Advanced Drug Delivery Reviews 141 (2019) 3–22

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Addressing barriers to effective cancer immunotherapy with nanotechnology: achievements, challenges, and roadmap to the next generation of nanoimmunotherapeutics

Enping Hong, Marina A. Dobrovolskaia *

Nanotechnology Characterization Laboratory, Cancer Research Technology Program, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD 21702

ARTICLE INFO

Article history:
Received 5 October 2017
Received in revised form 18 December 2017
Accepted 11 January 2018
Available online 12 January 2018

Keywords:
Nanoparticles
Vaccines
Adjuvant
Cell therapy
Drug delivery

ABSTRACT

Cancer is a complex systemic disorder that affects many organs and tissues and arises from the altered function of multiple cellular and molecular mechanisms. One of the systems malfunctioning in cancer is the immune system. Restoring and improving the ability of the immune system to effectively recognize and eradicate cancer is the main focus of immunotherapy, a topic which has garnered recent and significant interest. The initial excitement about immunotherapy, however, has been challenged by its limited efficacy in certain patient populations and the development of adverse effects such as therapeutic resistance and autoimmunity. At the same time, a number of advances in the field of nanotechnology have sought to address the challenges faced by modern immunotherapeutics and allow these therapeutic strategies to realize their full potential. This endeavour requires an understanding of not only the immunological barriers in cancer but also the mechanisms by which modern technologies and immunotherapeutics modulate the function of the immune system. Herein, we summarize the major barriers relevant to cancer immunotherapy and review current progress in addressing these obstacles using various approaches and clinically approved therapies. We then discuss the remaining challenges and how they can be addressed by nanotechnology. We lay out translational considerations relevant to the therapies described and propose a framework for the development of next-generation nanotechnology-enabled immunotherapies.

Contents

* Corresponding author at: Frederick National Laboratory for Cancer Research, P.O. Box B, 1050 Boyles Street, Frederick, MD 21702-1201, USA.
E-mail address: marina@mail.nih.gov (M.A. Dobrovolskaia).

https://doi.org/10.1016/j.addr.2018.01.005
0169-409X/

1. Introduction

A successful anti-tumor immune response consists of several components. First, tumor-associated antigens (TAAs) must be presented to effector immune cells by antigen-presenting cells (APCs), which activate the effector cells and prepare them for an immune response. Second, effector immune cells must make their way to the tumor, navigate the tumor vasculature, and penetrate the tumor tissue. Finally, these effector cells must bind to and recognize the TAAs on the tumor surface and kill the tumor cells presenting these antigens. This process is cyclical, wherein cancer cell death can release more antigens for processing by APCs and activate effector cells, thereby enhancing subsequent anti-tumor activity [1]. The anti-tumor immune response, however, is dysfunctional in cancer. Tumors can induce immune tolerance by avoiding or blocking the immune system from recognizing TAAs, or by actively suppressing activated effector T cells and natural killer (NK) cells to prevent them from eliminating tumor cells [2, 3]. Cancer immunotherapy aims at restoring the proper function of the anti-tumor immune response by overcoming mechanisms of tumor immune evasion or suppression. Recent advances in immunotherapy have invigorated the field by identifying and addressing the mechanisms by which tumors avoid being cleared by the immune system. These recent clinical successes and the approval of several promising immunotherapeutic drugs have spurred substantial scientific and public interest in the field. However, the clinical application of cancer immunotherapy still faces an array of challenges, including the lack of response or development of resistance to immunotherapy, toxicities associated with therapies such as cytokine or CAR-T cell infusion, and the limited efficacy of approaches such as cancer vaccines [4–7]. New generations of immunotherapies will have to address these challenges to achieve broader applicability and better patient outcomes.

Nanotechnology offers unique opportunities to tackle the clinical challenges faced by current immunotherapies. Depending on their physicochemical characteristics, nanoparticles can alter the pharmacokinetics, biodistribution, and activity of the therapeutic agents that are loaded within them. These properties allow investigators to engineer specific nanotechnology-enabled strategies for improving the therapeutic efficacy of anti-cancer drugs. Several nanoparticle drugs have already been approved for cancer therapy, such as liposomal doxorubicin (Doxil), albumin-bound paclitaxel (Abraxane), and liposomal irinotecan (Onivyde). In recent years, nanotechnology-enabled strategies for immunotherapy have been extensively investigated, yielding new strategies and insights for the development of more effective next-generation immunotherapies.

In this review, we summarize the barriers facing modern immunotherapy and examine the general cellular and biochemical barriers of the tumor microenviroment, the ways they are addressed by modern immunotherapies, and the specific barriers facing particular immunotherapeutic approaches. We also discuss how nanotechnology can directly address the general barriers to generating anti-tumor immunity and help overcome the specific barriers that limit current immunotherapies. We summarize translational considerations and lessons learned

Box 1
Barriers to effective immunotherapy.

Problems	Mechanisms/barriers	Selected references
Lack of tumor antigen processing and presentation	– Downregulation or loss of class I MHC – Disruption of antigen-processing machinery	[10]
Disabling of antigen-presenting cells in the tumor microenvironment	– Prevention of DC maturation using immunosuppressive mediators, e.g. VEGF – Induction of DC apoptosis	[38, 39]
Anergy and ineffectiveness of infiltrating immune cells	– Lack of expression of positive costimulatory ligands, e.g. CD80 (B7-1) and CD86 (B7-2) – Expression of negative costimulatory ligands, e.g. PD-L1, and upregulation thereof by immune mechanisms, e.g. IFN-γ – Generation of immunosuppressive cytokines and modulators in the tumor microenvironment, e.g. TGF-β, IL-10, IDO, PGE2 – Recruitment of immunosuppressive cell types to the tumor microenvironment, e.g. Tregs, MDSCs, M2 macrophages – Direct killing of T cells via Fas/FasL interactions	[2, 3, 22, 42, 43, 46]
Difficulty in transport and retention of drugs/nutrients in the tumor	– Elevated interstitial fluid pressure compresses and collapses tumor microvessels – High pressure gradient favors leakage of nutrients, drugs, and cells out of the tumor – Inefficient transport of nutrients leads to hypoxic regions and buildup of metabolic waste products	[49–51]
Inhibited trafficking and infiltration of immune cells to the tumor site	– Mismatch between chemokines produced from tumors and receptors expressed on immune cells – Downregulation or aberrant modification of chemokines – Tumor-associated ECs express low levels of adhesion molecules to inhibit T-cell transmigration and instead favor Treg trafficking – Expression of inhibitory molecules by ECs (e.g. PD-L1, FasL, TGF-β)	[30, 55–57]

from the various components of nanotechnology-enabled immunotherapies, including conventional immunotherapies, nucleic acids, nanotechnology, and biotechnology-based therapeutics. Finally, we lay out a framework for the development of the next generation of nanotechnology-enabled immunotherapies.

2. Barriers to effective anti-tumor immunotherapy

The interactions between cancer cells and their surrounding stroma and immune cells create an immunosuppressive network that inhibits anti-tumor immunity and allows cancer cells to flourish [8, 9]. For the purpose of this review, we separate the mechanisms of tumor immune evasion into three categories: biochemical, cellular, and vascular. These mechanisms and the barriers they create are summarized in Box 1 and reviewed further below.

2.1. Biochemical mechanisms of tumor immune escape

2.1.1. Dysfunction of antigen-processing machinery

Tumor cells have been observed disrupting the cellular machinery required for the transportation and processing of tumor antigens, particularly the downregulation or total loss of class I major histocompatibility complex (MHC) molecules needed for presenting these antigens [10]. Defects in the antigen-processing machinery (APM) include a downregulation of proteasome subunits [11, 12] needed for peptide generation from longer protein fragments. Other alterations involve transport proteins (TAP1 and TAP2) and processing proteins, which deliver peptides into the ER [13] and process proteins within the ER, respectively. Protein processing within the ER optimally trims and loads peptides onto class I MHC [14]. Alterations in these processes affect both antigen processing and presentation. The total loss of class I MHC is frequently observed in cancer, with loss of heterozygosity appearing to be the most common causal mechanism [15, 16]. Without proper presentation of TAAs by class I MHC, tumors can avoid recognition and lysis by cytotoxic T cells (CTLs). Class I MHC loss can render tumors sensitive to attack by NK cells and may lead to better prognosis [17]. However, evidence also exists that the abnormal release of class I MHC, either in soluble or exosome-bound form, may instead contribute to reduced sensitivity to killing by NK cells [18, 19].

2.1.2. Inhibitory ligands for the control of T-cell function

Tumor cells and their surrounding microenvironment can directly inhibit T-cell function by both contact-dependent and contact-independent mechanisms. Programmed cell death protein-1 (PD-1) is an inhibitory receptor on the surface of T cells that regulates T-cell exhaustion, and its engagement causes T cells to undergo loss of effector function regarding proliferation, cytotoxicity, and cytokine production [20, 21]. Some tumors can selectively upregulate programmed death ligand-1 (PD-L1), the ligand to PD-1 on T cells, in response to the effector cytokine interferon-γ (IFN-γ). Tumors are therefore able to hijack the PD-1 signaling pathway when they detect effector immune cell activity, strengthening their defense against immune attack [22]. Regulatory T cells (Tregs), a class of immunosuppressive cells, express high levels of the checkpoint inhibitor cytotoxic T-lymphocyte antigen-4 (CTLA-4), which competes with costimulatory ligands to dampen the immune response after T-cell activation. Treg-specific CTLA-4 deficiency was shown to promote anti-tumor immunity, demonstrating its involvement in suppressing the anti-tumor immune response [23]. Tumor cells can also directly kill T cells using the interaction between the death receptor Fas and its ligand FasL, either by the expression of FasL on tumor cells [24, 25] or tumor-derived FasL-bearing exosomes [26].

2.1.3. Immunosuppressive cytokines and mediators

The tumor and surrounding stroma also produce immunosuppressive cytokines and molecules that inhibit immune function without cell–cell contact. Signaling proteins such as transforming growth factor-β (TGF-β) and interleukin-10 (IL-10) are pleiotropic cytokines, able to induce a host of immunomodulatory effects, and they can suppress anti-tumor immunity through multiple mechanisms. TGF-β is expressed at high levels in many different tumors. It suppresses the effector functions of multiple immune cell types, including cytotoxicity and cytokine production by both T cells and NK cells, the proliferation of T cells, and the presentation of antigen by dendritic cells (DCs) (reviewed in [27]). IL-10 is a key regulator of inflammation that acts to restrain the immune response and prevent excessive damage [28]. In the tumor microenvironment, IL-10 production has been observed in tumor cells, T cells, and macrophages. Its actions also include downregulating tumor antigen processing and presentation, suppressing DC and T-cell function, and upregulating the checkpoint inhibitor ligand CTLA-4 on T cells (reviewed in [29]). Tumors can also disrupt chemokine signaling by aberrantly altering these signaling proteins or suppressing their production, impairing proper chemokine function and, consequently, inhibiting the homing of cytotoxic T cells to the tumor [30].

Several molecular metabolites also play immunosuppressive roles in the tumor microenvironment. Tryptophan metabolites produced by indoleamine 2,3-dioxygenase (IDO) are known to suppress T-cell and NK-cell function; in several tumor types, the expression of IDO in the tumor microenvironment and tumor-draining lymph nodes has been associated with poor prognosis (reviewed in [31]). Prostaglandin E2 (PGE_2), a metabolite of arachidonic acid and a product of the COX enzyme, is an active promoter of tumor inflammation and growth. It does so through several mechanisms. PGE_2 downregulates the chemokines MIP-1α and MIP-1β in macrophages and DCs as well as IL-8, IP-10, and MCP-1 in macrophages [32, 33]. These chemokines are essential for macrophage and lymphocyte trafficking during inflammation. It suppresses the activity of cytotoxic NK cells and $CD8^+$ T cells and downregulates Th1 cytokines (IL-2, IFN-γ, TNF-α), which favor tumor destruction [34, 35]. Finally, it favors the development of suppressive cells such as Tregs and myeloid-derived suppressor cells (MDSCs) [34]. Hence, tumors are able to mediate immunosuppression through a variety of soluble mediators, including cytokines, chemokines, and small molecular species.

2.2. Cellular mechanisms of tumor immune escape

Inherent to their genetic instability, transformed cells express abnormal proteins and peptides that can be recognized and eliminated by TAA-specific immune cells [36]. These TAA-specific T cells are primed by DCs, a class of APC highly specialized for antigen presentation and T-cell activation, which typically activate naïve antigen-inexperienced T cells in the periphery. Tumor-infiltrating T cells can then recognize antigens on the surface of tumor cells and target them for cytolysis. Within the tumor microenvironment, however, this interaction is rendered dysfunctional by several different mechanisms.

2.2.1. Dysregulation of DC function

Tumors have been shown to disrupt the trafficking and function of DCs in their microenvironment. DCs within the tumor microenvironment are frequently found to be dysfunctional; chronic inflammation and immunosuppressive signals result in poorly differentiated and immature DCs, which tend to be non-stimulatory or even tolerogenic, i.e., able to produce immune tolerance against the tumor [37, 38]. Tumor cells can induce apoptosis of DCs both *in vitro* and *in vivo*, thereby preventing these DCs from provoking an immune response [39]. Thus, in some cases, the DC infiltration of tumors can be used as an indicator of cancer progression as well as patient prognosis and survival [40, 41]. The function of DCs is therefore dysregulated through multiple mechanisms within the tumor microenvironment, preventing them from effectively activating cytotoxic T cells and coordinating an effective immune response.

2.2.2. Immunosuppressive cell populations

The tumor microenvironment contains a variety of immunosuppressive cell types. $CD4^+CD25^+FoxP3^+$ Tregs can suppress anti-tumor immune responses through the secretion of cytokines such as IL-10 and TGF-β and the presentation of inhibitory ligands such as CTLA-4, and they can inhibit and even kill DCs and other effector T cells and potentially compete with surrounding effector T cells for the immune-activating cytokine interleukin-2 (reviewed in [42]). MDSCs are immature but activated myeloid cells that are elevated in the bloodstream and tumors of cancer patients. They inhibit T-cell recruitment and activity through a variety of immunosuppressive mediators, including the production of nitric oxide, prostaglandin E2, and TGF-β (reviewed in [43]). Plasmacytoid DCs, a subset of DCs that typically secretes large amounts of inflammatory interferons in response to viral infection, can be subverted by the tumor microenvironment to become immunosuppressive or tolerogenic [44]. These cells have been observed to stimulate Treg activity in ovarian cancer patients [45]. Tumor-associated M2 macrophages have been implicated in a wide range of pro-tumorigenic activity, including the suppression of T-cell activity through the action of PD-L1, IL-10, and TGF-β; by recruiting Tregs; and by promoting tumor angiogenesis and metastasis (reviewed in [46]). The presence of these cells in the tumor and stroma create a strongly unfavorable environment for immune effector cell function.

2.3. Vascular

Tumor growth is sustained by rapid neovascularization and angiogenesis, resulting in a disorganized and malformed tumor vascular structure that differs significantly from physiologic vasculature [47]. A key characteristic of tumor blood vessels is that they are 'leaky' due to a loose association of pericytes and substantial gaps, up to 2 μm in diameter, between their endothelial cells. This architecture is attributed to the overproduction of vascular endothelial growth factor (VEGF) during tumor angiogenesis [48]. Due to solid stress on the tumor mass from both intratumoral growth and surrounding tumor tissue, blood and lymphatic vessels within the tumor are compressed. This condition results in elevated intratumoral interstitial fluid pressure (reviewed by Jain, Martin and Stylianopoulos [49]). Under conditions of sufficiently high interstitial fluid pressures, microvessels within the tumor can collapse, abolishing the transvascular delivery of bloodborne nutrients and drugs [50]. In addition, the high pressure gradient from the tumor to its periphery favors the leakage of nutrients, drugs, and cells out of the tumor [51, 52]. Overall, this leads to an uneven distribution of oxygen and nutrients within the tumor, which is accompanied by the formation of hypoxic regions and the buildup of metabolic waste products.

The dysregulated tumor vasculature contributes to tumor-mediated immune tolerance through the actions driven by VEGF, hypoxia, and the tumor endothelium [30]. VEGF is known to inhibit DC maturation and antigen presentation, and it upregulates PD-L1 expression on DCs [53]. Hypoxia is itself a driver of VEGF secretion by tumor cells and M2 macrophages, a mechanism intended to restore blood flow to oxygen-starved tissues, and it participates in immunosuppression through a variety of mechanisms. For example, it upregulates the expression of COX2 and production of PGE_2. Hypoxia also promotes TGF-β secretion by M2 macrophages and increases the expression of PD-L1 on cancer cells and CD86 (a ligand for CTLA-4) on DCs. It also inhibits the interaction between tumor cells and the costimulatory receptor NKG2D on NK cells and T cells (reviewed in [54]). Finally, the tumor endothelium also acts to significantly impair immune cell transport. Tumor-associated endothelial cells (ECs) express low levels of critical adhesion molecules such as E-selectin, ICAM1/2, and VCAM1, and they upregulate receptors that allow the transmigration of Tregs and other immunosuppressive populations (reviewed in [55]). These ECs also upregulate inhibitory molecules such as PD-L1, TGF-β, and the death ligand FasL, inducing the suppression or killing of cytotoxic cells [56–58]. The tumor vasculature therefore provides a highly unfavorable environment for the efficient penetration of drugs and immune cells and contributes to the suppression of anti-tumor immunity.

3. Addressing barriers with conventional immunotherapies

The current breakthrough successes in the field of cancer immunotherapy have built on sustained efforts to identify and dismantle barriers to effective anti-cancer immune responses. Herein, we review current progress in both clinical and preclinical immunotherapies, discussing how each of these therapies addresses the barriers we discussed earlier (Box 2), as well as the challenges that still remain (Box 3).

3.1. Cytokine therapy

Since the late 1980s, two cytokines—interleukin-2 (IL-2) and interferon alpha (IFN-α)—have been used as biologic drugs for activating the immune system to treat cancer. High-dose IL-2 cytokine therapy is currently used to treat metastatic melanoma and metastatic renal cell carcinoma [59]. Where tumor-infiltrating immune cells are hampered by tumor-induced anergy and disrupted trafficking, IL-2 activates and expands cytotoxic T cells and NK cells while maintaining their function, leading development of IL-2 as the first purely immunologic strategy that could cause tumor regression [60]. In clinical trials for metastatic melanoma and renal cell carcinoma, high-dose IL-2 induced overall responses in about 15% of patients [61, 62], along with long-term durable

Box 2
How modern immunotherapies address the barriers.

Barriers	Immunotherapeutic strategies for addressing barriers	Selected references
Lack of tumor antigen processing and presentation	– Enhance antigen presentation by tumor cells and increase DC cross-presentation of antigen, e.g. IFN-α – Engineer T cells that recognize a completely different antigen, e.g. CAR CD19-specific CAR-T cells – *In vivo* lysing of tumor cells and release of tumor antigens, e.g. oncolytic virotherapy (T-VEC)	[66, 90, 116]
Inhibited trafficking of immune cells to the tumor site	– Expand TILs *ex vivo* and re-infuse into the patient, e.g. adoptive TIL therapy – Expansion of T cells *in vivo*, e.g. IL-2 cytokine therapy	[60, 82]
Disabling of antigen-presenting cells in the tumor microenvironment	– Enhanced DC function, maturation, capacity to stimulate effector T cells, e.g. IFN-α therapy – Adoptive transfer of autologous DCs stimulated *ex vivo*, e.g. Sipuleucel-T – Stimulation of tumor-resident DCs by immunogenic cell death, as well as DC priming through virus-mediated GM-CSF expression, e.g. oncolytic T cell virus (T-VEC)	[66, 112, 116]
Anergy and ineffectiveness of infiltrating immune cells	– Nonspecific expansion and activation of T cells to attack the tumor, e.g. IL-2 therapy – Blockade of negative costimulatory molecules CTLA-4 and PD-1 to restore effector T-cell function, e.g. checkpoint inhibitors Yervoy (ipilimumab), Opdivo (nivolumab), and Keytruda (pembrolizumab) – Couple adoptive therapy with radioablative therapy to remove immunosuppressive Tregs and boost the efficacy of transferred TILs	[60, 82, 98, 102]
Difficulty in transport and retention of drugs/nutrients in the tumor	– Not addressed, although vascular normalization by treatment with VEGF has been demonstrated to improve drug delivery in preclinical models	[49]

Box 3
Challenges encountered by modern immunotherapies.

Immunotherapy	Challenges	Contributing factors	Selected references
Cytokine therapy	– Systemic toxicities and limited efficacy – Immunogenicity is a common safety issue	– Low serum half-lives of many cytokines necessitate high doses, leading to toxicities and off-target effects	[7, 71, 77, 80]
CAR-T cell therapy	– Cytokine release syndrome – Limited number of antigenic targets – Permanent loss of targeted cell population – Labor-intensive culture process	– Rapid expansion of infused CAR-T cells – Other promising tumor targets (e.g. ErbB2) are expressed at low levels on non-tumor tissues, and have caused patient deaths in clinical trials – CD19-targeting CAR-T cells can lead to B cell aplasia, requiring additional care	[4]
Adoptive TIL therapy	– Limited accessibility – Multiple rounds of expansion required – TILs have difficulty persisting after adoptive transfer – Difficulty in identifying antigen-specific T cells in cancer types besides melanoma	– Additional expansion methods (such as aAPCs) may be needed for more effective therapy – Limited number and immunosuppressed phenotype of isolated cells, inter-donor variability – Transferred TILs may be suppressed upon re-entering the tumor micro-environment – Persistence may be linked to telomere length, necessitating shorter times allowed for *ex vivo* expansion	[85]
Checkpoint inhibitor blockade	– Immune-related adverse events associated with treatment include colitis, arthritis, diabetes – Resistance to checkpoint inhibition has been observed	– On-target, off-tumor blockade induces autoimmune responses to therapy, which may result in treatment termination – Resistance possibly develops through treatment-induced selection of tumor cells expressing alternative immune checkpoints	[107–110]
Cancer vaccines	– Limited efficacy – Labor-intensive culture process for cellular vaccines	– Likely due to additional unaddressed barriers, e.g. tumor immunosuppression	[111, 113]

responses in some of those responders. IFN-α is approved for clinical use in the U.S. for several types of cancer, including hairy cell leukemia, AIDS-related Kaposi's sarcoma, melanoma after surgical resection, and renal cell carcinoma in combination with Avastin [63–65]. While IFN-α directly inhibits the proliferation and function of tumor cells, it also promotes a range of immune effects that favor anti-tumor immunity [66]. IFN-α treatment has been shown to increase TAA expression on tumor cells [67]. It also induces DC maturation and stimulates the cross-priming of cytotoxic T cells by DCs [68]. The pleiotropic nature of both IL-2 and IFN-α thus enables them to address tumor immunosuppression with multiple mechanisms.

Inefficient delivery remains a key drawback to cytokine therapy. Both IL-2 and IFN-α have short half-lives in circulation, necessitating high-dose infusions that cause significant systemic toxicities. For example, IL-2 may cause pulmonary edema and a potentially fatal capillary leak syndrome that necessitates careful patient management during therapy [7]. It may also contribute to the development of immunosuppression via regulatory T cells [69, 70]. IFN-α therapy is often associated with hematologic adverse events such as leukopenia and myelosuppression, liver toxicities, development of autoimmunity, and depression [71]. For patients with metastatic melanoma, adjuvant IFN treatment resulted in reduced health-related quality of life due to the side effects that followed [72]. Longer-circulating versions of these cytokines have thus been investigated to improve therapeutic efficacy while reducing side effects and off-target toxicities. Although PEGylated IFN-α2b was recently approved for treating melanoma patients after surgery [63], patients still showed a significant reduction in quality of life [73]. A clinical trial that followed high-dose IL-2 with the chronic maintenance of PEGylated IL-2 did not show any improved clinical benefit [74]. Another obstacle with cytokine therapy is the immunogenicity that may result. In some patients, repeated administration of therapeutic proteins may break the immune system's tolerance to self-antigens and result in the generation of antibodies that neutralize both the native protein and its therapeutic counterpart [75]. PEGylation helped to reduce the problem but did not overcome it, and the immunogenicity of therapeutic proteins and their PEGylated derivatives remains a significant hurdle [75]. For example, the development of antibodies to either IFN-α2a or IFN-α2b was reported in patients with hepatitis and neoplastic disease [76, 77]. Moreover, the antibodies generated in response to therapeutic IFN-α2a or IFN-α2b were cross-neutralizing for both products, their PEGylated versions, and native IFN-α1 [78, 79]. Similar challenges affecting pharmacokinetics, safety, and efficacy have been reported for recombinant IL-2 [80, 81].

3.2. Adoptive T-cell immunotherapy

The use of tumor-infiltrating lymphocytes (TILs) for adoptive immunotherapy, pioneered by Rosenberg and colleagues at the National Cancer Institute, presents a therapeutic strategy that addresses multiple immunologic barriers to effective therapy. TILs are lymphocytes that have already recognized and infiltrated the tumor but are suppressed and inactive. In this therapy, TILs are extracted from patient tumors, expanded *ex vivo* using cytokines and other stimuli, and re-infused into the patient, thus directly addressing the barrier of limited tumor-infiltrating effector cells [82]. Patients also undergo lymphodepletive chemotherapy and radiation regimens before TIL infusion, which removes endogenous lymphocytes that can compete with either infused TILs for activating cytokines or populations such as Tregs, which act to suppress anti-tumor immunity. These efforts have so far resulted in response rates of 50% or more in some clinical trials [83, 84].

The personalized nature of adoptive TIL therapy contributes to a number of challenges and complications. First, the therapy is not yet widely applicable. Second, patients must be able to endure a number of procedures, including tumor resection, lymphodepletive regimens, and IL-2 infusion [85]. Third, despite the ability to expand viable TILs, not all tumor samples yield immunologically active T cells [86]. TILs are usually grown with multiple rounds of expansion, and the data from responding and non-responding patients suggest that TILs with longer telomeres persist longer *in vivo* [87]. While *in vivo* persistence alone is insufficient to predict efficacy, TIL telomere length is being examined as a metric for identifying viable T cells, and methods for more rapidly culturing TILs are also being investigated. Finally, adoptive TIL therapy has so far only been widely applied to melanoma, while TILs capable of lysing autologous tumor cells are difficult to obtain from other tumor histologies [88]. Nevertheless, a patient with cholangiocarcinoma was successfully treated using a TIL approach after a tumor-reactive Th1 $CD4^+$ cell population was identified via exome sequencing [89]. Efforts remain underway to apply the successes of TIL therapy in melanoma to other cancer types.

3.3. Chimeric antigen receptor (CAR)-T cell therapy

The use of CAR-T cells for immunotherapy, particularly in the treatment of B-cell acute lymphocytic leukemia (ALL), is a recent advance in immunotherapy utilizing genetically modified T cells. In this therapy, T cells from patients are modified with chimeric antigen receptors

comprised of an antibody-derived targeting ligand that is fused to T cell receptor (TCR) signaling machinery, with next-generation CAR-T cells incorporating multiple signaling domains and additional costimulatory ligands [90]. This class of therapies entirely bypasses the barrier of dysregulated tumor antigen processing and presentation, as the antibody–antigen interaction is completely independent of MHC-restricted antigen recognition. CAR-T cells can thus eliminate cancerous B cells that have impaired or nonexistent MHC I expression, due to their recognition of the B-cell lineage marker CD19. In clinical trials, CAR-T cells targeting CD19 have achieved response rates of over 50–80% in refractory lymphoma [90], and the same technique has also been used for NY-ESO-1-targeting CAR-T cells to treat multiple myeloma [91] and synovial cell carcinoma [92].

Several obstacles hamper the widespread clinical application of CAR-T cell therapy. The activation and expansion of infused T cells cause a cytokine release syndrome (CRS) in patients, resulting in fever soon after infusion, organ dysfunction, neurologic symptoms, and other toxicities [4], with severe cases frequently requiring intensive medical intervention. CAR-T therapy–associated CRS is currently managed by corticosteroids and IL-6 receptor blockade by tocilizumab, which has had good results in the clinic. However, prolonged use of corticosteroids may reduce the effectiveness of the transferred T cells, as evidenced by patients who are treated with high-dose steroids and subsequently relapse despite previously achieving complete remission [93]. A second challenge is the selection of an appropriate antigenic target, as CAR-T cells can recognize and attack healthy cells expressing the target antigen (on-target/off-tumor recognition). In the case of CD19-specific CAR-T cells, the major on-target toxicity is B cell aplasia and resultant hypogammaglobulinemia [94]. CD19 was selected as a clinical target in part because these toxicities are medically manageable through immunoglobulin replacement therapy [94]. However, despite the ability to mitigate most infectious complications of B-cell aplasia by this approach, concerns still remain regarding potential long-term toxicities. CAR-T cells targeting other antigens have led to severe autoimmunity [95] and even death [96]. Finally, CAR-T cells have struggled to effectively treat solid tumors, likely due to the multiple mechanisms of immunosuppression common in the tumor microenvironment [97].

3.4. Checkpoint inhibitors

Immune checkpoint inhibitor therapies represent another recent advance in immunotherapy, the most successful of which have been antibodies directed against the T cell inhibitory ligands CTLA-4 and PD-1. As CTLA-4 and PD-1 serve to dampen the immune response, these antibodies enhance endogenous anti-tumor immune responses initiated by infiltrating cytotoxic T cells. Therapeutic CTLA-4 blockade raises the threshold for T-cell inhibition and prolongs T-cell activation [98], while PD-1 blockade prevents the exhaustion of T cells triggered by PD-L1 ligation. An antibody blocking PD-L1 was also recently approved for the treatment of metastatic Merkel cell carcinoma [99]. The application of checkpoint inhibitors in the clinic has resulted in long-term durable responses in patients with a variety of cancers, with median response durations of 1–3 years noted in objective responders [100]. Efforts are already underway to improve the clinical benefits demonstrated by checkpoint inhibitors. For example, the combination of both anti-CTLA-4 and anti-PD-1 is being considered because they act through non-overlapping immune pathways. Recent clinical reports have indicated overall response rates of the combination are around 40% for melanoma and renal cell carcinoma, with lower response rates observed for non-small cell lung cancer [101]. Currently, there is also significant interest in combining checkpoint inhibitors with a range of immunotherapeutic strategies, including adoptive cell therapy [102, 103], targeted therapies [104], and cancer vaccines [105], due to the ability of checkpoint inhibitors to amplify immune responses by inhibiting mechanisms of tumor immunosuppression.

However, not all patients respond to checkpoint inhibitors, and the search continues for predictive biomarkers of response to these therapeutics. For example, the tumor expression of PD-L1 does not definitively predict the clinical benefit of anti-PD-1 therapy, even though it may correlate with patient response in some cases [106]. In addition, resistance to checkpoint inhibition has also been observed and occurs possibly through the upregulation of alternative immune checkpoints such as TIM-3 [107]. The administration of checkpoint inhibitors is also commonly associated with immune-related adverse events (irAEs). While most of these are well-tolerated and can be managed symptomatically, irAEs can be robust enough, such as grade 3 or 4 diarrhea or colitis, to

Box 4
How nanotechnology can address barriers to effective immunotherapy.

Barriers	Nanotechnology-enabled strategies to address barriers	Selected references
Lack of tumor antigen processing and presentation	– Delivery and co-delivery of tumor lysates, antigens, neoantigens, and adjuvants to immune cells (examples include multiple platforms) – Improved lymphatic trafficking and delivery to the LN (examples include multiple platforms) – Ability to engage the immune cells both at the site of administration and at draining LN (examples include multiple platforms) – Systemic delivery and vaccination against identified tumor antigens – Enabling externally triggered disruption of tumor tissue by hyperthermia to release tumor antigens and combining with adjuvants for *in situ* vaccination (e.g. plasmonic gold nanostructures and SPION) – Enhancing chemotherapy-mediated ICD (e.g. Doxil and Abraxane)	[130, 131, 173, 174] [134, 138, 183]
Inhibited trafficking of immune cells to the tumor site	– Disruption of neovasculature to improve immune cell infiltration and release of danger signals (e.g. CYT-6091)	[201]
Disabling of antigen-presenting cells in the tumor microenvironment	– Combination delivery of antigen and adjuvant for enhanced DC priming (multiple platforms) – Targeted delivery to DCs (e.g. DEC205)	[130]
Anergy and ineffectiveness of infiltrating immune cells	– Delivery of inhibitors and oligonucleotides to the tumor microenvironment to counter tumor immunosuppression – Inhibition of immune checkpoint ligands by siRNA delivered to the TME (multiple platforms) – Co-delivery of agents to simultaneously abrogate immunosuppression and reactivate T cells (e.g. IL-2/TGFbi, CpG + anti-CD40, IL-2 + anti-CD137)	[121, 127–129]
Difficulty in transport and retention of drugs/nutrients in the tumor	– Tumor-specific accumulation of NPs via the EPR effect (examples include multiple platforms) – Magnetic guidance of NPs to the tumor site – Disruption of neovasculature (e.g. CYT-6091)	[120, 185, 201]

result in the permanent discontinuation of treatment [108]. In some cases, more serious autoimmune conditions, such as type I diabetes or autoimmune hypophysitis, may occur [109, 110].

3.5. Cancer vaccines

Therapeutic cancer vaccines are represented by a broad range of strategies to treat established cancers by educating the immune system against TAAs. These TAAs may be self-antigens that are overexpressed in the tumor or neoantigens generated by tumor-specific mutations that are absent in normal tissue. By delivering an antigen directly to the immune system, a successful cancer vaccine would thus overcome the barrier of limited tumor antigen presentation and possibly address additional barriers depending on vaccine design. However, it is clear that vaccination with peptide antigens is insufficient to evoke effective immune responses. In clinical trials between 1995 and 2004, the National Cancer Institute treated 440 patients with peptide vaccines that were either emulsified in an adjuvant, delivered by viruses, or encoded in DNA [111]. The overall response rate for all of these treatments was only 2.6%, most likely due to the extensive immunosuppressive mechanisms encountered by T cells in the tumor microenvironment.

Two cancer vaccines have so far been approved for clinical use. The autologous DC vaccine Sipuleucel-T was the first cellular immunotherapy approved for prostate cancer treatment, where patient DCs are extracted via leukapheresis for *ex vivo* treatment with an antigen-cytokine fusion protein before being reinfused as a vaccine [112]. Sipuleucel-T thus addresses the paucity of antigen-presenting cells in the tumor microenvironment by introducing large numbers of APCs educated *ex vivo*. This therapy proved to have limited efficacy because it only extended patient survival by four months in clinical trials and failed to improve progression-free survival [113, 114]. However, it did set an important precedent for DC vaccines that rely on *ex vivo* education of DCs without the need for *in vivo* targeting. Further efforts are underway to improve this therapy, including a trial in combination with indoximod, an inhibitor of the tumor immunosuppressor IDO [115]. The 2015 approval of Imlygic (T-VEC), the first oncolytic virus therapy, also represents a new class of cancer vaccines where viruses are repurposed to lyse tumor cells *in vivo*. T-VEC is derived from the type I herpes simplex virus, is engineered to target malignant cells, and carries the gene for GM-CSF, a cytokine that activates monocytes and DCs for the induction of immunity [116]. T-VEC therefore has the potential to debulk the tumor through virally induced lysis while exposing the immune system to TAAs under immune-activating conditions. In its Phase III trial, T-VEC achieved a 26.4% overall response rate in melanoma patients, and systemic immune responses were observed in patients at all disease stages [117]. T-VEC is likewise being tested alongside ipilimumab to investigate possible synergy with checkpoint inhibitors [118, 119].

4. Addressing barriers with nanotechnology

Nanotechnology has been extensively employed to enable immunotherapy both directly (by targeting barriers to immunotherapy), and indirectly (by improving conventional immunotherapies that are already used in the clinic) (Box 4).

4.1. Direct strategies to addressing barriers to immunotherapy

4.1.1. Offsetting the immunosuppressive tumor microenvironment by tumor-specific delivery

Nanoparticles are known to accumulate at sites of increased vascular permeability and reduced lymphatic drainage, such as the dysregulated vasculature of tumor beds. If circulating nanoparticles are sufficiently small to avoid renal clearance, they can leak into tumor tissue through the porous vasculature, and be subsequently trapped in the tumor bed. This phenomenon, known as the enhanced permeability and retention effect (EPR), is an important motivator for the use of nanotechnology for cancer therapy [120]. It turns the therapeutic barrier of dysregulated tumor vasculature into a viable target due to the potential of tumor-specific drug delivery enabled by nanoparticles. In addition, the loading of therapeutic agents within nanoparticles protects them from degradation, extending their circulation time in the body and allowing for a longer therapeutic window. In the clinic, nanoparticle systems such as Doxil and Onivyde have applied these strategies to the delivery of cytotoxic drugs, reducing systemic toxicities while maintaining therapeutic efficacy of the API.

Tumor-specific delivery is a useful strategy for targeting immunosuppressive mechanisms in the tumor microenvironment. In particular, the nanoparticle-mediated delivery of nucleic acid therapeutics to inhibit tumor immunosuppression has been reported by several investigators. As reviewed by Conde et al. [121], most of these reports are *in vitro* or *ex vivo* studies, though several demonstrate efficacy in animal models. Wang et al. designed 100 nm-polymersomes to deliver siRNA against CTLA-4, demonstrating nanoparticle uptake by $CD4^+$ and $CD8^+$ TILs after intravenous administration. Tumor-infiltrating T cells showed reduced expression of CTLA-4, along with enhanced tumor infiltration and anti-tumor activity [122]. Other reports have demonstrated the *in vitro* knockdown of the PD-1 ligands PD-L1 and PD-L2 in human T cells and tumor cells [123, 124], indicating that this approach can be extended to other immune checkpoint ligands as well. Huang et al. developed a dextran-based nanocomplex with a pH-responsive alginate element for delivery of CpG, anti-IL-10, and anti-IL-10 receptor oligodeoxynucleotides (ODNs) to tumor-associated macrophages. This formulation enabled ODN release in the acidic tumor microenvironment [125]. The nanocomplexes accumulated intratumorally after systemic intravenous administration and led to a reduction in tumor growth and angiogenesis. In another report, Conde et al. fabricated gold nanoparticles conjugated with anti-VEGF siRNA, which were delivered intratracheally in lung-tumor-bearing mice. The nanoparticles suppressed VEGF production in pulmonary macrophages and lung tissue, resulting in improved survival that was further extended with the addition of a macrophage-targeting ligand [126]. These reports show that nanoparticles can be used to deliver nucleic acid therapeutics directly to the tumor microenvironment for the inhibition of tumor immunosuppression.

Finally, nanotechnology has proven useful for combination immunotherapies, either by combining the effects of multiple immune-activating or immunosuppressive therapies or by simultaneously stimulating local immune cells while inhibiting mechanisms of tumor immunosuppression. Park et al. developed a hydrogel-encapsulated liposome delivering IL-2 and a TGF-β inhibitor [127]. This formulation was able to traffic to the tumor vasculature after intravenous administration, activate tumor-infiltrating T cells and NK cells, and improve survival in both subcutaneous and metastatic melanoma tumor models [127]. By complexing the excipient cyclodextrin into the hydrogel matrix, co-encapsulation and delivery of a hydrophilic protein together with a hydrophobic drug were achieved, demonstrating that nanoparticle chemistry can be designed to accommodate agents with very different chemical characteristics. The combination of soluble IL-2 and a TGF-β inhibitor failed to elicit any anti-tumor immunity, demonstrating the necessity of a nanotechnology platform for delivery of these therapeutic agents. Kwong et al. coated liposomes with anti-CD40 agonistic antibodies and CpG oligonucleotides. Liposomal anchoring of these agents allowed intratumoral retention of both CpG and anti-CD40, which enhanced their synergy and reduced their exposure to systemic circulation in treated animals after intratumoral injection, thereby significantly reducing toxicities associated with soluble CD40-specific antibodies [128]. To evoke better long-term responses, the same group developed liposomes with either IL-2 or anti-CD137 anchored on the particle surface. As before, intratumoral co-administration of both liposomes resulted in intratumoral retention of both agents, reduction of systemic toxicity compared to soluble agents, and long-term systemic anti-tumor immunity and resistance to rechallenge [129]. While these examples show the

induction of anti-tumor immunity without administration of a specific antigen, the principles outlined therein are similarly applicable to the cancer vaccine approaches discussed below.

4.1.2. Building more effective cancer vaccines with nanotechnology

Two key challenges in the area of cancer vaccines—the targeting of specific immune cell populations and the delivery of antigen and adjuvant to those cells—are problems well-suited for nanotechnology-based approaches. Whereas in other cancer drug delivery applications the clearance of drug-carrying nanoparticles by the MPS should be avoided to extend drug circulation times, the efficient uptake of antigen-carrying nanoparticles by DCs and other antigen-presenting cells becomes a desirable trait in vaccine applications. To enable improved vaccination strategies, nanoparticle systems can be designed to overcome physiological barriers in delivering vaccines to DCs for immune education and to carry combinatorial payloads for the stimulation of optimal immune responses (reviewed in [130]). Many of these strategies can also be used to improve DC-based cancer vaccines such as Sipuleucel-T.

4.1.3. Influence of nanoparticle physicochemical characteristics on lymph node targeting

Several strategies have been pursued to target nanoscale cancer vaccines to lymph nodes and other lymphoid organs, as well as to go after sites where anti-tumor T cells are primed. For nanoparticle-mediated strategies, nanocarrier size is known to affect nanoparticles' direct traffic to the lymphatic system. Some investigators have determined that smaller nanoparticles (with a hydrodynamic size of 30–50 nm) travel quickly through lymphatic vessels to lymph nodes upon subcutaneous injection, while their larger counterparts (with a hydrodynamic size equal to or greater than 100 nm) remain in the extracellular matrix of the injection site [131–133]. Sub-50-nm nanoparticles are therefore frequently used as vaccine candidates to target lymph-node-resident APCs. Notably, tumor-draining lymph nodes contain immunosuppressed, antigen-experienced T cells, which may be re-activated following immunotherapeutic stimuli. Jeanbart and colleagues found that administering nanoparticle vaccines to tumor-draining lymph nodes was more effective than targeting non-tumor-draining nodes and required nanoparticle-mediated delivery of antigen and adjuvant [134]. Protein-based nanoparticles, which are far smaller than the 50-nm size limit, have also demonstrated efficacy at lymph node targeting. For example, CpG adjuvants and peptide antigens modified with lipophilic albumin-binding domains converted the vaccine components into albumin nanoparticles upon contact with serum, which demonstrated improved anti-tumor efficacy and specific accumulation in lymph nodes [135]. Lee et al. also demonstrated the lymph node delivery and retention of ferritin-based protein nanoparticles, which were subsequently able to induce protective anti-tumor immunity [136]. Investigators have also shown that nanoparticles above the 50-nm size limit can be used to target lymph nodes via intravenous injection. This size limit only appears to confine itself to nanoparticle uptake via lymphatic vessels. A recent report directed intravenously administered microparticle constructs to lymph node high endothelial venules using the MECA-79 targeting antibody [137]. Kranz and colleagues optimized an mRNA cancer vaccine delivery system to target lymphoid organs and found that near-neutral or slightly negative RNA-lipid lipoplexes between 200–400 nm in diameter demonstrated exclusive splenic expression, dendritic cell uptake, and enhanced antitumor efficacy in mouse models. The study also revealed antigen-specific responses in patients tested in a Phase I clinical trial [138]. Another example of how nanotechnology can benefit vaccines through engineering nanoparticle size comes from the interbilayer-crosslinked multilamellar vesicles (ICMV) designed by Moon et al. [139, 140]. This concept allows for the encapsulation of a protein antigen between multiple layers of the core particle, exposure of the antigen on the particle surface, and co-delivery of various immunomodulatory agents [139, 140]. This design enabled stimulation of APCs both at the site of injection and in the resident lymph nodes through multistage particle dissolution and engagement of various size-dependent mechanisms of antigen delivery. As a result, the ICMVs stimulated a greater and longer-lasting humoral immune response than conventional vaccines [139, 140]. The successful targeting of cancer vaccines to lymphoid organs can therefore be achieved by a variety of strategies involving vehicle design and route of administration.

Nanoparticle surface charge is also known to impact lymphatic uptake, although here the picture is less clear. Several groups have demonstrated that, with an increase in negative surface charge, nanoparticle vaccines are taken up more effectively into lymph nodes after subcutaneous injection [141, 142]. However, other reports indicate that cationic gold– and hydrogel-based nanoparticles may also traffic more readily into draining lymph nodes than anionic nanoparticles [143–145]. These reports, however, involve the pulmonary delivery of nanoparticles, where cationic nanoparticles have been shown to be preferentially internalized by pulmonary DCs. The relationship between lymphatic uptake and nanoparticle surface charge therefore appears to depend on the route of administration.

4.1.4. Adjuvant delivery by nanoparticles

Nanoparticles are ideal systems for delivering appropriate combinations of antigen and adjuvant to immune cells. Such delivery is most frequently accomplished by incorporating pathogen-associated molecular patterns (PAMPs), evolutionarily conserved microbial components that are strong activators of immune cells and APCs, into the vaccine delivery vehicles alongside the desired antigen. As such, many different PAMPs have been used for this purpose, including LPS, Poly(I:C), and CpG oligonucleotides, largely encapsulated with antigen within the same nanoparticle platform (reviewed in [146]). The inherent physicochemical properties of nanoparticles have been used to promote immunity through activating inflammasomes [147, 148], triggering the complement pathway [131], or activating autophagy [149], which allows nanomaterials themselves to act as vaccine adjuvants. Investigators have a broad range of adjuvant candidates and material properties from which to select, and they should carefully choose appropriate combinations for the best results. A key challenge in this area is the avoidance of toxicities associated with the systemic administration of adjuvants, many of which have been linked to adverse inflammation [150–152]. Several investigators have developed nanoparticle-formulated adjuvants that traffic to lymph nodes and avoid systemic delivery. Two separate groups using different nanoparticle platforms to deliver a CpG adjuvant reported a stimulation of anti-tumor responses and the abolition of serum inflammatory cytokine responses to free CpG oligonucleotides [153, 154]. This behavior is not limited to oligonucleotide-based adjuvants and has also been demonstrated for the STING agonist cyclic di-GMP [155] as well as the TLR7/8 agonist R848 [156, 157]. Of note, several of these nanoparticle systems did not require co-encapsulation of antigen together with the adjuvant, indicating that the localization of immunomodulators to the lymph node environment is more important than localizing them to the same subcellular compartment by co-delivery within the same nanoparticle.

4.1.5. Tunable antigen delivery for cross-presentation and sustained release

The encapsulation of antigen in nanoparticles confers a host of advantages for vaccine delivery, including increased antigen uptake by APCs as well as the cross-presentation of antigens. DCs can capture antigen by the endolysosomal (class II) pathway, which stimulates T helper cell responses; they can also cross-present antigen via the class I pathway, which stimulates cytotoxic responses against cytosolic antigens. Capitalization on DC cross-presentation is therefore seen as a critical mechanism for stimulating cytotoxic T cell responses [158, 159]. To create a depot for sustained antigen delivery, antigens are frequently encapsulated in a biodegradable polymer such as poly(lactide-*co*-glycolide) (PLGA), where they are slowly released as the polymer degrades. The internalization of these antigen-loaded particles by DCs, or their presence near DCs as an

antigen depot, would therefore stimulate anti-tumor immunity by the uptake and cross-presentation of desired antigens. A variety of protein and peptide antigens have been employed for this purpose, including nanoparticles encapsulating tumor lysate, which have been investigated as vaccines that educate the immune system against the full range of tumor antigens and which can elicit immune responses from autologous patient samples [160, 161].

In experiments with cultured DCs, antigen encapsulated in PLGA nanoparticles was superior to its soluble counterpart in regard to both uptake and cross-presentation [162, 163], two key factors in eliciting strong DC-mediated T cell responses for anti-tumor immunity. To help nanoparticles undergo endosomal escape and enhance cross-presentation, several groups have incorporated polycationic components, such as polyethyleneimine (PEI), into the nanoparticle matrix [163, 164]. One group demonstrated this with a particle system composed fully of PEI [165]. However, the incorporation of the polycation PEI induced significant nanoparticle aggregation, a factor which may limit the translatability of these systems [166]. Other strategies for improving cross-presentation include the targeting of nanoparticles to DCs via the C-type lectin receptors DEC-205 or MGL, as well as intracellular targeting of antigen to lysosomal compartments via pH-sensitive linkers or lysosomal sorting signals [122, 167, 168].

Successful cancer immunotherapy requires the activation of both helper and cytotoxic T cells. Traditional adjuvants fail to fulfill this requirement. For example, alum, the only approved vaccine adjuvant, produces a strong antibody- and T_H2-biased immune response but is a poor inducer of cytotoxic T cell response [169]. Nanotechnology helps to overcome this problem by allowing investigators to tune antigen loading and release from nanoparticle carriers. Moreover, by optimizing the physicochemical properties of individual nanocarriers, one may achieve preferential induction of a desired immune response. For example, compared to liposomal vaccination, vaccination with PLGA nanoparticles was found to stimulate longer-lasting humoral responses as well as stronger $CD8^+$ T cell responses after a bacterial challenge. This effect was attributed to PLGA having slower antigen-release characteristics than liposomes. Both PLGA- and liposome-formulated vaccines were able to activate cell-mediated immunity, as opposed to alum, which only stimulated high antibody titers [170]. Similarly, polymersomes encapsulating antigens were better stimulators of $CD4^+$ T cell responses, while solid nanoparticles with surface-conjugated antigens engaged stronger $CD8^+$ T cell responses. The authors suggested that these differences are due to differential biodistribution and intracellular targeting, as the polymersomes were more efficiently targeted to DCs and the solid-core nanoparticles were mostly taken up by macrophages. An additional explanation could be a difference in particle size and the mechanism of the antigen release from the carriers, since polymersomes are 125 nm in size and release antigen after oxidation, while the solid-core nanoparticles are 30 nm and release antigen by reduction [171].

In using engineered nanosystems, investigators can select particular antigens and adjuvants that may be most relevant for a particular patient, opening the door for personalized nanoparticle vaccines. The advent of deep sequencing technologies has allowed for the identification of patient- and cancer-specific neoantigens that may be biomarkers and targets for effective cancer immunotherapy [172]. Using HDL-based nanodiscs with established clinical manufacturing procedures, Kuai and colleagues recently demonstrated for the first time a nanoparticle vaccine that is capable of eliciting immune responses against both single and multiple TAAs as well as an identified neoantigen in mouse models [173]. The nanodiscs co-delivered these antigens together with a nanodisc-bound CpG adjuvant, and the efficacy of the combined platform was not observed when the components were delivered separately. Combination therapy using the multi-epitope nanodisc vaccine and the checkpoint inhibitors anti-CTLA-4 and anti-PD-1 induced complete responses in almost all treated animals, demonstrating its further promise in combination with established immunotherapies.

4.1.6. Nanoparticle systems for externally induced immunotherapy

Nanoparticle systems can take advantage of unique materials and chemicals that are sensitive to external manipulation, allowing for localized intratumoral therapy and reduction of off-target effects. Such effects are typically achieved by imparting photosensitivity or magnetosensitivity to delivery systems, which can be externally stimulated after intratumoral accumulation. In particular, external excitation of some these nanosystems can cause localized tissue damage, inflammation, and the release of tumor antigens and danger signals. These nanoparticles can therefore initiate and enhance anti-tumor immune responses or act synergistically with other types of immunotherapies. External stimulation of nanoparticles can be used to address barriers to immunotherapy, including the local activation of APCs via hyperthermia- and phototherapy-induced cell death, as well as the magnetic guidance and targeting of nanoparticles to tumors.

Photosensitivity may be imparted to nanomaterials either through unique material properties or by incorporating known chemical photosensitizers into these nanoparticles. Specific nanosystems, such as gold nanorods and nanoshells [174], copper sulfide (CuS) [175], and Prussian Blue nanoparticles [176], have surface plasmon resonance properties that allow for strong adsorption of near infrared (NIR) light, resulting in efficient energy conversion for heat generation. This process is known as plasmonic photothermal therapy (PTT). On the other hand, nanoparticles may carry photosensitizers as part of their therapeutic payload, which are capable of generating singlet oxygen upon external excitation. This process is termed photodynamic therapy (PDT) [177]. By taking advantage of intratumoral nanoparticle accumulation, the application of external photostimulation localizes the area of effect specifically to the tumor, thus avoiding off-target effects. Recent studies investigating nanoparticle-induced phototherapy reveal that, while PTT and PDT excel at eliminating tumors at the site of photostimulation, these strategies struggle to induce systemic anti-tumor immunity when applied on their own. Therefore, several strategies have been used to enhance the immune responses instigated by nanoparticle-enabled phototherapy. Gold and copper sulfide nanoparticles have been coupled with CpG as an adjuvant, which has improved resulting immunotherapy even at distant tumor sites [178, 179]. Prussian Blue nanoparticles could also significantly enhance the anti-tumor efficacy of anti-CTLA-4 checkpoint inhibition [180]. While these studies involving PTT all required the intratumoral administration of nanoparticles, two recent nanoparticle PDT platforms were able to effect anti-tumor immunity after systemic intravenous administration in animal models. For example, PLGA nanoparticles co-encapsulating the photosensitizer indocyanine green (ICG) and the TLR7 agonist Imiquimod resulted in strong anti-tumor responses in two cancer models that were enhanced by anti-CTLA-4 [181]. He et al. developed a core-shell coordination polymer nanoparticle, delivering the chemotherapeutic oxaliplatin, as well as a porphyrin photosensitizer [182]. The oxaliplatin-generated immunogenic cell death, along with the cytotoxic reactive oxygen species generated upon porphyrin photostimulation, resulted in a substantial anti-tumor immune response that synergized with anti-PD-L1 therapy. These results demonstrate that nanoparticle-enabled phototherapy, both locally and systemically administered, can be used alongside adjuvants and other immunotherapies to boost systemic anti-tumor immunity.

Hyperthermia and apoptosis induction within the tumor also result in the release of tumor antigens and danger signals that activate the immune system. Iron oxide nanoparticles (IONPs) can induce intratumoral hyperthermia due to heating effects generated by hysteresis loss in an alternating magnetic field (AMF). As with phototherapy, IONP-mediated hyperthermia in tumors can also provoke anti-tumor immune responses. For example, hyperthermia treatment using 100 nm IONPs conferred resistance to a subsequent tumor challenge that was antigen-specific and dependent on $CD8^+$ T cells [183]. Hyperthermia mediated by lipid-coated magnetite nanoparticles synergized with IL-2 or

GM-CSF immunotherapy in a murine melanoma model [184]. Magnetic nanoparticles offer another advantage by guiding particle accumulation in the tumor area. This property was successfully used for the guidance and delivery of IFN-γ to tumors by cytokine-loaded IONPs [185]. Combined magnetic guidance and hyperthermia strategies have previously been applied in non-immunogenic cancer models and may be found useful in immunotherapeutic strategies as well.

4.2. Improving conventional immunotherapies

Nanotechnology offers multiple strategies for improving conventional immunotherapies (Box 5). Examples and remaining challenges are further considered below.

4.2.1. Nanoparticles for physiologically relevant cytokine delivery

Cytokine delivery presents a unique challenge for nanoparticle-enabled delivery systems. Physiologically, cytokines act in a paracrine fashion to target cells and function within a complex signaling network, and, as discussed earlier, their systemic administration for cancer therapy frequently leads to dose-limiting toxicities. In this arena, nanomedicine has most commonly been employed to alter cytokine biodistribution and to act as depots for sustained cytokine release *in vivo*, as reviewed elsewhere [186]. Liposomes and polymeric particles represent the most frequently used carriers for cytokine delivery, while IL-2, IL-12, TNF-α, GM-CSF, and IFN-γ are some of the most commonly studied APIs [187–190].

The benefits of using nanotechnology for cytokine delivery include the ability to formulate cytokines in physiologically relevant ways that enhance their stimulatory capacity, as well as the ability to achieve the sustained release of cytokines from carriers to mimic the paracrine release of these proteins from cells. For example, the encapsulation of IL-2 in nanoparticles and microparticles used for antigen presentation produced a far stronger expansion stimulus than IL-2 given at exogenous concentrations of up to 1,000-fold higher [191]. Biophysical modeling suggested that this effect was due to the sustained release of IL-2 in a paracrine fashion and its accumulation at the particle-cell interface, a phenomenon that is further enhanced by the embedding of nanoparticles in the cell membrane [192, 193]. Upon intratumoral injection, and when accompanied with surface-bound antibodies against CD3 and CD28, IL-2-loaded microparticles delayed tumor growth in a B16 tumor model, compared to antibody-coated particles with no cytokine [194]. Although this study did not use nanoparticles, the comparable immunostimulatory ability of IL-2-loaded nanoparticles suggests that similar results might be seen.

Nanoparticles have also been used as scaffolds to support surface-presented cytokines, such as IL-15 and TNF-α. IL-15 is a cytokine closely related to IL-2; however, it does not contribute to the development of Tregs. Unlike IL-2, the biological activity of IL-15 is achieved in a more complex fashion. This cytokine is presented on the surface of activated DCs and monocytes via its high-affinity receptor IL-15Rα, which acts as a superagonist for the cytokine and presents it to the IL-15Rβ that is expressed either on the same cell or on other cells. Therefore, leveraging the positive properties of this cytokine for immunotherapy requires co-delivery with its receptor α. Such co-delivery has been achieved using antigen-encapsulating PLGA nanoparticles, where the IL-15:IL-15Rα complex presented on the particle surface was a more potent activator of $CD8^+$ T cells than if the complex was delivered separately [195].

The cytokine TNF-α has also been investigated for cancer therapy due to its ability to directly induce the cell death of tumorigenic or virally infected cells, but its clinical administration was not approved due to systemic toxicity [196]. TNF-α on the surface of PEGylated colloidal gold nanoparticles (CYT-6091) has demonstrated anti-tumor efficacy in animal models [197], reduced systemic toxicity, and successfully passed a Phase I clinical trial [198]. TNF-α protein exists in two forms—soluble and membrane-bound—which differentially activate the TNF receptors TNFR1 and TNFR2. Both forms activate TNFR1, but only the membrane-bound form activates TNFR2 [199]. TNF-α bound to the surface of silica nanoparticles activated TNFR2-based

Box 5
How nanotechnology can improve conventional immunotherapies.

Immunotherapeutic approach	Challenges faced	Nanotechnology-based strategies to enhance immunotherapy	Selected references
Cytokine therapy	– Systemic toxicities and limited efficacy – Enhancing cytokine effectiveness through biophysical presentation	– Nanoparticle-mediated delivery of cytokine proteins and genes, allowing for improved half-lives (e.g. IL-2), tumor-specific targeting (e.g. IL-12), physiological presentation (e.g. IL-2, IL-15, TNF-α), reduced systemic toxicity (TNF-α)	[186, 195, 200, 201]
Adoptive TIL therapy	– TILs are not always accessible and expandable to the large numbers needed for adoptive therapy – Multiple rounds of expansion required, as well as the immunosuppressed phenotype of isolated TILs, may limit the efficacy of these cells – TILs have difficulty persisting after adoptive transfer – Difficulty in identifying antigen-specific T cells in cancer types besides melanoma	– Artificial antigen-presenting cell platforms can rapidly expand large number of TILs (e.g.) – T cell–conjugated nanoparticles enhance their survival post-injection by delivering critical cytokines, such as IL-15 and IL-21 – *In vivo* tracking and targeting of transferred TILs	[194, 213, 215–218, 220]
CAR-T cell therapy	– Cytokine release syndrome – Limited number of antigenic targets – Loss of targeted cell population – Labor-intensive culture process	– *In vivo* tracking of transferred CAR-T cells – Redirecting FITC-targeting CAR-T cells using FITC-folate or other conjugates – *In vivo* CAR gene delivery to peripheral T cells using nanoparticles	[215, 217–222]
Checkpoint inhibitor blockade	– Antitumor response is not universal in patients – Immune-related adverse events associated with treatment include colitis, arthritis, diabetes – Resistance to checkpoint inhibition has been observed	– Combination with a broad variety of nanotechnologies, including cancer vaccine and hyperthermia platforms to enhance efficacy (multiple examples in this review) – Nanoparticle-loaded microneedle patch for localized delivery of checkpoint inhibitors – Reporter NPs for early detection of effectiveness or resistance to checkpoint blockade – NPs such as Doxil can act as adjuvant therapies for checkpoint inhibitors	[173, 181, 182, 223, 229, 230]
Cancer vaccines	– Limited efficacy – Toxicity and ineffectiveness of adjuvants – Labor-intensive culture process for cellular vaccines	– Combination delivery of antigen and adjuvant in the same nanoparticle system for enhanced DC priming – Reduced toxicity by increasing specificity of delivery – Tuning NP adjuvanticity by changing particle PCC	[153, 154, 162, 163, 167, 168, 170, 171]

responses, indicating that nanoparticle presentation enables physiologically relevant signals that deliver the soluble that cytokine alone cannot provide [200]. CYT-6091, a PEGylated colloidal gold–formulated TNF-α, was shown to utilize TNFR1 on tumor endothelial cells to induce tumor neovasculature permeability efficiently, rapidly, and selectively [201], which is essential for enhancing both drug and immune cell infiltration into the tumor space. While TNFR2 signaling is necessary for T-cell function and DC survival, it also can stabilize Treg populations in inflammatory environments and the accumulation of MDSCs in the periphery [202–205]. Therefore, one may hypothesize that nanoparticle-formulated TNF-α may share this property. In the case of CYT-6091, preclinical studies demonstrated an accumulation of cytotoxic T cells without enhancement of Tregs in tumors of genetically engineered mice with pancreatic ductal adenocarcinomas after they were treated with this nanoformulation (Dobrovolskaia M and Tamarkin L, unpublished observation). These data show promising results and further emphasize the need for the thorough investigation and understanding of nanoparticle properties to determine either the beneficial or negative impacts of the carrier on the biological properties of TNF-α nanotherapeutics.

4.2.2. Enhancing adoptive immunotherapy

Immunotherapies based on adoptive transfer strategies, such as the use of expanded tumor-infiltrating lymphocytes and CAR-T cells, involve highly complex and expensive procedures that limit their translational application. Large numbers of effector T cells are generally required, and they must be expanded either from TILs that are isolated from the resected tumor or from the genetically modified CAR-T cells generated from a patient's blood. The *in vivo* tracking and monitoring of transferred cells remains a challenging task, and transferred cells still have to overcome the immunosuppressive microenvironment at the tumor site. Nanomedicine-based technologies have the potential to enhance cell-based immunotherapies at multiple stages of their application and thus contribute to the development of next-generation personalized immunotherapies.

4.2.2.1. Ex vivo expansion of T cells for adoptive immunotherapy. Nanoparticle platforms possess unique advantages as platforms for expanding large populations of anti-tumor cells. Engineered acellular systems provide a flexible, modular, and off-the-shelf approach for T-cell expansion [194]. These are termed artificial APC (aAPC) platforms and are typically equipped with various ligand combinations, including an MHC molecule and one or more costimulatory ligands whose type and density can be tuned for optimal T-cell activation and growth. Considerations for this technology include the degree of T-cell expansion afforded, the quality of T cells generated, and the separation of undesired components after expansion.

To date, multiple acellular, nanoparticle-based platforms for T-cell expansion have been developed in preclinical settings. Materials used for aAPC nanoparticles have included iron-dextran, PLGA, liposomes, and quantum dots [191, 195, 206–208]. Several strategies have been employed to enhance the proliferative capacity of aAPCs. As described in the previous section, aAPCs may be designed to encapsulate and release cytokines, providing essential signals for T-cell activation. Fadel et al. recently developed nanoparticle aAPCs releasing paracrine IL-2 into a magnetically separable carbon nanotube/PLGA nanoparticle composite. In this concept, the MHC I molecules and anti-CD28 were presented on a high-surface-area CNT scaffold, and IL-2 was released in paracrine fashion from magnetite-loaded PLGA nanoparticles [209]. T cells could thus be efficiently expanded using this system and cleanly separated after expansion. The shape of aAPCs and geometries of ligand clustering have also been manipulated to enhance T-cell expansion. Meyer et al. reported that ellipsoidal nanoparticles used as aAPCs were able to stimulate more efficient T-cell expansion than spherical nanoparticles and had the added benefit of improved pharmacokinetics after systemic administration [210]. To alter ligand distribution, Perica et al. constructed paramagnetic nanoscale aAPCs, which cluster on the T-cell membrane under a magnetic field and enhance T-cell signaling [211]. The resulting cells proliferated robustly and induced a greater anti-tumor response, compared to cells that did not see a magnetic field. Many of these systems reported an *ex vivo* expansion of T cells between 20- and 200-fold over a period of 7–14 days [191, 206, 209]. aAPCs themselves have also been tested as *in vivo* therapies, with Perica and colleagues demonstrating anti-tumor protection in the B16 tumor model after the intravenous administration and trafficking of nano-aAPCs to lymph nodes [206]. Nanoparticle-based platforms have thus been used to engineer systems for quickly generating large numbers of activated effector cells.

4.2.2.2. Nanoparticles for the improved survival and tracking of transferred T cells. Nanosystems can also aid in the survival and tracking of effector cells after adoptive transfer. In adoptive therapy with expanded tumor-infiltrating lymphocytes, IL-2 is often given alongside transferred cells to enhance their survival, subjecting patients to the systemic toxicities associated with cytokine therapy [212]. To overcome toxicity while promoting T-cell expansion and effector function, multilamellar lipid vesicles loaded with IL-15:IL-15Rα and IL-21were conjugated to the surface of transferred T cells [213]. These nanoparticles were stably tethered to the cell surface, did not affect T-cell effector function, and vastly improved the efficacy of adoptive T-cell therapy in mice, as compared to adoptive transfer with systemically administered cytokines. The same surface-conjugated nanoparticles were observed to co-localize with the immunological synapse between tumor-specific T cells and their targets, allowing for the nanoparticle-mediated, synapse-specific delivery of a phosphatase inhibitor to enhance T-cell expansion after delivery [214]. Labeling T cells with nanoparticles also presents a useful strategy for the *in vivo* tracking of transferred effector cells, a critical challenge for the understanding and advancement of modern adoptive transfer and CAR-T cell therapies. To date, T cells have been labeled with nanoparticles made of gold and iron oxide, allowing for their tracking in animal models by CT, MRI, or PET [215–219]. The nanoparticles themselves may be used as imaging agents or may be conjugated with moieties, such as radioisotopes, for non-invasive imaging. Liposomes have also been used to target T cells after adoptive transfer, using either antibodies specific for the transferred T cells or an IL-2-Fc fusion protein targeting activated T cells [220]. While translational issues exist for this system, including the potential lack of targeting ligands on patient-derived T cells and non-specific targets of IL-2-Fc, it nevertheless demonstrates a proof of concept that transferred T cells can be targeted with nanoparticles post-transfer.

4.2.2.3. Nanoparticles for improved CAR-T cell therapy. While nanotechnology-based strategies have not yet been extensively applied to CAR-T therapy, many of the strategies described earlier for adoptive TIL therapy (e.g. enhancing the expansion and survival of transferred T cells) also apply to CAR-T cells. Several recent reports describe the use of nanotechnology to directly improve CAR-T cell therapy. Bhatnagar et al. used gold nanoparticles functionalized with $^{64}Cu^{2+}$ to allow for positron emission tomography–enabled imaging of CAR-T cells after adoptive transfer in animal models [219]. To reduce on-target, off-tumor, and other systemic effects of CAR-T cells, Kim and colleagues engineered CAR-T cells to recognize an irrelevant target (i.e. fluorescein isothiocyante, or FITC), then directed them to attack cancer cells using a nanoscale bifunctional linker bearing FITC and folate [221], whose receptor is upregulated across multiple cancer types. Finally, a recent report used nanoparticle gene transfer carriers to edit T cells *in vivo*, introducing CAR expression within a patient's T cells without laborious *ex vivo* cell manipulation and gene transduction. Smith and colleagues utilized CD3e-targeted poly(β-amino ester) to deliver plasmids encoding the leukemia-targeting 194-1BBz CAR into T-cell nuclei [222]. These nanoparticles successfully transfected circulating T cells in mouse models, which then proliferated and induced robust anti-tumor immunity. This report represents the first proof of concept of *in vivo* CAR gene delivery to T cells using nanoparticles, directly addressing the cost barrier involving T cell therapies.

4.2.2.4. Enhancing checkpoint inhibitor therapy with nanotechnology. The clinical success of checkpoint inhibitors has spurred efforts to broaden their use in cancer therapy. Checkpoint inhibitors are thought to be most effective in PD-L1-expressing, immune-infiltrated tumors—a "hot" tumor microenvironment, where anti-tumor immune responses have arisen and become suppressed. As certain kinds of chemotherapy can cause immunogenic cell death in tumor cells, checkpoint inhibition may amplify the resulting immune responses, initiating therapeutic immune responses in patients who normally do not respond to checkpoint inhibitor therapy. Nanotechnology-formulated oncology drugs, such as PEGylated liposomal doxorubicin (Doxil), have shown promise in this regard. Doxil was shown to synergize with the checkpoint inhibitors anti-CTLA-4 or anti-PD-1 in two separate syngeneic tumor models, including both prophylactic and therapeutic settings. Crucially, the study showed that Doxil was more effective than free doxorubicin in delaying tumor growth and that the benefit due to Doxil was abolished in an immunodeficient setting [223]. While the existing data strongly argue for the immune system's involvement in this phenomenon, the mechanism is yet poorly understood. A proposed clinical trial already aims at testing the combination of Doxil with anti-PD-1 for platinum-resistant cancers [224]. There is also evidence that Doxil may work well with cytokine-based immunotherapies. Tumor cells that survive Doxil treatment reportedly upregulate MHC-I and Fas ligand, enhancing their immunogenicity and rendering them vulnerable to interleukin-18 (IL-18) immunotherapy [225], a cytokine that was halted in Phase II trials due to lack of efficacy in a monotherapeutic setting. Doxil has further been shown to improve the survival of tumor-bearing mice upon combination with free or liposomal IL-2, to a greater extent than any of the agents on their own [226]. Another nanotechnology formulation, nanoalbumin-bound paclitaxel (Abraxane), has also demonstrated a synergy with checkpoint inhibitors. Two ongoing studies currently seek to verify the combination of this formulation with anti-PD-1 or anti-PD-L1 [227, 228]. Therefore, the potential remains for clinically approved nanomedicines such as Doxil and Abraxane to be used in combination with immunotherapeutic approaches.

Additional strategies for assisting checkpoint inhibitor therapy include a "reporter nanoparticle" system containing a response element with an emitter-quencher FRET pair linked by a caspase-3 sensitive peptide sequence. Nanoparticle-delivered paclitaxel or the checkpoint inhibitor anti-PD-L1 induces tumor apoptosis, which in turn cleaves the reporter element and activates the fluorescence signal. Apoptosis-triggered fluorescence was observed as early as eight hours after the commencement of therapy, far earlier than differences found between tumor growth kinetics [229]. This observation demonstrates the utility of this system in reporting improved sensitivity or resistance to apoptosis-inducing checkpoint blockade therapies. A second strategy uses dextran nanoparticles to deliver encapsulated anti-PD-1, as well as a microneedle patch to localize antibody delivery to the tumor. This system was able to achieve retention and sustained release of anti-PD-1 at the tumor site, improving the anti-tumor efficacy over that of free anti-PD-1 alone [230]. These delivery systems provide new strategies for improving on existing checkpoint immunotherapies through more effective diagnostics and sustained delivery.

5. Translational considerations

The successful translation of many of these concepts to the clinic should lean heavily on lessons learned from the drug development of the relevant APIs, as well as the fields of nanotechnology and immunotherapy. Here, we summarize important translational considerations from each of these areas.

5.1. Lessons learned from biotherapeutics

The immunogenicity of protein-based therapeutics represents a significant barrier to both their translation and clinical use. Anti-drug antibodies (ADA) may affect the pharmacokinetics, safety, and efficacy of recombinant protein therapeutics by neutralizing the drugs themselves (e.g. anti-Eprex ADAs neutralize Eprex as well as the other recombinant erythropoietin drugs Neocormon and Aranesp), their native counterparts (e.g. anti-Eprex ADAs also neutralize endogenous erythropoietin), and ligands (e.g. idiotypic antibodies against the ligand can also neutralize the receptor) [231–233]. Strategies for overcoming this barrier include the identification and removal or masking of immunogenic epitopes within the protein product, the PEGylation of the protein, the humanization or generation of fully human therapeutic antibodies, and the reformulation of these drugs using novel technologies, including nanotechnology [234]. Although PEGylation is widely used for improved solubility, pharmacokinetics, and protection from immune recognition, there are many challenges associated with this approach. Linkers used to attach PEG to a protein may be immunogenic *per se*, while methods used for protein PEGylation may create activated polymers and co-product(s) with undesirable properties and toxicities [235]. The stability and type of the chemical bond(s) between the therapeutic protein, the PEG moiety, the selected chemical linker connecting the PEG to the protein, the coupling conditions, and the influence of PEGylation on surface charge are among the critical parameters responsible for the qualitative differences in PEGylated protein products [235]. Furthermore, accumulating evidences suggest the occurrence of natural anti-PEG antibodies in the plasma of healthy individuals [236]. Such pre-existing antibodies, when present in plasma of patients, may affect both the safety and the efficacy of the PEGylated products. Accidental contamination of protein therapeutics with nanomaterials (e.g. tungsten, silicon oil, cellulose fibers) or the presence of trace amounts of endotoxin contributes to the immunogenicity of these products and represents another significant hurdle in this therapeutic area [234]. It is important to distinguish between the nanomaterials accidentally contaminating the protein-based formulations and the nanoparticles engineered to deliver the proteins. While the presence of accidental particles in formulations contributes to their immunogenicity by promoting protein aggregation and exaggerating adjuvant properties of trace amounts of endotoxin, engineered nanocarriers may help to protect the protein therapeutics and prevent the immunogenicity.

"Bio-optimization" is an important approach in the preclinical development phase to screen out protein-based therapeutics with undesirable biological properties. Both *in vitro* and *in vivo* models are helpful in this effort. Moreover, some *in vitro* assays are more predictive of product safety than traditional animal models, which is an important lesson learned from biotechnology therapeutics. In the case of the anti-CD28 super-agonist antibody TGN1412, a cytokine secretion test using PBMC from healthy donors was more predictive of cytokine storm than animal studies conducted in rats and non-human primates [237]. A thorough evaluation of the lessons learned from the development of a product helps to overcome barriers and develop a protein therapeutic successfully even after its predecessor's failure [237].

5.2. Lessons learned from therapeutic oligonucleotides

Preclinical and clinical studies of traditional oligonucleotide-based therapeutics have revealed numerous translational barriers impeding favorable pharmacokinetics, toxicology, and stability of these products in the blood [238–245]. The toxicity arising from undesirable effects of therapeutic oligonucleotides on the immune system includes a broad spectrum of immunosuppressive, immunostimulatory, and immunomodulatory effects: myelosuppression, neutropenia, thrombocytopenia, lymphadenopathy, suppression of the immune response to common antigens, anaphylaxis, delayed-type hypersensitivity reactions, autoimmune hemolytic anemia, cytokine release syndrome, systemic inflammatory response syndrome, anemia, and thrombosis. The type of toxicity depends on the chemical composition and physicochemical properties of the individual product and has been discussed extensively elsewhere [246]. Many of these hurdles can be addressed

through chemical modifications of the oligonucleotide backbone, sequence optimization, change in route of administration, or alterations in the dose regimen (reviewed in [242, 243, 247]. Additional strategies, including in-silico screening for potential off-target toxicities and reformulation using nanotechnology carriers, have also been discussed [248, 249].

5.3. Lessons learned from nanotechnology-based combination products and complex drug formulations

The nanoparticle physicochemical properties critical for the biological performance of these materials include particle size, polydispersity, zeta potential, surface properties, composition, density of surface ligands, stability, solubility, architecture, and other material-dependent parameters such as aspect ratios for nanorods and fibers. Characterization of these properties often requires complex instrumentation and approaches different from those traditionally used for small molecules and biotechnology products. Establishing synthetic procedures and controls for ensuring the batch-to-batch consistency of nanotechnology-based products represents a significant challenge and is often material-specific [250, 251]. Once understood, these properties can be linked to biological effects. For example, nanomaterials with cationic surfaces tend to interact with platelets and are prone to thrombogenic side-effects, while anionic materials tend to inhibit coagulation [252, 253]. Other common structure-activity relationship patterns have been discussed elsewhere [252, 253]. Knowing such relationships will inform the design of delivery vehicles with optimal properties. The biological characterization of nanomaterials requires careful selection of appropriate models;

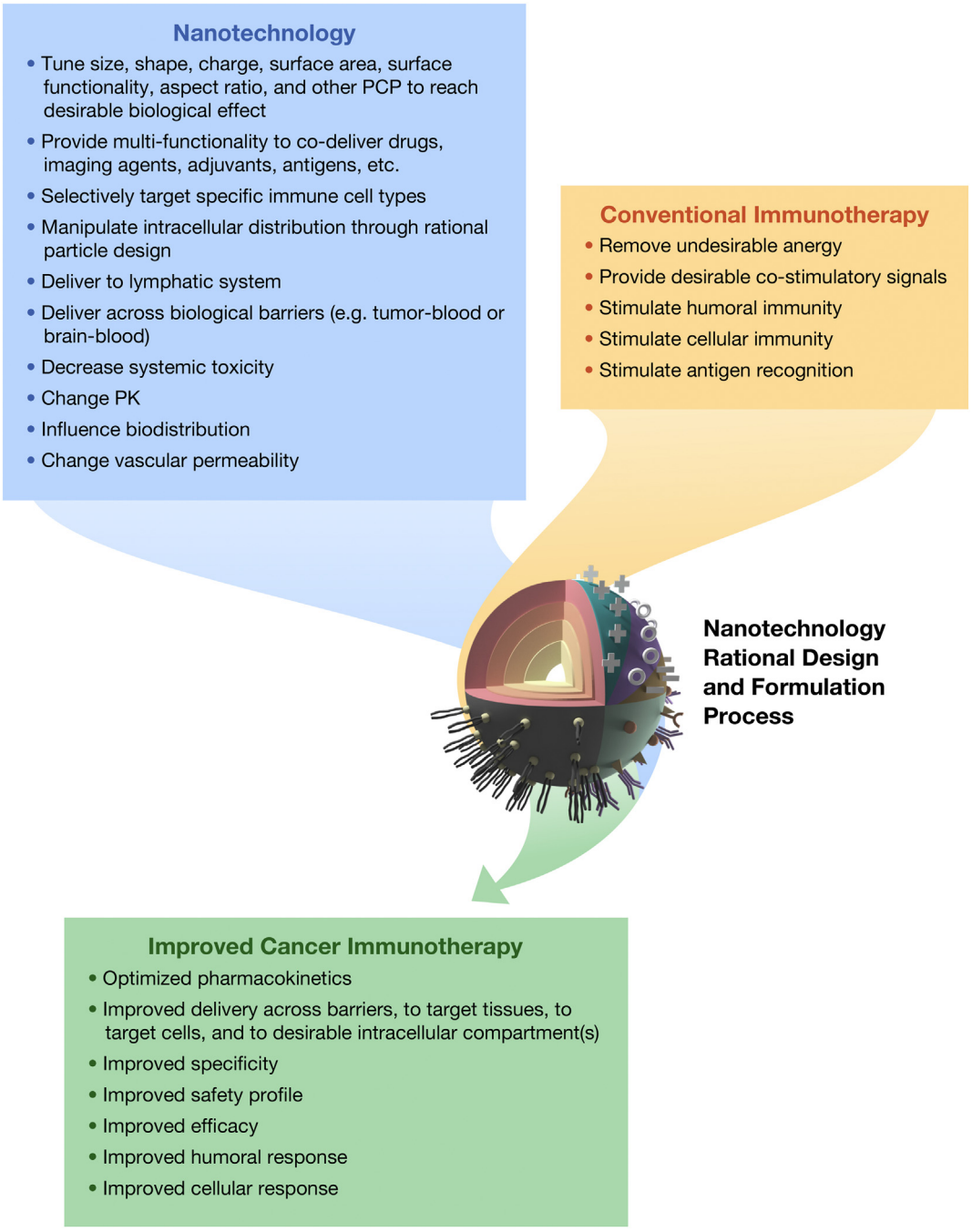

Fig. 1. General strategy for creating improved immunotherapies. The approach is suggested which leverages the benefits of traditional immunotherapeutics and nanotechnology. On a case-by-case basis and through the rational nanoparticle design it combines optimal properties of each component to create an improved generation of the immunotherapeutics.

understanding of both acute and long-term toxicities; estimation of drug loading and its stability, release, and conjugation; as well as quantification and determination of the biological activity of targeting moieties [254]. It is important to recognize that a drug reformulation using nanocarriers may result in a change in the drug biodistribution. For example, the cardiotoxicity of doxorubicin was overcome by reformulation using PEGylated liposomes. However, this reformulation also led to drug accumulation in the skin and subsequent palmar-plantar erythrodysesthesia (PPE), which created certain limitations for the use of PEGylated liposomal doxorubicin [255]. When studying nanotechnology-formulated drugs, it is also important to track all components of the formulations, including the platform and the drug, as well as to estimate both the particle-bound and free drug in biological matrices [256, 257]. To avoid compromised results in immunotoxicity and immunological efficacy studies, it is critical to accurately detect and quantify endotoxin in particle formulation [250, 258–271]. Sterilization and depyrogenation of nanomaterials also represent significant challenges [270, 272, 273]. When available, *in vitro* methods with good *in vivo* correlation profiles can be used to quickly screen for nanoformulation with the desirable biological property. Such assays for immunotoxicity studies have been discussed previously and, among other tests, include cytokine detection in human primary PBMC cultures, which is similar to the assay used in the characterization of biotechnology products [274–276]. The presence of chemical impurities and toxic excipients are common reasons for particle-based product failure in preclinical stages [250]. Often, these impurities are unknown at the initiation of the particle characterization study. Therefore, the importance of both in-study and post-study characterization is hard to underestimate. Since PEGylation is the most common approach for masking nanoparticles from immune recognition, the same challenges of PEG immunogenicity and pre-existing anti-PEG antibodies, as discussed above in "Lessons Learned from Biotherapeutics," apply to PEGylated nanomaterials. Many nanotechnology platforms utilize high molecular weight PEG, which may create additional hurdles, and thus warrant careful consideration during nanocarrier design stage. Despite its solubility in water, PEG's metabolism and clearance rates decrease as its molecular weight increases [277]. As such, it may create additional safety concerns for patients with abnormal renal clearance. These and other challenges in chemistry, efficacy, pharmacology, toxicology, and immunology, as well as some strategies to overcome them, have been reviewed elsewhere [249, 250, 252, 253, 257, 278]. Guidance for conducting the immunotoxicity studies required for the regulatory approval of the final nanotechnology-formulated drug product have also been discussed [279].

5.4. Lessons learned from conventional immunotherapy

Both the improvement of tumor-specific immune responses and the correction of the immunological environment of the tumor are important factors for successful tumor elimination. Clinical experience has shown that both factors need to be addressed in combination approaches for optimal anti-tumor immunity, which is already being tested in clinical trials [280, 281]. Despite understanding the need for improvement, optimization of the anti-tumor immune response with conventional immunotherapeutics is associated with many challenges. Due to the relatively low number of clinical responders to immunotherapy, the field is actively searching for predictive markers to identify these patients beforehand and improve the precision of these therapies [282]. In addition, while mouse models are the primary tool for advancing the field of immune-oncology [283], they fail to predict the range of heterogenous patient responses and toxicities experienced in the clinic, and the use of young and inbred mice overlooks the important factors of age, obesity, and gut microbiota [284]. For cellular immunotherapies, a key challenge is the need to identify, isolate, and characterize these cells post-transfer in order to better understand their behavior *in vivo*. This challenge requires new, sensitive technologies for single-cell tracking, isolation, and analysis, including both genotyping and phenotyping approaches. Both on-target and off-target effects are common with different types of immunotherapies. Addressing these limitations may benefit from the targeted delivery and real-time tunability of novel immunotherapeutics, the development of which, in turn, depends on highly sensitive, rapid technologies for cell identification and characterization.

6. Roadmap to the next generation of nanotechnology-enabled immunotherapies

Successful cancer immunotherapy requires the simultaneous removal of multiple barriers to anti-tumor immunity. This has been

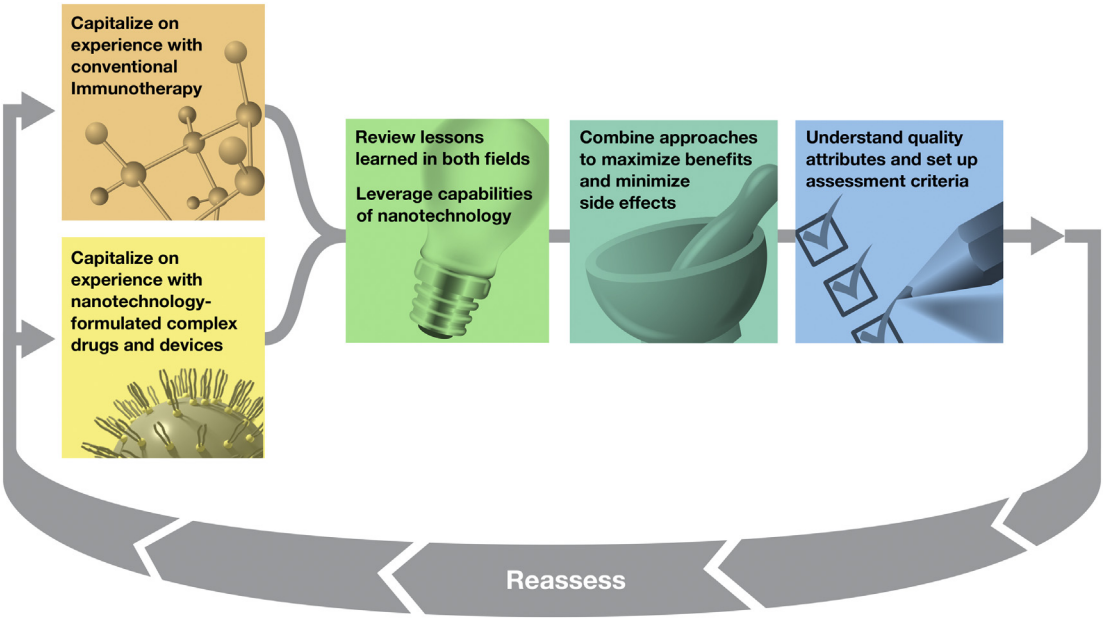

Fig. 2. A roadmap to the next generation of immunotherapeutics. The roadmap starts from capitalizing on the experiences with nanotechnology and immunotherapy. The product design is based on analyzing lessons learned from various fields, combining beneficial properties to maximize benefits and reduce side effects, identifying critical quality attributes, re-assessing multiple parameters to verify that the desired performance is achieved and adjusting if necessary.

borne out in the clinical experience with immunotherapy so far, as well as in the literature reviewed here. This is not a foreign concept to nanotechnology, as the tumor-specific delivery of nanoparticles also faces multiple barriers that have to be simultaneously addressed [285]. To this end, nanotechnology offers not only a carrier for desired therapeutics, but sophisticated delivery systems that can alter drug–drug, drug–tumor, and drug–immune cell interactions to amplify anti-tumor immunity. We have summarized the ability of nanotechnology to address the biological barriers to anti-tumor immunity and their capacity to enhance current immunotherapeutic strategies as well. In some cases, they can provide additional capabilities, such as external stimulation and specialized diagnostics. The success of nanotechnology in addressing these barriers bears witness to their potential in making significant contributions to the oncologist's toolkit for immunotherapy.

We envision the road to developing new immunotherapies as a cyclical process: drawing from scientific experience with past strategies and technologies, carefully reviewing lessons learned, and developing new approaches that deliver the best combinations of efficacy, translatability, and safety (Figure 1). Our review of the existing literature underscores the notion that monotherapies are generally inefficient for treating systemic disorders like cancer. With an understanding of how current immunotherapies affect the cancer immunity cycle, the field is already rapidly moving to test combination therapies in efforts to capitalize on recent successes [1, 286]. These efforts are ongoing in academia and small enterprises (multiple references in this review), as well as larger pharmaceutical companies [287]. Nanotechnology offers many advantages towards this effort, allowing investigators to optimize pharmacokinetics; improve delivery across physiological barriers to reach target tissues, target cells, and/or desirable intracellular compartment(s); and improve specificity, safety profile, and efficacy at both humoral and cellular levels (Figure 2). As discussed in this review, in order to amplify anti-tumor immunity, investigators should consider how nanotechnology may enhance current immunotherapy strategies or enable new ones and how these approaches can address multiple barriers to immunotherapy or steps in the cancer immunity cycle. The careful consideration of lessons learned from individual therapeutic areas to be combined will also impact the success of these novel combinatorial approaches. Finally, by understanding the biological quality attributes of each component separately and as a part of the combination, this framework will help set up acceptance criteria to enable translation of optimized products from bench to clinic. The evidence-driven re-evaluation and update of such quality attributes are critical contributors to the future success of these therapeutics (Figure 1). For example, investigators developing a novel formulation with one or more APIs should consider not only the desired target indication and all relevant considerations but also known challenges for similar formulations and APIs being tested. In testing for preclinical and clinical efficacy, the performance of the candidate nanotechnology should consistently be compared against "gold standard" treatment(s) (both immunotherapies and non-immunological therapies) for the selected indication, as well as those of similar formulations and APIs that have advanced or failed in the clinic. Throughout this process, care should be taken to maintain consistency in the nanotechnology across physicochemical characteristics and immunotherapeutic efficacy in suitable models. This is generally achieved only with a good understanding of the physicochemical attributes of the formulation and its biological/immunological effects, such that they can be appropriately measured, monitored, and adjusted where necessary. These efforts further contribute to our understanding of how nanotechnological attributes impact immunological phenomena, which in turn will iteratively inform the design of new nanotechnology-enabled immunotherapies. Nanoparticle attributes that enhance qualities such as lymphatic trafficking, synergy between immunomodulatory APIs, and *in vivo* survival and activation of effector cells can thus be further characterized and incorporated into future nanoparticle designs, as was done in several studies reviewed herein.

Ultimately, by bringing together combination immunotherapy strategies to address multiple barriers to tumor immunity, capitalizing on the multifunctional capabilities of nanotechnology, and drawing from translational considerations with conventional proteins, nucleic acids, and immune- and nanotechnology-based therapeutics, the research seeks to enable the creation of combination strategies to maximize benefits and minimize undesirable effects. Nanotechnology has significant potential to expand the impact and benefit of cancer immunotherapy, and we believe the intersection of these fields to hold great promise for the future.

Acknowledgments

The study was supported in whole or in part by Federal funds from the National Cancer Institute, National Institutes of Health, under contract HHSN261200800001E. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

References

[1] D.S. Chen, I. Mellman, Oncology meets immunology: the cancer-immunity cycle, Immunity 39 (2013) 1–10.
[2] C.G. Drake, E. Jaffee, D.M. Pardoll, Mechanisms of Immune Evasion by Tumors, Adv. Immunol. 90 (2006) 51–81.
[3] T.F. Gajewski, H. Schreiber, Y.X. Fu, Innate and adaptive immune cells in the tumor microenvironment, Nat. Immunol. 14 (2013) 1014–1022.
[4] C.L. Bonifant, H.J. Jackson, R.J. Brentjens, K.J. Curran, Toxicity and management in CAR T-cell therapy, Molecular Therapy — Oncolytics 3 (2016) 16011.
[5] C.N. Baxevanis, S.A. Perez, Cancer vaccines: limited success but the research should remain viable, Expert Rev Vaccines 15 (2016) 677–680.
[6] S. Kelderman, T.N. Schumacher, J.B. Haanen, Acquired and intrinsic resistance in cancer immunotherapy, Mol. Oncol. 8 (2014) 1132–1139.
[7] R.N. Schwartz, L. Stover, J.P. Dutcher, Managing toxicities of high-dose interleukin-2, Oncology 16 (2002) 11–20.
[8] G.A. Rabinovich, D. Gabrilovich, E.M. Sotomayor, Immunosuppressive strategies that are mediated by tumor cells, Annu. Rev. Immunol. 25 (2007) 267–296.
[9] W. Zou, Immunosuppressive networks in the tumour environment and their therapeutic relevance, Nat. Rev. Cancer 5 (2005) 263–274.
[10] P. Leone, E.C. Shin, F. Perosa, A. Vacca, F. Dammacco, V. Racanelli, MHC class I antigen processing and presenting machinery: organization, function, and defects in tumor cells, J. Natl. Cancer Inst. 105 (2013) 1172–1187.
[11] S.C. Tripathi, H.L. Peters, A. Taguchi, H. Katayama, H. Wang, A. Momin, M.K. Jolly, M. Celiktas, J. Rodriguez-Canales, H. Liu, C. Behrens, I.I. Wistuba, E. Ben-Jacob, H. Levine, J.J. Molldrem, S.M. Hanash, E.J. Ostrin, Immunoproteasome deficiency is a feature of non-small cell lung cancer with a mesenchymal phenotype and is associated with a poor outcome, Proc. Natl. Acad. Sci. U. S. A. 113 (2016) E1555–1564.
[12] M. Matsui, S. Machida, T. Itani-Yohda, T. Akatsuka, Downregulation of the proteasome subunits, transporter, and antigen presentation in hepatocellular carcinoma, and their restoration by interferon-gamma, J. Gastroenterol. Hepatol. 17 (2002) 897–907.
[13] B. Seliger, M.J. Maeurer, S. Ferrone, TAP off - tumors on, Immunol. Today 18 (1997) 292–299.
[14] E. Reeves, E. James, The Role of Endoplasmic Reticulum Aminopeptidase 1 Biology in Immune Evasion by Tumours, Journal of Vaccines and Immunology 1 (2015) 028–035.
[15] R. Mendez, T. Rodriguez, A. Del Campo, E. Monge, I. Maleno, N. Aptsiauri, P. Jimenez, S. Pedrinaci, G. Pawelec, F. Ruiz-Cabello, F. Garrido, Characterization of HLA class I altered phenotypes in a panel of human melanoma cell lines, Cancer Immunol. Immunother. 57 (2008) 719–729.
[16] P. Jimenez, J. Canton, A. Collado, T. Cabrera, A. Serrano, L.M. Real, A. Garcia, F. Ruiz-Cabello, F. Garrido, Chromosome loss is the most frequent mechanism contributing to HLA haplotype loss in human tumors, Int. J. Cancer 83 (1999) 91–97.
[17] Z. Madjd, I. Spendlove, S.E. Pinder, I.O. Ellis, L.G. Durrant, Total loss of MHC class I is an independent indicator of good prognosis in breast cancer, Int. J. Cancer 117 (2005) 248–255.
[18] M. Campoli, S. Ferrone, Tumor escape mechanisms: potential role of soluble HLA antigens and NK cells activating ligands, Tissue Antigens 72 (2008) 321–334.
[19] O. Ashiru, P. Boutet, L. Fernandez-Messina, S. Aguera-Gonzalez, J.N. Skepper, M. Vales-Gomez, H.T. Reyburn, Natural killer cell cytotoxicity is suppressed by exposure to the human NKG2D ligand MICA*008 that is shed by tumor cells in exosomes, Cancer Res. 70 (2010) 481–489.
[20] Y. Jiang, Y. Li, B. Zhu, T-cell exhaustion in the tumor microenvironment, Cell Death Dis. 6 (2015), e1792. .
[21] E.J. Wherry, T cell exhaustion, Nat. Immunol. 131 (2011) 492–499.
[22] W. Zou, L. Chen, Inhibitory B7-family molecules in the tumour microenvironment, Nat. Rev. Immunol. 8 (2008) 467–477.
[23] K. Wing, Y. Onishi, P. Prieto-Martin, T. Yamaguchi, M. Miyara, Z. Fehervari, T. Nomura, S. Sakaguchi, CTLA-4 control over Foxp3+ regulatory T cell function, Science 322 (2008) 271–275.
[24] A.E. Ryan, F. Shanahan, J. O'Connell, A.M. Houston, Addressing the "Fas counterattack" controversy: blocking fas ligand expression suppresses tumor immune evasion of colon cancer in vivo, Cancer Res. 65 (2005) 9817–9823.

[25] J. O'Connell, G.C. O'Sullivan, J.K. Collins, F. Shanahan, The Fas counterattack: Fas-mediated T cell killing by colon cancer cells expressing Fas ligand, J. Exp. Med. 184 (1996) 1075–1082.
[26] A.J. Abusamra, Z. Zhong, X. Zheng, M. Li, T.E. Ichim, J.L. Chin, W.P. Min, Tumor exosomes expressing Fas ligand mediate CD8+ T-cell apoptosis, Blood Cells Mol. Dis. 35 (2005) 169–173.
[27] S.H. Wrzesinski, Y.Y. Wan, R.A. Flavell, Transforming growth factor-beta and the immune response: implications for anticancer therapy, Clin. Cancer Res. 13 (2007) 5262–5270.
[28] P.J. Murray, Understanding and exploiting the endogenous interleukin-10/STAT3-mediated anti-inflammatory response, Curr. Opin. Pharmacol. 6 (2006) 379–386.
[29] T. Sato, M. Terai, Y. Tamura, V. Alexeev, M.J. Mastrangelo, S.R. Selvan, Interleukin 10 in the tumor microenvironment: a target for anticancer immunotherapy, Immunol. Res. 51 (2011) 170–182.
[30] E. Donnadieu, in: E. Donnadieu (Ed.), Defects in T cell trafficking and resistance to cancer immunotherapy, Springer International Publishing, Switzerland, 2016 (pp. VIII, 199).
[31] D.H. Munn, A.L. Mellor, Indoleamine 2,3-dioxygenase and tumor-induced tolerance, J. Clin. Invest. 117 (2007) 1147–1154.
[32] H. Jing, E. Vassiliou, D. Ganea, Prostaglandin E2 inhibits production of the inflammatory chemokines CCL3 and CCL4 in dendritic cells, J. Leukoc. Biol. 74 (2003) 868–879.
[33] K. Takayama, G. Garcia-Cardena, G.K. Sukhova, J. Comander, M.A. Gimbrone Jr., P. Libby, Prostaglandin E2 suppresses chemokine production in human macrophages through the EP4 receptor, J. Biol. Chem. 277 (2002) 44147–44154.
[34] M. Nakanishi, D.W. Rosenberg, Multifaceted roles of PGE2 in inflammation and cancer, Semin. Immunopathol. 35 (2013) 123–137.
[35] D. Wang, R.N. Dubois, Prostaglandins and cancer, Gut 55 (2006) 115–122.
[36] T. Boon, J.-C. Cerottini, B. van den Eynde, P. van der Bruggen, A. van Pel, Tumor antigens recognized by T lymphocytes, Annu. Rev. Immunol. 12 (1994) 337–365.
[37] S.K. Watkins, Z. Zhu, E. Riboldi, K.A. Shafer-Weaver, K.E. Stagliano, M.M. Sklavos, S. Ambs, H. Yagita, A.A. Hurwitz, FOXO3 programs tumor-associated DCs to become tolerogenic in human and murine prostate cancer, J. Clin. Invest. 121 (2011) 1361–1372.
[38] A. Pinzon-Charry, T. Maxwell, J.A. Lopez, Dendritic cell dysfunction in cancer: a mechanism for immunosuppression, Immunol. Cell Biol. 83 (2005) 451–461.
[39] C. Esche, A. Lokshin, G.V. Shurin, B.R. Gastman, H. Rabinowich, S.C. Watkins, M.T. Lotze, M.R. Shurin, Tumor's other immune targets: dendritic cells, J. Leukoc. Biol. 66 (1999) 336–344.
[40] M.R. Shurin, M.T. Lotze, Dendritic cells in cancer: emergence of the discipline, in: M.R. Shurin, R.D. Salter (Eds.), Dendritic Cells in Cancer, Springer Science & Business Media, New York, NY 2009, pp. 11–30.
[41] M.T. Lotze, Getting to the source: dendritic cells as therapeutic reagents for the treatment of patients with cancer, Ann. Surg. 226 (1997) 1–5.
[42] A. Facciabene, G.T. Motz, G. Coukos, T-regulatory cells: key players in tumor immune escape and angiogenesis, Cancer Res. 72 (2012) 2162–2171.
[43] Y.S. Khaled, B.J. Ammori, E. Elkord, Myeloid-derived suppressor cells in cancer: recent progress and prospects, Immunol. Cell Biol. 91 (2013) 493–502.
[44] S. Demoulin, M. Herfs, P. Delvenne, P. Hubert, Tumor microenvironment converts plasmacytoid dendritic cells into immunosuppressive/tolerogenic cells: insight into the molecular mechanisms, J. Leukoc. Biol. 93 (2013) 343–352.
[45] C. Conrad, J. Gregorio, Y.H. Wang, T. Ito, S. Meller, S. Hanabuchi, S. Anderson, N. Atkinson, P.T. Ramirez, Y.J. Liu, R. Freedman, M. Gilliet, Plasmacytoid dendritic cells promote immunosuppression in ovarian cancer via ICOS costimulation of Foxp3(+) T-regulatory cells, Cancer Res. 72 (2012) 5240–5249.
[46] T. Chanmee, P. Ontong, K. Konno, N. Itano, Tumor-associated macrophages as major players in the tumor microenvironment, Cancers (Basel) 6 (2014) 1670–1690.
[47] C.H. Blood, B.R. Zetter, Tumor interactions with the vasculature: angiogenesis and tumor metastasis, Biochim. Biophys. Acta 1032 (1990) 89–118.
[48] L. Munn, Aberrant vascular architecture in tumors and its importance in drug-based therapies, Drug Discov. Today 8 (2003) 396–403.
[49] R.K. Jain, J.D. Martin, T. Stylianopoulos, The role of mechanical forces in tumor growth and therapy, Annu. Rev. Biomed. Eng. 16 (2014) 321–346.
[50] Y. Boucher, R.K. Jain, Microvascular pressure is the principal driving force for interstitial hypertension in solid tumors: implications for vascular collapse, Cancer Res. 52 (1992) 5110–5114.
[51] R.K. Jain, R.T. Tong, L.L. Munn, Effect of vascular normalization by antiangiogenic therapy on interstitial hypertension, peritumor edema, and lymphatic metastasis: insights from a mathematical model, Cancer Res. 67 (2007) 2729–2735.
[52] Y. Boucher, L.T. Baxter, R.K. Jain, Interstitial pressure gradients in tissue-isolated and subcutaneous tumors: implications for therapy, Cancer Res. 50 (1990) 4478–4484.
[53] B.F. Johnson, T.M. Clay, A.C. Hobeika, H.K. Lyerly, M.A. Morse, Vascular endothelial growth factor and immunosuppression in cancer: current knowledge and potential for new therapy, Expert. Opin. Biol. Ther. 7 (2007) 449–460.
[54] I.B. Barsoum, M. Koti, D.R. Siemens, C.H. Graham, Mechanisms of hypoxia-mediated immune escape in cancer, Cancer Res. 74 (2014) 7185–7190.
[55] S.J. Turley, V. Cremasco, J.L. Astarita, Immunological hallmarks of stromal cells in the tumour microenvironment, Nat. Rev. Immunol. 15 (2015) 669–682.
[56] G.T. Motz, S.P. Santoro, L.P. Wang, T. Garrabrant, R.R. Lastra, I.S. Hagemann, P. Lal, M.D. Feldman, F. Benencia, G. Coukos, Tumor endothelium FasL establishes a selective immune barrier promoting tolerance in tumors, Nat. Med. 20 (2014) 607–615.
[57] M.M. Mazanet, C.C.W. Hughes, B7-H1 is expressed by human endothelial cells and suppresses T cell cytokine synthesis, J. Immunol. 169 (2002) 3581–3588.
[58] H.O. Akman, H. Zhang, M.A.Q. Siddiqui, W. Solomon, E.L.P. Smith, O.A. Batuman, Response to hypoxia involves transforming growth factor-b2 and Smad proteins in human endothelial cells, Blood 98 (2001) 3324–3331.
[59] A. Alva, G.A. Daniels, M.K. Wong, H.L. Kaufman, M.A. Morse, D.F. McDermott, J.I. Clark, S.S. Agarwala, G. Miletello, T.F. Logan, R.J. Hauke, B. Curti, J.M. Kirkwood, R. Gonzalez, A. Amin, M. Fishman, N. Agarwal, J.N. Lowder, H. Hua, S. Aung, J.P. Dutcher, Contemporary experience with high-dose interleukin-2 therapy and impact on survival in patients with metastatic melanoma and metastatic renal cell carcinoma, Cancer Immunol. Immunother. 65 (2016) 1533–1544.
[60] S.A. Rosenberg, IL-2: the first effective immunotherapy for human cancer, J. Immunol. 192 (2014) 5451–5458.
[61] M.B. Atkins, M.T. Lotze, J.P. Dutcher, R.I. Fisher, G. Weiss, K. Margolin, J. Abrams, M. Sznol, D. Parkinson, M. Hawkins, C. Paradise, L. Kunkel, S.A. Rosenberg, High-dose recombinant interleukin 2 therapy for patients with metastatic melanoma: analysis of 270 patients treated between 1985 and 1993, J. Clin. Oncol. 17 (1999) 2105–2116.
[62] G. Fyfe, R.I. Fisher, S.A. Rosenberg, M. Sznol, D. Parkinson, A.C. Louie, Results of treatment of 255 patients with metastatic renal cell carcinoma who received high-dose recombinant interleukin-2 therapy, J. Clin. Oncol. 13 (1995) 688–696.
[63] T.M. Herndon, S.G. Demko, X. Jiang, K. He, J.E. Gootenberg, M.H. Cohen, P. Keegan, R. Pazdur, U.S. food and drug administration approval: peginterferon-alfa-2b for the adjuvant treatment of patients with melanoma, Oncologist 17 (2012) 1323–1328.
[64] J. Summers, M.H. Cohen, P. Keegan, R. Pazdur, FDA drug approval summary: bevacizumab plus interferon for advanced renal cell carcinoma, Oncologist 15 (2010) 104–111.
[65] S. Ahmed, K.R. Rai, Interferon in the treatment of hairy-cell leukemia, Best Pract. Res. Clin. Haematol. 16 (2003) 69–81.
[66] B.S. Parker, J. Rautela, P.J. Hertzog, Antitumour actions of interferons: implications for cancer therapy, Nat. Rev. Cancer 16 (2016) 131–144.
[67] J.W. Greiner, P.H. Hand, P. Noguchi, P.B. Fisher, S. Pestka, J. Schlom, Enhanced expression of surface tumor-associated antigens on human breast and colon tumor cells after recombinant human leukocyte a-interferon treatment, Cancer Res. 44 (1984) 3208–3214.
[68] A. Le Bon, D.F. Tough, Type I interferon as a stimulus for cross-priming, Cytokine Growth Factor Rev. 19 (2008) 33–40.
[69] M. Ahmadzadeh, S.A. Rosenberg, IL-2 administration increases CD4+ CD25(hi) Foxp3+ regulatory T cells in cancer patients, Blood 107 (2006) 2409–2414.
[70] T.R. Malek, The main function of IL-2 is to promote the development of T regulatory cells, J. Leukoc. Biol. 74 (2003) 961–965.
[71] S. Sleijfer, M. Bannink, A.R. Van Gool, W.H.J. Kruit, G. Stoter, Side effects of interferon-alpha therapy, Pharm. World Sci. 27 (2005) 423–431.
[72] S. Ziefle, F. Egberts, S. Heinze, M. Volkenandt, M. Schmid-Wendtner, W. Tilgen, R. Linse, J. Boettjer, T. Vogt, K. Spieth, T. Eigentler, N.H. Brockmeyer, A. Heinz, A. Hauschild, M. Schaefer, Health-related quality of life before and during adjuvant interferon-α treatment for patients with malignant melanoma (DeCOG-Trial), J. Immunother. 34 (2011) 403–408.
[73] A. Bottomley, C. Coens, S. Suciu, M. Santinami, W. Kruit, A. Testori, J. Marsden, C. Punt, F. Sales, M. Gore, R. Mackie, Z. Kusic, R. Dummer, P. Patel, D. Schadendorf, A. Spatz, U. Keilholz, A. Eggermont, Adjuvant therapy with pegylated interferon alfa-2b versus observation in resected stage III melanoma: a phase III randomized controlled trial of health-related quality of life and symptoms by the European Organisation for Research and Treatment of Cancer Melanoma Group, J. Clin. Oncol. 27 (2009) 2916–2923.
[74] J.C. Yang, S.L. Topalian, D.J. Schwartzentruber, D.R. Parkinson, F.M. Marincola, J.S. Weber, C.A. Seipp, D.E. White, S.A. Rosenberg, The use of polyethylene glycol-modified interleukin-2 (PEG-IL-2) in the treatment of patients with metastatic renal cell carcinoma and melanoma. A phase I study and a randomized prospective study comparing IL-2 alone versus IL-2 combined with PEG-IL-2, Cancer 76 (1995) 687–694.
[75] A.S. Rosenberg, D. Verthelyi, B.W. Cherney, Managing uncertainty: a perspective on risk pertaining to product quality attributes as they bear on immunogenicity of therapeutic proteins, J. Pharm. Sci. 101 (2012) 3560–3567.
[76] G. Antonelli, G. Giannelli, M. Currenti, E. Simeoni, S. Del Vecchio, F. Maggi, M. Pistello, L. Roffi, G. Pastore, L. Chemello, F. Dianzani, Antibodies to interferon (IFN) in hepatitis C patients relapsing while continuing recombinant IFN-alpha2 therapy, Clin. Exp. Immunol. 104 (1996) 384–387.
[77] M.G. Tovey, C. Lallemand, Safety, Tolerability, and Immunogenicity of Interferons, Pharmaceuticals (Basel, Switzerland), vol 3, 2010 1162–1186.
[78] C. Lallemand, J.F. Meritet, R. Erickson, S.E. Grossberg, E. Roullet, O. Lyon-Caen, P. Lebon, M.G. Tovey, Quantification of neutralizing antibodies to human type I interferons using division-arrested frozen cells carrying an interferon-regulated reporter-gene, Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research 28 (2008) 393–404.
[79] C. Jorns, D. Holzinger, R. Thimme, H.C. Spangenberg, M. Weidmann, J. Rasenack, H. E. Blum, O. Haller, G. Kochs, Rapid and simple detection of IFN-neutralizing antibodies in chronic hepatitis C non-responsive to IFN-alpha, J. Med. Virol. 78 (2006) 74–82.
[80] E.L. Hanninen, J. Knuver-Hopf, J. Atzpodien, Immunogenicity of recombinant human interleukin-2: biological features and clinical relevance, biotherapy (Dordrecht, Netherlands), vol 6, 1993 251–261.
[81] A.L. Hjelm Skog, M. Wadhwa, M. Hassan, B. Gharizadeh, C. Bird, P. Ragnhammar, R. Thorpe, H. Mellstedt, Alteration of interleukin 2 (IL-2) pharmacokinetics and function by IL-2 antibodies induced after treatment of colorectal carcinoma patients with a combination of monoclonal antibody 17-1A, granulocyte macrophage colony-stimulating factor, and IL-2, Clin. Cancer Res. 7 (2001) 1163–1170.
[82] N.P. Restifo, M.E. Dudley, S.A. Rosenberg, Adoptive immunotherapy for cancer: harnessing the T cell response, Nat. Rev. Immunol. 12 (2012) 269–281.

[83] S.A. Rosenberg, J.C. Yang, R.M. Sherry, U.S. Kammula, M.S. Hughes, G.Q. Phan, D.E. Citrin, N.P. Restifo, P.F. Robbins, J.R. Wunderlich, K.E. Morton, C.M. Laurencot, S. M. Steinberg, D.E. White, M.E. Dudley, Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy, Clin. Cancer Res. 17 (2011) 4550–4557.
[84] M.J. Besser, R. Shapira-Frommer, A.J. Treves, D. Zippel, O. Itzhaki, L. Hershkovitz, D. Levy, A. Kubi, E. Hovav, N. Chermoshniuk, B. Shalmon, I. Hardan, R. Catane, G. Markel, S. Apter, A. Ben-Nun, I. Kuchuk, A. Shimoni, A. Nagler, J. Schachter, Clinical responses in a phase II study using adoptive transfer of short-term cultured tumor infiltration lymphocytes in metastatic melanoma patients, Clin. Cancer Res. 16 (2010) 2646–2655.
[85] R. Houot, L.M. Schultz, A. Marabelle, H. Kohrt, T-cell-based immunotherapy: adoptive cell transfer and checkpoint inhibition, Cancer Immunol Res 3 (2015) 1115–1122.
[86] S.L. Goff, F.O. Smith, J.A. Klapper, R. Sherry, J.R. Wunderlich, S.M. Steinberg, D. White, S.A. Rosenberg, M.E. Dudley, J.C. Yang, Tumor infiltrating lymphocyte therapy for metastatic melanoma: analysis of tumors resected for TIL, J. Immunother. 33 (2010) 840–847.
[87] J. Zhou, X. Shen, J. Huang, R.J. Hodes, S.A. Rosenberg, P.F. Robbins, Telomere length of transferred lymphocytes correlates with in vivo persistence and tumor regression in melanoma patients receiving cell transfer therapy, J. Immunol. 175 (2005) 7046–7052.
[88] J.R. Yannelli, C. Hyatt, S. McConnell, K. Hines, L. Jacknin, L. Parker, M. Sanders, S.A. Rosenberg, Growth of tumor-infiltrating lymphocytes from human solid cancers: summary of a 5-year experience, Int. J. Cancer 65 (1996) 413–421.
[89] E. Tran, S. Turcotte, A. Gros, P.F. Robbins, L. Yong-Chen, M.E. Dudley, J.R. Wunderlich, R.P. Somerville, K. Hogan, C.S. Hinrichs, M.R. Parkhurst, J.C. Yang, S. A. Rosenberg, Cancer immunotherapy based on mutation-specific CD4+ T cells in a patient with epithelial cancer, Science 344 (2014) 641–645.
[90] A.D. Fesnak, C.H. June, B.L. Levine, Engineered T cells: the promise and challenges of cancer immunotherapy, Nat. Rev. Cancer 16 (2016) 566–581.
[91] A.P. Rapoport, E.A. Stadtmauer, G.K. Binder-Scholl, O. Goloubeva, D.T. Vogl, S.F. Lacey, A.Z. Badros, A. Garfall, B. Weiss, J. Finklestein, I. Kulikovskaya, S.K. Sinha, S. Kronsberg, M. Gupta, S. Bond, L. Melchiori, J.E. Brewer, A.D. Bennett, A.B. Gerry, N.J. Pumphrey, D. Williams, H.K. Tayton-Martin, L. Ribeiro, T. Holdich, S. Yanovich, N. Hardy, J. Yared, N. Kerr, S. Philip, S. Westphal, D.L. Siegel, B.L. Levine, B.K. Jakobsen, M. Kalos, C.H. June, NY-ESO-1-specific TCR-engineered T cells mediate sustained antigen-specific antitumor effects in myeloma, Nat. Med. 21 (2015) 914–921.
[92] P.F. Robbins, R.A. Morgan, S.A. Feldman, J.C. Yang, R.M. Sherry, M.E. Dudley, J.R. Wunderlich, A.V. Nahvi, L.J. Helman, C.L. Mackall, U.S. Kammula, M.S. Hughes, N. P. Restifo, M. Raffeld, C.C. Lee, C.L. Levy, Y.F. Li, M. El-Gamil, S.L. Schwarz, C. Laurencot, S.A. Rosenberg, Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1, J. Clin. Oncol. 29 (2011) 917–924.
[93] M.L. Davila, I. Riviere, X. Wang, S. Bartido, J. Park, K. Curran, S.S. Chung, J. Stefanski, O. Borquez-Ojeda, M. Olszewska, J. Qu, T. Wasielewska, Q. He, M. Fink, H. Shinglot, M. Youssif, M. Satter, Y. Wang, J. Hosey, H. Quintanilla, E. Halton, Y. Bernal, D.C.G. Bouhassira, M.E. Arcila, M. Gonen, G.J. Roboz, P. Maslak, D. Douer, M.G. Frattini, S. Giralt, M. Sadelain, R. Brentjens, Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia, Sci. Transl. Med. 6 (2014), 224ra225.
[94] S.L. Maude, D.T. Teachey, D.L. Porter, S.A. Grupp, CD19-targeted chimeric antigen receptor T-cell therapy for acute lymphoblastic leukemia, Blood 125 (2015) 4017–4023.
[95] M.R. Parkhurst, J.C. Yang, R.C. Langan, M.E. Dudley, D.A. Nathan, S.A. Feldman, J.L. Davis, R.A. Morgan, M.J. Merino, R.M. Sherry, M.S. Hughes, U.S. Kammula, G.Q. Phan, R.M. Lim, S.A. Wank, N.P. Restifo, P.F. Robbins, C.M. Laurencot, S.A. Rosenberg, T cells targeting carcinoembryonic antigen can mediate regression of metastatic colorectal cancer but induce severe transient colitis, Mol. Ther. 19 (2011) 620–626.
[96] R.A. Morgan, J.C. Yang, M. Kitano, M.E. Dudley, C.M. Laurencot, S.A. Rosenberg, Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2, Mol. Ther. 18 (2010) 843–851.
[97] K. Newick, E. Moon, S.M. Albelda, Chimeric antigen receptor T-cell therapy for solid tumors, Mol Ther Oncolytics 3 (2016) 16006.
[98] A. Tarhini, E. Lo, D.R. Minor, Releasing the brake on the immune system: ipilimumab in melanoma and other tumors, Cancer Biother. Radiopharm. 25 (2010) 601–613.
[99] A. Stark, FDA approves first treatment for rare form of skin cancer, US Food and Drug Administration, Silver Spring (MD), 2017.
[100] P.A. Ott, F.S. Hodi, C. Robert, CTLA-4 and PD-1/PD-L1 blockade: new immunotherapeutic modalities with durable clinical benefit in melanoma patients, Clin. Cancer Res. 19 (2013) 5300–5309.
[101] M.K. Callahan, M.A. Postow, J.D. Wolchok, CTLA-4 and PD-1 pathway blockade: combinations in the clinic, Front. Oncol. 4 (2014) 385.
[102] W. Zou, J.D. Wolchok, L. Chen, PD-L1 (B7-H1) and PD-1 pathway blockade for cancer therapy: Mechanisms, response biomarkers, and combinations, Sci. Transl. Med. 8 (2016), 328rv324. .
[103] L.B. John, C. Devaud, C.P. Duong, C.S. Yong, P.A. Beavis, N.M. Haynes, M.T. Chow, M.J. Smyth, M.H. Kershaw, P.K. Darcy, Anti-PD-1 antibody therapy potently enhances the eradication of established tumors by gene-modified T cells, Clin. Cancer Res. 19 (2013) 5636–5646.
[104] P.E. Hughes, S. Caenepeel, L.C. Wu, Targeted Therapy and Checkpoint Immunotherapy Combinations for the Treatment of Cancer, Trends Immunol. 37 (2016) 462–476.
[105] J. Kleponis, R. Skelton, L. Zheng, Fueling the engine and releasing the break: combinational therapy of cancer vaccines and immune checkpoint inhibitors, Cancer Biol Med 12 (2015) 201–208.
[106] P. Sharma, J.P. Allison, The future of immune checkpoint therapy, Science 348 (2015) 56–61.
[107] S. Koyama, E.A. Akbay, Y.Y. Li, G.S. Herter-Sprie, K.A. Buczkowski, W.G. Richards, L. Gandhi, A.J. Redig, S.J. Rodig, H. Asahina, R.E. Jones, M.M. Kulkarni, M. Kuraguchi, S. Palakurthi, P.E. Fecci, B.E. Johnson, P.A. Janne, J.A. Engelman, S.P. Gangadharan, D.B. Costa, G.J. Freeman, R. Bueno, F.S. Hodi, G. Dranoff, K.K. Wong, P.S. Hammerman, Adaptive resistance to therapeutic PD-1 blockade is associated with upregulation of alternative immune checkpoints, Nat. Commun. 7 (2016) 10501.
[108] C. Boutros, A. Tarhini, E. Routier, O. Lambotte, F.L. Ladurie, F. Carbonnel, H. Izzeddine, A. Marabelle, S. Champiat, A. Berdelou, E. Lanoy, M. Texier, C. Libenciuc, A.M. Eggermont, J.C. Soria, C. Mateus, C. Robert, Safety profiles of anti-CTLA-4 and anti-PD-1 antibodies alone and in combinationNat Rev Clin Oncol 2016.
[109] T. Dillard, C.G. Yedinak, J. Alumkal, M. Fleseriu, Anti-CTLA-4 antibody therapy associated autoimmune hypophysitis: serious immune related adverse events across a spectrum of cancer subtypes, Pituitary 13 (2010) 29–38.
[110] C. Gaudy, C. Clevy, S. Monestier, N. Dubois, Y. Preau, S. Mallet, M.A. Richard, J.J. Grob, R. Valero, S. Beliard, Anti-PD1 pembrolizumab can induce exceptional fulminant Type 1 diabetes, Diabetes Care 38 (2015) e182–183.
[111] S.A. Rosenberg, J.C. Yang, N.P. Restifo, Cancer immunotherapy: moving beyond current vaccines, Nat. Med. 10 (2004) 909–915.
[112] L.G. Gomella, F. Gelpi-Hammerschmidt, C. Kundavram, Practical guide to immunotherapy in castration-resistant prostate cancer: the use of sipuleucel-T immunotherapy, Can. J. Urol. 21 (2014) 48–56.
[113] J.N. Graff, E.D. Chamberlain, Sipuleucel-T in the treatment of prostate cancer: an evidence-based review of its place in therapy, Core Evid 10 (2015) 1–10.
[114] C.S. Higano, P.F. Schellhammer, E.J. Small, P.A. Burch, J. Nemunaitis, L. Yuh, N. Provost, M.W. Frohlich, Integrated data from 2 randomized, double-blind, placebo-controlled, phase 3 trials of active cellular immunotherapy with sipuleucel-T in advanced prostate cancer, Cancer 115 (2009) 3670–3679.
[115] U.o.M. Masonic Cancer Center, Phase II Study of Sipuleucel-T and Indoximod for Patients With Refractory Metastatic Prostate Cancer, ClinicalTrials.gov [Internet] National Library of Medicine (US), Bethesda (MD), 2012.
[116] C. Grigg, Z. Blake, R. Gartrell, A. Sacher, B. Taback, Y. Saenger, Talimogene laherparepvec (T-Vec) for the treatment of melanoma and other cancers, Semin. Oncol. 43 (2016) 638–646.
[117] R.H. Andtbacka, H.L. Kaufman, F. Collichio, T. Amatruda, N. Senzer, J. Chesney, K.A. Delman, L.E. Spitler, I. Puzanov, S.S. Agarwala, M. Milhem, L. Cranmer, B. Curti, K. Lewis, M. Ross, T. Guthrie, G.P. Linette, G.A. Daniels, K. Harrington, M.R. Middleton, W.H. Miller Jr., J.S. Zager, Y. Ye, B. Yao, A. Li, S. Doleman, A. VanderWalde, J. Gansert, R.S. Coffin, Talimogene Laherparepvec Improves Durable Response Rate in Patients With Advanced Melanoma, J. Clin. Oncol. 33 (2015) 2780–2788.
[118] I. Puzanov, M.M. Milhem, R.H.I. Andtbacka, D.R. Minor, O. Hamid, A. Li, M. Chastain, K. Gorski, A. Anderson, A.M. Vanderwalde, J. Chou, H. Kaufman, Primary analysis of a phase 1b multicenter trial to evaluate safety and efficacy of talimogene laherparepvec (T-VEC) and ipilimumab (ipi) in previously untreated, unresected stage IIIB-IV melanoma, J. Clin. Oncol. 32 (2014).
[119] Amgen, Ipilimumab With or Without Talimogene Laherparepvec in Unresected Melanoma, ClinicalTrials.gov Internet National Library of Medicine (US), Bethesda (MD), 2012.
[120] A. Schroeder, D.A. Heller, M.M. Winslow, J.E. Dahlman, G.W. Pratt, R. Langer, T. Jacks, D.G. Anderson, Treating metastatic cancer with nanotechnology, Nat. Rev. Cancer 12 (2012) 39–50.
[121] J. Conde, C.E. Arnold, F. Tian, N. Artzi, RNAi nanomaterials targeting immune cells as an anti-tumor therapy: the missing link in cancer treatment? Mater. Today 19 (2016) 29–43.
[122] S.Y. Li, Y. Liu, C.F. Xu, S. Shen, R. Sun, X.J. Du, J.X. Xia, Y.H. Zhu, J. Wang, Restoring anti-tumor functions of T cells via nanoparticle-mediated immune checkpoint modulation, J. Control. Release 231 (2016) 17–28.
[123] P.Y. Teo, C. Yang, L.M. Whilding, A.C. Parente-Pereira, J. Maher, A.J. George, J.L. Hedrick, Y.Y. Yang, S. Ghaem-Maghami, Ovarian cancer immunotherapy using PD-L1 siRNA targeted delivery from folic acid-functionalized polyethylenimine: strategies to enhance T cell killing, Adv Healthc Mater 4 (2015) 1180–1189.
[124] K. Iwamura, T. Kato, Y. Miyahara, H. Naota, J. Mineno, H. Ikeda, H. Shiku, siRNA-mediated silencing of PD-1 ligands enhances tumor-specific human T-cell effector functions, Gene Ther. 19 (2012) 959–966.
[125] Z. Huang, Z. Zhang, Y. Jiang, D. Zhang, J. Chen, L. Dong, J. Zhang, Targeted delivery of oligonucleotides into tumor-associated macrophages for cancer immunotherapy, J. Control. Release 158 (2012) 286–292.
[126] J. Conde, C. Bao, Y. Tan, D. Cui, E.R. Edelman, H.S. Azevedo, H.J. Byrne, N. Artzi, F. Tian, Dual targeted immunotherapy via in vivo delivery of biohybrid RNAi-peptide nanoparticles to tumour-associated macrophages and cancer cells, Adv. Funct. Mater. 25 (2015) 4183–4194.
[127] J. Park, S.H. Wrzesinski, E. Stern, M. Look, J. Criscione, R. Ragheb, S.M. Jay, S.L. Demento, A. Agawu, P. Licona Limon, A.F. Ferrandino, D. Gonzalez, A. Habermann, R.A. Flavell, T.M. Fahmy, Combination delivery of TGF-beta inhibitor and IL-2 by nanoscale liposomal polymeric gels enhances tumour immunotherapy, Nat. Mater. 11 (2012) 895–905.
[128] B. Kwong, H. Liu, D.J. Irvine, Induction of potent anti-tumor responses while eliminating systemic side effects via liposome-anchored combinatorial immunotherapy, Biomaterials 32 (2011) 5134–5147.
[129] B. Kwong, S.A. Gai, J. Elkhader, K.D. Wittrup, D.J. Irvine, Localized immunotherapy via liposome-anchored Anti-CD137 + IL-2 prevents lethal toxicity and elicits local and systemic antitumor immunity, Cancer Res. 73 (2013) 1547–1558.
[130] D.J. Irvine, M.C. Hanson, K. Rakhra, T. Tokatlian, Synthetic nanoparticles for vaccines and immunotherapy, Chem. Rev. 115 (2015) 11109–11146.

[131] S.T. Reddy, A.J. van der Vlies, E. Simeoni, V. Angeli, G.J. Randolph, C.P. O'Neil, L.K. Lee, M.A. Swartz, J.A. Hubbell, Exploiting lymphatic transport and complement activation in nanoparticle vaccines, Nat. Biotechnol. 25 (2007) 1159–1164.
[132] V. Manolova, A. Flace, M. Bauer, K. Schwarz, P. Saudan, M.F. Bachmann, Nanoparticles target distinct dendritic cell populations according to their size, Eur. J. Immunol. 38 (2008) 1404–1413.
[133] J.K. Eby, K.Y. Dane, C.P. O'Neil, S. Hirosue, M.A. Swartz, J.A. Hubbell, Polymer micelles with pyridyl disulfide-coupled antigen travel through lymphatics and show enhanced cellular responses following immunization, Acta Biomater. 8 (2012) 3210–3217.
[134] L. Jeanbart, M. Ballester, A. de Titta, P. Corthesy, P. Romero, J.A. Hubbell, M.A. Swartz, Enhancing efficacy of anticancer vaccines by targeted delivery to tumor-draining lymph nodes, Cancer Immunol Res 2 (2014) 436–447.
[135] H. Liu, K.D. Moynihan, Y. Zheng, G.L. Szeto, A.V. Li, B. Huang, D.S. Van Egeren, C. Park, D.J. Irvine, Structure-based programming of lymph-node targeting in molecular vaccines, Nature 507 (2014) 519–522.
[136] B.R. Lee, H.K. Ko, J.H. Ryu, K.Y. Ahn, Y.H. Lee, S.J. Oh, J.H. Na, T.W. Kim, Y. Byun, I.C. Kwon, K. Kim, J. Lee, Engineered human ferritin nanoparticles for direct delivery of tumor antigens to lymph node and cancer immunotherapy, Sci. Rep. 6 (2016) 35182.
[137] J. Azzi, Q. Yin, M. Uehara, S. Ohori, L. Tang, K. Cai, T. Ichimura, M. McGrath, O. Maarouf, E. Kefaloyianni, S. Loughhead, J. Petr, Q. Sun, M. Kwon, S. Tullius, U.H. von Andrian, J. Cheng, R. Abdi, Targeted Delivery of Immunomodulators to Lymph Nodes, Cell Rep. 15 (2016) 1202–1213.
[138] L.M. Kranz, M. Diken, H. Haas, S. Kreiter, C. Loquai, K.C. Reuter, M. Meng, D. Fritz, F. Vascotto, H. Hefesha, C. Grunwitz, M. Vormehr, Y. Husemann, A. Selmi, A.N. Kuhn, J. Buck, E. Derhovanessian, R. Rae, S. Attig, J. Diekmann, R.A. Jabulowsky, S. Heesch, J. Hassel, P. Langguth, S. Grabbe, C. Huber, O. Tureci, U. Sahin, Systemic RNA delivery to dendritic cells exploits antiviral defence for cancer immunotherapy, Nature 534 (2016) 396–401.
[139] D.J. Irvine, J.J. Moon, Lipid vesicle compositions and methods of use, 2014.
[140] J.J. Moon, H. Suh, A.V. Li, C.F. Ockenhouse, A. Yadava, D.J. Irvine, Enhancing humoral responses to a malaria antigen with nanoparticle vaccines that expand Tfh cells and promote germinal center induction, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 1080–1085.
[141] T.R. Bagby, S. Duan, S. Cai, Q. Yang, S. Thati, C. Berkland, D.J. Aires, M.L. Forrest, Lymphatic trafficking kinetics and near-infrared imaging using star polymer architectures with controlled anionic character, Eur. J. Pharm. Sci. 47 (2012) 287–294.
[142] D.A. Rao, M.L. Forrest, A.W. Alani, G.S. Kwon, J.R. Robinson, Biodegradable PLGA based nanoparticles for sustained regional lymphatic drug delivery, J. Pharm. Sci. 99 (2010) 2018–2031.
[143] E. Seydoux, L. Rodriguez-Lorenzo, R.A. Blom, P.A. Stumbles, A. Petri-Fink, B.M. Rothen-Rutishauser, F. Blank, C. von Garnier, Pulmonary delivery of cationic gold nanoparticles boost antigen-specific CD4+ T Cell Proliferation, Nanomedicine 12 (2016) 1815–1826.
[144] C.A. Fromen, T.B. Rahhal, G.R. Robbins, M.P. Kai, T.W. Shen, J.C. Luft, J.M. DeSimone, Nanoparticle surface charge impacts distribution, uptake and lymph node trafficking by pulmonary antigen-presenting cells, Nanomedicine 12 (2016) 677–687.
[145] C.A. Fromen, G.R. Robbins, T.W. Shen, M.P. Kai, J.P.Y. Ting, J.M. DeSimone, Controlled analysis of nanoparticle charge on mucosal and systemic antibody responses following pulmonary immunization, Proc. Natl. Acad. Sci. U. S. A. 112 (2014) 488–493.
[146] S.L. Demento, A.L. Siefert, A. Bandyopadhyay, F.A. Sharp, T.M. Fahmy, Pathogen-associated molecular patterns on biomaterials: a paradigm for engineering new vaccines, Trends Biotechnol. 29 (2011) 294–306.
[147] M. Zhu, R. Wang, G. Nie, Applications of nanomaterials as vaccine adjuvants, Hum Vaccin Immunother 10 (2014) 2761–2774.
[148] J. Harris, F.A. Sharp, E.C. Lavelle, The role of inflammasomes in the immunostimulatory effects of particulate vaccine adjuvants, Eur. J. Immunol. 40 (2010) 634–638.
[149] H. Li, Y. Li, J. Jiao, H.M. Hu, Alpha-alumina nanoparticles induce efficient autophagy-dependent cross-presentation and potent antitumour response, Nat. Nanotechnol. 6 (2011) 645–650.
[150] S.M. Opal, Endotoxins and other sepsis triggers, in: R. C, P. P, R. MH (Eds.), Endotoxemia and Endotoxin Shock: Disease, Diagnosis and Therapy, Karger AG, Basel 2010, pp. 14–24.
[151] A. Batista-Duharte, E.B. Lindblad, E. Oviedo-Orta, Progress in understanding adjuvant immunotoxicity mechanisms, Toxicol. Lett. 203 (2011) 97–105.
[152] D. Verthelyi, D.M. Klinman, CpG-ODN—safety considerations, in: E. Raz (Ed.), Microbial DNA and Host Immunity, Humana Press, Totowa, NJ 2002, pp. 385–396.
[153] A. de Titta, M. Ballester, Z. Julier, C. Nembrini, L. Jeanbart, A.J. van der Vlies, M.A. Swartz, J.A. Hubbell, Nanoparticle conjugation of CpG enhances adjuvancy for cellular immunity and memory recall at low dose, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 19902–19907.
[154] C. Bourquin, D. Anz, K. Zwiorek, A.L. Lanz, S. Fuchs, S. Weigel, C. Wurzenberger, P. von der Borch, M. Golic, S. Moder, G. Winter, C. Coester, S. Endres, Targeting CpG Oligonucleotides to the Lymph Node by Nanoparticles Elicits Efficient Antitumoral Immunity, J. Immunol. 181 (2008) 2990–2998.
[155] M.C. Hanson, M.P. Crespo, W. Abraham, K.D. Moynihan, G.L. Szeto, S.H. Chen, M.B. Melo, S. Mueller, D.J. Irvine, Nanoparticulate STING agonists are potent lymph node-targeted vaccine adjuvants, J. Clin. Invest. 125 (2015) 2532–2546.
[156] G.M. Lynn, R. Laga, P.A. Darrah, A.S. Ishizuka, A.J. Balaci, A.E. Dulcey, M. Pechar, R. Pola, M.Y. Gerner, A. Yamamoto, C.R. Buechler, K.M. Quinn, M.G. Smelkinson, O. Vanek, R. Cawood, T. Hills, O. Vasalatiy, K. Kastenmuller, J.R. Francica, L. Stutts, J.K. Tom, K.A. Ryu, A.P. Esser-Kahn, T. Etrych, K.D. Fisher, L.W. Seymour, R.A. Seder, In vivo characterization of the physicochemical properties of polymer-linked TLR agonists that enhance vaccine immunogenicity, Nat. Biotechnol. 33 (2015) 1201–1210.
[157] P.O. Ilyinskii, C.J. Roy, C.P. O'Neil, E.A. Browning, L.A. Pittet, D.H. Altreuter, F. Alexis, E. Tonti, J. Shi, P.A. Basto, M. Iannacone, A.F. Radovic-Moreno, R.S. Langer, O.C. Farokhzad, U.H. von Andrian, L.P. Johnston, T.K. Kishimoto, Adjuvant-carrying synthetic vaccine particles augment the immune response to encapsulated antigen and exhibit strong local immune activation without inducing systemic cytokine release, Vaccine 32 (2014) 2882–2895.
[158] O.P. Joffre, E. Segura, A. Savina, S. Amigorena, Cross-presentation by dendritic cells, Nat. Rev. Immunol. 12 (2012) 557–569.
[159] C.M. Fehres, W.W. Unger, J.J. Garcia-Vallejo, Y. van Kooyk, Understanding the biology of antigen cross-presentation for the design of vaccines against cancer, Front. Immunol. 5 (2014) 149.
[160] C.L. Chiang, G. Coukos, L.E. Kandalaft, Whole Tumor Antigen Vaccines: Where Are We? Vaccines (Basel) 3 (2015) 344–372.
[161] S. Prasad, V. Cody, J.K. Saucier-Sawyer, W.M. Saltzman, C.T. Sasaki, R.L. Edelson, M. A. Birchall, D.J. Hanlon, Polymer nanoparticles containing tumor lysates as antigen delivery vehicles for dendritic cell-based antitumor immunotherapy, Nanomedicine 7 (2011) 1–10.
[162] H. Shen, A.L. Ackerman, V. Cody, A. Giodini, E.R. Hinson, P. Cresswell, R.L. Edelson, W.M. Saltzman, D.J. Hanlon, Enhanced and prolonged cross-presentation following endosomal escape of exogenous antigens encapsulated in biodegradable nanoparticles, Immunology 117 (2006) 78–88.
[163] R. Han, J. Zhu, X. Yang, H. Xu, Surface modification of poly(D,L-lactic-co-glycolic acid) nanoparticles with protamine enhanced cross-presentation of encapsulated ovalbumin by bone marrow-derived dendritic cells, J. Biomed. Mater. Res. A 96 (2011) 142–149.
[164] C. Song, Y.W. Noh, Y.T. Lim, Polymer nanoparticles for cross-presentation of exogenous antigens and enhanced cytotoxic T-lymphocyte immune response, Int. J. Nanomedicine 11 (2016) 3753–3764.
[165] J. Chen, Z. Li, H. Huang, Y. Yang, Q. Ding, J. Mai, W. Guo, Y. Xu, Improved antigen cross-presentation by polyethyleneimine-based nanoparticles, Int. J. Nanomedicine 6 (2011) 77–84.
[166] A. Petrizzo, C. Conte, M. Tagliamonte, M. Napolitano, K. Bifulco, V. Carriero, A. De Stradis, M.L. Tornesello, F.M. Buonaguro, F. Quaglia, L. Buonaguro, Functional characterization of biodegradable nanoparticles as antigen delivery system, J. Exp. Clin. Cancer Res. 34 (2015) 114.
[167] S.S. Saluja, D.J. Hanlon, F.A. Sharp, E. Hong, D. Khalil, E. Robinson, R. Tigelaar, T.M. Fahmy, R.L. Edelson, Targeting human dendritic cells via DEC-205 using PLGA nanoparticles leads to enhanced cross-presentation of a melanoma-associated antigen, Int. J. Nanomedicine 9 (2014) 5231–5246.
[168] T.H. Kang, J.H. Lee, H.C. Bae, K.H. Noh, J.H. Kim, C.K. Song, B.C. Shin, C.F. Hung, T.C. Wu, J.S. Park, T.W. Kim, Enhancement of dendritic cell-based vaccine potency by targeting antigen to endosomal/lysosomal compartments, Immunol. Lett. 106 (2006) 126–134.
[169] T.R. Ghimire, The mechanisms of action of vaccines containing aluminum adjuvants: an in vitro vs in vivo paradigm, Spring 4 (2015) 181.
[170] S.L. Demento, W. Cui, J.M. Criscione, E. Stern, J. Tulipan, S.M. Kaech, T.M. Fahmy, Role of sustained antigen release from nanoparticle vaccines in shaping the T cell memory phenotype, Biomaterials 33 (2012) 4957–4964.
[171] A. Stano, E.A. Scott, K.Y. Dane, M.A. Swartz, J.A. Hubbell, Tunable T cell immunity towards a protein antigen using polymersomes vs. solid-core nanoparticles, Biomaterials 34 (2013) 4339–4346.
[172] T.N. Schumacher, R.D. Schreiber, Neoantigens in cancer immunotherapy, Science 348 (2015) 69–74.
[173] R. Kuai, L.J. Ochyl, K.S. Bahjat, A. Schwendeman, J.J. Moon, Designer vaccine nanodiscs for personalized cancer immunotherapy, Nat. Mater. 16 (2016) 489–496.
[174] X. Huang, P.K. Jain, I.H. El-Sayed, M.A. El-Sayed, Plasmonic photothermal therapy (PPTT) using gold nanoparticles, Lasers Med. Sci. 23 (2007) 217.
[175] Y. Li, W. Lu, Q. Huang, M. Huang, C. Li, W. Chen, Copper sulfide nanoparticles for photothermal ablation of tumor cells, Nanomedicine (London) 5 (2010) 1161–1171.
[176] G. Fu, W. Liu, S. Feng, X. Yue, Prussian blue nanoparticles operate as a new generation of photothermal ablation agents for cancer therapy, Chem. Commun. (Camb.) 48 (2012) 11567–11569.
[177] A.P. Castano, P. Mroz, M.R. Hamblin, Photodynamic therapy and anti-tumour immunity, Nat. Rev. Cancer 6 (2006) 535–545.
[178] Y. Tao, E. Ju, Z. Liu, K. Dong, J. Ren, X. Qu, Engineered, self-assembled near-infrared photothermal agents for combined tumor immunotherapy and chemo-photothermal therapy, Biomaterials 35 (2014) 6646–6656.
[179] L. Guo, D.D. Yan, D. Yang, Y. Li, X. Wang, O. Zalewski, B. Yan, W. Lu, Combinatorial photothermal and immuno cancer therapy using chitosan-coated hollow copper sulfide nanoparticles, ACS Nano 8 (2014) 5670–5681.
[180] J. Cano-Mejia, R.A. Burga, E.E. Sweeney, J.P. Fisher, C.M. Bollard, A.D. Sandler, C.R. Cruz, R. Fernandes, Prussian Blue Nanoparticle-based Photothermal Therapy Combined with Checkpoint Inhibition for Photothermal Immunotherapy of NeuroblastomaNanomedicine 2016.
[181] Q. Chen, L. Xu, C. Liang, C. Wang, R. Peng, Z. Liu, Photothermal therapy with immune-adjuvant nanoparticles together with checkpoint blockade for effective cancer immunotherapy, Nat. Commun. 7 (2016) 13193.
[182] C. He, X. Duan, N. Guo, C. Chan, C. Poon, R.R. Weichselbaum, W. Lin, Core-shell nanoscale coordination polymers combine chemotherapy and photodynamic therapy to potentiate checkpoint blockade cancer immunotherapy, Nat. Commun. 7 (2016) 12499.
[183] S. Toraya-Brown, M.R. Sheen, P. Zhang, L. Chen, J.R. Baird, E. Demidenko, M.J. Turk, P.J. Hoopes, J.R. Conejo-Garcia, S. Fiering, Local hyperthermia treatment of tumors induces CD8(+) T cell-mediated resistance against distal and secondary tumors, Nanomedicine 10 (2014) 1273–1285.

[184] A. Ito, K. Tanaka, K. Kondo, M. Shinkai, H. Honda, K. Matsumoto, T. Saida, T. Kobayashi, Tumor regression by combined immunotherapy and hyperthermia using magnetic nanoparticles in an experimental subcutaneous murine melanoma, Cancer Sci. 94 (2003) 308–313.
[185] R. Mejias, S. Perez-Yague, L. Gutierrez, L.I. Cabrera, R. Spada, P. Acedo, C.J. Serna, F.J. Lazaro, A. Villanueva, P. Morales Mdel, D.F. Barber, Dimercaptosuccinic acid-coated magnetite nanoparticles for magnetically guided in vivo delivery of interferon gamma for cancer immunotherapy, Biomaterials 32 (2011) 2938–2952.
[186] D.A. Christian, C.A. Hunter, Particle-mediated delivery of cytokines for immunotherapy, Immunotherapy 4 (2012) 425–441.
[187] P.M. Anderson, D.C. Hanson, D.E. Hasz, M.R. Halet, B.R. Blazar, A.C. Ochoa, Cytokines in liposomes: preliminary studies with IL-1, IL-2, IL-6, GM-CSF and Interferon-gamma, Cytokine 6 (1994) 92–101.
[188] T. Shimizu, T. Kishida, U. Hasegawa, Y. Ueda, J. Imanishi, H. Yamagishi, K. Akiyoshi, E. Otsuji, O. Mazda, Nanogel DDS enables sustained release of IL-12 for tumor immunotherapy, Biochem. Biophys. Res. Commun. 367 (2008) 330–335.
[189] P.M. Anderson, E. Katsanis, A.S. Leonard, D. Schow, C.M. Loeffler, M.B. Goldstein, A. C. Ochoa, Increased local antitumor effects of interleukin 2 liposomes in mice with MCA-106 sarcoma pulmonary metastases, Cancer Res. 50 (1990) 1853–1856.
[190] E. Kedar, O. Palgi, G. Golod, I. Babai, Y. Barenholz, Delivery of cytokines by liposomes. III. Liposome-encapsulated GM-CSF and TNF-alpha show improved pharmacokinetics and biological activity and reduced toxicity in mice, J. Immunother. 20 (1997) 180–193.
[191] E.R. Steenblock, T.M. Fahmy, A comprehensive platform for ex vivo T-cell expansion based on biodegradable polymeric artificial antigen-presenting cells, Mol. Ther. 16 (2008) 765–772.
[192] E.R. Steenblock, T. Fadel, M. Labowsky, J.S. Pober, T.M. Fahmy, An artificial antigen-presenting cell with paracrine delivery of IL-2 impacts the magnitude and direction of the T cell response, J. Biol. Chem. 286 (2011) 34883–34892.
[193] M. Labowsky, J. Lowenthal, T.M. Fahmy, An in silico analysis of nanoparticle/cell diffusive transfer: application to nano-artificial antigen-presenting cell:T-cell interaction, Nanomedicine 11 (2015) 1019–1028.
[194] E.R. Steenblock, S.H. Wrzesinski, R.A. Flavell, T.M. Fahmy, Antigen presentation on artificial acellular substrates: modular systems for flexible, adaptable immunotherapy, Expert. Opin. Biol. Ther. 9 (2009) 451–464.
[195] E. Hong, I.M. Usiskin, C. Bergamaschi, D.J. Hanlon, R.L. Edelson, S. Justesen, G.N. Pavlakis, R.A. Flavell, T.M. Fahmy, Configuration-dependent Presentation of Multivalent IL-15:IL-15Ralpha Enhances the Antigen-specific T Cell Response and Antitumor ImmunityJ Biol Chem 2015.
[196] F. Balkwill, Tumour necrosis factor and cancer, Nat. Rev. Cancer 9 (2009) 361–371.
[197] G.F. Paciotti, L. Myer, D. Weinreich, D. Goia, N. Pavel, R.E. McLaughlin, L. Tamarkin, Colloidal gold: a novel nanoparticle vector for tumor directed drug delivery, Drug delivery 11 (2004) 169–183.
[198] S.K. Libutti, G.F. Paciotti, A.A. Byrnes, H.R. Alexander Jr., W.E. Gannon, M. Walker, G. D. Seidel, N. Yuldasheva, L. Tamarkin, Phase I and pharmacokinetic studies of CYT-6091, a novel PEGylated colloidal gold-rhTNF nanomedicine, Clin. Cancer Res. 16 (2010) 6139–6149.
[199] M. Grell, E. Douni, H. Wajant, M. Lohden, M. Clauss, B. Maxeiner, S. Georgopoulos, W. Lesslauer, G. Kollias, K. Pfizenmaier, P. Scheurich, The Transmembrane Form of Tumor Necrosis Factor Is the Prime Activating Ligand of the 80 kDa Tumor Necrosis Factor Receptor, Cell, 83, 1995.
[200] S. Bryde, I. Grunwald, A. Hammer, A. Krippner-Heidenreich, T. Schiestel, H. Brunner, G.E.M. Tovar, K. Pfizenmaier, P. Scheurich, Tumor necrosis factor (TNF)-functionalized nanostructured particles for the stimulation of membrane TNF-specific cell responses, Bioconjug. Chem. 16 (2005) 1459–1467.
[201] J.M. Farma, M. Puhlmann, P.A. Soriano, D. Cox, G.F. Paciotti, L. Tamarkin, H.R. Alexander, Direct evidence for rapid and selective induction of tumor neovascular permeability by tumor necrosis factor and a novel derivative, colloidal gold bound tumor necrosis factor, Int. J. Cancer 120 (2007) 2474–2480.
[202] N.J. Maney, G. Reynolds, A. Krippner-Heidenreich, C.M. Hilkens, Dendritic cell maturation and survival are differentially regulated by TNFR1 and TNFR2, J. Immunol. 193 (2014) 4914–4923.
[203] X. Chen, X. Wu, Q. Zhou, O.M. Howard, M.G. Netea, J.J. Oppenheim, TNFR2 is critical for the stabilization of the CD4+Foxp3+ regulatory T. cell phenotype in the inflammatory environment, J. Immunol. 190 (2013) 1076–1084.
[204] X. Zhao, L. Rong, X. Zhao, X. Li, X. Liu, J. Deng, H. Wu, X. Xu, U. Erben, P. Wu, U. Syrbe, J. Sieper, Z. Qin, TNF signaling drives myeloid-derived suppressor cell accumulation, J. Clin. Invest. 122 (2012) 4094–4104.
[205] T. Calzascia, M. Pellegrini, H. Hall, L. Sabbagh, N. Ono, A.R. Elford, T.W. Mak, P.S. Ohashi, TNF-alpha is critical for antitumor but not antiviral T cell immunity in mice, J. Clin. Invest. 117 (2007) 3833–3845.
[206] K. Perica, A. De Leon Medero, M. Durai, Y.L. Chiu, J.G. Bieler, L. Sibener, M. Niemoller, M. Assenmacher, A. Richter, M. Edidin, M. Oelke, J. Schneck, Nanoscale artificial antigen presenting cells for T cell immunotherapy, Nanomedicine 10 (2014) 119–129.
[207] R. Zappasodi, M. Di Nicola, C. Carlo-Stella, R. Mortarini, A. Molla, C. Vegetti, S. Albani, A. Anichini, A.M. Gianni, The effect of artificial antigen-presenting cells with preclustered anti-CD28/-CD3/-LFA-1 monoclonal antibodies on the induction of ex vivo expansion of functional human antitumor T cells, Haematologica 93 (2008) 1523–1534.
[208] B. Prakken, M. Wauben, D. Genini, R. Samodal, J. Barnett, A. Mendivil, L. Leoni, S. Albani, Artificial antigen-presenting cells as a tool to exploit the immune 'synapse', Nat. Med. 6 (2000) 1406–1410.
[209] T.R. Fadel, F.A. Sharp, N. Vudattu, R. Ragheb, J. Garyu, D. Kim, E. Hong, N. Li, G.L. Haller, L.D. Pfefferle, S. Justesen, K.C. Herold, T.M. Fahmy, A carbon nanotube-polymer composite for T-cell therapy, Nat. Nanotechnol. 9 (2014) 639–647.
[210] R.A. Meyer, J.C. Sunshine, K. Perica, A.K. Kosmides, K. Aje, J.P. Schneck, J.J. Green, Biodegradable nanoellipsoidal artificial antigen presenting cells for antigen specific T-cell activation, Small 11 (2015) 1519–1525.
[211] K. Perica, A. Tu, A. Richter, J.G. Bieler, M. Edidin, J.P. Schneck, Magnetic field-induced T cell receptor clustering by nanoparticles enhances T cell activation and stimulates antitumor activity, ACS Nano 8 (2014) 2252–2260.
[212] S.A. Feldman, Y. Assadipour, I. Kriley, S.L. Goff, S.A. Rosenberg, Adoptive cell therapy—tumor-infiltrating lymphocytes, T-cell receptors, and chimeric antigen receptors, Semin. Oncol. 42 (2015) 626–639.
[213] M.T. Stephan, J.J. Moon, S.H. Um, A. Bershteyn, D.J. Irvine, Therapeutic cell engineering with surface-conjugated synthetic nanoparticles, Nat. Med. 16 (2010) 1035–1041.
[214] M.T. Stephan, S.B. Stephan, P. Bak, J. Chen, D.J. Irvine, Synapse-directed delivery of immunomodulators using T-cell-conjugated nanoparticles, Biomaterials 33 (2012) 5776–5787.
[215] R. Meir, K. Shamalov, O. Betzer, M. Motiei, M. Horovitz-Fried, R. Yehuda, A. Popovtzer, R. Popovtzer, C.J. Cohen, Nanomedicine for cancer immunotherapy: tracking cancer-specific T-cells *in vivo* with gold nanoparticles and CT imaging, ACS Nano 9 (2015) 6363–6372.
[216] L.C. Kennedy, A.S. Bear, J.K. Young, N.A. Lewinski, J. Kim, A.E. Foster, R.A. Drezek, T cells enhance gold nanoparticle delivery to tumors *in vivo*, Nanoscale Res. Lett. 6 (2011) 283–293.
[217] D.E. Hu, M.I. Kettunen, K.M. Brindle, Monitoring T-lymphocyte trafficking in tumors undergoing immune rejection, Magn. Reson. Med. 54 (2005) 1473–1479.
[218] M.F. Kircher, J.R. Allport, E.E. Graves, V. Love, L. Josephson, A.H. Lichtman, R. Weissleder, *In Vivo* High Resolution Three-Dimensional Imaging of Antigen-Specific Cytotoxic T-Lymphocyte Trafficking to Tumors, Cancer Res, 63, 2003.
[219] P. Bhatnagar, Z. Li, Y. Choi, J. Guo, F. Li, D.Y. Lee, M. Figliola, H. Huls, D.A. Lee, T. Zal, K.C. Li, L.J.N. Cooper, Imaging of genetically engineered T cells by PET using gold nanoparticles complexed to Copper-64, Integr. Biol. 5 (2013) 231–238.
[220] Y. Zheng, M.T. Stephan, S.A. Gai, W. Abraham, A. Shearer, D.J. Irvine, In vivo targeting of adoptively transferred T-cells with antibody- and cytokine-conjugated liposomes, J. Control. Release 172 (2013) 426–435.
[221] M.S. Kim, J.S. Ma, H. Yun, Y. Cao, J.Y. Kim, V. Chi, D. Wang, A. Woods, L. Sherwood, D. Caballero, J. Gonzalez, P.G. Schultz, T.S. Young, C.H. Kim, Redirection of genetically engineered CAR-T cells using bifunctional small molecules, J. Am. Chem. Soc. 137 (2015) 2832–2835.
[222] T.T. Smith, S.B. Stephan, H.F. Moffett, L.E. McKnight, W. Ji, D. Reiman, E. Bonagofski, M.E. Wohlfahrt, S.P.S. Pillai, M.T. Stephan, In situ programming of leukaemia-specific T cells using synthetic DNA nanocarriersNat Nanotechnol 2017.
[223] J. Rios-Doria, N. Durham, L. Wetzel, R. Rothstein, J. Chesebrough, N. Holoweckyj, W. Zhao, C.C. Leow, R. Hollingsworth, Doxil synergizes with cancer immunotherapies to enhance antitumor responses in syngeneic mouse models, Neoplasia 17 (2015) 661–670.
[224] D.-F.C. Institute, Pembrolizumab Combined With PLD For Recurrent Platinum Resistant Ovarian, Fallopian Tube Or Peritoneal Cancer, ClinicalTrials.gov [Internet] National Library of Medicine, Bethesda (MD), 2016.
[225] I. Alagkiozidis, A. Facciabene, C. Carpenito, F. Benencia, Z. Jonak, S. Adams, R.G. Carroll, P.A. Gimotty, R. Hammond, G.A. Danet-Desnoyers, C.H. June, D.J. Powell Jr., G. Coukos, Increased immunogenicity of surviving tumor cells enables cooperation between liposomal doxorubicin and IL-18, J. Transl. Med. 7 (2009) 104.
[226] A. Cabanes, S. Even-Chen, J. Zimberoff, Y. Barenholz, E. Kedar, A. Gabizon, Enhancement of antitumor activity of polyethylene glycol-coated liposomal doxorubicin with soluble and liposomal interleukin 2, Clin. Cancer Res. 5 (1999) 687–693.
[227] C. Corporation, Safety Study of Nivolumab With Nab-Paclitaxel Plus or Minus Gemcitabine in Pancreatic Cancer, Nab-Paclitaxel/Carboplatin in Stage IIIB/IV Non-Small Cell Lung Cancer or Nab-Paclitaxel in Recurrent Metastatic Breast Cancer, ClinicalTrials.gov Internet National Library of Medicine (US), Bethesda (MD), 2014.
[228] Hoffman-LaRoche, A Study of Atezolizumab in Combination With Nab-Paclitaxel Compared With Placebo With Nab-Paclitaxel for Participants With Previously Untreated Metastatic Triple-Negative Breast Cancer (IMpassion130), ClinicalTrials.gov Internet National Library of Medicine (US), Bethesda (MD), 2015.
[229] A. Kulkarni, P. Rao, S. Natarajan, A. Goldman, V.S. Sabbisetti, Y. Khater, N. Korimerla, V. Chandrasekar, R.A. Mashelkar, S. Sengupta, Reporter nanoparticle that monitors its anticancer efficacy in real time, Proc. Natl. Acad. Sci. U. S. A. 113 (2016) E2104–E2113.
[230] C. Wang, Y. Ye, G.M. Hochu, H. Sadeghifar, Z. Gu, Enhanced cancer immunotherapy by microneedle patch-assisted delivery of anti-PD1 antibody, Nano Lett. 16 (2016) 2334–2340.
[231] S.K. Gershon, H. Luksenburg, T.R. Cote, M.M. Braun, Pure red-cell aplasia and recombinant erythropoietin, N. Engl. J. Med. 346 (2002) 1584–1586 author reply 1584-1586.
[232] S. Hermeling, H. Schellekens, D.J. Crommelin, W. Jiskoot, Micelle-associated protein in epoetin formulations: a risk factor for immunogenicity? Pharm. Res. 20 (2003) 1903–1907.
[233] P. Chamberlain, A.R. Mire-Sluis, An overview of scientific and regulatory issues for the immunogenicity of biological products, Dev. Biol. (Basel) 112 (2003) 3–11.
[234] A.N. Ilinskaya, M.A. Dobrovolskaia, Understanding the immunogenicity and antigenicity of nanomaterials: past, present and future, Toxicol. Appl. Pharmacol. 299 (2016) 70–77.
[235] G.E. Francis, D. Fisher, C. Delgado, F. Malik, A. Gardiner, D. Neale, PEGylation of cytokines and other therapeutic proteins and peptides: the importance of biological optimisation of coupling techniques, Int. J. Hematol. 68 (1998) 1–18.

[236] B.M. Chen, Y.C. Su, C.J. Chang, P.A. Burnouf, K.H. Chuang, C.H. Chen, T.L. Cheng, Y.T. Chen, J.Y. Wu, S.R. Roffler, Measurement of pre-existing IgG and IgM antibodies against polyethylene glycol in healthy individuals, Anal. Chem. 88 (2016) 10661–10666.
[237] D. Tyrsin, S. Chuvpilo, A. Matskevich, D. Nemenov, P.S. Romer, P. Tabares, T. Hunig, From TGN1412 to TAB08: the return of CD28 superagonist therapy to clinical development for the treatment of rheumatoid arthritis, Clin. Exp. Rheumatol. 34 (2016) 45–48.
[238] S.P. Henry, R.S. Geary, R. Yu, A.A. Levin, Drug properties of second-generation antisense oligonucleotides: how do they measure up to their predecessors? Curr. Opin. Investig. Drugs 2 (2001) 1444–1449.
[239] S.P. Henry, D. Monteith, F. Bennett, A.A. Levin, Toxicological and pharmacokinetic properties of chemically modified antisense oligonucleotide inhibitors of PKC-alpha and C-raf kinase, Anticancer Drug Des. 12 (1997) 409–420.
[240] S.P. Henry, D. Monteith, A.A. Levin, Antisense oligonucleotide inhibitors for the treatment of cancer: 2. Toxicological properties of phosphorothioate oligodeoxynucleotides, Anticancer Drug Des. 12 (1997) 395–408.
[241] J.T. Holmlund, B.P. Monia, T.J. Kwoh, F.A. Dorr, Toward antisense oligonucleotide therapy for cancer: ISIS compounds in clinical development, Curr. Opin. Mol. Ther. 1 (1999) 372–385.
[242] A.A. Levin, A review of the issues in the pharmacokinetics and toxicology of phosphorothioate antisense oligonucleotides, Biochim. Biophys. Acta 1489 (1999) 69–84.
[243] D.K. Monteith, S.P. Henry, R.B. Howard, S. Flournoy, A.A. Levin, C.F. Bennett, S.T. Crooke, Immune stimulation—a class effect of phosphorothioate oligodeoxynucleotides in rodents, Anticancer Drug Des. 12 (1997) 421–432.
[244] D. Schubert, A.A. Levin, D. Kornbrust, C.L. Berman, J. Cavagnaro, S. Henry, R. Seguin, N. Ferrari, S.B. Shrewsbury, The oligonucleotide safety working group (OSWG), Nucleic Acid Ther 22 (2012) 211–212.
[245] D.C. Evans, A.P. Watt, D.A. Nicoll-Griffith, T.A. Baillie, Drug-protein adducts: an industry perspective on minimizing the potential for drug bioactivation in drug discovery and development, Chem. Res. Toxicol. 17 (2004) 3–16.
[246] M.A. Dobrovolskaia, S.E. McNeil, Immunological and hematological toxicities challenging clinical translation of nucleic acid-based therapeutics, Expert. Opin. Biol. Ther. 15 (2015) 1023–1048.
[247] A.D. Keefe, S. Pai, A. Ellington, Aptamers as therapeutics, Nat. Rev. Drug Discov. 9 (2010) 537–550.
[248] M. Lindow, H.P. Vornlocher, D. Riley, D.J. Kornbrust, J. Burchard, L.O. Whiteley, J. Kamens, J.D. Thompson, S. Nochur, H. Younis, S. Bartz, J. Parry, N. Ferrari, S.P. Henry, A.A. Levin, Assessing unintended hybridization-induced biological effects of oligonucleotides, Nat. Biotechnol. 30 (2012) 920–923.
[249] M.A. Dobrovolskaia, S.E. McNeil, Strategy for selecting nanotechnology carriers to overcome immunological and hematological toxicities challenging clinical translation of nucleic acid-based therapeutics, Expert opinion on drug delivery 12 (2015) 1163–1175.
[250] R.M. Crist, J.H. Grossman, A.K. Patri, S.T. Stern, M.A. Dobrovolskaia, P.P. Adiseshaiah, J.D. Clogston, S.E. McNeil, Common pitfalls in nanotechnology: lessons learned from NCI's Nanotechnology Characterization Laboratory, Integr Biol (Camb) 5 (2013) 66–73.
[251] J.D. Clogston, A.K. Patri, Importance of physicochemical characterization prior to immunological studies, in: M.A. Dobrovolskaia, S.E. McNeil (Eds.), Handbook of immunological properties of engineered nanomaterials, World Scientific Publishing Ltd, Singapore 2013, pp. 25–51.
[252] M.A. Dobrovolskaia, Pre-clinical immunotoxicity studies of nanotechnology-formulated drugs: challenges, considerations and strategy, J. Control. Release 220 (2015) 571–583.
[253] M.A. Dobrovolskaia, M. Shurin, A.A. Shvedova, Current understanding of interactions between nanoparticles and the immune system, Toxicol. Appl. Pharmacol. 299 (2016) 78–89.
[254] P.P. Adiseshaiah, J.B. Hall, S.E. McNeil, Nanomaterial standards for efficacy and toxicity assessment, Wiley Interdiscip Rev Nanomed Nanobiotechnol 2 (2010) 99–112.
[255] D. Lorusso, A. Di Stefano, V. Carone, A. Fagotti, S. Pisconti, G. Scambia, Pegylated liposomal doxorubicin-related palmar-plantar erythrodysesthesia ('hand-foot' syndrome), Annals of oncology: official journal of the European Society for Medical Oncology 18 (2007) 1159–1164.
[256] S.T. Stern, P.P. Adiseshaiah, R.M. Crist, Autophagy and lysosomal dysfunction as emerging mechanisms of nanomaterial toxicity, Part Fibre Toxicol 9 (2012) 20.
[257] S.T. Stern, J.B. Hall, L.L. Yu, L.J. Wood, G.F. Paciotti, L. Tamarkin, S.E. Long, S.E. McNeil, Translational considerations for cancer nanomedicine, J. Control. Release 146 (2010) 164–174.
[258] K.A. Afonin, W.W. Grabow, F.M. Walker, E. Bindewald, M.A. Dobrovolskaia, B.A. Shapiro, L. Jaeger, Design and self-assembly of siRNA-functionalized RNA nanoparticles for use in automated nanomedicine, Nat. Protoc. 6 (2011) 2022–2034.
[259] M. Di Gioacchino, C. Petrarca, F. Lazzarin, L. Di Giampaolo, E. Sabbioni, P. Boscolo, R. Mariani-Costantini, G. Bernardini, Immunotoxicity of nanoparticles, Int. J. Immunopathol. Pharmacol. 24 (2011) 65S–71S.
[260] M.A. Dobrovolskaia, B.W. Neun, J.D. Clogston, H. Ding, J. Ljubimova, S.E. McNeil, Ambiguities in applying traditional Limulus amebocyte lysate tests to quantify endotoxin in nanoparticle formulations, Nanomedicine (London) 5 (2010) 555–562.
[261] M.A. Dobrovolskaia, B.W. Neun, J.D. Clogston, J.H. Grossman, S.E. McNeil, Choice of method for endotoxin detection depends on nanoformulation, Nanomedicine (London) 9 (2014) 1847–1856.
[262] M.A. Dobrovolskaia, A.K. Patri, T.M. Potter, J.C. Rodriguez, J.B. Hall, S.E. McNeil, Dendrimer-induced leukocyte procoagulant activity depends on particle size and surface charge, Nanomedicine (London) 7 (2012) 245–256.
[263] A.N. Ilinskaya, S. Man, A.K. Patri, J.D. Clogston, R.M. Crist, R.E. Cachau, S.E. McNeil, M.A. Dobrovolskaia, Inhibition of phosphoinositol 3 kinase contributes to nanoparticle-mediated exaggeration of endotoxin-induced leukocyte procoagulant activity, Nanomedicine (London) 9 (2014) 1311–1326.
[264] Y. Li, P. Italiani, E. Casals, N. Tran, V.F. Puntes, D. Boraschi, Optimising the use of commercial LAL assays for the analysis of endotoxin contamination in metal colloids and metal oxide nanoparticles, Nanotoxicology 9 (2015) 462–473.
[265] G.J. Oostingh, E. Casals, P. Italiani, R. Colognato, R. Stritzinger, J. Ponti, T. Pfaller, Y. Kohl, D. Ooms, F. Favilli, H. Leppens, D. Lucchesi, F. Rossi, I. Nelissen, H. Thielecke, V.F. Puntes, A. Duschl, D. Boraschi, Problems and challenges in the development and validation of human cell-based assays to determine nanoparticle-induced immunomodulatory effects, Part Fibre Toxicol 8 (2011) 8.
[266] E.J. Petersen, T.B. Henry, J. Zhao, R.I. MacCuspie, T.L. Kirschling, M.A. Dobrovolskaia, V. Hackley, B. Xing, J.C. White, Identification and avoidance of potential artifacts and misinterpretations in nanomaterial ecotoxicity measurements, Environ. Sci. Technol. 48 (2014) 4226–4246.
[267] T. Pfaller, R. Colognato, I. Nelissen, F. Favilli, E. Casals, D. Ooms, H. Leppens, J. Ponti, R. Stritzinger, V. Puntes, D. Boraschi, A. Duschl, G.J. Oostingh, The suitability of different cellular in vitro immunotoxicity and genotoxicity methods for the analysis of nanoparticle-induced events, Nanotoxicology 4 (2010) 52–72.
[268] S. Smulders, J.P. Kaiser, S. Zuin, K.L. Van Landuyt, L. Golanski, J. Vanoirbeek, P. Wick, P.H. Hoet, Contamination of nanoparticles by endotoxin: evaluation of different test methods, Part Fibre Toxicol 9 (2012) 41.
[269] H. Vallhov, J. Qin, S.M. Johansson, N. Ahlborg, M.A. Muhammed, A. Scheynius, S. Gabrielsson, The importance of an endotoxin-free environment during the production of nanoparticles used in medical applications, Nano Lett. 6 (2006) 1682–1686.
[270] M.A. Vetten, C.S. Yah, T. Singh, M. Gulumian, Challenges facing sterilization and depyrogenation of nanoparticles: effects on structural stability and biomedical applications, Nanomedicine 10 (2014) 1391–1399.
[271] M.A. Dobrovolskaia, S.E. McNeil, Nanoparticles and endotoxin, in: M.A. Dobrovolskaia, S.E. McNeil (Eds.), Handbook of Immunological Properties of Engineered Nanomaperials, World Scientific Publishing Ltd, Singapore 2013, pp. 77–115.
[272] J. Zheng, J.D. Clogston, A.K. Patri, M.A. Dobrovolskaia, S.E. McNeil, Sterilization of silver nanoparticles using standard gamma irradiation procedure affects particle integrity and biocompatibility, J Nanomed Nanotechnol 2011 (2011) 001.
[273] N. Subbarao, Impact of nanoparticle sterilization on analytical characterization, in: M.A. Dobrovolskaia, S.E. McNeil (Eds.), Handbook of Immunological Properties of Engineered nanomaterials, World Scientific Publishing Ltd, Singapore 2013, pp. 53–71.
[274] M.A. Dobrovolskaia, S.E. McNeil, Understanding the correlation between in vitro and in vivo immunotoxicity tests for nanomedicines, J. Control. Release 172 (2013) 456–466.
[275] M.A. Dobrovolskaia, S.E. McNeil, In vitro assays for monitoring nanoparticle intercation with components of the immune system, in: M.A. Dobrovolskaia, S.E. McNeil (Eds.), Handbook of immunological properties of engineered nanomaterials, World Scientific Publishing Ltd, Singapore 2013, pp. 581–634.
[276] M.J. Smith, C.E. McLouglin, W. K.L. Jr., G. D., Evaluating the adverse effects of nanomaterials on the immune system with animal models, in: M.A. Dobrovolskaia, S.E. McNeil (Eds.), Handbook of immunological properties of engineered nanomaterials, World Scientific Publishing Ltd, Singapore 2013, pp. 639–663.
[277] A. Baumann, D. Tuerck, S. Prabhu, L. Dickmann, J. Sims, Pharmacokinetics, metabolism and distribution of PEGs and PEGylated proteins: quo vadis? Drug Discov. Today 19 (2014) 1623–1631.
[278] T.J. Anchordoquy, Y. Barenholz, D. Boraschi, M. Chorny, P. Decuzzi, M.A. Dobrovolskaia, Z.S. Farhangrazi, D. Farrell, A. Gabizon, H. Ghandehari, B. Godin, N. M. La-Beck, J. Ljubimova, S.M. Moghimi, L. Pagliaro, J.H. Park, D. Peer, E. Ruoslahti, N. J. Serkova, D. Simberg, Mechanisms and Barriers in Cancer Nanomedicine: Addressing Challenges, Looking for Solutions, ACS Nano 11 (2017) 12–18.
[279] S. Bancos, K.M. Tyner, J.L. Weaver, Immunotoxicity testing of drug-nanoparticle conjugates: regulatory considerations, in: M.A. Dobrovolskaia, S.E. McNeil (Eds.), Handbook of immunological properties of engineered nanomaterials, World Scientific Publishing Ltd, Singapore 2013, pp. 671–685.
[280] E.L.a. Company, A Study of Galunisertib (LY2157299) in Combination With Nivolumab in Advanced Refractory Solid Tumors and in Recurrent or Refractory NSCLC, or Hepatocellular Carcinoma, ClinicalTrials.gov Internet National Library of Medicine (US), Bethesda (MD), 2015.
[281] M.A.C. Center, Nivolumab vs Nivolumab + Bevacizumab vs Nivolumab + Ipilimumab in Metastatic Renal Cell Carcinoma (mRCC), ClinicalTrials.gov Internet National Library of Medicine (US), Bethesda (MD), 2014.
[282] J. Yuan, P.S. Hegde, R. Clynes, P.G. Foukas, A. Harari, T.O. Kleen, P. Kvistborg, C. Maccalli, H.T. Maecker, D.B. Page, H. Robins, W. Song, E.C. Stack, E. Wang, T.L. Whiteside, Y. Zhao, H. Zwierzina, L.H. Butterfield, B.A. Fox, Novel technologies and emerging biomarkers for personalized cancer immunotherapy, J Immunother Cancer 4 (2016) 3.
[283] L. Zitvogel, J.M. Pitt, R. Daillere, M.J. Smyth, G. Kroemer, Mouse models in oncoimmunology, Nat. Rev. Cancer 16 (2016) 759–773.
[284] L.E. Klevorn, R.M. Teague, Adapting Cancer Immunotherapy Models for the Real World, Trends Immunol. 37 (2016) 354–363.
[285] E. Blanco, H. Shen, M. Ferrari, Principles of nanoparticle design for overcoming biological barriers to drug delivery, Nat. Biotechnol. 33 (2015) 941–951.
[286] K.M. Morrissey, T. Yuraszeck, C.C. Li, Y. Zhang, S. Kasichayanula, Immunotherapy and Novel Combinations in Oncology- Current Landscape, Challenges and Opportunities, Clin Transl Sci, 2016.
[287] AstraZeneca, Annual Report, 2016.