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Progress and opportunities for enhancing the delivery and efficacy of checkpoint inhibitors for cancer immunotherapy

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Abstract

Despite the advent of immune checkpoint blockade for effective treatment of advanced malignancies, only a minority of patients respond to therapy and significant immune-related adverse events remain to be minimized. Innovations in engineered drug delivery systems and controlled release strategies can improve drug accumulation at and retention within target cells and tissues in order to enhance therapeutic efficacy while simultaneously reducing drug exposure in off target tissues to minimize the potential for treatment-associated toxicities. This review will outline basic principles of the immune physiology of checkpoint signaling, the existing knowledge of dose-efficacy relationships in checkpoint inhibition, the influence of administration route on treatment efficacy, as well as the resulting checkpoint inhibitor antibody biodistribution profiles amongst target versus systemic tissues. It will also highlight recent successes in the application of drug delivery principles and technologies towards augmenting checkpoint blockade therapy in cancer. Delivery strategies that have been developed for other therapeutic and immunotherapy applications with as-of-yet underexplored potential in checkpoint inhibition therapy will also be discussed.

Keywords

cytotoxic T lymphocyte antigen-4; programmed cell death-1; cancer immunotherapy; tumor immunology; lymph node; drug delivery systems; controlled release; therapeutic antibody; immune-related associated toxicity

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1. Introduction

Immune checkpoint blockade with anti-cytotoxic T-lymphocyte-associated protein (CTLA)-4, anti-programmed cell death (PD)-1, and anti-PD-ligand (PD-L) monoclonal antibody (mAb) drugs have emerged as a successful treatment approach that induces durable objective responses in patients with advanced melanoma, squamous cell lung cancer, renal cell carcinoma, and classical Hodgkin lymphoma due to role of these molecules in costimulatory signaling to T cells [1] that suppresses anti-tumor immunity in human cancers. However, despite clinical successes, objective tumor responses are achieved in only a minority of patients. Several complementary/overlapping tiers of immune regulation can contribute to anti-tumor immune suppression [2] that may limit treatment efficacy. Accordingly, biomarkers are in development to identify individuals most likely to benefit from checkpoint blockade [3,4]. Furthermore, considerable preclinical and clinical research focuses on how the efficacy of checkpoint inhibition may be improved when used in combination with agents with orthogonal but synergistic signaling activity, for example targeted therapies [5,6] and cancer vaccines [7], which expand the population of tumor antigen-specific lymphocytes. Significant immune-related adverse events (irAE) and toxicities associated with treatment with checkpoint inhibitors when used alone or in combination (e.g. vemurafenib and ipimumab [8]) also remain to be minimized [9–11].

To this end, an emerging area of investigation aiming to augment checkpoint blockade therapy is the development of engineered delivery systems and controlled release innovations to improve mAb accumulation and retention within target cells and tissues in order to enhance immunotherapeutic efficacy and reduce off-target effects. This review will highlight such methods and their successes and, within the context of the basic principles of the immune physiology of checkpoint signaling, the known effects of delivered mAb dose and route of administration on treatment efficacy, as well as checkpoint inhibitor mAb biodistribution amongst target versus systemic tissues, delivery strategies that have been developed for other therapeutic applications with underexplored potential in checkpoint inhibition therapy.

2. Checkpoints and their tissues of action

CTLA-4 and PD-1 as well as their ligands exhibit discrete expression profiles, signaling pathways, and molecular mechanisms that underlie their physiological and pathophysiological roles [12,13] (Figure 1). CTLA-4 attenuates T cell responses largely by inhibiting co-stimulatory signaling through CD28. This is facilitated in part by its out-competing CD28 binding to CD80 and CD86 [14], molecule's whose expression is restricted to antigen presenting cells. Accordingly, CTLA-4's suppression of anti-tumor immunity is considered to largely occur within secondary lymphoid organs (i.e. lymph nodes and spleen), tissues where T cell activation occurs [15–18] rather than within the tumor microenvironment. Since CTLA-4 is not expressed on the surface of naive and resting memory T cells [19], costimulation can occur upon antigen recognition. Augmented CD8 T cell responses resulting from anti-CTLA-4 treatment likely occurs through increased activation of CD4⁺ cells since CTLA-4 is also predominantly expressed on CD4 helper rather than CD8 T cells [20]. CTLA-4 also drives the suppressive function of regulatory T

cells [21,22], which can locally inhibit anti-tumor immunity through their accumulation within the tumor microenvironment. Supporting the concept that CTLA-4's effects are not solely localized to secondary lymphoid tissues and can also function within the tumor itself, CTLA-4 blockade exhibits anti-tumor effects even when lymphocyte egress from lymph nodes is inhibited [23].

Like CTLA-4, resting naive and memory T cells lack PD-1 expression, which increases upon T cell receptor engagement and results in inhibition of T cell receptor-mediated effector functions. During T cell response to infection, PD-1 functions to restrain collateral tissue damage [24]. Accordingly, therapeutic PD-1 blockade in the context of tumor immunotherapy is thought to work predominantly within the tumor microenvironment, where PD-1 is highly expressed on tumor-infiltrating lymphocytes [25,26] and both tumor cells and tumor infiltrating leukocytes often over express its ligands [27]. Many human cancer types have been shown to overexpress PD-1 ligands [28] via mechanisms of either intrinsic resistance (resulting from genetic alterations or signaling pathway activation) or adaptive resistance (an adaptation of tumor cells to an inflammatory immune microenvironment) [12]. On the other hand, PD-1 also plays a role in early fate decisions of T cells recognizing antigens presented in the lymph node, affecting both the size of the proliferative T cell population upon antigen recognition and partially converting T cell tolerance to effector differentiation [29]. The proliferative burst of virus-specific CD8 T cells after PD-1 blockade in mice chronically infected with lymphocytic choriomeningitis virus also only occurs within lymphoid tissues [30].

3. Dosing Effects on Checkpoint Blockade Efficacy and Toxicity

The dosage of mAb administered is an important criterion that can greatly affect therapeutic response. Accordingly, clinical studies have established a dose-toxicity relationship for anti-CTLA-4 therapy indicating that higher doses lead to better response rates but with concurrent increases in iRAE. In a study with patients with advanced melanoma, anti-CTLA-4 mAb ipilimumab was administered at doses of 0.3, 3, or 10 mg/kg with the highest tested dose resulting in better overall response rates as well as higher total lymphocyte counts, a measurement used as a biomarker for anti-CTLA-4 therapy efficacy/pharmacodynamics [31]. In that same study, as the dose was increased, blood serum concentration of ipilimumab and iRAE also increased in a linear fashion, however at all three doses, manageable safety profiles were achieved [31]. From a mechanistic standpoint, it has been shown in both preclinical and clinical models that CTLA-4 blockade leads to proliferation and activation of both regulatory and effector T cells, however, at lower doses in patients, regulatory T cells appear to be more sensitive to anti-CTLA4 treatment and therefore higher doses may be needed to affect effector T cells to result in anti-tumor immunity [32,33].

In contrast to CTLA-4, there are conflicting reports on the dose effects on the clinical efficacy and toxicity of PD-1 blockade. In patients with prostate, lung, and advanced melanoma, doses of anti-PD-1 nivolumab up to 10 mg/kg were well tolerated with no signs or indications of a dose-efficacy or dose-toxicity relationship [10,34]. However, in another trial in patients with advanced melanoma, a dose of 10 mg/kg anti-PD-1 mAb

lambrolizumab every two weeks resulted in a superior rate of response (52%) compared to those achieved by lower doses (2 mg/kg every three weeks) or the same dose, just on a less frequent schedule (10 mg/kg every three weeks) (25% and 27% respectively) [35]. However, these dose-related improvements in response rates were accompanied by higher frequencies of iRAE, 23% for the group receiving 10 mg/kg every two weeks compared to 4% and 9% for the groups receiving 10 mg/kg every 3 weeks and 2 mg/kg every three weeks, respectively [35]. Similarly, in a preclinical study using the B16F10 melanoma model, improvements in tumor reduction were seen with increasing dose of mAb blocking PD-1 [36].

In contrast to checkpoint blockade with anti-CTLA-4 mAb and the conflicting reports on checkpoint blockade with anti-PD-1 mAb, the rate of patient response and frequency of iRAE with anti-PD-L1 treatment appears to be relatively dose independent. In a study involving patients with various advanced cancer types, clinical activity was seen with a dose as low as 1 mg/kg, although there appeared to be an improvement in response at higher doses, albeit not to a statistically significant level [37]. However, like anti-PD-1 mAb, preclinical studies have indicated a slight improvement in survival and tumor suppression with increasing anti-PD-L1 mAb dose [36,38], although toxicity effects have not to our knowledge been well studied in rodent models.

Other consistent themes seen in clinical testing are the dose-dependent blood pharmacokinetics and dose-independent blood pharmacodynamics or blood lymphocyte PD-1 or PD-L1 occupancy rates [34,35,37]. Specifically, as the dose of administered mAb increases, serum mAb concentration also increases in a direct manner. However, PD-1/PD-L1 expressing blood lymphocytes appear to be saturated at the lowest of tested dose (0.3 mg/kg) suggesting that as the dose of mAb is increased and the serum concentration increases, unwanted accumulation in off-target tissues may result, leading to higher likelihoods of iRAE.

With these monotherapy studies in mind, the potential for combination therapy with anti-CTLA-4 and anti-PD-1 mAb drugs to achieve improved effects using lower doses compared to monotherapy was evaluated. In a study in patients with advanced melanoma, escalating doses of both nivolumab and ipilimumab were concurrently administered [39]. Doses at or above 3 mg/kg of nivolumab and 3 mg/kg of ipilimumab surpassed the maximum tolerated dose [39]. However, when reduced to 1 mg/kg nivolumab and 3 mg/kg ipilimumab, iRAE were reduced to acceptable levels while still achieving substantial rates of favorable responses (53%) [39]. In fact, all nine of the patients who responded to combination therapy exhibited tumor regression of 80% or more compared to less than 3% of patients who received nivolumab or ipilimumab monotherapy at a dose of 3 mg/kg [39]. These phase I results suggest that combination therapy with nivolumab and ipilimumab can achieve high rates of patient response while maintaining manageable safety profiles depending on dose.

4. Biodistribution of Non-specific and Checkpoint Inhibitor mAb

Given the established dose-response relationships for some checkpoint inhibitor mAb with respect to both therapeutic and side effects, mAb biodistribution profiles within target versus

off-target tissues may critically influence their effects both locally in addition to distant tissues resulting from the abscopal effects intrinsic to immunotherapy (Figure 1). When intravenously (i.v.) administered, IgG rapidly distributes throughout the body leading to accumulation primarily within blood-rich systemic organs including the liver, heart, kidneys, lungs, and spleen [40,41]. It also exhibits a long *in vivo* half-life, approximately 21 days which is due to recycling by the neonatal Fc receptor [42]. This long half-life can be advantageous in sustaining the effects of therapeutic mAb, but can also lead to significant exposure in non-target cells and tissues. As an alternative to i.v. infusion, Epenetos et al. investigated the effects of intratumoral (i.t.) injection on the accumulation and retention of mAb within tumors. Not surprisingly, i.t. administration led to tumor concentrations of mAb approximately 10 times greater than those achieved by i.v. injection up to 18 days post injection [43]. This may be favorable for tumor mAb retention, however depending on the tumor location, it may be infeasible, therefore requiring different administration routes. As with i.t. injection, IgG is largely retained at the administration site when administered subcutaneously (s.c.) resulting in slow and very low accumulation levels in systemic organs compared to i.v. injections [44]. Filipe et al. found s.c. injection required approximately 24 hours to achieve appreciable levels of IgG accumulation in systemic tissues, as opposed to several minutes with an i.v. infusion [44]. Moreover, no accumulation was observed or reported in the lymph nodes irrespective of administration route [44]. In addition to these studies, it is important to consider the therapeutic mAb closely when evaluating the half-life and distribution as these parameters can vary with the IgG isotype and host in part through their effects on neonatal Fc receptor affinity [45].

In addition to route of administration and IgG isotype, mAb distributions are drastically affected by target specificity. As a result, in addition to accumulating within systemic organs, checkpoint blockade mAb have been shown to distribute appreciably to secondary lymphoid organs, specifically lymph nodes and the spleen in addition to the tumor itself when administered i.v. and at levels dramatically higher than that seen with non-specific mAb (Figure 2). For example, Higashikawa et al. demonstrated that anti-CTLA-4 mAb exhibits enhanced accumulation in CT26 tumors compared to a control non-specific IgG antibody as a result of its binding to CTLA-4 expressing T cells [46] (Figure 2). Natarajan et al. also demonstrated highest accumulation levels of anti-PD-1 in the spleen, liver, blood, and tumor 24 hours post injection with this same trend continuing 48 hours post injection using a melanoma mouse xenograft and radiolabeling anti-PD-1 [47]. Moreover, when unlabeled anti-PD-1 was administered just prior to infusion of labeled anti-PD-1, significantly less labeled anti-PD-1 mAb was found to accumulate in the spleen and tumor, indicating specificity towards PD-1-expressing lymphocytes [47]. Interestingly, anti-PD-L1 shows similar biodistribution profiles to that of anti-PD-1 mAb when injected i.v., with high levels of accumulation within the liver, lungs, and kidneys [48,49]. Anti-PD-L1 mAb tissue distribution appears concentration dependent, an effect primarily attributed to the large abundance of PD-L1-expressing splenocytes. Thus the spleen acts as a sink for anti-PD-L1 mAb and as the dose increases, splenocytes become saturated, allowing anti-PD-L1 mAb to instead accumulate in other PD-L1-expressing tissues such as tumors [48–50]. Using a B16F10 mouse melanoma model, Hettich et al. evaluated the biodistribution of anti-PD-1 and anti-PD-L1 mAb using PD-1 or PD-L1-deficient mice as well as PD-L1-deficient

B16F10 melanoma cells [51] (Figure 2). In both naïve and tumor bearing mice, anti-PD-1 accumulated significantly more in draining lymph nodes and the spleen compared to experiments where PD-1 was blocked by treatment with unlabeled anti-PD-1 mAb or when PD-1-deficient mice were used, indicating specificity towards PD-1 and confirming expression in these tissues [51] (Figure 2). Similar trends were also observed for anti-PD-L1 mAb distributions presumably due to PD-L1 expressing tumor cells and lymphocytes in the tumor, lymph nodes, and spleen, again confirming specificity towards PD-L1 [51] (Figure 2). However, in addition to the appreciable accumulation in secondary lymphoid tissues, anti-PD-L1 was also found to accumulate in the lungs and brown adipose tissue due to the expression of PD-L1 in these tissues [51]. Overall, mAb tend to accumulate in blood rich organs including the liver, lungs, and kidneys, however checkpoint blockade inhibitors alter the distribution to tissues with high expression of checkpoint receptors, mainly the spleen, tumor, and lymph nodes.

5. Route of Administration Effects on the Efficacy of Checkpoint Inhibition Cancer Therapy

The route of administration is another important parameter with potential to influence the effects of mAb therapy. Therapeutic mAb are administered i.v. clinically, however i.t., peritumoral (p.t.), and s.c. injection routes have been shown to improve mAb immunotherapy efficacy both by enhancing mAb delivery locally to the tumor as well as reducing systemic accumulation in preclinical models. For example, Fransen *et al.* showed that s.c. injection of anti-CTLA-4 mAb led to an effective anti-tumor response using a slow-release Montanide formulation [52]. Specifically, this administration route led to stimulation of T cells localized to the site of injection while using a mAb concentration four times lower than the intraperitoneal (i.p.) administration dose of 200 ug used in the majority of preclinical mouse studies [52]. This route of administration also greatly reduced mAb serum concentrations thereby preventing systemic toxicities associated with anti-CTLA-4 therapy [52]. In a similar study investigating the effect of checkpoint inhibitor route of administration on therapeutic efficacy, van Hooren *et al.* found i.t. injection to result in lower serum mAb concentrations while still slowing tumor growth compared to i.v. or s.c. (non-tumor bearing flank) injection [53]. When combining anti-PD-1 with anti-CTLA-4 mAb treatment, improvements in survival were achieved using p.t. injections of 30 ug per mouse at comparable rates to that induced by 100 ug i.v. injection of anti-PD-1 mAb [53]. Sandin *et al.* showed in a pancreatic adenocarcinoma model that p.t. injection of 30 ug anti-CTLA-4 mAb was just as effective as a 200 ug i.p. treatment in reducing tumor size while simultaneously resulting in a drastically lower serum concentrations of anti-CTLA-4 mAb [54]. Interestingly, this 30 ug peritumoral injection did not increase the regulatory T cell frequency in tumor-draining lymph nodes or spleen while the 200 ug i.p. injection significantly increased regulatory T cell frequencies in these tissues. It is also interesting to note that as the dose administered p.t. was increased to 90 ug, serum concentrations rose, leading to an increase in the frequency of regulatory T cells in the spleen, indicating that the 90 ug p.t. injection of anti-CTLA-4 had saturated available binding sites within the tumor [54]. Overall, these results suggest a local s.c., i.t., or p.t. injection route can be used to reduce serum levels of therapeutic mAb while still achieving an effective anti-tumor

response, albeit in a dose dependent fashion, though to our knowledge this concept in the context of anti-PD-1 monotherapy has yet to be established.

6. Drug Delivery Systems Improving Checkpoint Blockade mAb Delivery to Target Tissues

Due to iRAE and the requirement for repeated dosing in clinical checkpoint blockade therapeutic protocols, drug delivery platforms that improve mAb delivery to the tumor and achieve sustained release have garnered recent interest (Table 1). To this end, microparticle-based formulations aiming to prolong the retention of therapeutic agent at the site of injection have emerged as an attractive strategy since increasing carrier size enhances and prolongs retention at the site of injection [55,56] (Figure 3). We also recently demonstrated for the first time that this principle is conserved in malignant tissues, resulting in sustained retention within the tumor after i.t injection, despite the remodeled, irregular, and leaky tumor vasculature (Figure 3) [18]. Material systems can additionally be engineered to control the release of agent from its carrier in order to prolong its therapeutic effects. For example, Rahimian *et al.* engineered microparticles ~10–25 μm in diameter composed of a biodegradable poly(lactic-co-hydroxymethyl-glycolic-acid) polymer that could be modified to vary mAb release kinetics and loading efficiency [57]. The formulation the authors deemed optimal allowed for a burst release of about 20% of loaded mAb followed by a sustained release of the remaining 80% of mAb over approximately 30 days [57]. When compared to the same dose of mAb formulated in an incomplete Freund's adjuvant formulation, the mAb-loaded microparticle system resulted in serum mAb levels that were significantly lower (5–10 fold) than the mAb administered in incomplete Freund's adjuvant, although tumor-bearing animal survival was comparable for formulations [57]. Lei *et al.* engineered functionalized mesoporous silica based microparticles (12–15 μm) for controlled mAb release [58]. By tuning particle functional groups ($-\text{COOH}$, $-\text{SO}_3\text{H}$, $-\text{SH}$, etc.) and pore sizes up to ~30 nanometers, the entrapment and release of mAb was controlled [58]. This microparticle-based delivery platform resulted in improved mAb tumor retention and sustained release in a mouse melanoma model that slowed tumor growth and prolonged animal survival [58].

In addition to the use of s.c. administered mAb-loaded microparticles, microneedle (MN)-based platforms may be an appealing administration strategy to improve mAb retention due to its ease of administration on superficial tumors such as those of the skin, and potential to more homogeneously distribute therapeutic agent throughout the target tissue. Wang *et al.* and Ye *et al.* utilized MN to deliver nanoparticles composed of hyaluronic acid or dextran-alginate that encapsulate anti-PD-1 mAb and other small molecule therapeutics [59,60]. In this engineered MN system, the release of anti-PD-1 mAb from the nanoparticles was stimuli responsive to either hyaluronidase, an enzyme overexpressed in the tumor microenvironment [61] or glucose-triggered reductions in local pH. These MN-based approaches resulted in sustained anti-PD-1 mAb retention within the tumor, leading to a more robust immunotherapeutic response indicated by enhanced T cell infiltration into the tumor, reductions in tumor growth, and prolonged animal survival [59,60].

Another approach is the use of hydrogel-based platforms to improve release kinetics of delivered mAb. Wang et al. recently demonstrated that a s.c. injected alginate hydrogel improved the anti-tumor activity of both celecoxib and anti-PD-1 mAb when used individually or combined [36]. It is interesting to note that this system did not lower the serum concentration of anti-PD-1 mAb compared to an i.p. injection of free mAb [36]. However, the accumulation within the B16F10 melanoma was much higher compared to i.p. infusion [36].

Another approach developed to improve immune checkpoint mAb accumulation within tumors and resulting therapeutic effects is piggybacking on the intrinsic capacity of platelets to accumulate at wound sites, such as those created with tumor resection [62,63]. Wang et al. decorated platelets via a bifunctional maleimide linker and demonstrated the release of anti-PD-L1 mAb via platelet-derived microparticles following platelet activation or adhesion [64]. This approach improved survival and tumor suppression compared to i.v. injection of free anti-PD-L1 mAb in both melanoma and breast cancer models [64].

Lastly, combining checkpoint blockade inhibitors with a photothermal therapy through the use of nanoparticles or nanotubes has also led to improved checkpoint blockade therapy including enhanced immunological memory and systemic immunity towards metastatic cancer cells [65–67]. This is an attractive approach as the nanoparticles or nanotubes ablate the primary tumor resulting in released tumor-associated antigens that stimulate an anti-tumor immune response [66]. Ablation of the tumor also leads to infiltration of lymphocytes to the primary tumor site and when combined with an i.v. or i.p. injection of anti-CTLA-4, a substantial increase in CD8⁺ effector T cells infiltrating the tumor is achieved [65–67]. Taken together, these drug delivery approaches show great promise in improving checkpoint blockade by enhancing payload delivery without the need for repeated injections and potential for reducing systemic toxicity.

7. Opportunities and Potential Strategies for Improving Checkpoint Blockade Cancer Immunotherapy

Despite recent successes, enhancing checkpoint inhibitor mAb delivery to target tissues remains challenging. There are several excellent review papers that outline the challenges in mAb delivery to tumors that the reader is referred to [68,69] with two prevailing schools of thought that will be highlighted. First, tumors undergo significant remodeling that results in high levels of variation in the composition of the tumor vasculature and interstitium. Specifically, the tumor is comprised of a heterogeneous population of cells with a leaky, irregular vasculature, resulting in heterogeneous oxygenation of the tumor interstitium as well as an increase interstitial fluid pressure that can limit extravasation into the tumor from the blood [70]. Moreover, due to high cell densities and a highly cross-linked and dense extracellular matrix, the overall porosity of the tumor interstitium can be very low and result in the constriction of both tumor lymphatics and tumor blood vessels. Pending on the target antigen abundance, this can also greatly affect mAb distribution profiles within the tumor since mAb bind their targets strongly and therefore the total delivered dose may saturate peripheral cells prior to penetrating deeper within the tumor, an effect referred to as the

“binding site barrier” [71]. Second, parameters of the mAb itself may influence their capacity to accumulate, penetrate, and evenly distribute within tumors. For example, the relatively large size of mAb can restrict diffusive tumor transport [72]. Antigen affinity and neonatal Fc receptor recycling also play key roles in altering tumor distribution via restraining mAb to the point of tumor entry and altering circulation time leading to clearance prior to tumor entry, respectively [73,74].

With the aforementioned challenges in mind, rational strategies and drug delivery systems have been engineered to improve mAb delivery and distribution. The reader is referred to reference [75] for an excellent review paper regarding drug delivery to tumors, of which we will only highlight a few examples. First, in a manner conceptually similar to the use of photothermal tumor therapy to enhance checkpoint inhibition, direct modulation of the tumor microenvironment has been explored to improve drug delivery to tumors, specifically by normalizing the tumor matrix using extracellular matrix-degrading enzymes such as collagenase or extracellular matrix antagonists to improve the diffusivity and penetration of agents within the tumor [76–78]. Normalization of the tumor microvasculature using anti-angiogenic therapies can also reduce tumor interstitial hypertension and matrix remodeling, leading to improved drug entry and penetration [79]. Nanoparticles have also emerged as an attractive tumor mAb delivery approach as they can either encapsulate or be decorated with mAb. And by virtue of their propensity for prolonged circulation times relative to microparticles as well as propensity to accumulate within tumors and for cellular uptake, nanoparticles can improve mAb delivery to tumors as well as be used to control mAb release [80–82]. However, nanoparticles may be stymied from penetration into the tumor interstitium due to their size, accumulate in non-target cells, and may negatively affect the mAb structure/binding.

In addition to solid tumors as tissue targets, checkpoint inhibitor mAb delivery to secondary lymphoid tissues is also attractive in the context of tumor therapy for several reasons. First, many cancers metastasize via the lymphatic system, resulting in metastatic tumors within tumor-draining lymph nodes. Therefore, targeting anti-cancer therapeutics to lymph nodes may be of great interest to eradicate lymph node metastases. Lymph nodes in addition to the spleen harbor liquid tumor cells, providing further rationale for targeting. Second, one of the major tissues responsible for the priming of anti-tumor immune responses are tumor-draining lymph nodes [17]. This is because tumor antigens drain from the primary tumor microenvironment to the tumor-draining lymph node via the lymphatic vasculature [17,18,55]. Furthermore, tumor-draining lymph nodes often display altered immunological microenvironments relative to non-tumor associated lymph nodes, suggesting active regulation of the tumor-draining lymph node microenvironment by the tumor to suppress anti-tumor immunity. We and others have noted lymph node remodeling associated with tumor lymphatic drainage [17,18,83–85], effects that may alter antigen, cytokine, growth factor, or even therapeutic agent accumulation within and distribution throughout tumor-draining lymph nodes to alter their signaling activity [18]. Furthermore, increased frequencies of regulatory T cells [86] and low densities of CD169⁺ subcapsular sinus macrophages [87] within tumor-draining lymph nodes predicts poor patient outcome in melanoma [86]. Importantly, CTLA-4 and PD-1 play roles in directing early fate decisions of T cells recognizing antigens presented within lymph nodes [29].

Accordingly, technologies and/or methodologies targeting checkpoint inhibitor mAb to tumor-draining lymph nodes, such as those that have been used for delivery of other classes of immunotherapeutic agents and demonstrated the ability to enhance their immunotherapeutic effects while simultaneously reducing accumulation in off-target tissues [88,89], have potential to enhance checkpoint inhibitor therapeutic effects. Due to the nature and anatomy of the lymphatic system [18,90], localization of high concentrations of immunomodulatory agents to lymph nodes is difficult to obtain through the administration of free drug or by conventional microparticle-based formulations (Figure 4). However, lymphatic uptake and accumulation within draining lymph nodes after intradermal, s.c., and intramuscular injection is optimum for formulations roughly tens of nanometers in hydrodynamic diameter [91,92] (Figure 4), which may facilitate the use of a significantly lower total administered dose to reduce treatment-associated toxicities [93] by reducing levels of accumulation in off target tissues [83,93,56] (Figure 4). This principle has been explored for lymph node targeting by our group and others using nanoformulations comprised of synthetic materials including Pluronic-stabilized poly(propylene sulfide) [94,95], polymer dendrimers [96,97] and poly(γ -glutamic acid)-L-phenylalanine ethylester [98] as well as biologically derived biopolymers such as hyaluronic acid [99] and albumin [93]. These formulations have been used to enhance the delivery and bioactivity of small molecules [94,97], oligonucleotides [93,94,100], as well as proteins and peptides [93,101,102] within lymph nodes. However, as the result of extracellular matrix and vascular remodeling within the tumor microenvironment, lymphatic transport in solid tumors is significantly attenuated [103,104], which has the potential to undermine a lymphatic mediated drug delivery strategy to tumor-draining lymph nodes via i.t. administration. This may undermine the feasibility of lymphatic-targeting delivery systems to enhance lymph node delivery for melanoma immunotherapy. However, size-based principles of lymph node-targeting [90] were recently shown by our group to be conserved in malignant skin (Figure 4) [55]. In particular, although the extent of accumulation of nanoscale dextran tracers administered i.t. within tumor-draining lymph nodes substantially diminished as melanomas advanced, significant levels of accumulation of 30 nm, but not 5 nm or 50–500 nm tracers, within tumor-draining lymph nodes was still appreciable (Figure 4) [55]. Furthermore, when analyzed for their relative distribution amongst local tumor-draining lymph node versus systemic tissues, only tracers 30 nm in hydrodynamic diameter exhibited tumor-draining lymph node-selective accumulation profiles whereas all other tested sizes resulted instead in significant levels of systemic accumulation that increased with tumor stage (Figure 4) [55]. These results suggest that delivery systems ~30 nm in hydrodynamic size are ideal for lymphatic exposure even in cancers that are known to induce significant remodeling of the lymphatic vasculature. Therefore, 30 nm based formulations for immunotherapeutic delivered have the potential to simultaneously localize high levels of payload to both the tumor (Figure 3) as well as its draining lymph nodes (Figure 4) while minimizing exposure in off target tissues to minimize the risk of treatment-associated toxicities when administered i.t.. In addition to size, we also recently demonstrated carrier flexibility versus rigidity to have effects on lymph node accumulation after intradermal administration [56]. Notably, flexible dextrans accumulated at levels roughly an order of magnitude higher than size-matched rigid polystyrene spheres [56]. Alternatively direct injection into lymph nodes has

also been explored as a means to localize payload activity to lymph nodes, an approach amenable to controlled release strategies to tune therapeutic activity [105].

Lastly, the route of administration may be one of the most important parameters to consider when trying to optimize mAb therapy [90]. I.v. injection leads to significant accumulation within the primary tumor and spleen and may be particularly attractive in treating metastatic cancers but will also lead to accumulation within other systemic tissues, potentially resulting in more iRAE. I.t. injection on the other hand may improve tumor retention and enhance drainage to tumor-draining lymph nodes, but it may not be feasible pending the location of the tumor. Finally, in addition to different profiles of accumulation within various tissues, the distribution within the tumor may vary significantly pending the administration route. Whereas an i.t. injection would more likely result in mAb localized primarily to the core of the tumor, an i.v. injection would instead most likely result in levels of mAb accumulation being the highest immediately surrounding the tumor blood vasculature, potentially resulting in mAb interactions with different tumor-resident cell populations.

8. Conclusions

Engineered drug delivery systems offer the significant advantages of enabling more finely tuned control of tissue and cell targeting as well as rate of therapeutic agent release within target tissues to improve the immunotherapeutic effects of checkpoint inhibitor mAb drugs. The success of such systems will likely be defined as either increasing the proportion of patients who respond to treatment or enhancing drug safety profiles, though ideally both. With checkpoint inhibition likely to be approved for an even broader array of malignancies pending the success of innumerable ongoing clinical trials, the number of translational targets and opportunities for engineers as well as materials and formulation scientists to innovative solutions within this nascent but growing field is sure to continue to grow.

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Figure 1. In the context of cancer, checkpoints are active within tumors and secondary lymphoid tissues. Left, Canonical and non-canonical checkpoint signaling in tumors and secondary lymphoid tissues. Right, Routes of drug administration and drug delivery systems that improve mAb delivery to target tissues with their relative advantages and limitations in conferring enhanced drug bioactivity and potential for toxicity. I.V., intravenous; I.T., intratumoral; S.C., subcutaneous; LN, lymph node. Green and purple syringe, mAb and lines roughly indicate distribution profiles resulting from i.v. and i.t. administration of mAb, respectively.

Figure 2.

Checkpoint expression and checkpoint blockade mAb biodistribution in naïve and tumor bearing mice. (A) PET images of BALB/c mice implanted with CT26 tumors 48 hours post administration of radiolabeled anti-CTLA-4 mAb (top row) or radiolabeled control IgG (bottom row). Coronal view (a) and sagittal view (b) are shown. (B) ImmunopET/CT sections using radiolabeled anti-PD-1 mAb 24 hours post injection in naïve wildtype mice (C57BL/6) (WT), PD-1 blocked (Blocked) and PD deficient mice (PD-1^{-/-}). White ticks in the coronal sections indicate the position of the Transverse section. (C) Quantification of B in various organs. * indicates significance relative to the Block and/or PD-1^{-/-} groups using one-way ANOVA with Tukey's multiple comparison test. P-values < 0.05 considered

significant. (D) 10 days following implantation of C57BL/6 mice with wildtype CD133-expressing B16F10 melanoma cells on the left and CD133-expressing, PD-L1 knockout B16F10 melanoma cells on the right, radiolabeled anti-PD-L1 mAb was administered. ImmunoPET/CT images 24 hours post injection are shown here (coronal, transverse, and sagittal). (D) Reproduced from REF 46 (A) and REF 51 (B–D) with permission.

Figure 3.

Retention in skin is size dependent and remains relatively unchanged by tumor growth and progression. (A) Total exposure (AUC calculated from measured tissue concentrations) of tracers injected into naïve skin of C57Bl6 mice increases with increasing hydrodynamic size. * indicates significant for 5–12 vs 25, 50, and 500 nm tracers by one-way ANOVA and posthoc Fisher's LSD tests. (B) Tracer retention and resulting exposure within the skin or melanoma site of injection is relatively unchanged by tumor growth. (C) Micro-computed tomography 3D reconstructions of the remodeling tumor blood vasculature in naïve skin and B16F10 melanomas. Day refers to day post B16F10 implant. Reproduced from REF 55 (B,C) and REF 56 (A) with permission.

Figure 4.

Size-based principles of lymph node drug targeting are conserved in tumors. Time-resolved accumulation of tracers in draining lymph nodes (dLN) (A) and ratio of accumulating tracer concentrations within dLN to systemic tissues (B). * indicates significance relative to all other tracers at the same time point by two-way ANOVA and post-hoc Tukey's tests. § indicates significance for 500 nm tracer relative to all other time points, ‡ for 50 nm tracer relative to all other time points, † for 30 nm tracer vs all other time points by one-way ANOVA and post-hoc Fisher's LSD tests. One, two, three, and four symbols denoting statistical significance represent $p < 0.05$, 0.01 , 0.001 , and 0.0001 , respectively. Tumor growth reduces tracer exposure within dLN (C) and increases systemic tracer accumulation in the spleen, lungs, and liver, and kidneys (D). C, * $p < 0.05$ and ** $p < 0.01$ relative to all other tracers within same tumor day group by one-way ANOVA with post-hoc Fisher's LSD test.

D, *** indicates significance for 5 nm tracer at day 9 in kidney vs all other groups by two-way ANOVA and post-hoc Tukey's tests. (E) Despite these distribution and transport changes resulting from tumor growth, the relative enrichment of 30 nm but not 5, 50, or 500 nm exposure within dLN relative to systemic tissues (AUC calculated from measured levels of concentrations of injected tracers in individual tissues 1–72 h p.i) seen in naïve skin is conserved. Reproduced from REF 55 with permission.

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Table 1

Drug delivery systems improving checkpoint blockade mAb delivery and therapy.

Approach	Article	Drug Delivery System	Results
Microparticles for sustained mAb release	Lei et al. [58]	Silica microparticles	Functionalizing mesoporous silica and modifying the pore sizes within the microparticles resulted in prolonged release of mAb that improved antitumor immunity resulting from immunotherapy.
	Rahimtan et al. [57]	Polymeric microparticles	Varying the polymer composition of microparticles influenced mAb release kinetics to enable tumor-localized sustained release over 30 days but resulted in comparable animal survival relative to a control formulation.
Hydrogel for sustained mAb release	Li et al. [36]	Alginate Hydrogel	Hydrogel-mediated sustained release of anti-PD-1 mAb into serum and retention in the tumor resulted in increased levels of CD4 and CD8 T cells while reducing regulatory T cells in the spleen, tumor-draining lymph node, and tumor.
Microneedle (MN)-mediated sustained and local mAb release	Wang et al. 2016 [60]	Glucose triggered pH-reductions release mAb from MN-delivered nanoparticles	MN administration of nanoparticles encapsulating anti-PD-1 mAb improved anti-tumor immunity via enhanced tumor retention of mAb causing local infiltration of CD8 ⁺ T cells as well as improved survival and tumor reduction.
	Ye et al. [59]	Hyaluronidase triggered degradation of MN-delivered nanoparticles	By improving the retention and sustained release of mAb within the tumor, a single administration of mAb loaded nanoparticles via a MN patch improved anti-PD-1 mAb therapy, inducing enhanced tumor infiltration of CD8 ⁺ T cells and prolonged animal survival.
Therapeutic mAb "hitchhiking" for tumor-targeted delivery	Wang et al. 2017 [64]	Platelet-based Carrier	Decorating platelets with anti-PD-L1 mAb enabled its "hitchhiking" to the tumor and triggered release following platelet adhesion with in tumors, resulting in enhanced accumulation of mAb within the tumor and an increased CD8 ⁺ to Treg ratio compared to i.v. administration which prolonged animal survival.