren L, & Nie G (2018). Sequentially Responsive Therapeutic Peptide Assembling Nanoparticles for Dual-Targeted Cancer Immunotherapy. Nano Letters, 18(5), 3250–3258. 10.1021/acs.nanolett.8b01071 [PubMed: 29683683]

Cheng Y, Lemke-Miltner CD, Wongpattaraworakul W, Wang Z, Chan CHF, Salem AK, Weiner GJ, & Simons AL (2020). In situ immunization of a TLR9 agonist virus-like particle enhances anti-PD1 therapy. Journal for ImmunoTherapy of Cancer, 8(2), e000940. 10.1136/jitc-2020-000940 [PubMed: 33060147]

Chiang C-S, Lin Y-J, Lee R, Lai Y-H, Cheng H-W, Hsieh C-H, Shyu W-C, & Chen S-Y (2018). Combination of fucoidan-based magnetic nanoparticles and immunomodulators enhances tumour-localized immunotherapy. Nature Nanotechnology, 13(8), 746–754. 10.1038/s41565-018-0146-7

Curis, Inc. (2020). A Phase 1, Open-Label, Dose Escalation and Dose Expansion Trial Evaluating the Safety, Pharmacokinetics, Pharmacodynamics, and Clinical Effects of Orally Administered

CA-170 in Patients With Advanced Tumors and Lymphomas (Clinical Trial Registration No. NCT02812875). clinicaltrials.gov. https://clinicaltrials.gov/ct2/show/NCT02812875

Da Silva CG, Rueda F, Löwik CW, Ossendorp F, & Cruz LJ (2016). Combinatorial prospects of nano-targeted chemoimmunotherapy. Biomaterials, 83, 308–320. 10.1016/j.biomaterials.2016.01.006 [PubMed: 26796043]

Du Y, Liang X, Li Y, Sun T, Jin Z, Xue H, & Tian J (2017). Nuclear and Fluorescent Labeled PD-1-Liposome-DOX-64Cu/IRDye800CW Allows Improved Breast Tumor Targeted Imaging and Therapy. Molecular Pharmaceutics, 14(11), 3978–3986. 10.1021/acs.molpharmaceut.7b00649 [PubMed: 29016143]

Duan X, Chan C, Guo N, Han W, Weichselbaum RR, & Lin W (2016). Photodynamic Therapy Mediated by Nontoxic Core–Shell Nanoparticles Synergizes with Immune Checkpoint Blockade To Elicit Antitumor Immunity and Antimetastatic Effect on Breast Cancer. Journal of the American Chemical Society, 138(51), 16686–16695. 10.1021/jacs.6b09538 [PubMed: 27976881]

Duan X, Chan C, Han W, Guo N, Weichselbaum RR, & Lin W (2019). Immunostimulatory nanomedicines synergize with checkpoint blockade immunotherapy to eradicate colorectal tumors. Nature Communications, 10(1), 1–15. 10.1038/s41467-019-09221-x

Dumortier H (2013). When carbon nanotubes encounter the immune system: Desirable and undesirable effects. Advanced Drug Delivery Reviews, 65(15), 2120–2126. 10.1016/j.addr.2013.09.005 [PubMed: 24056183]

Emens LA, Ascierto PA, Darcy PK, Demaria S, Eggermont AMM, Redmond WL, Seliger B, & Marincola FM (2017). Cancer immunotherapy: Opportunities and challenges in the rapidly evolving clinical landscape. European Journal of Cancer, 81, 116–129. 10.1016/j.ejca.2017.01.035 [PubMed: 28623775]

Engeland CE, Grossardt C, Veinalde R, Bossow S, Lutz D, Kaufmann JK, Shevchenko I, Umansky V, Nettelbeck DM, Weichert W, Jäger D, von Kalle C, & Ungerechts G (2014). CTLA-4 and PD-L1 Checkpoint Blockade Enhances Oncolytic Measles Virus Therapy. Molecular Therapy, 22(11), 1949–1959. 10.1038/mt.2014.160 [PubMed: 25156126]

Etxeberria I, Glez-Vaz J, Teijeira Á, & Melero I (2020). New emerging targets in cancer immunotherapy: CD137/4–1BB costimulatory axis. ESMO Open, 4(Suppl 3). 10.1136/esmoopen-2020-000733

Fadel TR, & Fahmy TM (2014). Immunotherapy applications of carbon nanotubes: From design to safe applications. Trends in Biotechnology, 32(4), 198–209. 10.1016/j.tibtech.2014.02.005 [PubMed: 24630474]

Fahmueller YN, Nagel D, Hoffmann R-T, Tatsch K, Jakobs T, Stieber P, & Holdenrieder S (2013). Immunogenic cell death biomarkers HMGB1, RAGE, and DNAse indicate response to radioembolization therapy and prognosis in colorectal cancer patients. International Journal of Cancer, 132(10), 2349–2358. 10.1002/ijc.27894 [PubMed: 23047645]

Feng B, Zhou F, Hou B, Wang D, Wang T, Fu Y, Ma Y, Yu H, & Li Y (2018). Binary Cooperative Prodrug Nanoparticles Improve Immunotherapy by Synergistically Modulating Immune Tumor Microenvironment. Advanced Materials, 30(38), 1803001. 10.1002/adma.201803001

Ferris RL, Blumenschein G, Fayette J, Guigay J, Colevas AD, Licitra L, Harrington K, Kasper S, Vokes EE, Even C, Worden F, Saba NF, Iglesias Docampo LC, Haddad R, Rordorf T, Kiyota N, Tahara M, Monga M, Lynch M, … Gillison ML (2016). Nivolumab for Recurrent Squamous-Cell Carcinoma of the Head and Neck. New England Journal of Medicine, 375(19), 1856–1867. 10.1056/NEJMoa1602252

Fiering S (2017). Cancer immunotherapy: Making allies of phagocytes. Nature Nanotechnology, 12(7), 615–616. 10.1038/nnano.2017.89

Finn OJ (2008). Cancer Immunology. New England Journal of Medicine, 358(25), 2704–2715. 10.1056/NEJMra072739

Gallacher D, Armoiry X, Auguste P, Court R, Mantopoulos T, Patterson J, De Santis M, Cresswell J, & Mistry H (2019). Pembrolizumab for Previously Treated Advanced or Metastatic Urothelial Cancer: An Evidence Review Group Perspective of a NICE Single Technology Appraisal. PharmacoEconomics, 37(1), 19–27. 10.1007/s40273-018-0689-3 [PubMed: 30030817]

Galluzzi L, Buqué A, Kepp O, Zitvogel L, & Kroemer G (2017). Immunogenic cell death in cancer and infectious disease. Nature Reviews Immunology, 17(2), 97–111. 10.1038/nri.2016.107

Galon J, & Bruni D (2019). Approaches to treat immune hot, altered and cold tumours with combination immunotherapies. Nature Reviews Drug Discovery, 18(3), 197–218. 10.1038/s41573-018-0007-y [PubMed: 30610226]

Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pagès C, Tosolini M, Camus M, Berger A, Wind P, Zinzindohoué F, Bruneval P, Cugnenc P-H, Trajanoski Z, Fridman W-H, & Pagès F (2006). Type, Density, and Location of Immune Cells Within Human Colorectal Tumors Predict Clinical Outcome. Science, 313(5795), 1960–1964. 10.1126/science.1129139 [PubMed: 17008531]

Gandhapudi SK, Ward M, Bush JPC, Bedu-Addo F, Conn G, & Woodward JG (2019). Antigen Priming with Enantiospecific Cationic Lipid Nanoparticles Induces Potent Antitumor CTL Responses through Novel Induction of a Type I IFN Response. The Journal of Immunology, 202(12), 3524–3536. 10.4049/jimmunol.1801634 [PubMed: 31053626]

Ganesh S, Shui X, Craig KP, Park J, Wang W, Brown BD, & Abrams MT (2018). RNAi-Mediated β-Catenin Inhibition Promotes T Cell Infiltration and Antitumor Activity in Combination with Immune Checkpoint Blockade. Molecular Therapy, 26(11), 2567–2579. 10.1016/j.ymthe.2018.09.005 [PubMed: 30274786]

Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, Eder JP, Patnaik A, Aggarwal C, Gubens M, Horn L, Carcereny E, Ahn M-J, Felip E, Lee J-S, Hellmann MD, Hamid O, Goldman JW, Soria J-C, Dolled-Filhart M, … Gandhi L (2015). Pembrolizumab for the Treatment of Non–Small-Cell Lung Cancer. New England Journal of Medicine, 372(21), 2018–2028. 10.1056/NEJMoa1501824

Grzywa TM, Paskal W, & Włodarski PK (2017). Intratumor and Intertumor Heterogeneity in Melanoma. Translational Oncology, 10(6), 956–975. 10.1016/j.tranon.2017.09.007 [PubMed: 29078205]

Hargadon KM, Johnson CE, & Williams CJ (2018). Immune checkpoint blockade therapy for cancer: An overview of FDA-approved immune checkpoint inhibitors. International Immunopharmacology, 62, 29–39. 10.1016/j.intimp.2018.06.001 [PubMed: 29990692]

Hashiguchi S, Yamaguchi Y, Takeuchi O, Akira S, & Sugimura K (2010). Immunological basis of M13 phage vaccine: Regulation under MyD88 and TLR9 signaling. Biochemical and Biophysical Research Communications, 402(1), 19–22. 10.1016/j.bbrc.2010.09.094 [PubMed: 20875795]

Hellmann MD, Friedman CF, & Wolchok JD (2016). Chapter Six—Combinatorial Cancer Immunotherapies. In Schreiber RD (Ed.), Advances in Immunology (Vol. 130, pp. 251–277). Academic Press. 10.1016/bs.ai.2015.12.005 [PubMed: 26923003]

Hickey JW, Vicente FP, Howard GP, Mao H-Q, & Schneck JP (2017). Biologically Inspired Design of Nanoparticle Artificial Antigen-Presenting Cells for Immunomodulation. Nano Letters, 17(11), 7045–7054. 10.1021/acs.nanolett.7b03734 [PubMed: 28994285]

Hoopes PJ, Moodie KL, Petryk AA, Petryk JD, Sechrist S, Gladstone DJ, Steinmetz NF, Veliz FA, Bursey AA, Wagner RJ, Rajan A, Dugat D, Crary-Burney M, & Fiering SN (2017). Hypo-fractionated radiation, magnetic nanoparticle hyperthermia and a viral immunotherapy treatment of spontaneous canine cancer (Ryan TP, Ed.; p. 1006605). 10.1117/12.2256213

Hoopes PJ, Wagner RJ, Duval K, Kang K, Gladstone DJ, Moodie KL, Crary-Burney M, Ariaspulido H, Veliz FA, Steinmetz NF, & Fiering SN (2018). Treatment of Canine Oral Melanoma with Nanotechnology-Based Immunotherapy and Radiation. Molecular Pharmaceutics, 15(9), 3717–3722. 10.1021/acs.molpharmaceut.8b00126 [PubMed: 29613803]

Hubbell JA, & Langer R (2013). Translating materials design to the clinic. Nature Materials, 12(11), 963–966. 10.1038/nmat3788 [PubMed: 24150414]

Imai K, Matsuyama S, Miyake S, Suga K, & Nakachi K (2000). Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: An 11-year follow-up study of a general population. The Lancet, 356(9244), 1795–1799. 10.1016/S0140-6736(00)03231-1

Incyte Corporation. (2020). A Phase 3 Randomized, Double-Blind, Placebo-Controlled Study of Pembrolizumab (MK-3475) in Combination With Epacadostat or Placebo in Subjects With Unresectable or Metastatic Melanoma (Keynote-252 / ECHO-301) (Clinical Trial Registration results/NCT02752074). clinicaltrials.gov. https://clinicaltrials.gov/ct2/show/results/NCT02752074

Jin J-O, Zhang W, Du J-Y, Wong K-W, Oda T, & Yu Q (2014). Fucoidan Can Function as an Adjuvant In Vivo to Enhance Dendritic Cell Maturation and Function and Promote Antigen-Specific T Cell Immune Responses. PLOS ONE, 9(6), e99396. 10.1371/journal.pone.0099396 [PubMed: 24911024]

Kawata A, Une Y, Hosokawa M, Uchino J, & Kobayashi H (1992). Tumor-infiltrating Lymphocytes and Prognosis of Hepatocellular Carcinoma. Japanese Journal of Clinical Oncology, 22(4), 256–263. 10.1093/oxfordjournals.jjco.a039559 [PubMed: 1331571]

Kerstetter-Fogle A, Shukla S, Wang C, Beiss V, Harris PLR, Sloan AE, & Steinmetz NF (2019). Plant Virus-Like Particle In Situ Vaccine for Intracranial Glioma Immunotherapy. Cancers, 11(4). 10.3390/cancers11040515

Khair DO, Bax HJ, Mele S, Crescioli S, Pellizzari G, Khiabany A, Nakamura M, Harris RJ, French E, Hoffmann RM, Williams IP, Cheung A, Thair B, Beales CT, Touizer E, Signell AW, Tasnova NL, Spicer JF, Josephs DH, … Karagiannis SN (2019). Combining Immune Checkpoint Inhibitors: Established and Emerging Targets and Strategies to Improve Outcomes in Melanoma. Frontiers in Immunology, 10. 10.3389/fimmu.2019.00453

Kim BYS, Rutka JT, & Chan WCW (2010). Nanomedicine. The New England Journal of Medicine, 363(25), 2434–2443. 10.1056/NEJMra0912273 [PubMed: 21158659]

Kosmides AK, Sidhom J-W, Fraser A, Bessell CA, & Schneck JP (2017). Dual Targeting Nanoparticle Stimulates the Immune System To Inhibit Tumor Growth. ACS Nano, 11(6), 5417–5429. 10.1021/acsnano.6b08152 [PubMed: 28589725]

Labadie BW, Bao R, & Luke JJ (2019). Reimagining IDO Pathway Inhibition in Cancer Immunotherapy via Downstream Focus on the Tryptophan–Kynurenine–Aryl Hydrocarbon Axis. Clinical Cancer Research, 25(5), 1462–1471. 10.1158/1078-0432.CCR-18-2882 [PubMed: 30377198]

Lang T, Liu Y, Zheng Z, Ran W, Zhai Y, Yin Q, Zhang P, & Li Y (2019). Cocktail Strategy Based on Spatio-Temporally Controlled Nano Device Improves Therapy of Breast Cancer. Advanced Materials, 31(5), 1806202. 10.1002/adma.201806202

Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, Schadendorf D, Dummer R, Smylie M, Rutkowski P, Ferrucci PF, Hill A, Wagstaff J, Carlino MS, Haanen JB, Maio M, Marquez-Rodas I, McArthur GA, Ascierto PA, … Wolchok JD (2015). Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. The New England Journal of Medicine, 373(1), 23–34. 10.1056/NEJMoa1504030 [PubMed: 26027431]

Larkin J, Chiarion-Sileni V, Gonzalez R, Grob J-J, Rutkowski P, Lao CD, Cowey CL, Schadendorf D, Wagstaff J, Dummer R, Ferrucci PF, Smylie M, Hogg D, Hill A, Márquez-Rodas I, Haanen J, Guidoboni M, Maio M, Schöffski P, … Wolchok JD (2019). Five-Year Survival with Combined Nivolumab and Ipilimumab in Advanced Melanoma. New England Journal of Medicine. 10.1056/NEJMoa1910836

Larkin J, Lao CD, Urba WJ, McDermott DF, Horak C, Jiang J, & Wolchok JD (2015). Efficacy and Safety of Nivolumab in Patients With *BRAF* V600 Mutant and *BRAF* Wild-Type Advanced Melanoma: A Pooled Analysis of 4 Clinical Trials. JAMA Oncology, 1(4), 433. 10.1001/jamaoncol.2015.1184 [PubMed: 26181250]

Lebel M-È, Chartrand K, Tarrab E, Savard P, Leclerc D, & Lamarre A (2016a). Potentiating Cancer Immunotherapy Using Papaya Mosaic Virus-Derived Nanoparticles. Nano Letters, 16(3), 1826–1832. 10.1021/acs.nanolett.5b04877 [PubMed: 26891174]

Lebel M-È, Chartrand K, Tarrab E, Savard P, Leclerc D, & Lamarre A (2016b). Potentiating Cancer Immunotherapy Using Papaya Mosaic Virus-Derived Nanoparticles. Nano Letters, 16(3), 1826–1832. 10.1021/acs.nanolett.5b04877 [PubMed: 26891174]

Lee KL, Murray AA, Le DHT, Sheen MR, Shukla S, Commandeur U, Fiering S, & Steinmetz NF (2017). Combination of Plant Virus Nanoparticle-Based in Situ Vaccination with Chemotherapy Potentiates Antitumor Response. Nano Letters, 17(7), 4019–4028. 10.1021/acs.nanolett.7b00107 [PubMed: 28650644]

Lemke-Miltner CD, Blackwell SE, Yin C, Krug AE, Morris AJ, Krieg AM, & Weiner GJ (2020). Antibody Opsonization of a TLR9 Agonist–Containing Virus-like Particle Enhances In Situ Immunization. The Journal of Immunology, 204(5), 1386–1394. 10.4049/jimmunol.1900742 [PubMed: 31953355]

Li G, Gao Y, Gong C, Han Z, Qiang L, Tai Z, Tian J, & Gao S (2019). Dual-Blockade Immune Checkpoint for Breast Cancer Treatment Based on a Tumor-Penetrating Peptide Assembling Nanoparticle. ACS Applied Materials & Interfaces, 11(43), 39513–39524. 10.1021/acsami.9b13354 [PubMed: 31599562]

Li L, Knutsdottir H, Hui K, Weiss MJ, He J, Philosophe B, Cameron AM, Wolfgang CL, Pawlik TM, Ghiaur G, Ewald AJ, Mezey E, Bader JS, & Selaru FM (2019, January 24). Human primary liver cancer organoids reveal intratumor and interpatient drug response heterogeneity. 10.1172/jci.insight.121490

Li S-Y, Liu Y, Xu C-F, Shen S, Sun R, Du X-J, Xia J-X, Zhu Y-H, & Wang J (2016). Restoring anti-tumor functions of T cells via nanoparticle-mediated immune checkpoint modulation. Journal of Controlled Release, 231, 17–28. 10.1016/j.jconrel.2016.01.044 [PubMed: 26829099]

Liu Z, Ravindranathan R, Kalinski P, Guo ZS, & Bartlett DL (2017). Rational combination of oncolytic vaccinia virus and PD-L1 blockade works synergistically to enhance therapeutic efficacy. Nature Communications, 8(1), 1–12. 10.1038/ncomms14754

Lizotte PH, Wen AM, Sheen MR, Fields J, Rojanasopondist P, Steinmetz NF, & Fiering S (2016). *In situ* vaccination with cowpea mosaic virus nanoparticles suppresses metastatic cancer. Nature Nanotechnology, 11(3), 295–303. 10.1038/nnano.2015.292

Long GV, Dummer R, Hamid O, Gajewski TF, Caglevic C, Dalle S, Arance A, Carlino MS, Grob J-J, Kim TM, Demidov L, Robert C, Larkin J, Anderson JR, Maleski J, Jones M, Diede SJ, & Mitchell TC (2019). Epacadostat plus pembrolizumab versus placebo plus pembrolizumab in patients with unresectable or metastatic melanoma (ECHO-301/KEYNOTE-252): A phase 3, randomised, double-blind study. The Lancet Oncology, 20(8), 1083–1097. 10.1016/S1470-2045(19)30274-8 [PubMed: 31221619]

Longo V, Brunetti O, Gnoni A, Licchetta A, Delcuratolo S, Memeo R, Solimando AG, & Argentiero A (2019). Emerging Role of Immune Checkpoint Inhibitors in Hepatocellular Carcinoma. Medicina, 55(10). 10.3390/medicina55100698

Makarenkova ID, Logunov D. Yu., Tukhvatulin AI, Semenova IB, Besednova NN, & Zvyagintseva TN (2012). Interactions between Sulfated Polysaccharides from Sea Brown Algae and Toll-Like Receptors on HEK293 Eukaryotic Cells In Vitro. Bulletin of Experimental Biology and Medicine, 154(2), 241–244. 10.1007/s10517-012-1922-2 [PubMed: 23330135]

Marabelle A, Tselikas L, de Baere T, & Houot R (2017). Intratumoral immunotherapy: Using the tumor as the remedy. Annals of Oncology, 28(suppl_12), xii33–xii43. 10.1093/annonc/mdx683 [PubMed: 29253115]

Meng X, Du G, Ye L, Sun S, Liu Q, Wang H, Wang W, Wu Z, & Tian J (2017). Combinatorial antitumor effects of indoleamine 2,3-dioxygenase inhibitor NLG919 and paclitaxel in a murine B16-F10 melanoma model. International Journal of Immunopathology and Pharmacology, 30(3), 215–226. 10.1177/0394632017714696 [PubMed: 28604143]

Mi Y, Smith CC, Yang F, Qi Y, Roche KC, Serody JS, Vincent BG, & Wang AZ (2018). A Dual Immunotherapy Nanoparticle Improves T-Cell Activation and Cancer Immunotherapy. Advanced Materials, 30(25), 1706098. 10.1002/adma.201706098

Min Y, Roche KC, Tian S, Eblan MJ, McKinnon KP, Caster JM, Chai S, Herring LE, Zhang L, Zhang T, DeSimone JM, Tepper JE, Vincent BG, Serody JS, & Wang AZ (2017). Antigen-capturing nanoparticles improve the abscopal effect and cancer immunotherapy. Nature Nanotechnology, 12(9), 877–882. 10.1038/nnano.2017.113

Mohsen MO, Speiser DE, Knuth A, & Bachmann MF (2020). Virus-like particles for vaccination against cancer. Wiley Interdisciplinary Reviews. Nanomedicine and Nanobiotechnology, 12(1). 10.1002/wnan.1579

Mokhtari RB, Homayouni TS, Baluch N, Morgatskaya E, Kumar S, Das B, & Yeger H (2017). Combination therapy in combating cancer. Oncotarget, 8(23), 38022–38043. 10.18632/oncotarget.16723 [PubMed: 28410237]

Muller AJ, Manfredi MG, Zakharia Y, & Prendergast GC (2019). Inhibiting IDO pathways to treat cancer: Lessons from the ECHO-301 trial and beyond. Seminars in Immunopathology, 41(1), 41–48. 10.1007/s00281-018-0702-0 [PubMed: 30203227]

Murray AA, Wang C, Fiering S, & Steinmetz NF (2018). In Situ Vaccination with Cowpea vs Tobacco Mosaic Virus against Melanoma. Molecular Pharmaceutics, 15(9), 3700–3716. 10.1021/acs.molpharmaceut.8b00316 [PubMed: 29798673]

Nauts HC, Swift WE, & Coley BL (2007). The Treatment of Malignant Tumors by Bacterial Toxins as Developed by the Late William I 3.

Nikpoor AR, Tavakkol-Afshari J, Sadri K, Jalali SA, & Jaafari MR (2017). Improved tumor accumulation and therapeutic efficacy of CTLA-4-blocking antibody using liposome-encapsulated antibody: In vitro and in vivo studies. Nanomedicine: Nanotechnology, Biology and Medicine, 13(8), 2671–2682. 10.1016/j.nano.2017.08.010

Noelle RJ, Ceeraz S, LeMercier I, Nowak E, Lines J, Wang L, & Spaller M (2016). Vista antagonist and methods of use (United States Patent No. US20160331803A1). https://patents.google.com/patent/US20160331803A1/en

Ou W, Thapa RK, Jiang L, Soe ZC, Gautam M, Chang J-H, Jeong J-H, Ku SK, Choi H-G, Yong CS, & Kim JO (2018). Regulatory T cell-targeted hybrid nanoparticles combined with immuno-checkpoint blockage for cancer immunotherapy. Journal of Controlled Release, 281, 84–96. 10.1016/j.jconrel.2018.05.018 [PubMed: 29777794]

Palmer AC, & Sorger PK (2017). Combination cancer therapy can confer benefit via patient-to-patient variability without drug additivity or synergy. Cell, 171(7), 1678–1691.e13. 10.1016/j.cell.2017.11.009 [PubMed: 29245013]

Palomäki J, Välimäki E, Sund J, Vippola M, Clausen PA, Jensen KA, Savolainen K, Matikainen S, & Alenius H (2011). Long, Needle-like Carbon Nanotubes and Asbestos Activate the NLRP3 Inflammasome through a Similar Mechanism. ACS Nano, 5(9), 6861–6870. 10.1021/nn200595c [PubMed: 21800904]

Pardoll DM (2012). The blockade of immune checkpoints in cancer immunotherapy. Nature Reviews Cancer, 12(4), 252–264. 10.1038/nrc3239 [PubMed: 22437870]

Patel R, Czapar AE, Fiering S, Oleinick NL, & Steinmetz NF (2018). Radiation Therapy Combined with Cowpea Mosaic Virus Nanoparticle in Situ Vaccination Initiates Immune-Mediated Tumor Regression. ACS Omega, 3(4), 3702–3707. 10.1021/acsomega.8b00227 [PubMed: 29732445]

Postow MA, Chesney J, Pavlick AC, Robert C, Grossmann K, McDermott D, Linette GP, Meyer N, Giguere JK, Agarwala SS, Shaheen M, Ernstoff MS, Minor D, Salama AK, Taylor M, Ott PA, Rollin LM, Horak C, Gagnier P, … Hodi FS (2015). Nivolumab and Ipilimumab versus Ipilimumab in Untreated Melanoma. New England Journal of Medicine, 372(21), 2006–2017. 10.1056/NEJMoa1414428

Qin S, Xu L, Yi M, Yu S, Wu K, & Luo S (2019). Novel immune checkpoint targets: Moving beyond PD-1 and CTLA-4. Molecular Cancer, 18. 10.1186/s12943-019-1091-2

Ramanayake Mudiyanselage TMR, Michigami M, Ye Z, Uyeda A, Inoue N, Sugiura K, Fujii I, & Fujiwara D (2020). An Immune-Stimulatory Helix–Loop–Helix Peptide: Selective Inhibition of CTLA-4–B7 Interaction. ACS Chemical Biology, 15(2), 360–368. 10.1021/acschembio.9b00743 [PubMed: 31841301]

Ribas A, Hamid O, Daud A, Hodi FS, Wolchok JD, Kefford R, Joshua AM, Patnaik A, Hwu W-J, Weber JS, Gangadhar TC, Hersey P, Dronca R, Joseph RW, Zarour H, Chmielowski B, Lawrence DP, Algazi A, Rizvi NA, … Robert C (2016). Association of Pembrolizumab With Tumor Response and Survival Among Patients With Advanced Melanoma. JAMA, 315(15), 1600–1609. 10.1001/jama.2016.4059 [PubMed: 27092830]

Ribas A, & Wolchok JD (2018). Cancer immunotherapy using checkpoint blockade. Science, 359(6382), 1350–1355. 10.1126/science.aar4060 [PubMed: 29567705]

Riley RS, June CH, Langer R, & Mitchell MJ (2019). Delivery technologies for cancer immunotherapy. Nature Reviews Drug Discovery, 18(3), 175. 10.1038/s41573-018-0006-z [PubMed: 30622344]

Robert C, Long GV, Brady B, Dutriaux C, Maio M, Mortier L, Hassel JC, Rutkowski P, McNeil C, Kalinka-Warzocha E, Savage KJ, Hernberg MM, Lebbé C, Charles J, Mihalcioiu C, Chiarion-Sileni V, Mauch C, Cognetti F, Arance A, … Ascierto PA (2015, January 21). Nivolumab in Previously Untreated Melanoma without BRAF Mutation (world) [Research-article]. Massachusetts Medical Society. 10.1056/NEJMoa1412082

Ruoslahti E, Bhatia SN, & Sailor MJ (2010). Targeting of drugs and nanoparticles to tumors. Journal of Cell Biology, 188(6), 759–768. 10.1083/jcb.200910104

Russell L, Peng KW, Russell SJ, & Diaz RM (2019). Oncolytic Viruses: Priming Time for Cancer Immunotherapy. Biodrugs, 33(5), 485–501. 10.1007/s40259-019-00367-0 [PubMed: 31321623]

Saha D, Martuza RL, & Rabkin SD (2017). MACROPHAGE POLARIZATION CONTRIBUTES TO GLIOBLASTOMA ERADICATION BY COMBINATION IMMUNOVIROTHERAPY AND IMMUNE CHECKPOINT BLOCKADE. Cancer Cell, 32(2), 253–267.e5. 10.1016/j.ccell.2017.07.006 [PubMed: 28810147]

Saha D, Martuza RL, & Rabkin SD (2018). Oncolytic herpes simplex virus immunovirotherapy in combination with immune checkpoint blockade to treat glioblastoma. Immunotherapy, 10(9), 779–786. 10.2217/imt-2018-0009 [PubMed: 30008259]

Sasikumar PG, & Ramachandra M (2020). Peptide and peptide-inspired checkpoint inhibitors: Protein fragments to cancer immunotherapy. Medicine in Drug Discovery, 8, 100073. 10.1016/j.medidd.2020.100073

Sasikumar PG, Ramachandra RK, Adurthi S, Dhudashiya AA, Vadlamani S, Vemula K, Vunnum S, Satyam LK, Samiulla DS, Subbarao K, Nair R, Shrimali R, Gowda N, & Ramachandra M (2019). A Rationally Designed Peptide Antagonist of the PD-1 Signaling Pathway as an Immunomodulatory Agent for Cancer Therapy. Molecular Cancer Therapeutics, 18(6), 1081–1091. 10.1158/1535-7163.MCT-18-0737 [PubMed: 31015307]

Saxena M, & Bhardwaj N (2018). Re-Emergence of Dendritic Cell Vaccines for Cancer Treatment. Trends in Cancer, 4(2), 119–137. 10.1016/j.trecan.2017.12.007 [PubMed: 29458962]

Schadendorf D, Hodi FS, Robert C, Weber JS, Margolin K, Hamid O, Patt D, Chen T-T, Berman DM, & Wolchok JD (2015). Pooled Analysis of Long-Term Survival Data From Phase II and Phase III Trials of Ipilimumab in Unresectable or Metastatic Melanoma. Journal of Clinical Oncology, 33(17), 1889–1894. 10.1200/JCO.2014.56.2736 [PubMed: 25667295]

Sharma S, Kotamraju VR, Mölder T, Tobi A, Teesalu T, & Ruoslahti E (2017). Tumor-penetrating nanosystem strongly suppresses breast tumor growth. Nano Letters, 17(3), 1356–1364. 10.1021/acs.nanolett.6b03815 [PubMed: 28178415]

Sheen MR, & Fiering S (2019). In situ vaccination: Harvesting low hanging fruit on the cancer immunotherapy tree. Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology, 11(1), e1524. 10.1002/wnan.1524 [PubMed: 29667346]

Shi Y, & Lammers T (2019). Combining Nanomedicine and Immunotherapy. Accounts of Chemical Research, 52(6), 1543–1554. 10.1021/acs.accounts.9b00148 [PubMed: 31120725]

Shoeb E, & Hefferon K (2019). Future of cancer immunotherapy using plant virus-based nanoparticles. Future Science OA, 5(7), FSO401. 10.2144/fsoa-2019-0001 [PubMed: 31428448]

Shukla S, Jandzinski M, Wang C, Gong X, Bonk KW, Keri RA, & Steinmetz NF (2019). A Viral Nanoparticle Cancer Vaccine Delays Tumor Progression and Prolongs Survival in a HER2 + Tumor Mouse Model. Advanced Therapeutics, 2(4), 1800139. 10.1002/adtp.201800139 [PubMed: 33855164]

Sindhwani S, Syed AM, Ngai J, Kingston BR, Maiorino L, Rothschild J, MacMillan P, Zhang Y, Rajesh NU, Hoang T, Wu JLY, Wilhelm S, Zilman A, Gadde S, Sulaiman A, Ouyang B, Lin Z, Wang L, Egeblad M, & Chan WCW (2020). The entry of nanoparticles into solid tumours. Nature Materials, 19(5), 566–575. 10.1038/s41563-019-0566-2 [PubMed: 31932672]

Singh M, & Overwijk WW (2015). Intratumoral immunotherapy for melanoma. Cancer Immunology, Immunotherapy, 64(7), 911–921. 10.1007/s00262-015-1727-z [PubMed: 26050024]

Song Y, Margolles-Clark E, Bayer A, & Buchwald P (2014). Small-molecule modulators of the OX40–OX40 ligand co-stimulatory protein–protein interaction. British Journal of Pharmacology, 171(21), 4955–4969. 10.1111/bph.12819 [PubMed: 24930776]

Topalian SL, Drake CG, & Pardoll DM (2015). Immune Checkpoint Blockade: A Common Denominator Approach to Cancer Therapy. Cancer Cell, 27(4), 450–461. 10.1016/j.ccell.2015.03.001 [PubMed: 25858804]

Tran S, DeGiovanni P-J, Piel B, & Rai P (2017). Cancer nanomedicine: A review of recent success in drug delivery. Clinical and Translational Medicine, 6(1), 44. 10.1186/s40169-017-0175-0 [PubMed: 29230567]

Tundo GR, Sbardella D, Lacal PM, Graziani G, & Marini S (2019). On the Horizon: Targeting Next-Generation Immune Checkpoints for Cancer Treatment. Chemotherapy, 64(2), 62–80. 10.1159/000500902 [PubMed: 31387102]

Wang C, Fiering SN, & Steinmetz NF (2019). Cowpea Mosaic Virus Promotes Anti-Tumor Activity and Immune Memory in a Mouse Ovarian Tumor Model. Advanced Therapeutics, 2(5), 1900003. 10.1002/adtp.201900003 [PubMed: 33969181]

Wang C, & Steinmetz NF (2019). CD47 Blockade and Cowpea Mosaic Virus Nanoparticle In Situ Vaccination Triggers Phagocytosis and Tumor Killing. Advanced Healthcare Materials, 8(8), 1801288. 10.1002/adhm.201801288

Wang C, & Steinmetz NF (2020). A Combination of Cowpea Mosaic Virus and Immune Checkpoint Therapy Synergistically Improves Therapeutic Efficacy in Three Tumor Models. Advanced Functional Materials, 30(27), 2002299. 10.1002/adfm.202002299 [PubMed: 34366758]

Wang C, Xu L, Liang C, Xiang J, Peng R, & Liu Z (2014). Immunological Responses Triggered by Photothermal Therapy with Carbon Nanotubes in Combination with Anti-CTLA-4 Therapy to Inhibit Cancer Metastasis. Advanced Materials, 26(48), 8154–8162. 10.1002/adma.201402996 [PubMed: 25331930]

Wei SC, Duffy CR, & Allison JP (2018). Fundamental Mechanisms of Immune Checkpoint Blockade Therapy. Cancer Discovery, 8(9), 1069–1086. 10.1158/2159-8290.CD-18-0367 [PubMed: 30115704]

Wen AM, & Steinmetz NF (2016). Design of virus-based nanomaterials for medicine, biotechnology, and energy. Chemical Society Reviews, 45(15), 4074–4126. 10.1039/C5CS00287G [PubMed: 27152673]

Wilhelm S, Tavares AJ, Dai Q, Ohta S, Audet J, Dvorak HF, & Chan WCW (2016). Analysis of nanoparticle delivery to tumours. Nature Reviews Materials, 1(5), 1–12. 10.1038/natrevmats.2016.14

Woller N, Gürlevik E, Fleischmann-Mundt B, Schumacher A, Knocke S, Kloos AM, Saborowski M, Geffers R, Manns MP, Wirth TC, Kubicka S, & Kühnel F (2015). Viral Infection of Tumors Overcomes Resistance to PD-1-immunotherapy by Broadening Neoantigenome-directed T-cell Responses. Molecular Therapy, 23(10), 1630–1640. 10.1038/mt.2015.115 [PubMed: 26112079]

Xu J, Xu L, Wang C, Yang R, Zhuang Q, Han X, Dong Z, Zhu W, Peng R, & Liu Z (2017). Near-Infrared-Triggered Photodynamic Therapy with Multitasking Upconversion Nanoparticles in Combination with Checkpoint Blockade for Immunotherapy of Colorectal Cancer. ACS Nano, 11(5), 4463–4474. 10.1021/acsnano.7b00715 [PubMed: 28362496]

Yap TA, Papadopoulos KP, LoRusso P, Wong DJL, Hu-Lieskovan S, & Holz J-B (2019). A first-in-human phase I study of FS118, an anti-LAG-3/PD-L1 bispecific antibody in patients with solid tumors that have progressed on prior PD-1/PD-L1 therapy. Journal of Clinical Oncology, 37(15_suppl), TPS2652–TPS2652. 10.1200/JCO.2019.37.15_suppl.TPS2652

Yu Z, Geng J, Zhang M, Zhou Y, Fan Q, & Chen J (2014). Treatment of osteosarcoma with microwave thermal ablation to induce immunogenic cell death. Oncotarget, 5(15), 6526–6539. [PubMed: 25153727]

Zang X, Zhao X, Hu H, Qiao M, Deng Y, & Chen D (2017). Nanoparticles for tumor immunotherapy. European Journal of Pharmaceutics and Biopharmaceutics, 115, 243–256. 10.1016/j.ejpb.2017.03.013 [PubMed: 28323111]

Zhai W, Zhou X, Wang H, Li W, Chen G, Sui X, Li G, Qi Y, & Gao Y (2020). A novel cyclic peptide targeting LAG-3 for cancer immunotherapy by activating antigen-specific CD8+ T cell responses. Acta Pharmaceutica Sinica B, 10(6), 1047–1060. 10.1016/j.apsb.2020.01.005 [PubMed: 32642411]

Zhang L, Wang L, Shahzad KA, Xu T, Wan X, Pei W, & Shen C (2017). Paracrine release of IL-2 and anti-CTLA-4 enhances the ability of artificial polymer antigen-presenting cells to expand antigen-specific T cells and inhibit tumor growth in a mouse model. Cancer Immunology, Immunotherapy: CII, 66(9), 1229–1241. 10.1007/s00262-017-2016-9 [PubMed: 28501941]

Zhou F, Feng B, Yu H, Wang D, Wang T, Ma Y, Wang S, & Li Y (2019). Tumor Microenvironment-Activatable Prodrug Vesicles for Nanoenabled Cancer Chemoimmunotherapy Combining Immunogenic Cell Death Induction and CD47 Blockade. Advanced Materials, 31(14), 1805888. 10.1002/adma.201805888

Zhou X, Zuo C, Li W, Shi W, Zhou X, Wang H, Chen S, Du J, Chen G, Zhai W, Zhao W, Wu Y, Qi Y, Liu L, & Gao Y (2020). A Novel d-Peptide Identified by Mirror-Image Phage Display Blocks TIGIT/PVR for Cancer Immunotherapy. Angewandte Chemie International Edition, 59(35), 15114–15118. 10.1002/anie.202002783 [PubMed: 32386245]

Zou M-Z, Liu W-L, Li C-X, Zheng D-W, Zeng J-Y, Gao F, Ye J-J, & Zhang X-Z (2018). A Multifunctional Biomimetic Nanoplatform for Relieving Hypoxia to Enhance Chemotherapy and Inhibit the PD-1/PD-L1 Axis. Small, 14(28), 1801120. 10.1002/smll.201801120

Zylberberg C, & Matosevic S (2016). Pharmaceutical liposomal drug delivery: A review of new delivery systems and a look at the regulatory landscape. Drug Delivery, 23(9), 3319–3329. 10.1080/10717544.2016.1177136 [PubMed: 27145899]

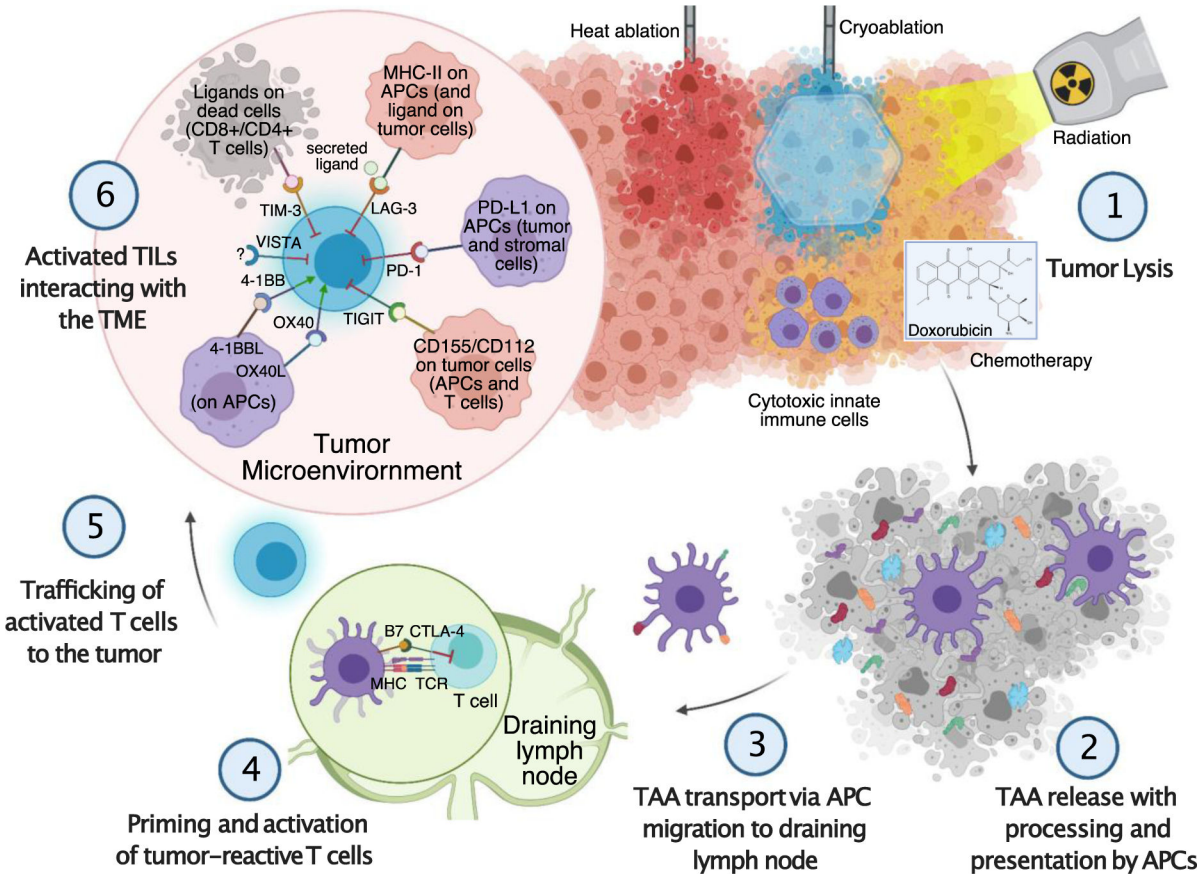

Figure 1.

Cancer-immunity cycle and checkpoint immunotherapy targets

Schematic view of the cyclic process through which the immune system generates a response to cancer. Checkpoints within this cycle provide regulatory negative (inhibitory checkpoints, red inhibition symbols) or positive (stimulatory checkpoints, green arrows) feedback mechanisms to attenuate or augment to anti-tumor immune response. Key steps include tumor lysis (1), which can be induced by cytotoxic immune cells or by cancer therapies (radiation, chemotherapy, heat or cryoablation are shown here). Damaged tumor cells release TAAs into the tumor microenvironment. With local inflammation, APCs process and transport TAAs to draining lymph nodes (2, 3). Cross-presentation of antigens to T cells leads to activation of tumor-reactive T cells (4). Circulating activated tumor-reactive T cells migrate to and infiltrate tumors (5, 6). Interactions with the TME may suppress or promote their anti-tumor effector functions, mediated through immune checkpoint signaling. Abbreviations are as follows: APC, antigen presenting cell; B7, B7 co-stimulatory protein; CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; LAG-3, lymphocyte-activation gene 3; MHC, major histocompatibility complex molecule; OX40, OX40 co-stimulatory receptor; OX40L, OX40 ligand; PD-1, programmed cell death-1 receptor; PD-L1, programmed cell death-1 ligand; TAA, tumor-associated antigen; TIM-3, T cell immunoglobulin and mucin-domain containing molecule 3; TIGIT, T cell immunoreceptor with immunoglobulin and ITIM domains; TCR, T cell receptor; TIL, tumor-infiltrating lymphocyte; VISTA, V-domain Ig-containing Suppressor of T-cell

Activation; 4–1BB, 4–1BB co-stimulatory receptor; 4–1BBL, 4–1BB ligand. Created with BioRender.com

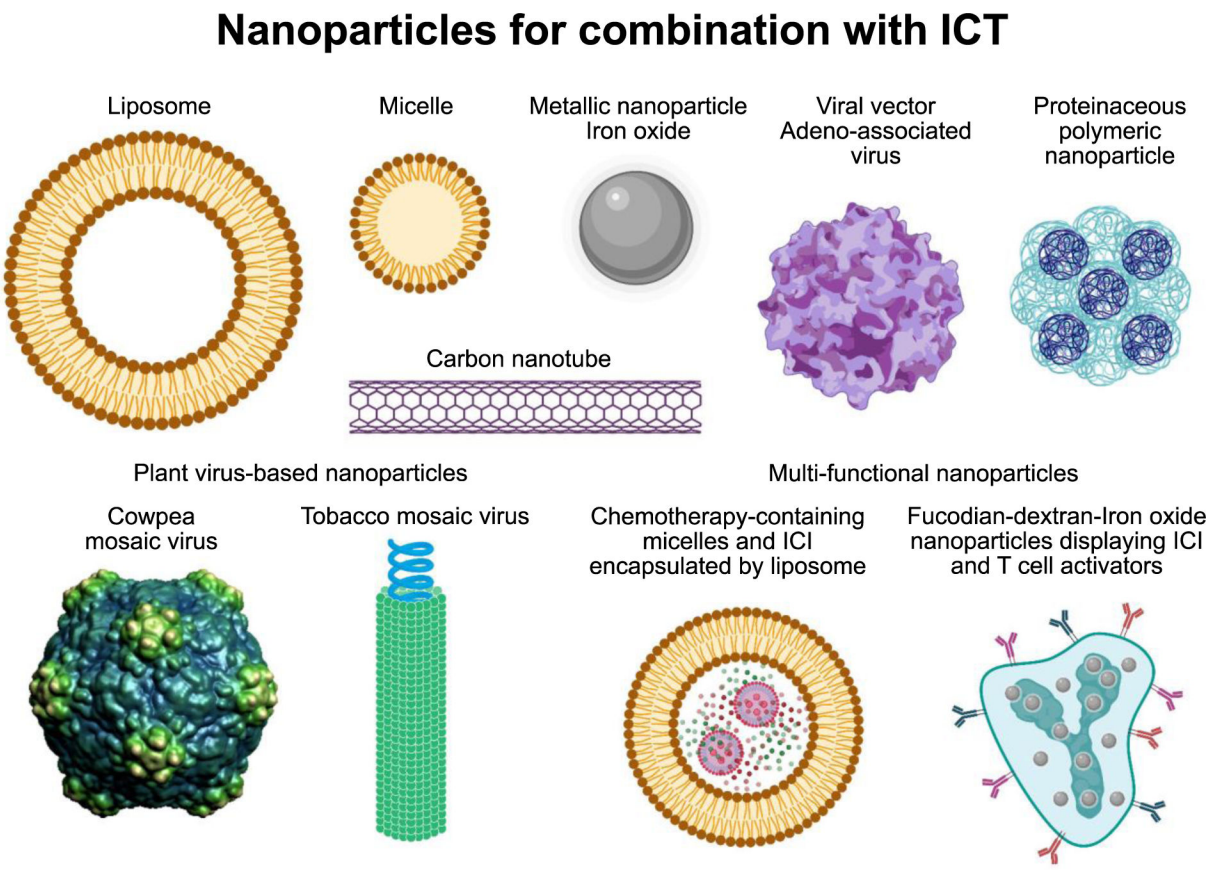

Figure 2.

An overview of several nanoparticles in development for combination ICT applications. A wide array of nanoparticles and design features are being developed for combination ICT. Synthetic: polymeric lipid and proteinaceous nanoparticles, metallic nanoparticles, liposomes, micelles, and carbon nanotubes. Viral vectors: Adeno-associated virus. Plant virus-based nanoparticles: cowpea mosaic virus and tobacco mosaic virus. Multi-functional nanoparticles: multi-layered nanoparticle containing ICI and chemotherapy-loaded micelles and magnetic fucodian-dextran-iron oxide nanoparticle displaying ICI and T cell activator antibodies. Schematic images are not to scale. Abbreviations are as follows: ICI, immune checkpoint inhibitor; ICT, immune checkpoint therapy. Image of cowpea mosaic virus was reproduced from the VIPER database (www.viperdb.scripps.edu). Created with BioRender.com

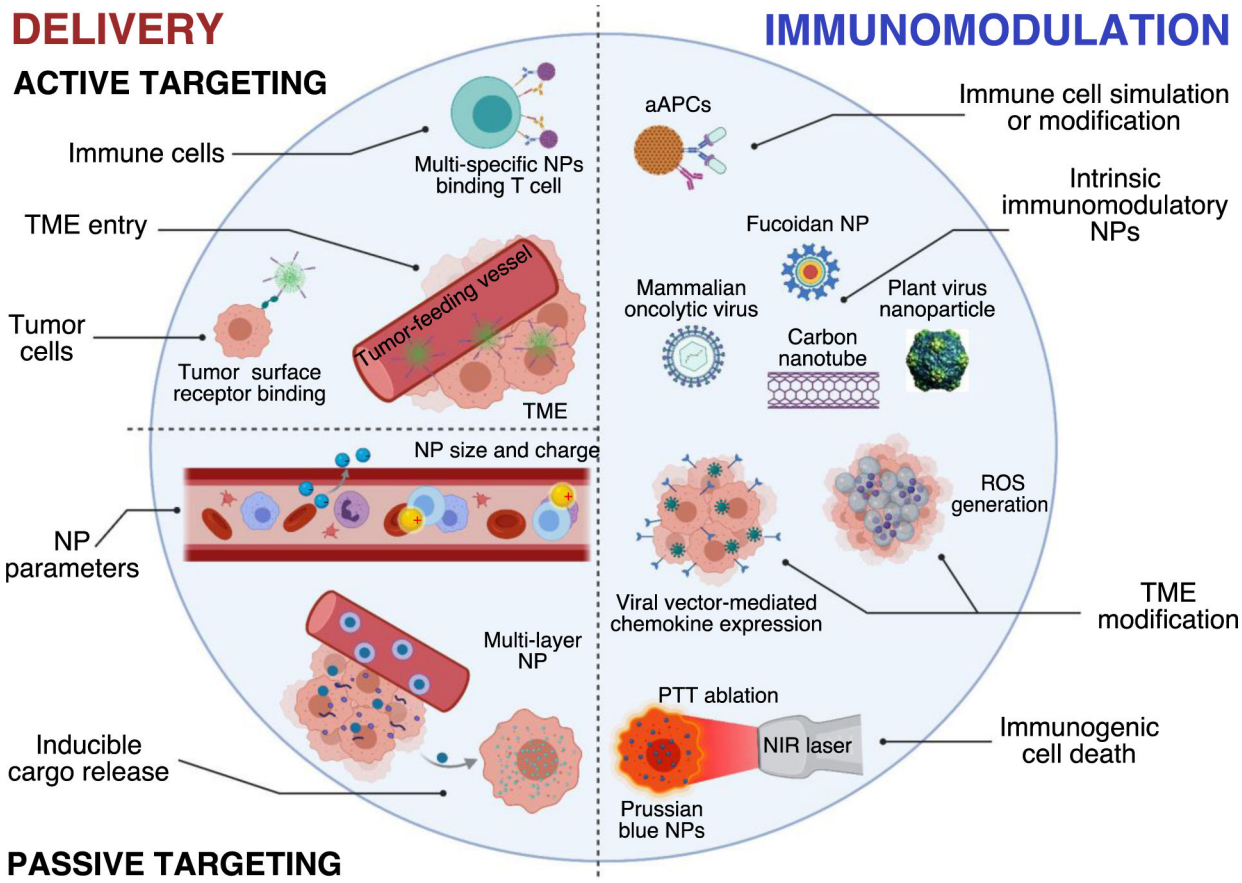

Figure 3.

Schematic overview of nanoparticle strategies for ICT combination.

Nanoparticle strategies can be primarily categorized as delivery and immunomodulation approaches. Within delivery strategies, nanoparticles may be designed with active targeting and passive targeting capabilities. Nanoparticles with immunomodulatory function may mimic or modify immune cell function, possess inherent immunogenicity, modify the TME, or induce immunogenic cell death. Abbreviations are as follows: aAPCs; artificial antigen-presenting cells; NIR, near infrared; NP, nanoparticle; PTT, photothermal therapy; ROS, reactive oxygen species; TME, tumor microenvironment. Image of plant virus nanoparticle (cowpea mosaic virus) was reproduced from the VIPER database (www.viperdb.scripps.edu). Created with BioRender.com.

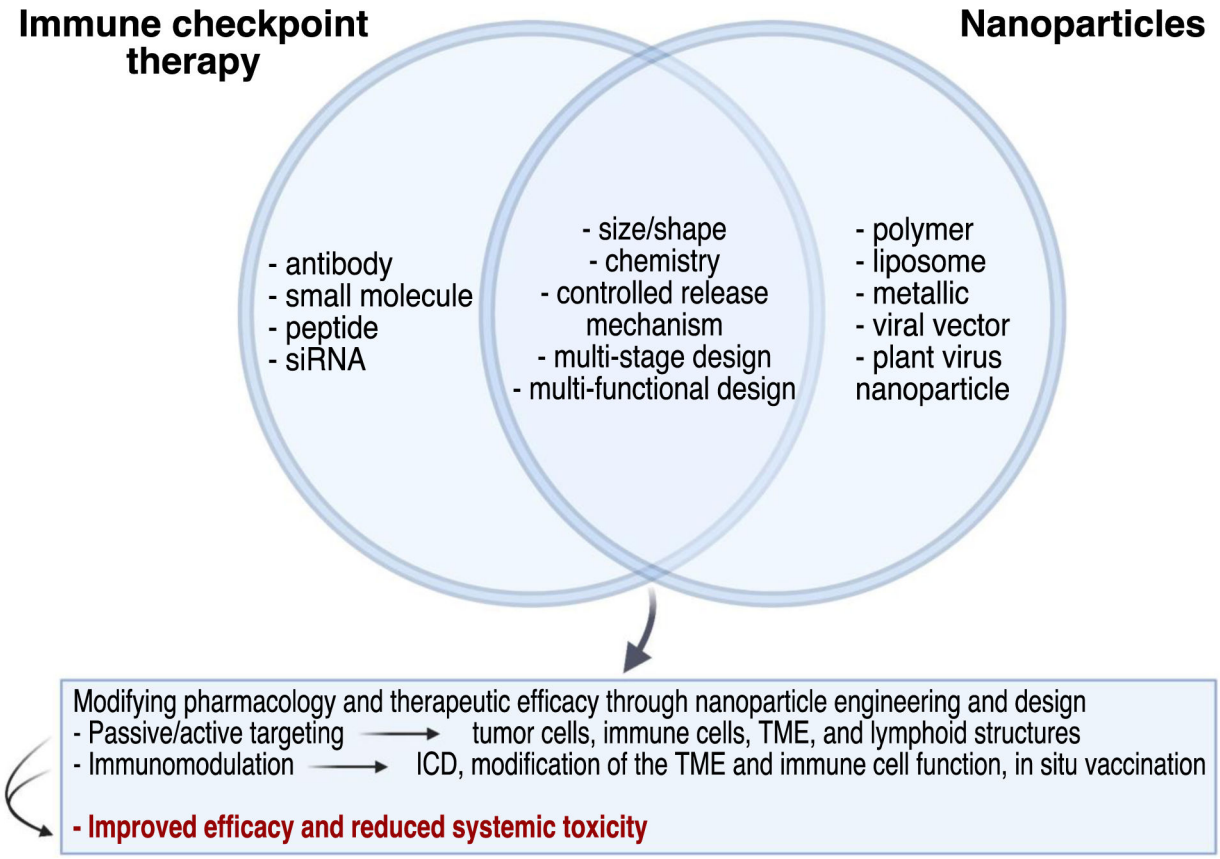

Figure 4.

Nanomedicine and ICT combination strategies aim to improve therapeutic efficacy and reduce systemic toxicity of ICT.

Different types of ICT (left circle) can be integrated with a variety of nanoparticle types (right circle) utilizing key design features (intersection of circles). These combinations approaches can use passive targeting, active targeting, and immunomodulation to improve efficacy and decrease toxicity. Abbreviations are as follows: siRNA, small interfering RNA; ICD, immunogenic cell death; TME, tumor microenvironment. Created with BioRender.com.

Table 1:

Immune checkpoint therapeutics in clinical use or development

Immune checkpoint	Antibody	Small molecule	Peptide
PD-1/PD-L1	Pembrolizumab (Merck), Nivolumab (Bristol-Myers Squibb/Ono), Cemiplimab (Sanofi/Regeneron), Atezolizumab (Roche), Avelumab (Merck/Pfizer), Durvalumab (AstraZeneca)	CA-170 (Curis/ Aurigene), CA-327 (Curis/Aurigene)	AUNP-12 (Aurigene), ^{D}PPA-1 (Chang et al., 2015), BMS-986189 (Bristol-Myers Squibb)
CTLA-4	Ipilimumab (Bristol-Myers Squibb)		ERY2–4 (Ramanayake Mudiyanselage et al., 2020)
TIM-3	TSR-022 (Tesaro), LY3321367 (Eli Lilly and Company), Sym023 (Symphogen), BGBA425 (BeiGene), ICAGN02390 (Incyte)		
TIGIT	Tiragolumab (Genentech/Roche), EOS-448 (iTeos Therapeutics)		^{D}TBP-3 (X. Zhou et al., 2020)
LAG-3	BMS-986016 (Bristol-Myers Squibb), GSK2831781 (GlaxoSmithKline)		C25 (Zhai et al., 2020)
VISTA	CI-8993 (Curis), JNJ-510588 (Jassen Research & Development)	CA-170 (Curis/ Aurigene), CA-327 (Curis/Aurigene)	AP1049 (Noelle et al., 2016)
OX40	PF-04518600 (Pfizer), INBRX 106 (INBRX), BMS 986178 (Bristol-Myers Squibb)	DB36, DB71, DB15, CVN (Song et al., 2014)	
4–1BB	BMS-663513 (Bristol-Myers Squibb), PF-05082566 (Pfizer)		