



Nanotechnology-based immunotherapies to combat cancer metastasis

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Abstract

Emerging concepts in nanotechnology have gained particular attention for their clinical translation of immunotherapies of cancer, autoimmune and infectious diseases. Several nanoconstructs have been engineered with unique structural, physico-chemical, and functional features as robust alternatives for conventional chemotherapies. Traditional cancer therapies like chemotherapy, radiotherapy, and ultimately surgery are the most widely practiced in biomedical settings. Biomaterials and nanotechnology have introduced vehicles for drug delivery and have revolutionized the concept of the modern immunotherapeutic paradigm. Various types of nanomaterials, such as nanoparticles and, more specifically, drug-loaded nanoparticles are becoming famous for drug delivery applications because of safety, patient compliance, and smart action. Such therapeutic modalities have acknowledged regulatory endorsement and are being used in twenty-first-century clinical settings. Considering the emerging concepts and landscaping potentialities, herein, we spotlight and discuss nanoparticle-based immunotherapies as a smart and sophisticated drug delivery approach to combat cancer metastasis. The introductory part of this manuscript discusses a broad overview of cancer immunotherapy to understand better the tumor microenvironment and nanotechnology-oriented immunomodulatory strategies to cope with advanced-stage cancers. Following that, most addressable problems allied with conventional immunotherapies are given in comparison to nanoparticle-based immunotherapies. The later half of this work comprehensively highlights the requisite delivery of various bioactive entities with particular cases and examples. Finally, this review also encompasses a comprehensive concluding overview and future standpoints to strengthen a successful clinical translation of nanoparticle-based immunotherapies as a smart and sophisticated drug delivery approach.

Keywords Nanoparticles · Immunotherapeutic · Cancer battle · Tumor metastasis · Drug delivery · Biomedical

Introduction

Noncommunicable diseases (NCDs) have a massive contribution to global deaths, and cancer is regarded as the most common cause of deaths. NCDs share 63% of global deaths,

while cancers contribute 9% of global deaths [1, 2]. The incidence of cancer is growing sharply, and cancer-associated deaths are believed to be the main barrier to elevating life expectancy during the initial decades of the twenty-first century [2–4]. The tumor undergoes several molecular-level

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evolutionary processes during growth that consequently yield specific changes, allowing the immune response of the tumor cells. Similarly, tumor cells escape from the tumor niche, invade the neighboring tissues, enter through the circulatory pathways, and are implanted into distant tissues and organs by forming premetastatic niches that subsequently form metastatic lesions. It is driven by genetic and epigenetic factors of tumor cells and certain biomolecules produced in the tumor microenvironment (TME) [5–7]. Tumor metastasis is one of the main factors driving cancer-related fatalities [5, 6]. Metastasis is a complex nonlinear series of events and is considered to be the most crucial factor responsible for drug resistance and a potential barrier in the successful therapy of cancers. Besides, it can cause organ damage under critical conditions. Several authors organize detailed mechanisms of metastasis, readers are referred to go through these reviews to understand further metastasis and its impact on therapeutic outcome [5–9]. An overview of the complex and concurrent routes of metastasis is shown in Fig. 1 [6].

For a long time, oncologists have relied only on three types of therapies: surgery, chemotherapy, and radiotherapy for cancers [10]. Later, cancer treatments then evolved, and new therapeutic modalities were developed [11]. Nowadays, various types of therapies and strategies are being used to treat cancers, overcome the barrier of metastasis and eradicate cancer cells from the human body [12]. Most of the

therapies lack discrimination between normal and cancer cells ideal immunotherapies should be able to tolerate normal cells and target cancer cells and are associated with off-target toxicities. Evidence indicates that the immune system can manage tumors effectively. For instance, revocation of the tumor without treatment, presence of various types of tumor-specific immune cells like cytotoxic T cells (CTCs), monocytes, lymphocytes and plasmacytic infiltrates, higher incidence of cancer in immunocompromised patients and cancer remission with immunomodulators can augment the role of the immune system in cancers [13]. Immunotherapies have shown the fastest growth during recent years and have proven to be the most promising in oncology and have a good impact on human health [14]. Immune system studies suggest that the efficacy of immunomodulatory approaches depends on activation of baseline immune response and untethering the pre-existing immunity [15]. However, effector T cells-induced immune response plays a pivotal role [14]. Recent progress in technology has allowed researchers to explore the mechanisms and role of tumor immune environment and immune systems in tumor growth and metastasis (Fig. 2) [13, 14]. Immunomodulatory approaches have offered opportunities and extended arms towards clinical translation of immunotherapies for cancer treatment. Optimization of emerging “cancer immunotherapy” is regarded as one of the major pillars in treating malignancies along with surgery, chemotherapy and radiotherapy. This review

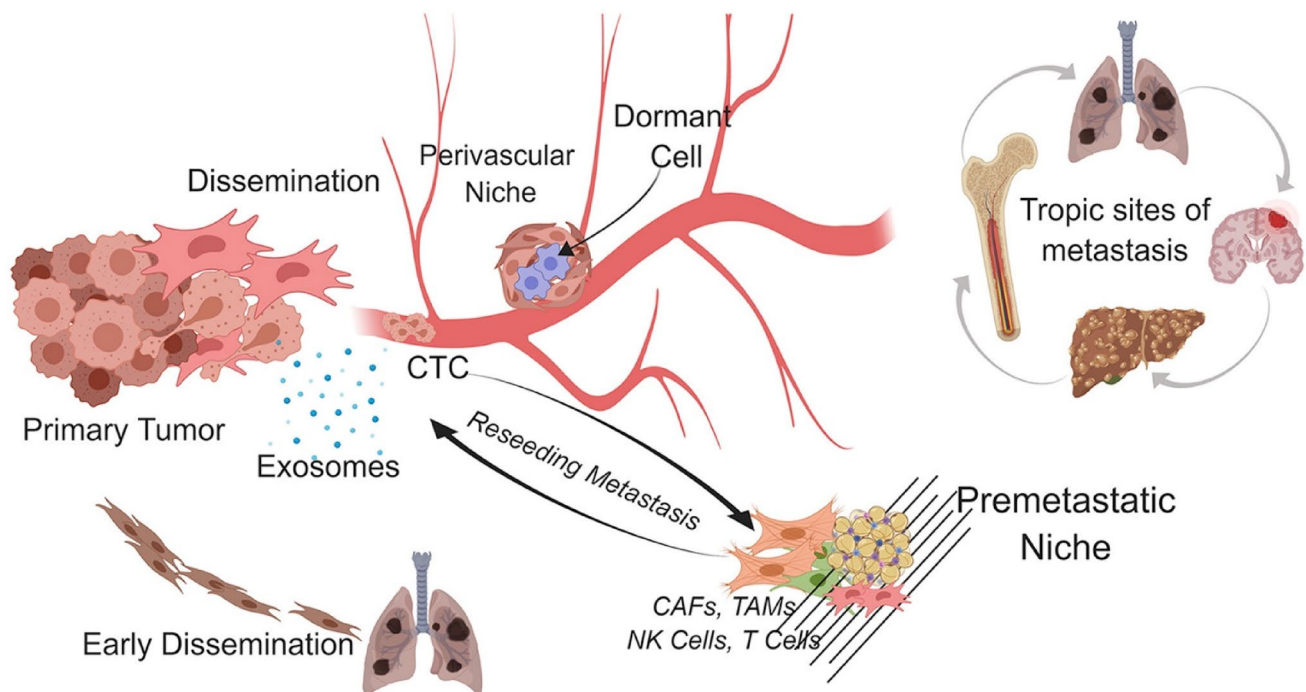


Fig. 1 Overview of the complex and concurrent routes of metastasis. Reprinted from Suhail et al. [6] with permission under a Creative Commons license

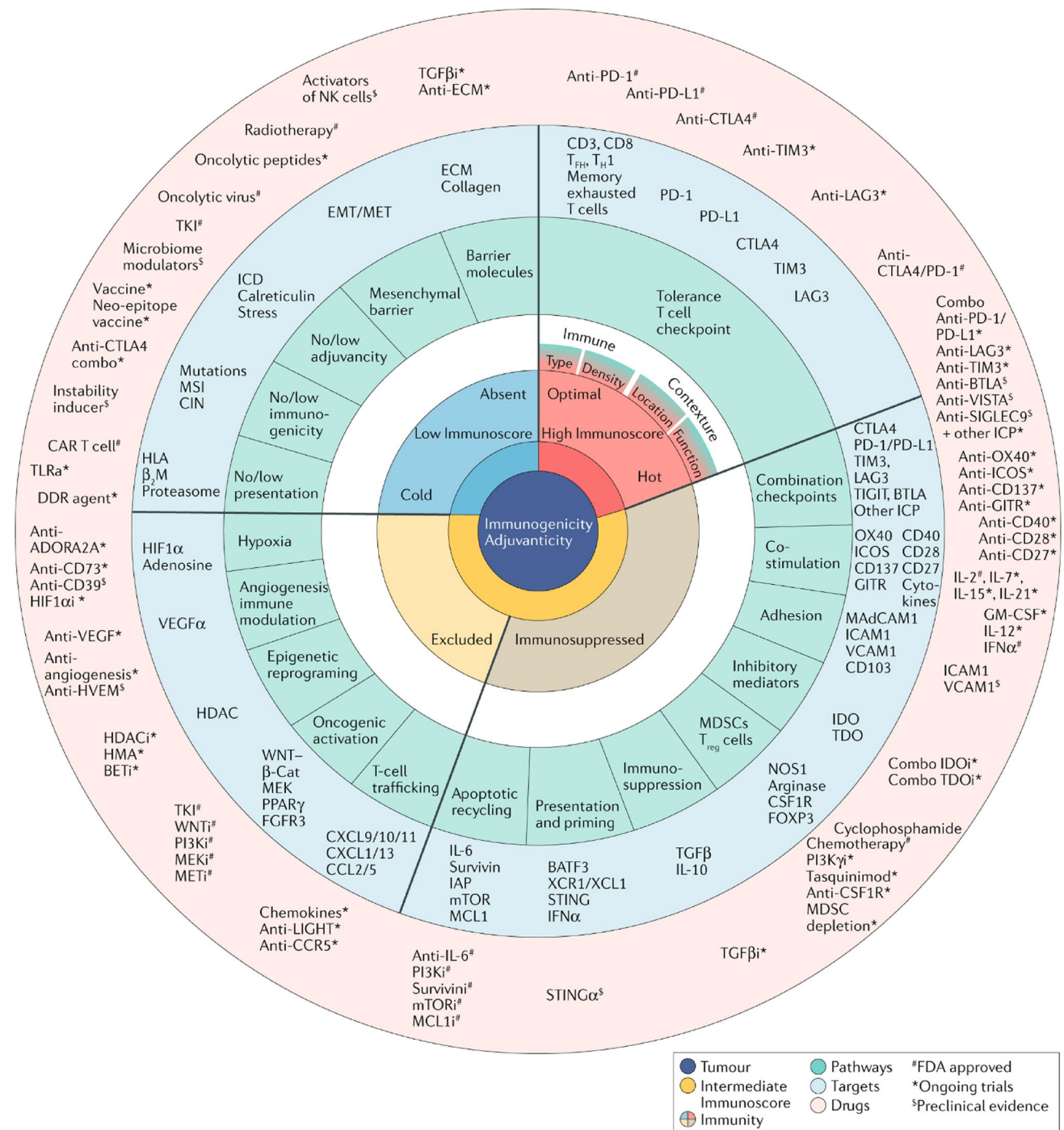


Fig. 2 The tumor–immune classification cycle as a tool to direct anticancer therapy. Reprinted from Galon and Bruni [14], with permission from Springer Nature. License Number: 5121760526516

discusses the recent progress in understanding the tumor microenvironment and nanotechnology-oriented immunomodulatory strategies to treat initial and advanced-stage cancers. Besides, it also gives a comprehensive overview of the limitation of immunotherapies and current challenges associated with this nanotechnology.

Immunotherapies—potential therapeutic modalities

Immunotherapies are the immunomodulatory therapeutic regimens that boost up the immune system and bears

specificity, potency, and memory as the potential as most important features. Inspired by these features immunotherapies are being considered for development in a wide range of different types of immune disease, infectious diseases and cancers at preclinical and clinical [16, 17]. The concept of cancer immunotherapies was probably hypothesized around the 1890s by William Coley, when he used bacterial toxins to treat cancer patients [11, 18]. This evidence was further endorsed by Thomas and Burnet theory of tumor immune surveillance. In the field of oncology, immunotherapies have seen alternate intervals of success and failure [18]. Previously, immunotherapies were restricted by severe toxicities and limited efficacy, while the recent breakthrough advances in understanding fundamental immunology and translational immunotherapy in cancer research have opened a new era of immunotherapies with a positive, encouraging impact in advanced stage cancers [19–21].

The immune system can eliminate cancers via two possible ways; (1) Natural or innate immune response or (1) acquired or adaptive immunity. But the tumor immune escape mechanism involves three phases of reaction; in the first phase immune system recognize the cancer cells and remove them from the body, the second phase immune system maintain a balance between cancer cells and immune cells and finally cancer cells immunoediting give rise the synthesis of immunocompatible cancer cells which have immune escape ability. Subsequently, these types of cells survive and develop tumors [22]. To mount an effective immunotherapeutic response, (1) released tumor cell antigens must be taken up by dendritic cells (DCs) must take up the tumor cell antigens with proper maturation signals to prompt differentiation and antigen presentation. (2) DCs should also be able to activate antitumor T cell responses driving their differentiation into tumor-specific activation of CTCs activating Natural Killer cells (NKC) or Natural Killer T cells (NKT) response and heightening T helper cells 1 response. Subsequently, antitumor T cells must penetrate the tumor and elicit an antitumor response [23].

Immunotherapies are aimed to reprogram the tumor immune microenvironment, increase the cytotoxicity of cellular immunity by the CTCs and NKCs via administration of stimulators and strengthen the immune system to recognize the threat of cancers to eliminate it (Fig. 3) [24]. Immunotherapies mediate the activation, proliferation, differentiation and survival of antitumor lymphocytes and are elicit antitumor responses to eliminate tumor cells [25]. For instance, cytokines play an important role in regulation of anti-tumor CTCs or NKCs functions and significant evidence augments that interleukin-15 (IL-15) signaling must be optimal to maintain the full potential of NKCs antitumor activity [25]. Triggered by signals from surface receptors like NKG2D, DNAM1, Nkp30, Nkp44 and Nkp46, NKCs release cytotoxic granules on target. NKCs bearing Fc-receptor CD-16

does not need activation signal by other NKCs, it mediates antibody-dependent cellular toxicity upon it interaction with antibody-coated cells. NKCs can also kill the target cell by tumor necrosis factor (TNF) related apoptosis-inducing ligand receptor (TRAILR) pathway and FAS–FAS ligand (also known as CD-95–CD-95 ligand) pathway [24]. While other specific monoclonal antibodies-based immunotherapies block the programmed death-1 (PD-1 or its receptors PD-L1/PD-L2) pathway [26]. For detailed mechanisms of action of immunotherapies, readers are referred to read the relevant literature.

Immunotherapies includes various modalities to deal with cancers including cytoimmunotherapies [23], growth factors or growth factor inhibitors [27, 28], vaccination [11], immunomodulatory strategies [14], cytokines [25], antigens and neoantigens [29], antibodies [30], etc. Among them, autologous tumor-directed T-cell therapy also known as adoptive cell therapy has shown high proportion of complete response in metastatic melanoma and other hematologic malignancies [19–21]. Immune checkpoint blockade therapy; administration of an antibody (ipilimumab) that hamper the inhibitory receptor of human cytotoxic T cell antigen-4 (CTCA-4) or PD-1/PD-L1 has expressed complete response in 20% of patients with advanced melanoma [31, 32]. Published literature shows that antibodies therapeutics possibly constitute a substantial part of biological drugs. A total of 79 antibodies have been approved by the United States FDA [33]. While upon combining both of the therapies (CTCA-4 and PD1) has shown quick and complete tumor regression in 10 weeks, thus indicating their synergistic therapeutic effects [30, 34]. Larkin et al. reported more than 5 years' overall survival was 52% in combination of ipilimumab and nivolumab, nivolumab group showed 44% as compared with 26% overall survival of ipilimumab group [35]. Similarly, Hodi et al. report that median overall survival did not reach after 4 years follow up report of clinical trials [36] and Wolchok et al. observed that after 3 years overall survival rates were 58% in combination, 52% in nivolumab and 34% in ipilimumab administered patients [37].

Problems allied with conventional immunotherapies

After the complete treatment, follow-up care is critical to monitor for treatment relapse leading to secondary malignancies and post-therapeutic long-term complications [38]. The most important questions that were previously associated with immunotherapies are effectiveness and toxicity. However, the recent breakthroughs have given a landscaping direction for their success. At the same time, it has opened new challenges for immunotherapy of the modern era. Safe and accurate delivery of therapeutic agents at the target site

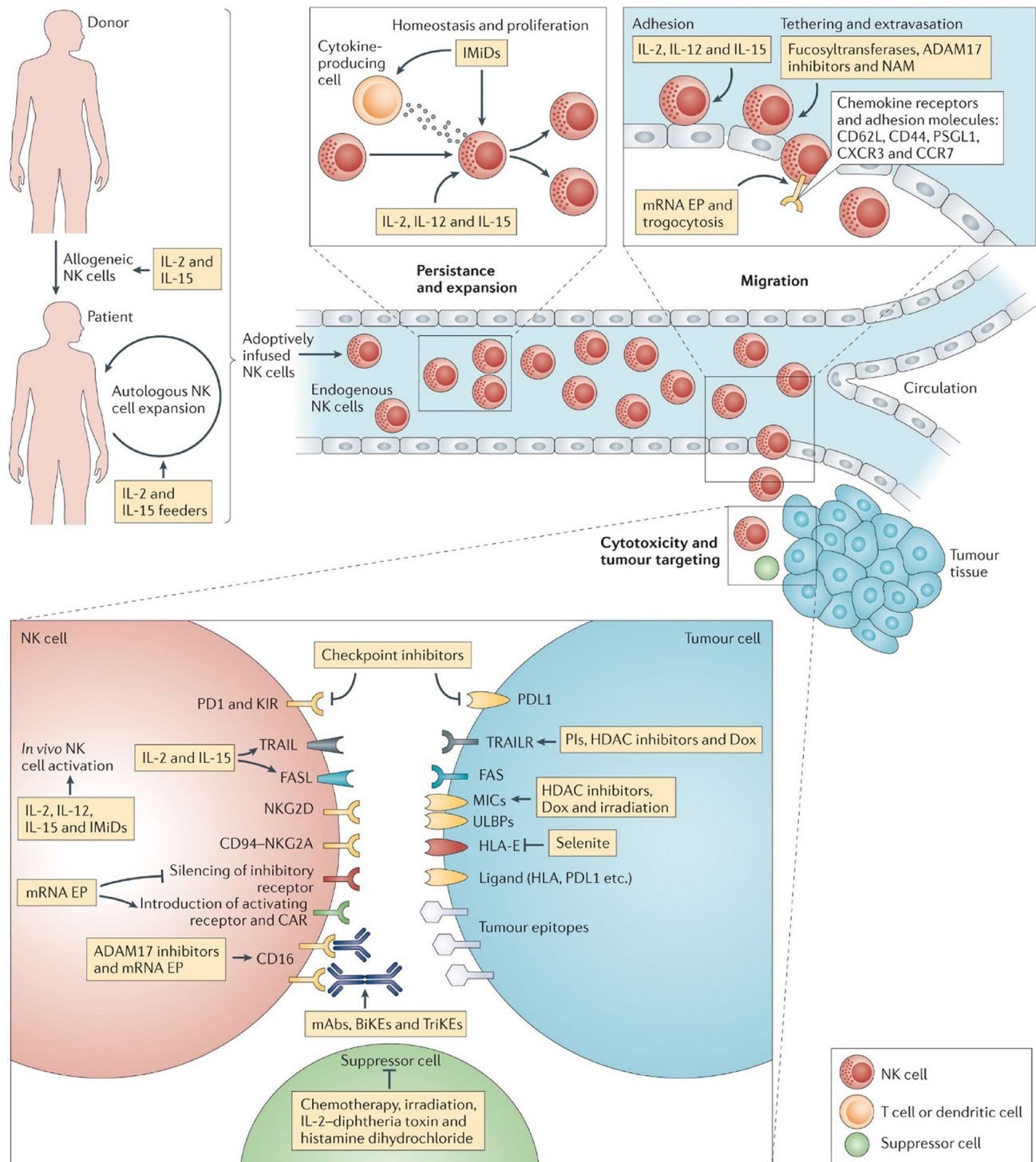


Fig. 3 Schematic overview of drugs that bolster NK cell antitumour immunity and their interaction points. Reprinted from Childs and Carlsten [24], with permission from Springer Nature. License Number: 5121760703231

is nanotechnology-based therapies' main aim, which possibly can maintain the efficacy of therapy and optimize the output while minimizing toxicities [39–41]. After injection, nanomedicine is subjected to different obstacles inside the

body that can restrict drug from reaching the target. Various organs like liver, spleen, lymph nodes, lungs, and skin are considered the most important barriers among them [42]. Besides organs, significant data augments that various

physiological and pathological anatomical structures like pleural fluid (surfactant) [43], blood–brain barrier [44–46], ocular structure [47–49], abnormal tumoral vasculature [50, 51], high intratumoral pressure [51], etc. are important barriers. Poor lymphatic drainage of the tumor area results in the accumulation of various substances at the regional or local level which are responsible for vascular permeability (such as angiotensin, bradykinins, angiotensin-converting enzyme inhibitors, nitric oxide, etc.) is also a hurdle in the successful delivery of nanoparticles [52–54]. Each entity regarded as a barrier in delivery has its mechanism, which unfavored the successful transportation of the carrier at the target site. Interested readers can go through the relevant literature, some of the manuscripts we have cited in this section at the appropriate place as well.

Since 1986 the concept of enhanced permeability and retention (EPR) effect has been a central paradigm in cancer nanomedicine. It is believed that increased vascular permeability optimize transport of high molecular weight drugs and prodrugs [55–57]. While, recent investigations by Sindhwani et al. report that although there are gaps in endothelium of tumor tissue size 2 μm . Still, the endothelium is mostly intact and active transport mechanism is followed for the remittance of nanoparticles through endothelial cells. He further shows that these interendothelial gaps are responsible for the extravasation of a limited (only 3%) amount nanoparticles into tumor tissue [58]. These challenges need to pay special attention to develop systematically developed nanotechnology-based targeted immunotherapies.

Besides anatomical and physiological barriers of nanotechnology-based systems of drug delivery, immunotherapy-related toxicities, resistance to immunotherapies and limited clinical benefits are major challenges associated explicitly with immunotherapies. Toxicities of immunotherapies can be explicated may range from general symptoms like fever or fatigue to organ damage like colitis, rash, adrenal and thyroid insufficiency [59]. Immunotherapies are believed to have fewer long-term toxicities than radiotherapy and chemotherapies, especially in pediatrics. Immunomodulatory agents such as cytokines and monoclonal antibodies (mAbs) are well tolerated. Fever myalgia's, chills, headache, fatigues are common symptoms associated with cytokines while acute infusion-related reactions are associated with mAbs. Infusion-related acute reactions are well managed by administering antihistamines, antipyretics, and corticosteroids [60, 61]. Body of mAbs target bearing cells are indiscriminately diminished by the mAbs. For instance, rituximab depletes B cells and induce humoral immunosuppression, which can lead to higher risk of infection [60].

The main concern of the adoptive T-cell transfer is their lack of discrimination between cancerous and normal cells, so they also diminish the target bearing cells (even with low expression) in normal tissue. Cytokine release syndrome is another

severe and fatal entity associated with adoptive T-cell therapy and may lead to organ failure and death [62, 63]. Checkpoint inhibitors show mild to moderate reactions such as hepatitis, pneumonitis, colitis and pancreatitis, thyroiditis, in young age patients treated with ipilimumab [64], while the PD-1/PD-L1 blockade showed hepatitis, pneumonitis, colitis, thyroiditis, dermatitis and hypophysitis and was comparably less toxic than ipilimumab [65–67]. However, these side effects were effectively managed by short course of steroids or interruption of therapy [68]. Michot et al. observed hemophagocytic syndrome in a patient after 8 weeks of immune checkpoint inhibitors administration, which was treated with corticosteroids. Unluckily, the patient developed a brain hemorrhage and died off later on [69]. In spite of significant advances and durable response of Immune checkpoint inhibitors, a significant proportion of patients do not benefit due to metastasis or acquired resistance due to intrinsic and extrinsic environmental factors [59]. Figure 4 shows these molecules and their targets [59].

Resistance to immunotherapy

Complete response in patients is the forefront aim of therapies. Resistance to various therapies is a big challenge in clinical practice that leads to remission of disease, extended hospital stays, increased morbidity, mortality, and significantly affects the health budget [70]. Abundant literature justifies the rising tide of drug resistance in various types of cancers and against various therapeutic modalities derived by intrinsic and extrinsic factors [71]. Evidence of heterogeneous responses in different lesions in same patients exists. Tumor antigen disappearance, refashioned pathway of antigen presenting machinery like mutation in beta-2 microglobulin, downregulation or deletion of major histocompatibility complex (MHC), inhibition of immune cell infiltration or functions like mutation in PI3K, mitogen-activated protein kinase pathway and various mutations interferon gamma pathway are tumor intrinsic pathways responsible for resistance to therapies [72, 73]. Other extrinsic factors such as regulatory T cells (Tregs), myeloid derived suppressor cells, M2 macrophages, alongside other inhibitory immune checkpoints can also be important contributors in the way of antitumor immune response [74]. Disclosure of accurate molecular immune microenvironment of tumor such as PD-L1 expression, mutational loads, genes involved in chromatin remodeling, loss of various genetic information's, etc. could be helpful in planning the efficient dosage administration and schedule of multiple immunotherapies while lowering the risk of disease remission or failure of therapy [75, 76].

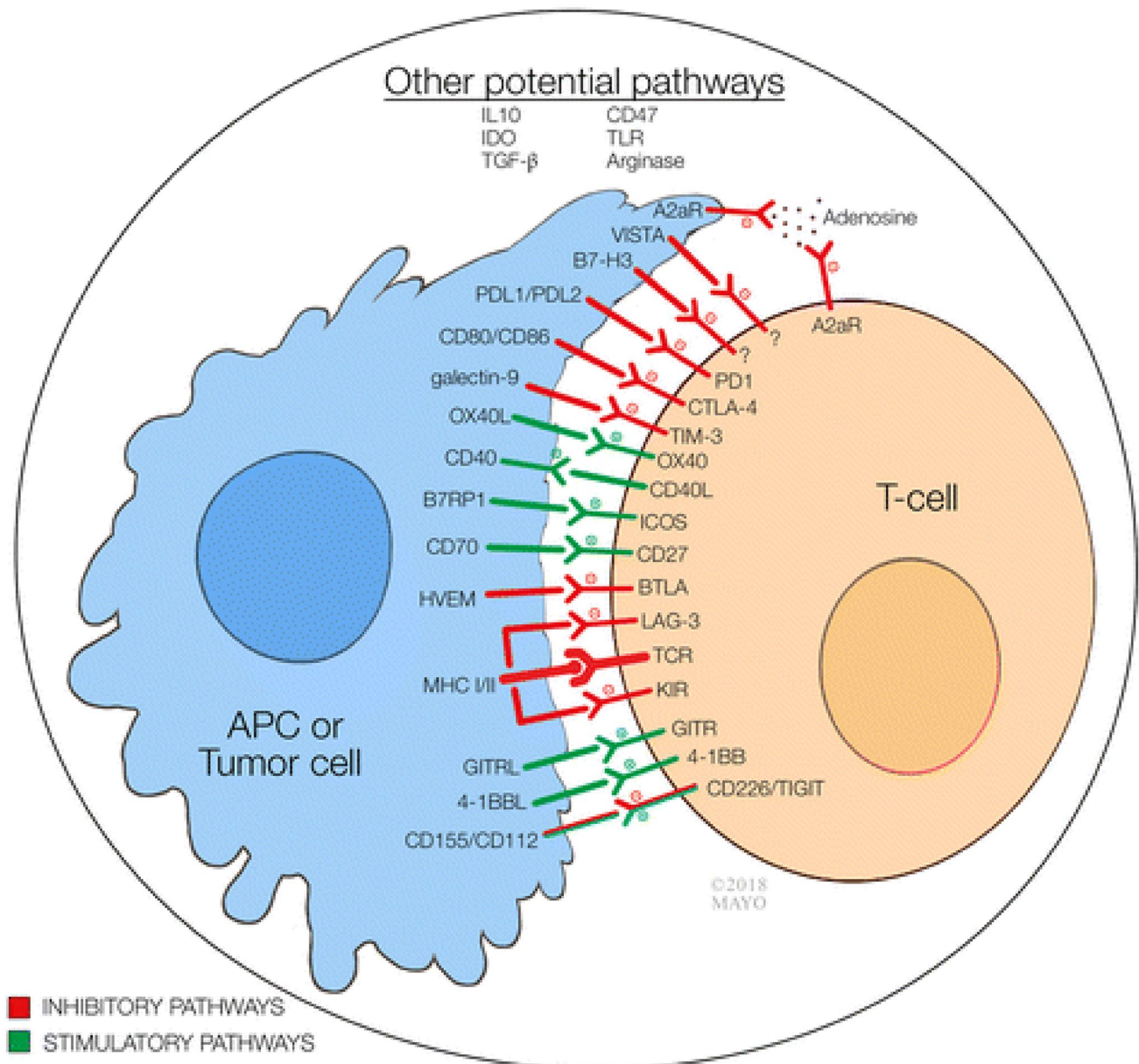


Fig. 4 Immune interactions involving antigen presenting cells or tumor cells, T cells, and tumor microenvironment. Reprinted from Marin-Acedo et al. [59] with permission under a Creative Commons license

Activation of dendritic cells through nanoparticles

Dendritic cells, B cells, and macrophages are the major components of the immune system and are prime representatives of antigen-presenting cells (APCs). These cells stimulate the adaptive immune response and through this stimulation exert antitumor efficacy [77]. These APCs possess the capabilities to phagocytize the antigens as well as damage-associated molecular patterns. When these APCs are stimulated, they present antigens on the major histocompatibility complexes

to other immune cells like cytotoxic T-lymphocytes (CTLs) and downstream cytokines excretion [78]. Due to the key role of APCs in stimulating such responses, substantial efforts have focused on targeting the APCs with several kinds of nanoparticles having immunomodulating agents.

DCs are professional antigen-presenting cells that are skilled in propagating an antigen-specific immune response against pathogens. Hence, immunotherapeutic approaches exploit DCs to present antigens as a source of cell-mediated therapeutic vaccination in patients having advanced levels of malignancies. In this connection, ex vivo DCs are

trained with antigens and then these are transferred back into patients for vaccination [79]. Although, a higher antigen-specific CTLs response is produced by this strategy at the metastatic tumor site, however, this approach lacks therapeutic efficacy in clinical trials. Additionally, this approach is also technically challenging and expensive as well. Thus, in situ targeting of DCs with adjuvant and antigen-loaded nanoparticles can significantly upsurge the clinical applications of dendritic cell-mediated immunotherapy.

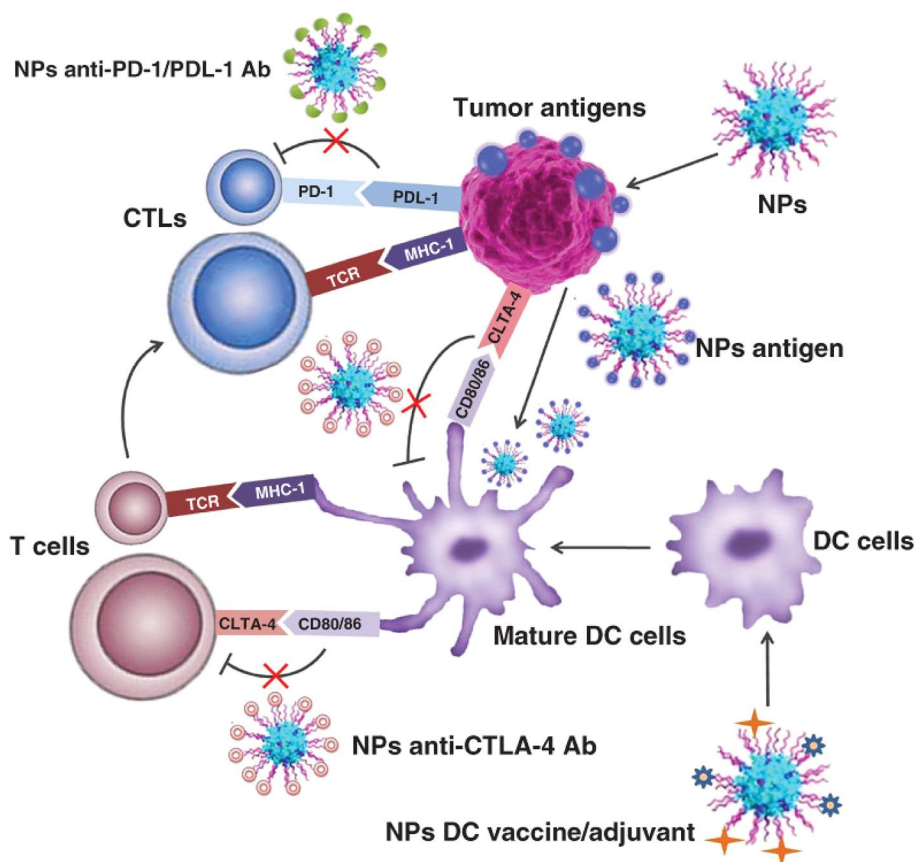
Delivery of antigens

As compared with soluble formulation, loading of antigens into the nanoparticles presents greater advantages. Firstly, nanoparticles protect the antigens from degradation and deliver them to the target in a more precise and sustained manner than free antigens. Secondly, nanoparticles prevent the entry of the loaded antigens into the blood circulation thus reduced the off-target toxicity on one hand and provides targeted delivery to the resident immune cells on the other hand. The most important advantage of loading antigens into the nanoparticles is that the DCs tend to cross-present the antigens more effectively when conjugated with nanoparticles. Thus, to increase the efficacy of

dendritic cells-mediated immunotherapy, several immunostimulatory and antigen-based compounds have been fabricated in nanoparticles to provide site-specific delivery in vivo [80].

In one study, Gao et al. formulated chitosan and carboxymethyl chitosan-based nanoparticles laden with extracellular material of *Vibrio anguillarum*. In this study, it was found that as compared with free extracellular material, the material laden within the nanoparticles exhibited enhanced innate as well as adaptive immune responses [81]. Similarly, Maji et al. evaluated the impact of cationic liposomes on the antigen-presenting and maturation capacity of the dendritic cells. From this study, it was found that as compares with anionic and neutral liposomes, the cationic liposomes were being taken up more efficiently by DCs and delivered to the target site for major histocompatibility complexes-based processing [82]. Rietscher et al. investigated the potential of PEG-b-PAGE-PLGA as a nanocarrier for prophylactic vaccination. They have also loaded ovalbumin as a model antigen within this nanocarrier. In this study, it was found that as compared with free and soluble ovalbumin antigen, the antigen that was loaded into nanoparticles exhibited significantly higher T-cell activation through antigen-presenting cells [83]. Several nanoparticles-based approaches for cancer immunotherapy are shown in Fig. 5 [84].

Fig. 5 Visual illustration of several nanoparticles-based approaches for cancer immunotherapy. Reprinted from Qiu et al. [84] with permission from John Wiley and Sons. License Number: 5121761002630



Delivery of adjuvant

Vaccines are important form of immunotherapy and activate immune system. Vaccines efficient for antigen processing and presentation. When co-delivered with chemotherapeutics, they generate a synergistic effect and heighten the efficacy of therapies [85]. In immunotherapy, the vaccine adjuvants stimulate the immune system against a specific antigen. These adjuvants imitate the particular set of pathogenically preserved molecules called pathogen-associated molecular patterns. One such immune-stimulating compound is lipopolysaccharide which is the component of the bacterial cell wall, and nucleic acid situated in the pathological environment. As the immune system is involved to detect these moieties, thus the conjugation of the adjuvant with antigens can significantly upsurge the activity of the lymphocytes, dendritic cells, and macrophages [86]. Nevertheless, these immunostimulators can also promote undesired side effects like toxic shocks when administered systemically. In this regard, the delivery of chemotherapies through nanocarriers has significantly reduced the toxicity of various drugs (i.e. amphotericin B, and doxorubicin) through site-specific delivery at lower doses [87].

In one study, Sokolova et al. examined the potential of cationic gelatin-based nanoparticles for delivery of CpG adjuvant (a short single-stranded DNA that effectively stimulates DCs via toll-like receptors inside the phagosomes). In this study, it was observed that as compared with free CpG, the CpG delivered through nanoparticles exhibited significantly higher antigen-specific T-cell response, greater antitumoral immunity, and targeted delivery in a murine melanoma model [88].

In addition to the delivery of a single adjuvant, the nanocarriers also possess the potential to deliver multiple adjuvants in a single nanocarrier. In this regard, Schlosser et al. formulated the PLGA-based microspheres loaded with OVA antigen and CpG adjuvant. This combination system exhibited much better CTL responses in mice than alone OVA or CpG [89]. Similarly, Hamdy et al. observed that combined delivery of tyrosine-related protein 2 (i.e. poor immunogenic melanoma antigen) and monophosphoryl lipid A (i.e. an adjuvant) in a PLGA-based nanocarrier exhibited antitumor activities [90]. In another study conducted by Li et al. mice immunization with autophagosomes extracted from cancer cells and α -Al₂O₃ as an adjuvant resulted in tumor regression. They also reported that the combined delivery of two adjuvants without conjugation was inefficient, hence conjugation is very important to get the desired results. Fen et al. demonstrated that coating of nanoparticles, having MPL as an adjuvant, with a layer of cancer cells membrane possess the potential to produce a Tumor antigen associated (TAA) specific immune response [91].

Dendritic cells targeting

Active targeting of nanoparticles and their cargos to the DCs offers a promising approach for targeted and efficient delivery of immunotherapeutics. The nanoparticles' surface tailored with certain ligands like fucose, mannose, anti-CD11c, N-acetyl glucosamine, and anti-DEC205 have shown preferential higher uptake of nanoparticles within the cell through receptor-mediated endocytosis [92]. Furthermore, binding of these nanoparticles to the specific receptors expressed on the DCs also increases the maturation and hence further improves the efficacy of the vaccine formulation [93]. In this regard, Kempf et al. fabricated ligand conjugated PLGA-based nanoparticles for targeted delivery to the dendritic cells. as compared with blank nanoparticles, ligand conjugated nanoparticles exhibited more efficient delivery of nanoparticles to the dendritic cells, higher expression of IL-2, and higher upregulation of DCs maturation markers like CD 86 and CD 83 [94].

In this connection, Qian et al. formulated a vaccine that was functionalized with tumor antigen peptides that reach the target site via scavenger receptor-mediated class B1 pathway. The self-assembly, smaller size, and targetability of the prepared nanovaccine illustrated significant loading of tumor antigen peptide, considerable accumulation in lymph nodes (LNs), and increased antigen presentation. They also established that nanovaccine could be used alone as well as in combination with CpG as a therapeutic or prophylactic nanovaccine. Moreover, they also concluded that the size of the nanoparticles also plays an important role in site-specific delivery [95]. Mostly, large size particles (500–2000 nm) uptake by LNs relies on the DCs uptake, while the small-sized nanoparticles (20–200 nm) are freely uptaken by the LNs and subsequently target the LN-resident DCs [96].

Recently, supramolecular peptides, protein and their derivatives are gaining special attention of researchers for immunological applications like vaccines and certain other immunotherapies [97]. Biomaterials created from Liu et al. administered CpG-DNA/peptide vaccines comprising an antigen and adjuvant cargo in tumor bearing mice. Authors observed 30 folds higher T cell priming, increased LN accumulation, lesser systemic disruption and optimized antitumor effect while minimizing toxicities [98]. However, the current approaches used in cancer vaccine delivery necessitate direct peptide alteration that affect the vaccine efficacy. Li et al. has recently reported more effective adsorption approach using polyethyleneimine in mesoporous silica microrods (MSR) vaccines to the immunogenoity of vaccine. This atrategy resulted better outcome than that of existing MSR and bolus vaccines to activate the host HCs and T cells antitumor response. It

eradicated established tumors in 80% mice and interestingly developed immunological memory [99].

Kokate et al. investigated that RNA-lipoplexes could be efficiently targeted *in vivo*. Authors found that the lipoplexes could be targeted to the site of action (i.e. dendritic cells, and spleen) by simply adjusting the net charge on the nanoparticles. These nanoparticles have shown promising results in various cancer models and currently, these are in phase I clinical trials. In the future, several targeting mechanisms can also lead to state-of-the-art ways of prompting dendritic cell manipulation through nanocarriers [100]. Dendritic cell based synthetic multi-epitope DNA vaccine delivery via liposomes was predicted by Yang et al.. Authors proposed that DNA vaccines were efficiently uptaken by DCs leading to significant tumor suppression and recruitment of CD8⁺ T cells at tumor cells [101].

The maturation of DC and infiltration of effector T cells in tumor tissue and tumor-draining lymph nodes (tdLN) are pivotal for immunotherapy. Transdermal delivery of non-vaccine pembrolizumab complex microneedles optimizing transdermal immunization in skin tumors has recently been reported by Zhou et al. [102]. The authors observed accumulation of regimens at tdLN, DCs maturation activation and optimization Th1 immune responses. Moreover, pembrolizumab significantly activated CTCs and recruitment while reducing the Tregs in tdLN. Overall, the results indicate the transformation of immunosuppressive TME to immunoactive TME.

Alterations in the tumor microenvironment through nanoparticles

There are certain characteristics associated with the tumor microenvironment like hypoxia, high proteolytic activity, irregular vascularization, and reduced extracellular pH, which can complicate immunotherapy. Moreover, the tumor microenvironment also produces an immunosuppressive environment through the release of several cytokines, tumor-associated macrophages, myeloid-derived suppressive cells, and Tregs. The presence of such an environment leads to treatment resistance and clinically poor prognosis [103]. Hence, there is a need for novel immunotherapeutic options that can control the tumor microenvironment on one hand and reverse the immunosuppressive conditions on the other hand. The utilization of nanoparticles-based immunotherapies presents an auspicious approach to remove this tumor persuaded immunosuppression.

In a current preclinical study, researchers developed nanolipogels in which the drug was loaded into cyclodextrin and cytokine-laden biodegradable polymers. The formulated nanolipogel exhibited the potential to deliver interleukin-2 and transforming growth factor-beta

inhibitors in a sustained manner to the tumor microenvironment. The mice group treated with nanolipogel displayed a delay in tumor growth, increased survival rate, enhanced activity of natural killer cells, and intratumoral activated CD8⁺ cells [104].

To target immunosuppressive cells, Sacchetti et al. evaluated the potential of Treg-specific receptor ligands to enhance tumor-specific internalization of PEG-modified carbon nanotubes. The nanotubes complexed with glucocorticoid-induced TNFR-based receptor (i.e. over-expressed in intratumoral Tregs) exhibited higher accumulation in the target site than the non-targeted sites [105]. Similarly, Zhu et al. formulated PEG and mannose modified nanoparticles to enhance targeted delivery. The prepared nanoparticles exhibited stealth properties and reached the targeted site where the PEG was cleaved and mannose groups were exposed. After administration through the intravenous route, it was also observed that as compared with non-functionalized nanoparticles, the mannose decorated nanoparticles exhibited improved accumulation in the tumor microenvironment [106].

The applications of chemotherapy can also modify the tumor microenvironment and in turn, upsurge the therapeutic efficacy of the subsequent immunotherapy [107]. In this regard, Lu et al. investigated that a combination of TRP2 antigen and curcumin-PEG-based nanomicelles generated synergistic antitumor activity in melanoma mice model than alone treatment. In the components of the immune system, the combined therapy also remarkably increased the CTL response as well as the production of interferon-gamma. However, in the tumor microenvironment, the combination therapy reduced the expression of immunosuppressive factors. This reduction in the immunosuppression is in line with increased levels of CD8⁺ T-cell population and pro-inflammatory cytokines levels [108]. Similarly, curcumin and loaded nanoparticles with encapsulated nanovaccines triggered residual tumor cells death and DCs recruitment, respectively. Besides, this hybrid delivery system also induced a strong T cells-specific immune response [109].

Immunotherapy can also be improved through physical manipulation. In combination with anti-CTLA-4 antibody therapy, some researchers investigated the potential of photothermal ablation of tumors with intratumorally injected PEG-conjugated single-walled carbon nanotubes. They found that the applied strategy had successfully modulated the adaptive immune responses, particularly cellular immunity against metastatic cancers. The mechanism through which it was achieved was based on photothermal-based cell death, which mediated the secretion of damage-associated molecular patterns and TAA that primes the immune system [110].

Immune modulating compounds delivery through nanoparticles

The EPR effect found in various disease conditions, including metastatic cancers, can be exploited for the targeted delivery of immune-modulating agents to the tumor site. Even though the concept of EPR is controversial in human beings, it is hypothesized that leaky blood vessels in the tumor site and the extended circulation time of the nanocarrier upsurgers the targeted uptake of the nanocarriers to the tumor site [111]. To achieve passive targeted delivery, the most important consideration is that the nanoparticles must resist the uptake by the reticuloendothelial system. To overcome this problem, nanoparticles should be designed in such a way that they should be anionic or neutral, and must have a size in the range of 8 and 200 nm, and must be protected by a stealthing agent like PEG, that prevents opsonization. Thus, nanoparticles having these properties can be efficiently used as a nanocarrier for passive targeted delivery of immune-modulating agents [54].

Delivery of antibodies

Therapies based on antibodies have now become a promising strategy to efficiently treat metastatic cancers. The major advantage of this approach involves the targeted delivery of the drug while preventing off-target delivery. The antibodies bind specifically and preferentially to the target proteins, which are particularly overexpressed in the tumor microenvironment [42]. However, there are certain drawbacks associated with these delivery approaches such as poor pharmacokinetics, inadequate tissue penetration, and compromised interaction with the components of the immune system [112]. Researchers have employed various strategies to overcome these limitations and to improve the delivery efficiency of various antibodies. In this connection, Kim et al. modified the assembly of polyion complex micelles to load charged antibody derivatives for increased stability, efficient delivery to the cytosol, and the recognition of the antigens within the cells (Fig. 6) [113]. They observed that an optimum ratio of block cationomers and homopolymers-based micelles loaded with antibodies significantly upsurgers the endosomal evasion on one hand and also increased the intracellular recognition of the antigens [113]. Chen et al. designed a nanocarrier consisting of PLGA having an anti-OX40-monoclonal antibody. As compared with the free monoclonal antibody, the antibody loaded into a PLGA-based nanocarrier exhibited significantly higher clinical activity when evaluated in a phase-I

clinical trial. Moreover, the prepared nanocarrier also showed substantially higher CTL-induced cell proliferation, antigen-specific cytotoxicity, and cytokine production than free antibodies [114].

In clinical trials, the most effective immunotherapeutic-based antibodies block the immunosuppressive pathways that lead towards the progression of cancers. Among these antibodies, programmed death-1 and CTL-linked protein-4 allows the CTLs to target and destroy the cancer cells and produce an efficient clinical response in various kinds of metastatic cancers [115]. However, some patients exhibit poor response to such kind of therapies as well. To upsurge the clinical response, Lei et al. formulated mesoporous silica nanoparticles having CTCA-4 via non-covalent linkages at a very high density to provide targeted and long-lasting antibody release. The prepared formulation exhibited higher therapeutic efficacy than the same amount administered systemically. Additionally, alterations in the functionalities of the nanoparticles could also modify the rate of antibody release [116].

The codelivery of antibodies along with cytokines via nanoparticles also exhibited promising results. In this regard, Kwong et al. engineered pegylated nanoparticles having IL-2Fc fusion protein and T-cell stimulatory anti-CD137. The administration of the prepared formulation through intratumoral injection in the murine B16F10 model cured the primary tumor significantly. The prepared nanoparticles also reduced the lethal inflammation-based toxicities and produced antitumor memory than the equivalent dose of soluble antibodies [117]. Likewise, Li et al. formulated an alginate-based hydrogel delivery system having PD-1 monoclonal antibody and celecoxib. The combined delivery system significantly enhanced antitumor effects than alone. These positive results appeared due to sustained and high concentrations of drugs and antibodies in the tumor microenvironment [118].

Surprisingly, the codelivery of PD-1 antibody and celecoxib increased the levels of CD8⁺IFN- γ ⁺ T-cells and CD4⁺IFN- γ ⁺ within the immune components and the tumor microenvironment. Additionally, the expression of CD4⁺FoxP3⁺ Tregs and MDSC in the tumor site showed an immunogenic response increment. Kosmides et al. formulated a nanoparticles-based delivery system having combined T-cell stimulatory signals, anti-PD-L1, and anti-4-1BB. This dually targeted delivery system redirected the responses of effector T-cells to identify target cells whereas simultaneous blockage of various inhibitory pathways. In vitro, this response generated a six-fold increment in IFN- γ production through CD8⁺ T cells in the presence of tumor cells. Furthermore, the expression of PD-1 and tumor growth declined up to 30% in the tumor-infiltrating lymphocytes [119].

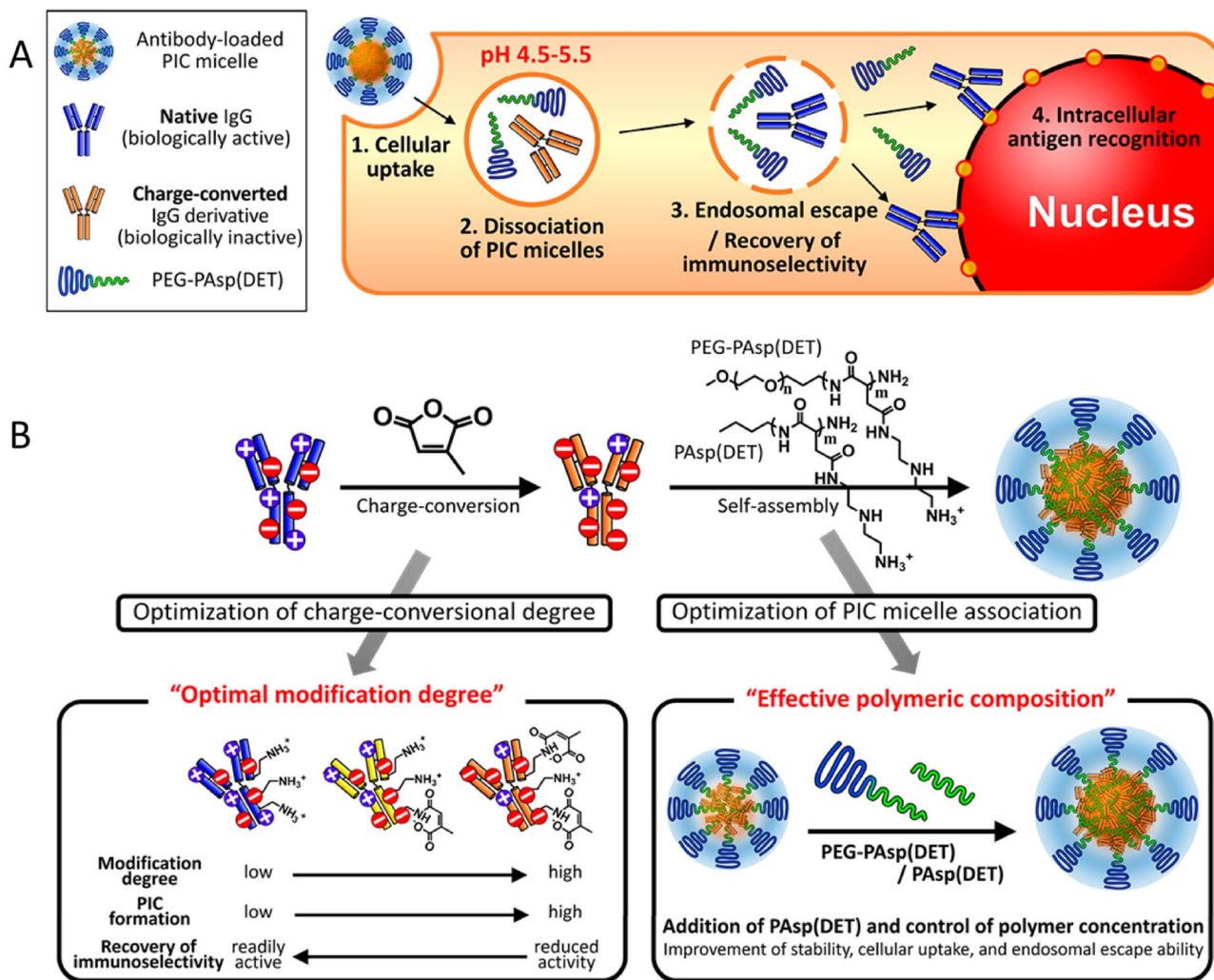


Fig. 6 **A** Pathways for successful intracellular antibody delivery with PIC micelles. **B** Formation of PIC micelles incorporating charge-converted igg antibody derivatives and strategies to engineer the systems

in this study. Reprinted from Kim et al. [113] with permission from American Chemical Society

Delivery of immune-modulatory compounds through nanoparticles

Delivery of genes via nanoparticles

The delivery of small interfering ribonucleic acid (siRNA) can provide better utility in nanoparticles mediated metastatic cancer immunotherapy. In a recent study, Li et al. formulated cationic lipid-based PEG-PLGA nanoparticles for the efficient delivery of CTCA-4 siRNA and exhibited that this carrier system can efficiently enter T cells both in vitro and in vivo. It was observed that the prepared nanoformulation was internalized by tumor-infiltrating lymphocytes and increased T cell proliferation [120]. Wang et al. synthesized cationic polymer-lipid based hybrid nanoparticles (nanovesicles) to deliver doxorubicin and siRNA. Adjuvant delivery

of doxorubicin with siRNA via nanovesicles showed antigen presentation and induced immunogenic cell death of B16 cells in multiple myeloma [121].

Similarly, Huang et al. also used cationic lipid based polymeric nanoparticles to deliver siRNA and certain chemotherapeutics, which induced tumor regression and restricted tumor metastasis in colorectal and pancreatic cancers [122]. Li et al. investigated the efficacy of lipid micelles for the codelivery of shikonin and siRNA and observed efficient tumor accumulation and cytoplasmic delivery. Authors reported that this micelles-based nanocarriers efficiently delivered cargo, which effectively elicited the recruitment of CTCs and induced immunogenic cell death [123]. Furthermore, Wang et al. reported the successful delivery of intratumoral CRISPR activation libraries eliciting sufficient antitumor response to clear the local and distant established tumors

by targeting mutated genes precisely [124]. Furthermore, the systemic delivery of the prepared nanoparticles also increased the number of effector cells, reduced the ratio of expression of CD4⁺ FOXP3⁺ Tregs, decreased the tumor growth, and significantly enhanced the survival time of mice bearing melanoma [120].

Chemical modification of nanoparticles with certain biomolecules like tyrosine has also been recently reported for the efficient delivery of siRNA, biocompatibility and favorable physical properties in various *in vitro*, *ex vivo* and *in vivo* models [125]. Teo et al. evaluated the sensitization of the epithelial lining of ovarian cancer cells to CTL killing through the delivery of PD-L1 siRNA by folic acid functionalized polyethyleneimine nanocarriers. The prepared nanocarriers enhanced the cellular uptake of the PD-L1 siRNA into SKOV-3-Luc cells and reduced the nonspecific uptake into the monocytes [126]. Roeven et al. investigated that transfecting the PD-L1 and PD-L2 siRNA with SAINT-RED consisting of cationic amphiphilic lipid SAINT-18, produced the long-term knockdown of the PD-1 ligands without disturbing the maturation and viability of the dendritic cells. Moreover, they also found that the prepared transfection system in combination with a peptide of histocompatibility antigens mRNA could produce clinical-grade dendritic cell vaccines to upsurge antitumor immunity [127].

To further enhance the efficiency of vaccines in advanced stages of metastatic cancers, Xu et al. designed liposome-protamine-hyaluronic acid nanoparticles for the delivery of TGF- β siRNA. The resulting TGF- β downregulation upsurged the vaccine efficiency and reduced the tumor growth up to 52% than control groups [128].

Delivery of cytokines through nanoparticles

The capability of cytokines to affect the components and responses of the immune system has motivated researchers to use them in immunotherapy. In this regard, several cytokines like IL-2, TNF- α , and interferon α/γ (IFN- α/γ) have been approved by the food and drug administration authority (FDA) for cancer therapy. However, these are rapidly metabolized and excreted from the body when administered in safe doses systemically. Hence, there is a need to administer higher doses to achieve desired therapeutic effects, however, higher doses are linked with higher adverse effects. Thus, there is a necessity to develop nanoparticles mediated carrier systems that can deliver cytokines specifically and preferentially to the target sites [129].

Considering the advantage of stealth liposomes, the delivery of IL-2 via the inhalational route in mice having mice with metastatic lung cancer can significantly decrease the tumor growth than free IL-2 [130]. Hagen et al. investigated that pegylated liposomes could be effective for systemic delivery of TNF- α along with liposomal chemotherapy for

advanced stages of solid tumors. In this regard, in phase-I clinical trial, patients with follicular lymphoma receiving liposome-based nanoformulation of TAA and IL-2 illustrated an increment in tumor lymphocytes infiltration and tumor growth reduction [131]. Lastly, work by Anderson et al. illustrated that liposomes could serve as an efficient nanocarrier for efficient delivery of IL-6, IL-2, IL-1a, and granulocyte and macrophage colony-stimulating factor (GM-CSF) against metastatic cancers [132]. Visual presentation of mechanism of immunosuppression is shown in Fig. 7 [133].

Conclusions and future prospects

Nanotechnology and its promises in cancer immunotherapies have been discussed in this review. But, exploring the complexity of TME, immunogenicity, and off-target toxicities are still challenging concerns of successful cancer immunotherapies. As costimulatory and inhibitory signals regulate tumor-specific CTCs. Currently immunotherapeutic approaches are based on reinstating their function by targeting their inhibitory pathways. However, various tumor cells such as tumor-infiltrating immune cells, tumor-associated stromal cells and cytokines and/or chemokines are crucial mediators of CTC functions. The immune system is a complex interaction of various farsighted stimulatory and inhibitory responses originating at subcellular, cellular and tissue levels. Under current challenging circumstances and limited technical understanding in immunotherapies, nanotechnology, especially nanomaterials, has a new epoch in immunotherapies and can have great promise and opportunities. Genetically engineered cells to harvest cellular nanocarriers expressing PD-1 to deliver immunological molecules have also been successfully used. These approaches are expected to explore new directions for personalized immunotherapy in medicine.

Bionanomaterials are believed to be the important player in delivering immunotherapeutic agents, but their compatibility is the main limiting factor. So it also necessitates the development of standard and optimized characterization and measurement techniques. Until now, limited data on the medical impact of bionanomaterials and their clinical transformation is available. Based on the current evidence, the effectiveness and selectivity of bio-nanocarriers hinder the target (tumor) site delivery of the drug. Hence restricting their large scale precise clinical translation. Which indicates further consideration of bionanomaterials biosafety as an immunotherapeutic carrier. Last but not least, *in vivo* pharmacokinetics and host immune responses of bionanomaterials needs to be further explored in detail.

Besides, revision of awareness protocols, refreshers course on evaluation of adverse reaction, and continued

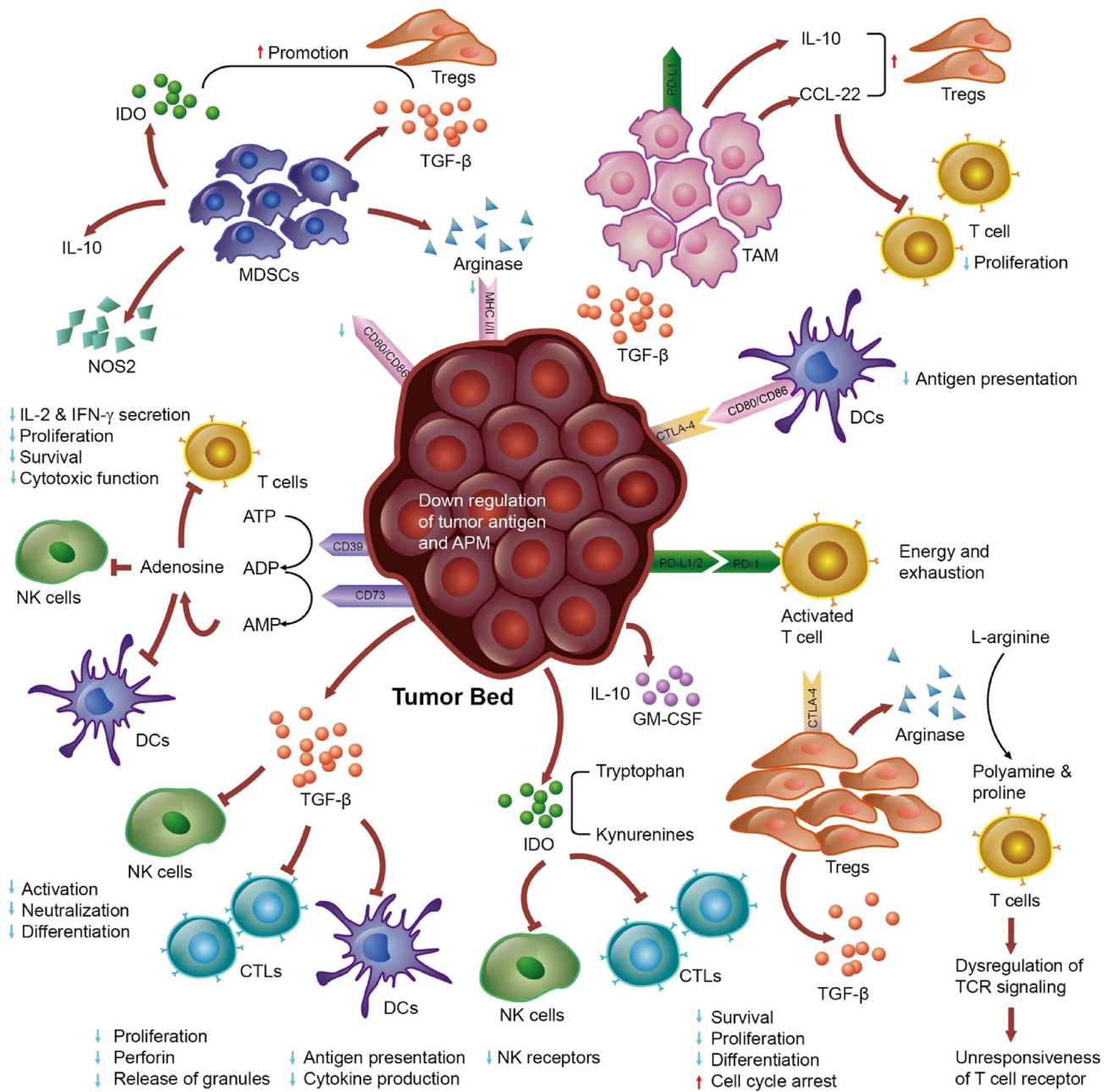


Fig. 7 Visual presentation of mechanism of immunosuppression. Reprinted from Kapadia et al. [133] with permission from Elsevier. License Number: 5121761230857

evaluation of quality clinical practices can effectively improve the outcomes [134, 135]. Although, there are several limitations in the clinical inauguration of bionanomaterials and fabrication. An improved design in light of deep clinical understanding will greatly help to establish effective and safe immunotherapies. As discussed earlier, systems of targeted delivery of drugs via carriers would be of great interest. At the same time, optimization of nanoparticles synthesis by exploring their interaction with various

drug-like biological drugs or vaccines could be helpful in their appropriated and optimum delivery designs. Besides, it could be helpful to avoid the unnecessary and harmful interactions with drugs, especially with biological drugs. Interdisciplinary cooperation can enhance the chance of more accurate design of drug carriers.

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Consent to participate Not applicable.

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