



Induction of anti-cancer T cell immunity by *in situ* vaccination using systemically administered nanomedicines

Geoffrey M. Lynn^{a,b}, Richard Laga^c, Christopher M. Jewell^{a,d,e,f,g,*}

^a Fischell Department of Bioengineering, A. James Clark Hall, Room 5110, 8278 Paint Branch Drive, College Park, MD, 20742, USA

^b Avidia Technologies, Baltimore, MD, 21205, USA

^c Institute of Macromolecular Chemistry, Czech Academy of Sciences, Heyrovského nám. 2, 162 06, Prague, Czech Republic

^d United States Department of Veterans Affairs, VA Maryland Health Care System, 10 North Greene Street, Baltimore, MD, 21201, USA

^e Robert E. Fischell Institute for Biomedical Devices, A. James Clark Hall, Room 5110, 8278 Paint Branch Drive, College Park, MD, 20742, USA

^f Department of Microbiology and Immunology, University of Maryland School of Medicine, 685 West Baltimore Street, HSF-I Suite 380, Baltimore, MD, 21201, USA

^g Marlene and Stewart Greenebaum Cancer Center, Executive Office, Suite N9E17, 22 S. Greene Street, Baltimore, MD, 21201, USA

ARTICLE INFO

Keywords:

Nanomedicine and biomaterials
Chemotherapeutic and immunostimulant
Nanoparticle and microparticle
Immunogenic cell death
Pattern recognition receptor

ABSTRACT

Patients with inadequate anti-cancer T cell responses experience limited benefit from immune checkpoint inhibitors and other immunotherapies that require T cells. Therefore, treatments that induce *de novo* anti-cancer T cell immunity are needed. One strategy – referred to as *in situ* vaccination – is to deliver chemotherapeutic or immunostimulatory drugs into tumors to promote cancer cell death and provide a stimulatory environment for priming T cells against antigens already present in the tumor. However, achieving sufficient drug concentrations in tumors without causing dose-limiting toxicities remains a major challenge. To address this challenge, nanomedicines based on nano-sized carriers (“nanocarriers”) of chemotherapeutics and immunostimulants are being developed to improve drug accumulation in tumors following systemic (intravenous) administration. Herein, we present the rationale for using systemically administrable nanomedicines to induce anti-cancer T cell immunity via *in situ* vaccination and provide an overview of synthetic nanomedicines currently used clinically. We also describe general strategies for improving nanomedicine design to increase tumor uptake, including use of micelle- and star polymer-based nanocarriers. We conclude with perspectives for how nanomedicine properties, host factors and treatment combinations can be leveraged to maximize efficacy.

1. Introduction: role of anti-cancer T cells in durable tumor regression

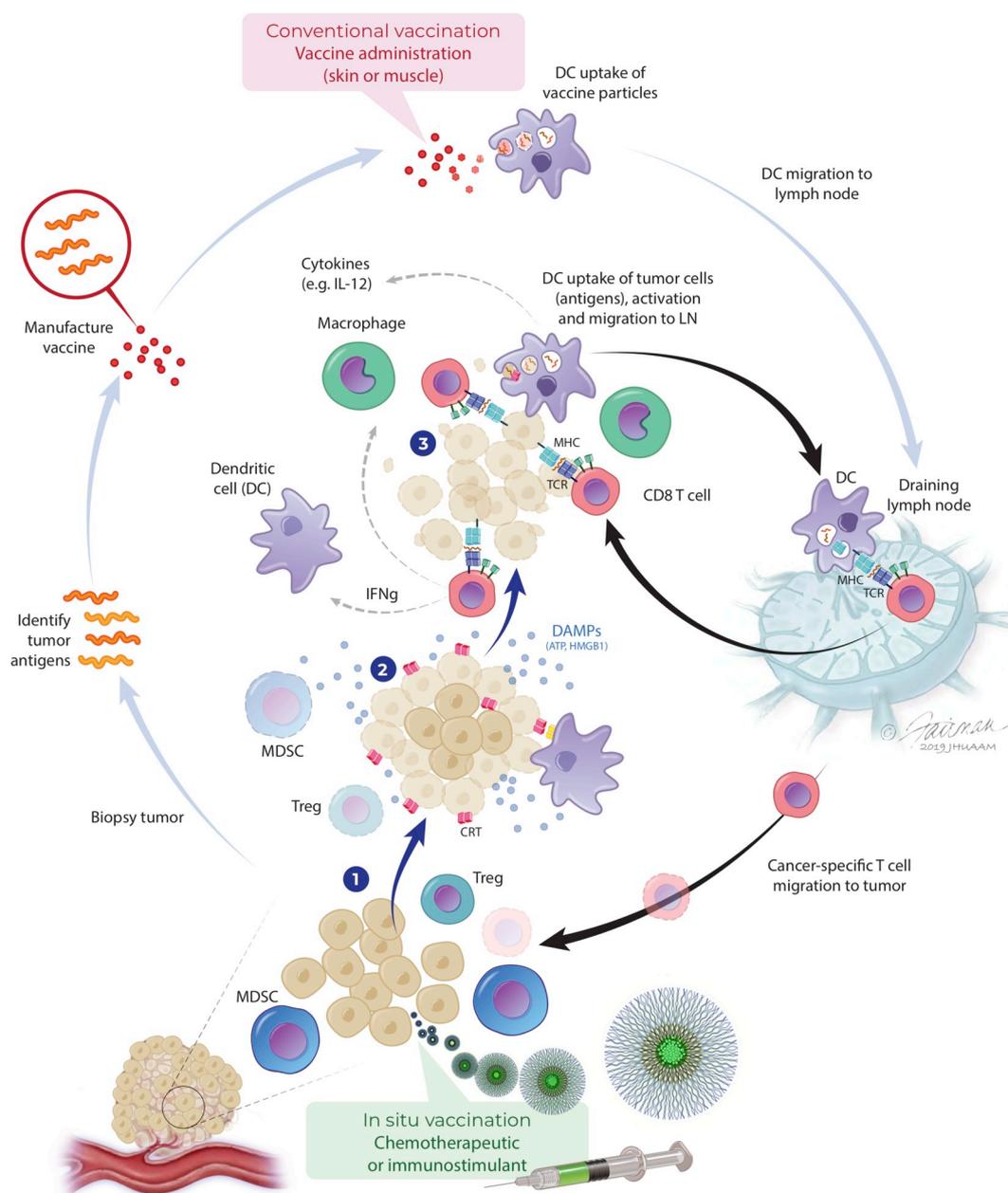
Immunotherapies can be highly effective for prolonging the survival of patients with advanced cancers [1,2]. Such strategies rely on T cells to mediate tumor-specific killing through T cell receptor (TCR) recognition of antigens presented as peptide-MHC complexes on cancer cell surfaces [3]. Analysis of the TCR specificity of tumor-infiltrating lymphocytes used in adoptive cell therapies (ACT) have revealed that both CD4 and CD8 T cells recognizing a variety of tumor antigens – including self-antigens and tumor-specific antigens (“neoantigens”) – are capable of mediating complete and durable tumor regression [4,5]. Moreover, the ability of checkpoint inhibitors (CPIs), such as anti-PD1 and anti-CTLA4, to mediate durable tumor regression has been shown to correlate with tumor mutational burden (*i.e.* number of neoantigens) and T cell infiltration into tumors [6,7]. A current challenge is that patients who lack anti-cancer T cells experience limited to no benefit

from ACT and CPIs [8]. Therefore, strategies for inducing *de novo* anti-cancer T cell immunity are needed.

One promising approach is to prime anti-cancer T cell responses using vaccines delivering tumor antigens. Conventional vaccination approaches include peptide-based tumor antigens combined with immuno-stimulants (adjuvants) [9]; nucleic acids [10], bacterial vectors [11] and viral vectors [12] expressing tumor antigens; and, antigen-presenting cells (APCs) pulsed with tumor antigens [13] (Fig. 1). Conventional vaccines are attractive because T cell immunity can be focused against specific antigens that are unique to cancer cells, thereby minimizing potential off-target toxicity. However, conventional vaccines require the identification and selection of suitable antigens, which can be a labor-intensive and challenging process given that tumor antigen composition and expression varies between patients and can even vary between different metastatic lesions within the same patient [14–16]. An alternative approach, sometimes referred to as *in situ* vaccination, is to use certain chemotherapies, immunostimulants, or

* Corresponding author. Fischell Department of Bioengineering, A. James Clark Hall, Room 5110, 8278 Paint Branch Drive, College Park, MD, 20742, USA.

E-mail address: cmjewell@umd.edu (C.M. Jewell).



Approach	Composition	Examples [Ref.]
Conventional vaccines	Protein/peptide vaccines	Peptide antigens combined with adjuvants [9]
	Genetic vaccines	Nucleic acids [10], bacteria [11] and viruses [12]
	Cell vaccines	Dendritic cells (DCs) pulsed with tumor antigens [13]
In situ vaccines	Small molecules	PRR agonists [19], cytotoxic chemotherapeutics [45]
	Biologics	Antibodies [20], cytokines [21], oncolytic viruses [22]

Fig. 1. Conventional and *in situ* vaccination can be used to induce anti-cancer T cell immunity. Conventional vaccines often use tumor antigens identified from biopsies, which are manufactured in one of several common formats (e.g., peptide/protein, nucleic acid, viral vector or dendritic cell-based) and then administered in the skin or muscle to prime anti-cancer T cells in lymph nodes. In contrast, *in situ* vaccination involves (1) delivery of immunostimulants or cytotoxic chemotherapeutics into tumors to (2) kill cancer cells, as well as deplete suppressor cells (MDSCs, Tregs) and/or promote immunogenic cancer cell death. Immunogenic cancer cell death is characterized by both the translocation of calreticulin (CRT) to cancer cell surfaces, which promotes their uptake by dendritic cells (DCs), as well as cancer cell release of danger associated molecular patterns (DAMPs) that recruit and activate immune cells. Activated DCs loaded with tumor antigens migrate to draining lymph nodes and prime anti-cancer T cells (3) that hone to tumors and kill cancer cells. The accompanying table summarizes different types of conventional and *in situ* vaccines that have been used for inducing T cell immunity.

even radiation therapy, to mediate tumor killing and convert tumors from an immuno-suppressive to immuno-supportive environment that makes existing tumor antigens accessible for T cell priming [17,18] (Fig. 1). By relying on the tumor as the source of antigens, *in situ*

vaccination provides the advantages that antigen identification is not required and that the same therapy can be applied to any patient.

In this review, we summarize data supporting the use of immunostimulants and chemotherapeutics for inducing anti-cancer T cell

immunity via *in situ* vaccination and describe how formulations of such drugs in nanocarriers can be used as systemically administrable nanomedicines for enhanced safety and efficacy. We then highlight emerging nanocarrier technologies that overcome limitations of conventional approaches in the clinic. Throughout, we offer a first-hand perspective on the topics addressed based on our own experience developing and evaluating nanomedicines for inducing T cell immunity.

2. Immunostimulants and chemotherapeutics can induce T cell immunity via *in situ* vaccination

2.1. Agonists of pattern recognition receptors as immunostimulants for inducing anti-cancer T cell immunity

A variety of immunostimulants with diverse physical properties and mechanisms of action have shown efficacy for mediating tumor regression by *in situ* vaccination. These include agonists of pattern recognition receptors (PRRs) [19], antibodies (e.g., anti-CD40) [20], cytokines [21], oncolytic viruses [22] and even micron-sized bacteria (e.g., Bacillus Calmette-Guerin or BCG) [23]. However, the following sections will limit discussion of immunostimulants to molecularly-defined agonists of PRRs, which are some of the most studied and – by virtue of their relatively small size – have the greatest need for formulations, e.g., nanocarriers, that can improve their uptake and retention in tumors.

PRRs are germ-line encoded receptors that function in the innate sensing of pathogens by a variety of cell types, particularly APCs [24]. Agonists of PRRs include a broad range of molecules that resemble or are derived from conserved structures of pathogens but are uncommon in humans. Among the various types of PRR agonists that have been identified, agonists of Toll-like receptor (TLR)-3 (dsRNA), TLR-4 (lipopolysaccharide and its synthetic analogs), TLR-7 (ssRNA and synthetic analogs of nucleotide bases), TLR-9 (CpG) and stimulator of interferon genes (STING) (e.g., cyclic dinucleotides (CDNs)) have been most extensively studied in the clinical setting [19] because these agonists induce type-I interferons (IFNs) needed for promoting Th1 CD4 and CD8 T cell immunity [25].

The most clinically advanced class of TLR agonists are imidazoquinoline-based TLR-7 agonists, which includes formulations of Imiquimod as a topical cream (Aldara[®]) used for treating cutaneous neoplasms and warts [26]. While several mechanisms have now been attributed to the anti-cancer activity of Imiquimod, the recognition that tumor regression is, in part, mediated by *de novo* priming of T cells against tumor antigens has provided a powerful proof-of-concept that *in situ* vaccination with immunostimulants is clinically relevant [27]. Agonists of TLR-3 and TLR-7 have also been administered intravenously for treating disseminated cancers [28–30]; however, toxicities (*i.e.* flu-like symptoms) related to systemic immune activation were found to be dose-limiting. Therefore, many groups have opted to administer agonists of TLR-3 [19], TLR-7 [31,32] and TLR-9 [33–36] by the intratumoral route to increase drug concentrations in the tumor while reducing systemic exposure.

Intratumoral administration of the TLR-9 agonist, CpG, in mice [34] and humans [37] has been shown to lead to the induction of anticancer T cell immunity that is synergistic with CPIs and leads to regression of distal, non-treated tumor nodules (Fig. 2A and B). Similarly, intratumoral administration of the TLR-4 agonist, G100, a synthetic analog of LPS, in a stable emulsion was shown in mice and humans to promote systemic T cell immunity that correlated with regression of treated and distal nodules [38,39].

STING agonists are another promising class of immunostimulants for *in situ* vaccination [40]. Intratumoral administration of CDN-based STING agonists has been shown to ablate large, established tumors through TNF-alpha mediated necrosis as well as T cell dependent mechanisms in mice [41] (Fig. 2C and D). These compelling data have motivated efforts to evaluate intratumoral administration of CDN-based

STING agonists in the clinical setting as well as motivated a broader search for the identification of novel agonists of STING [42,43].

2.2. Chemotherapeutics promote anti-cancer T cells by inducing immunogenic cancer cell death and/or depleting suppressor cells

Until recently, chemotherapies were believed to predominantly mediate tumor clearance through direct cytotoxic effects. However, it is increasingly recognized that tumor clearance with chemotherapies can involve multiple immune-mediated mechanisms that may act alone or in concert [44,45].

For instance, it has been shown that chemotherapy-induced immunogenic cancer cell death is a major mechanism for driving CD8 T cell immunity associated with the efficacy of various chemotherapies [46], particularly anthracyclines [47,48] (Fig. 2E and F). The mechanism involves translocation of calreticulin to tumor cell surfaces [48], which promotes their uptake by APCs, as well as tumor cell release of pro-inflammatory molecules (e.g., HMGB1 and ATP) that drive APC activation and migration to lymph nodes where T cell priming occurs [47] (Fig. 1). Chemotherapies can also promote tumor regression through the depletion or phenotypic conversion of different suppressor cell populations [44,49]. Accordingly, cyclophosphamide as well as combination therapies using 5-fluorouracil have been shown to reduce the number and/or functionality of regulatory T cells (Tregs) [50,51], while gemcitabine has been reported to deplete both Tregs and myeloid-derived suppressor cells (MDSCs) [52,53].

2.3. Nanocarriers are needed to limit systemic drug exposure and maximize tumor accumulation

A major challenge to using immunostimulants and chemotherapeutics for *in situ* vaccination is that such drugs often exhibit broad biodistribution and rapid clearance from the blood following parenteral administration due to their small size and amphiphilic characteristics [54–56]. This means that high or frequent doses are needed to ensure that sufficient drug concentrations are maintained in tumors to mediate a therapeutic effect; however, drug accumulation in other tissues can quickly give rise to toxicities that are dose-limiting [30,57].

To address this challenge, various particle-based formulations have been developed to physically restrict drug distribution as a means to limit systemic exposure (*i.e.* toxicity) and improve accumulation in tumors [58,59]. While intratumoral administration of formulated drugs can be effective in certain settings [60], the intravenous route of administration may be needed for treating disseminated disease or inaccessible tumors. However, each route of administration has unique challenges and opportunities. An important consideration for the intravenous route is the size of particles comprising the drug formulation. Though micron-sized, or larger, particles may be suitable for use by local (e.g., intratumoral) routes of administration, such formulations given intravenously often have poor tumor penetration (see: section 4.2) and can form toxic emboli that occlude arteries [61]. Therefore, nano-sized macromolecular and particle carriers, referred to as nanocarriers, are needed for the delivery of immunostimulant and chemotherapeutic drugs as systemically administrable nanomedicines. This class of materials is the focus of section 3.

3. Nanocarriers for systemic administration of chemotherapeutics: status and challenges

3.1. Nanocarriers can improve drug safety and efficacy by modulating pharmacokinetics and distribution

Nanocarriers can be broadly defined as any nano-sized material, typically between 5 and 200 nm in diameter, that is used to transport drug molecules to a target tissue [62]. Common types of nanocarriers include vesicles based on lipids (liposomes) [63]; polymers of different

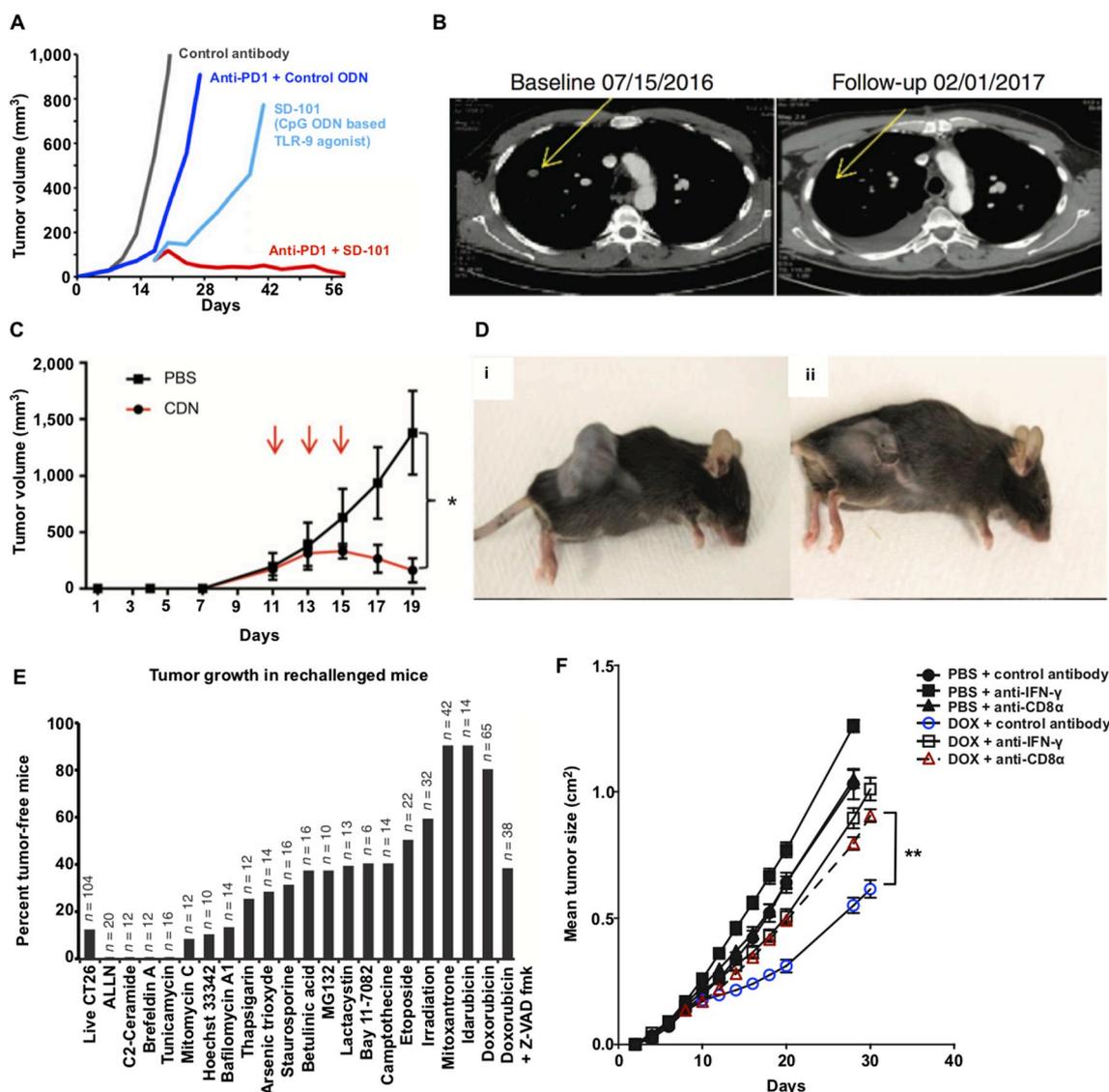


Fig. 2. *In situ* vaccination with immunostimulants and chemotherapeutics can promote durable tumor regression through immunological mechanisms. (A) Mice with established CT26 tumors received either intratumoral (i.t.) injections with a CpG oligodeoxynucleotide (ODN)-based TLR-9 agonist, SD-101, i.t. SD-101 + systemic anti-PD1, i.t. control (inactive) ODN + systemic anti-PD1 or i.t. control ODN alone. Tumor growth kinetics are shown and indicate that i.t. treatment with the TLR-9 agonist (SD-101) combined with anti-PD1 leads to optimal tumor regression. (B) CT images taken from a patient with metastatic melanoma that was treated with SD-101 and anti-PD1 show regression of a non-injected tumor, suggesting that the treatment induced systemic anti-cancer T cell immunity. (C, D) Intratumoral injection of a CDN-based STING agonist leads to acute rejection of B16F10 melanoma tumors. Mice were implanted with 5×10^5 B16F10 cells on day 0 and then treated with either CDN or PBS for a total of three treatments (red arrows). Tumor growth kinetic (C) and representative images (D) of mice treated with PBS (i) or CDN (ii) are shown. (E) CT26 tumor cells were cultured with the indicated chemotherapeutic agents (x-axis) for 24–48 h and then injected into the left flank of mice. Live tumor cells were injected into the right flank of the same mice 8 days later and the percentage of tumor-free mice was assessed 120 days later (note: n is the total number of mice used across multiple studies). Tumor rejection indicates that anti-cancer T cell immunity was primed by the treated cells, which suggests that the treatment promoted immunogenic cell death. (F) Mice with established MCA205 fibrosarcoma cells were treated with intratumoral doxorubicin or PBS and a subset of these mice were treated with either anti-IFN- γ or anti-CD8 antibodies to evaluate the impact of IFN- γ and CD8 T cell depletion on tumor regression by doxorubicin. Panel (A) adapted from Ref. [34]; (B) adapted from Ref. [37]; (C, D) adapted from Ref. [41]; (E) adapted from Ref. [48]; and, (F) adapted from Ref. [47]. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

architecture and hydrodynamic behavior (linear or branched random coils, polymer micelles, rigid polymer particles and polymersomes) [64]; dendrimers [65]; inorganic nanoparticles based on gold, iron oxide and silica [66]; carbon nanotubes [67]; and, recombinant proteins [68,69] and virus-like particles [70].

Nanocarriers offer a variety of potential benefits for the delivery of chemotherapeutic and immunostimulant drugs, including increased solubility; slower degradation; reduced rates of blood clearance; and, lower systemic drug exposure [59]. However, the capacity of nanocarriers to passively accumulate in tumors largely on the basis of their size has provided the strongest impetus for their use. For instance,

whereas low-molecular-weight drugs can be rapidly eliminated by the kidneys [71], nanocarriers (> 5 nm, diameter) evade renal elimination and can be designed for prolonged circulation. Longer circulation leads to greater exposure to tumor vasculature, which is characterized by slow blood flow [72] and irregular, “leaky” vessels [73,74] that permit the extravasation of nanocarriers into tumors that lack functional lymphatic capillaries [75], thereby leading to passive nanocarrier accumulation into tumors by a process referred to as enhanced permeability and retention (EPR) [76,77].

While a broad variety of nanocarriers have been developed for delivering drugs to tumors, only a limited number of these have been

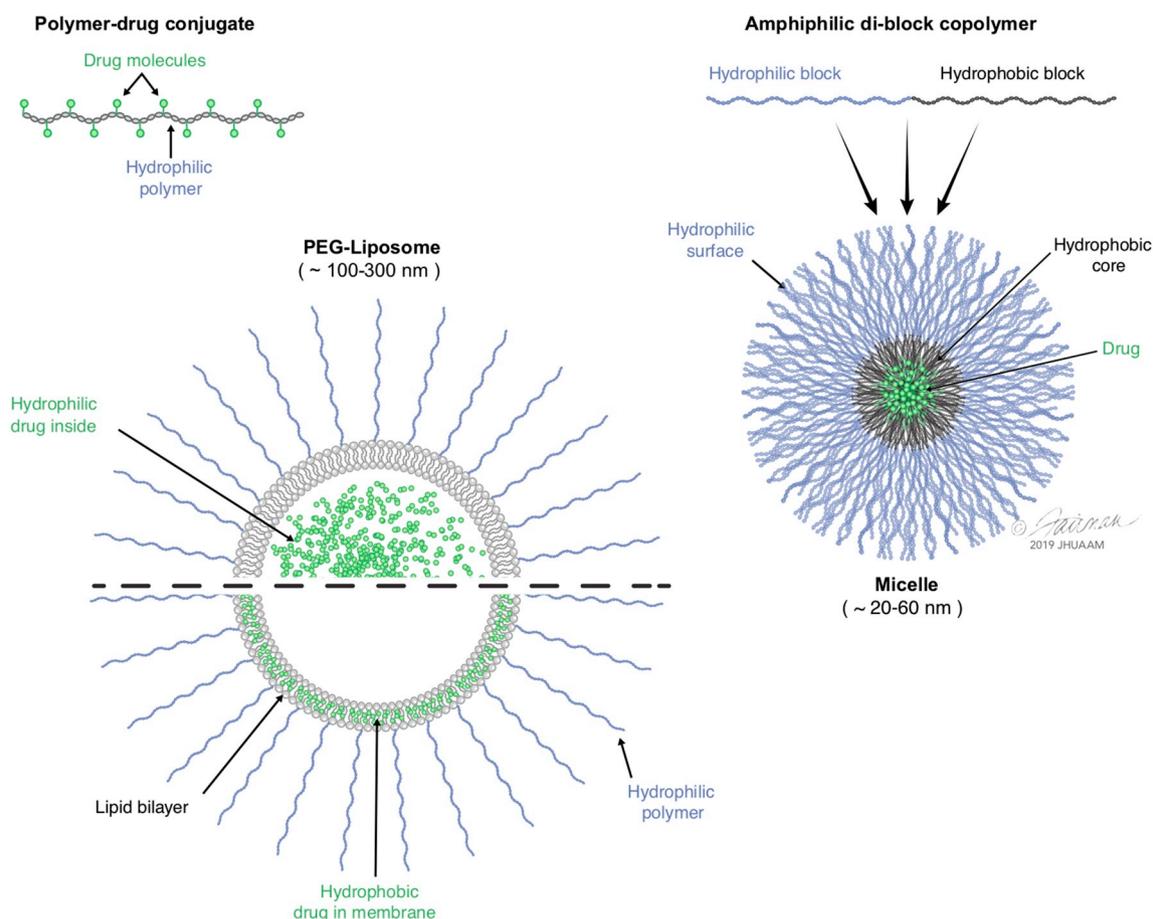


Fig. 3. Nanomedicines based on polymer-drug conjugates, micelles and liposomes. The polymer-drug conjugate represented in the cartoon schematic is a linear copolymer wherein multiple drug molecules are attached to the backbone of the polymer. The micelle particle represented in the schematic is comprised of multiple amphiphilic di-block polymers; the hydrophobic portion of the di-block polymer drives particle assembly and solubilizes hydrophobic drugs in the core of the particles, while the hydrophilic portion of the di-block polymer stabilizes the micelle. The liposome schematic shows a cross-section of a lipid bilayer vesicle coated with hydrophilic PEG chains; hydrophilic drugs can be encapsulated inside the liposomal particle, whereas hydrophobic drugs can insert into the bilayer membrane.

advanced to clinical trials and fewer still have been approved by regulatory authorities for human use [78]. The next section summarizes the status of the most clinically advanced synthetic nanocarriers, including liposomes, hydrophilic polymers and polymer micelles (Fig. 3), found in nanomedicines approved for clinical use. Readers are referred elsewhere (see [78–80]) for reviews that provide a more complete summary of nanomedicines that have been approved by regulatory authorities and/or are undergoing clinical testing.

3.2. Nanomedicines based on liposomes, hydrophilic polymers and polymer micelles

3.2.1. Liposomes

Liposomes are lipid bilayer vesicles, which are typically hollow spheres of 100–300 nm diameter and often include a hydrophilic polymer surface coating, *i.e.* poly(ethylene glycol) (PEG). The approval of a PEGylated liposomal formulation of doxorubicin (Doxil[®]) for the treatment of Kaposi's sarcoma by the FDA in 1995 was the first time a nanomedicine was approved for clinical use by a major regulatory authority [81,82]. Since then, several liposomal formulations of chemotherapeutics have gained FDA approval, including doxorubicin (Doxil[®] and Myocet[®]), vincristine (Marqibo[®]), daunorubicin (Dau-nOxome[®]), cytarabine (Depocyt[®]), irinotecan (Onivyde[®]) and a co-formulation of cytarabine and irinotecan (Vyxeos[®]) [80,83]. While liposomal formulations have demonstrated some benefit in altering the toxicity profile of certain chemotherapeutics, most have failed to demonstrate a significant improvement in efficacy [84,85].

3.2.2. Hydrophilic polymers

An alternative nanocarrier approach is to covalently link multiple chemotherapeutic drug molecules to synthetic, hydrophilic polymers to yield macromolecular polymer-drug conjugates [86]. The most clinically advanced polymer-drug conjugate, referred to as paclitaxel polyglumex (Opaxio[®]), comprises multiple paclitaxel molecules covalently linked to poly(glutamic acid) (PGA) polymers and is approved by the FDA for treating several cancer types, including non-small lung cancer (NSCLC) [87,88]. Other notable polymer-drug conjugates include platinum and anthracycline conjugates of linear N-(2-hydroxypropyl)(methacrylamide) (HPMA)-based copolymers [89,90] as well as cyclodextrin-containing polymers conjugated to camptothecin, referred to as CRLX101 [91]. Linear HPMA-based polymer-drug conjugates evaluated in the clinical setting have demonstrated lower toxicity and a higher maximum tolerated dose (MTD) compared with free drug but have failed to demonstrate an improvement in efficacy and are therefore no longer being developed [92]. Similarly, CRLX101 showed promise in preclinical animal models and early clinical testing but recently failed to demonstrate improved efficacy as compared with standard of care in renal cell cancer patients [93], though, several clinical studies evaluating the potential of CRLX101 for other indications are ongoing.

3.2.3. Polymer micelles

Another approach is to physically entrap hydrophobic chemotherapeutic drugs inside the core of micelles formed from amphiphilic polymers. The most clinically advanced micelle strategies use A-B type di-block copolymers consisting of hydrophilic PEG chains linked to

hydrophobic polymers based on polyesters, e.g., poly(lactic acid) (PLA [94]), or substituted poly(amino acids), such as poly(L-glutamic acid) [95,96]. PEG-PLA micelle formulations of paclitaxel (Genexol® PM) have been approved for several indications in South Korea and are currently undergoing clinical testing in other territories [80]. Clinical studies with current micelle approaches have demonstrated an improvement in the MTD with certain chemotherapies but have shown only modest survival benefit [94,97].

3.2.4. Other nanocarriers used in nanomedicines

Other notable nanomedicines that are approved, or are at the late stages of clinical development, include chemotherapeutic drugs covalently attached to recombinant proteins (e.g., albumin [68] and antibodies [69]). Such strategies have demonstrated similar safety and efficacy as compared with synthetic nanomedicines but are based on recombinant technologies and are not covered herein. Finally, while a number of inorganic nanocarriers based on gold, iron oxide and silica have been evaluated extensively in the preclinical setting [66], few have entered late stages of clinical testing for chemotherapeutic and/or immunostimulant delivery. Though, the safe use of iron oxide nanoparticles in patients for other applications (i.e. treatment of anemia and as a contrast agent) suggests that further investigation of inorganic nanocarriers is warranted.

3.3. Current nanomedicines are limited by relatively low uptake into tumors

Only modest improvements in safety and efficacy have been observed with current nanomedicines compared with free, unformulated drugs, in the clinical setting [98,99]. One observation to account for this shortcoming is that current nanomedicines typically provide no more than a 2-fold improvement in drug accumulation in tumors as compared with vital organs [100]. To characterize the full extent of this problem (i.e. low tumor accumulation), Wilhelm *et al.* performed a meta-analysis of 117 preclinical studies and found that the median amount of nanocarrier accumulation in tumors is 0.7% of the injected dose [101]. While these observations have challenged assumptions that EPR can be relied upon to uniformly enable passive targeting of nanomedicines to tumors [98], they have also fueled efforts to better understand how nanocarrier properties impact tumor accumulation as a means to enable improved design of nanomedicines. The following sections highlight some of the results of these efforts.

4. Nanocarrier design parameters that impact nanomedicine accumulation in tumors

4.1. Clearance mechanisms and physical barriers reduce nanomedicine accumulation in tumors

Improving nanomedicines requires an understanding of the mechanisms that limit their accumulation in tumors, which include blood clearance mechanisms, physical barriers that impede extravasation and high interstitial pressures within the tumor [102–104]. Clearance mechanisms include passive elimination by the kidneys as well as active uptake of nanomedicines by cells of the reticuloendothelial system (RES) located in the liver and spleen [105–107]. Accumulation of nanomedicines into the tumor can also be blocked by physical barriers, including the occlusion of tumor blood vessels due to the physical stress of rapidly proliferating tumor cells [108]; the relatively low vascular volume [109]; and, the small porosity of basement membrane and extracellular matrix (ECM) coverage that dictate the size limit for particle extravasation into the tumor [110,111]. Results of preclinical studies evaluating how different parameters (e.g., size, charge and shape) of nanomedicines can be modulated to reduce or slow clearance and maximize drug penetration into tumors are discussed below.

4.2. Sizes between 10 and 30 nm diameter enable prolonged circulation and deep tumor penetration

Hydrodynamic diameter is one of the principle factors dictating the kinetics of nanomedicine blood clearance as well as the extent of nanomedicine penetration into tumors [71,112,113]. Indeed, various studies have shown that particles less than ~5 nm diameter are rapidly eliminated from the circulation by the kidney but that tumor penetration is inversely proportional to size [114–117]. Accordingly, Popovic *et al.* showed that ~10 nm particles can effectively extravasate and penetrate tumor tissue, whereas 30 nm particles showed much slower rates of extravasation and 125 nm particles were unable to extravasate [115]. These findings suggest that nanomedicines based on particles between 10 and 30 nm in diameter are optimal for avoiding renal clearance and maximizing tumor tissue penetration, though it should be emphasized that size is only one of several parameters that impact tumor uptake [103].

4.3. Neutral charge prevents non-specific interactions and permits deep tumor penetration

Nanomedicine surface charge is another critical parameter affecting blood half-life and tumor penetration. Nanomedicines comprising positively charged particles are rapidly cleared from the blood due to their non-specific interactions with negatively charged serum proteins, which can result in the formation of large aggregates that are efficiently phagocytized by RES cells [103]. On the other hand, positively charged nanomedicines show high accumulation in tumor vasculature, possibly due to electrostatic interactions with negatively charged proteoglycans of the basement membrane that may be exposed in tumor vessels [118,119]. In contrast, particles with neutral and negative charge show higher blood circulation and overall less accumulation in tumors at a macroscopic level but improved extravasation as compared with cationic materials, with neutral materials limiting non-specific interactions and providing the best balance of tumor accumulation and penetration [118,120–122]. Therefore, nanocarriers used in nanomedicines should be designed with neutral, or near-neutral charge for optimal accumulation in tumors. One means of modulating nanomedicine charge is to assemble particles through electrostatic interactions of oppositely charged materials. Indeed, several self-assembled technology platforms are being developed based on electrostatic interactions of immunostimulatory drugs [123–125], which provides a facile approach for modulating charge to achieve optimal tumor penetration.

4.4. Coating nanomedicines with hydrophilic polymers (e.g., PEG) reduces blood protein binding and clearance by RES cells

Another mechanism that can increase clearance of nanomedicines from the blood is their adsorption or binding of blood proteins (e.g., complement proteins and antibodies) that promote phagocytosis by RES cells. Approaches to modify the surfaces of nanomedicines with hydrophilic polymers, such as PEG, have been developed to reduce blood protein binding and increase circulation time [126,127]. However, the density [128], functional group composition [129] and architecture [130] of the PEG surface coating have all been shown to have an impact on blood circulation time and remain areas of active investigation.

5. Optimizing nanomedicine design to maximize drug accumulation in tumors

5.1. Nanomedicines should be neutral and small, 10–30 nm, diameter, for optimal tumor uptake

The aforementioned studies suggest that nanomedicines should

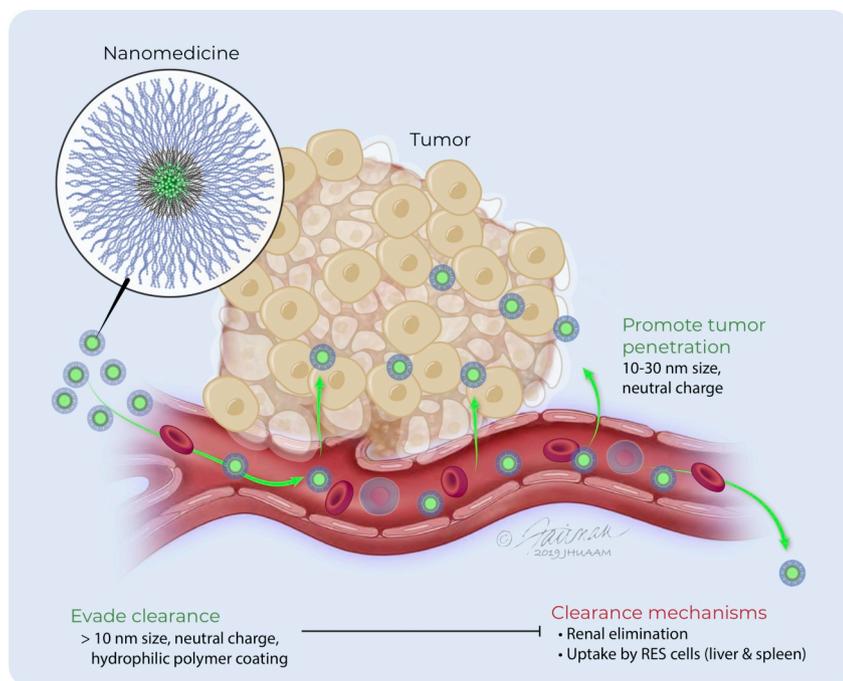


Fig. 4. Nanomedicine properties that impact tumor accumulation. Nanomedicines administered by the intravenous route must evade clearance by the kidneys and avoid capture by cells of the reticuloendothelial system (RES), principally located within the liver, spleen and lungs, to allow for prolonged circulation needed for tumor accumulation. Nanomedicines between 10 and 30 nm diameter with near neutral charge most efficiently extravasate and penetrate tumors.

have a particle size between 10 and 30 nm, near neutral charge and surfaces coated with high densities of hydrophilic polymer chains to maximize drug accumulation in tumors through passive mechanisms [131] (Fig. 4). Two types of nanocarriers suitable for addressing these requirements are described below.

5.2. Micelles offer high drug loading in small particles suitable for tumor targeting

In addition to micelle technologies used in nanomedicines approved for human use (see: section 3.2.3), various types of next generation systems are in preclinical and early stage clinical development. The potential advantages of micelles are that they permit high drug loading and are typically of a small, and tunable size range between 10 and 100 nm that is suitable for tumor accumulation [59]. Moreover, emerging technologies are systematically addressing historic challenges to using micelles. For instance, towards improving micelle stability, Lu *et al.* recently showed that micelles with ultra-low critical micellar concentrations (10^{-6} mM) could be achieved using lipid amphiphiles comprising “superhydrophilic” zwitterionic polymers [132]. To further simplify micelle design and improve drug loading, Shamay *et al.* recently showed that attachment of certain sulfated organic dyes directly to hydrophobic chemotherapeutics can result in an amphiphilic drug that self-assembles into micelles with ultra-high drug loading (up to 90%) [133]. Another way to improve drug molecule loading into the micelle is to covalently attach drug molecules directly to micelle-forming polymers through degradable linkers [134,135]. This allows for greater control over the extent of drug loading as well as the timing and location of drug release, e.g., in response to low pH in the tumor [135]. Amphiphilic materials can also be prepared with different architectures, such as telodendrimers [136], or with stimuli-responsive properties to control the conditions under which micelle formation occurs [137]. Overall, the small size, high drug loading and chemical programmability of micelle systems makes them attractive for use in next-generation nanomedicines.

5.3. Dendrimers and star polymers can be synthesized with size and charge optimal for tumor targeting

An alternative to using amphiphilic molecules that assemble into supramolecular structures is to chemically synthesize single macromolecules of a desirable size. Indeed, advances in coupling chemistry have made it possible to readily access chemically defined dendrimers with sizes > 10 nm diameter [65,138]. While dendrimers alone have been used as nanocarriers for a variety of drug delivery applications [139,140], further modification of dendrimers with the attachment of multiple hydrophilic polymer arms to produce “star polymers” can improve steric shielding and enable greater control over nanomedicine properties (e.g., size, drug loading, charge, etc.) that impact tumor accumulation and treatment efficacy [141] (Fig. 5A).

The most clinically advanced nanomedicine based on star polymers – which is currently in phase II clinical testing – is a PEGylated poly (lysine)-based dendrimer with multiple docetaxel molecules linked to the dendrimer core [142,143]. As an alternative to linking drug molecules to the dendrimer core, Etrych and colleagues have developed star polymers based on multivalent HPMA polymers linked to poly(amidoamine) (PAMAM) and 2,2-bis(hydroxymethyl)propionic acid (bis-MPA) dendrimers that enable attachment of multiple drug molecules to each of the polymer arms through pH-sensitive bonds designed to release drug in the acidic tumor microenvironment, thereby combining passive targeting with selectivity in terms of drug release [144,145]. Anthracycline conjugates of the HPMA-based star polymers (~26 nm, diameter) showed higher tumor accumulation and improved tumor regression as compared with linear polymer-drug conjugates (~9 nm, diameter) (Fig. 5B) [144,146], which suggests that star polymers may be helpful for overcoming limitations, *i.e.* low tumor accumulation, of earlier generation nanomedicines based on hydrophilic, linear polymer-drug conjugates [86,92].

5.4. Nanomedicines based on micelle and star polymer carriers of anthracyclines induce durable anti-cancer T cell immunity

Importantly, many next generation nanomedicines leveraging the aforementioned advances in nanocarrier design, including HPMA-based star polymers [147], have been shown to be highly effective for

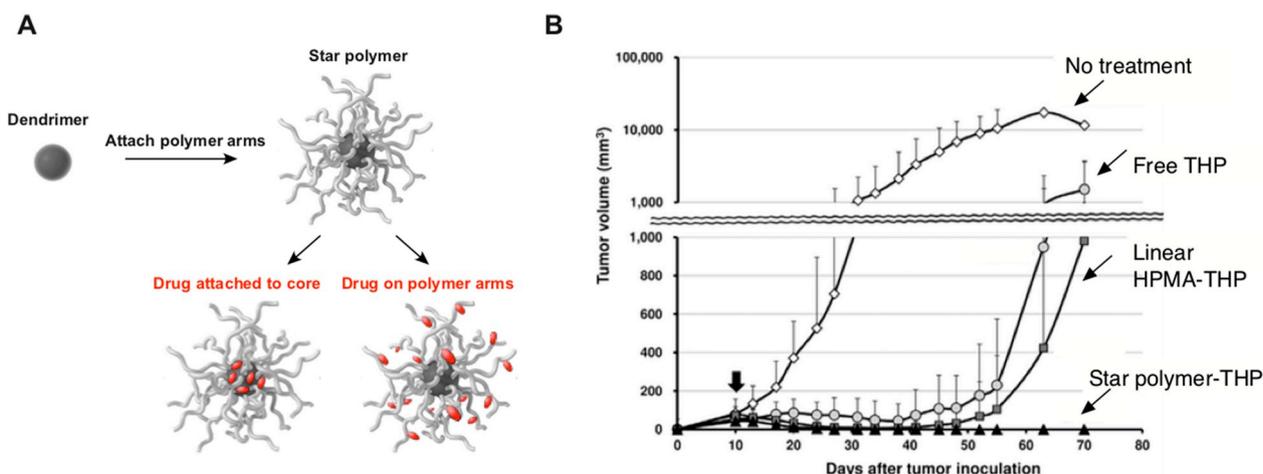


Fig. 5. Nanomedicines based on star polymers mediate durable tumor regression. (A) Star polymers can be prepared by the attachment of multiple hydrophilic polymer arms to dendrimer cores. Drug molecules are attached to the core and/or arms of the star polymer to produce star polymer-based nanomedicines. (B) Mice with established S-180 tumors were treated with 5 mg/kg equivalent dose of either the free anthracycline drug, THP (also referred to as Pirarubicin), THP conjugated to linear HPMA-based polymers (HPMA-THP) or THP conjugated to the arms of star polymers (star polymer-THP). Tumor volume was assessed at various timepoints thereafter. Panel (B) adapted from Ref. [139].

inducing anti-cancer T cell immunity associated with tumor regression. For instance, Mastria *et al.* recently showed that self-assembling nanoparticles based on peptide conjugates of doxorubicin administered intravenously improved tumor regression as compared with doxorubicin alone and that the enhanced efficacy was CD8 T cell dependent [148]. Moreover, while a variety of nanocarriers have been developed for delivering immunostimulants for use in vaccines to induce T cell immunity, many of these same systems can also be used in nanomedicines for cancer treatment via *in situ* vaccination [149–151]. Indeed, Kuai *et al.* recently showed that lipid-based nanodiscs used for vaccination could also be used as chemotherapeutic nanomedicines for inducing T cell immunity that is synergistic with CPIs [152,153]. These findings substantiate that nanomedicines optimized for tumor accumulation can be highly effective for mediating tumor clearance through the induction of durable anti-cancer T cell immunity.

6. Using host factors to improve nanomedicine accumulation into tumors and enhance T cell immunity

6.1. Leveraging the tumor microenvironment and host physiology to improve nanomedicine accumulation in tumors

A variety of strategies have been developed for improving nanocarrier accumulation into tumors through programmed responsiveness of the nanocarrier to properties that distinguish tumor tissue from normal tissue, such as pH [154], expression of certain enzymes [155] and hypoxia [156]. Targeting molecules, such as antibodies, aptamers or natural ligands of receptors that bind tumor-specific molecules (e.g., HER2) can also be used to trap materials in the tumor to improve accumulation and have shown some benefit for certain patients [157]. Nanomedicine accumulation into tumors can also be improved by altering host physiology [158]. Strategies include the reduction of nanocarrier blood clearance mechanisms (e.g., liver Kupffer cell depletion [107]) as well as the modulation of tumor blood flow and vessel leakiness [159–161]. Such strategies are orthogonal to – and therefore should be pursued in parallel with – approaches for improving the physical properties (e.g., size, charge, surface properties) of nanomedicines to improve drug accumulation in tumors.

6.2. Conditioning lymph nodes to enhance priming of memory T cells

Lymph nodes draining tumor tissue are sources of tumor antigens

and can be targeted with immunostimulants to promote the priming and expansion of T cell immunity [162,163]. As the conditions under which T cells are primed can impact the generation of memory T cells, which provide durable anti-cancer immunity needed to prevent relapse [164,165], it may also be beneficial to condition tumor-draining lymph nodes to modulate the quality of T cell responses primed [166]. In this regard, our lab recently showed that lymph node conditioning with microparticles carrying the immunomodulator rapamycin could alter the phenotypic quality of T cells generated against a melanoma antigen, and depending on the concentration of rapamycin, recently primed T cells could be directed towards a central memory, rather than effector, phenotype [167,168]. These findings suggest that host conditioning of the tumor microenvironment and/or tumor draining lymph nodes [169] may be an effective strategy for eliciting high magnitude and quality (*i.e.* memory phenotype) anti-cancer T cell responses needed for clearing large tumors [170]. Though, additional studies will be needed to explore how best to combine systemically administered nanomedicines with host lymph node conditioning strategies.

7. Uptake of nanomedicines by phagocytic cells for the selective depletion and/or phenotypic conversion of suppressors cells

While many nanomedicines are designed to evade capture by phagocytic cells of myeloid lineage, immunotherapy is a setting where preferential targeting of myeloid lineage cells, such as MDSCs, may be beneficial. For instance, Jeanbart *et al.* showed that pluronic-stabilized poly(propylene sulfide) (PPS) micelles carrying the cytotoxic drug 6-thioguanine could effectively deplete MDSCs in tumors as well as the periphery and that such depletion was associated with enhanced T cell mediated tumor clearance [171]. Rather than targeting their depletion, Rodell *et al.* recently showed that a TLR-7 agonist formulated in cross-linked cyclodextrin-based nanoparticles could be used to convert tumor-associated macrophages from a suppressive M2 to an anti-tumorigenic, M1-like phenotype [172]. It is also worth noting that a major focus of the vaccine delivery field has been to develop nanocarriers capable of targeting different phagocytic populations in lymph nodes, spleen and liver, and thus lessons from this work may be useful in the design of nanomedicines for targeting phagocytic cells for cancer treatment [151,173–178].

8. Future outlook and concluding remarks

While nanomedicines based on a broad range of chemotherapeutic and immunostimulant drugs have shown promise for mediating tumor regression through the induction of anti-cancer T cell immunity, low drug accumulation in tumors remains a significant challenge limiting the efficacy of such approaches. However, there is reason to be optimistic. Recent data suggests that doses of chemotherapeutic and immunostimulant drugs required for inducing anti-cancer T cells are lower than those required for tumor ablation [179,180]. Indeed, Sivick *et al.* recently showed that while high doses of a STING agonist immunostimulant ablated tumors, lower doses could be used to induce durable T cell immunity [180]. These findings – combined with the aforementioned improvements in nanomedicine design (see: section 5) – give reason to be optimistic that next generation nanomedicines will be able to reliably achieve sufficient drug concentrations in tumors needed for inducing anti-cancer T cell immunity while minimizing systemic drug exposure associated with toxicity. Optimizing the therapeutic potential of such next generation nanomedicines, though, will likely require combination with complementary therapies to unleash the full potential of T cells. Therefore, future studies should evaluate immunostimulant and/or chemotherapeutic nanomedicines alone and in combination with other therapies, particularly those that augment the action of T cells (*e.g.*, CPIs, adenosine receptor antagonists, anti-tumor antibodies, cytokines, oncolytic viruses, *etc.*), to identify optimal combinations that maximize treatment efficacy. In conclusion, the tremendous promise of systemically administrable nanomedicines for improving the safety and efficacy of immunotherapies warrants vigorous development by industry and the scientific community as well as continued support of these activities by funding agencies.

Author contributions

All authors contributed to writing and revising the manuscript.

Competing financial interests

GML is an employee and shareholder of Avidea Technologies, Inc., which is actively developing nanomedicines for cancer treatment. RL is a shareholder and advisor to Avidea Technologies, Inc. CMJ is an employee of the VA Maryland Health Care System. The views reported in this paper do not reflect the views of the Department of Veterans Affairs or the United States Government. CMJ has an equity position in Cellth Systems, LLC which is developing cancer diagnostic technologies.

Acknowledgements

This work was supported in part by the NIH (Award #R01EB027143), the Damon Runyon Cancer Research Foundation (Award # DRR3415), the United States Department of Veterans Affairs (Award # 1I01BX003690), the Czech Science Foundation (project 19-08176S) and the Ministry of Education, Youth and Sports of the Czech Republic within the National Sustainability Program II (project BIOCEV-FARLQ1604).

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