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Combining Targeted Nanomedicine and Cancer Immunotherapy

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Abstract

Cancer immunotherapy can be improved significantly by nanomedicine. A nanomedicine is a drug delivery system with a size between 1-100 nm-sized used primarily for improving the efficiency and toxicity of conjugated or entrapped chemotherapy drugs. Clinical performance of cancer nanomedicines has been somewhat disappointing, primarily because there are no tools and technologies to stratify patients. Immunotherapy, on the other hand, has led to complete cures and long-term survival in patients with advanced stages of cancer. There are, however, relatively few patients who benefit from immunotherapy. There is increasing evidence that combing nanomedicine and immunotherapy can enhance therapeutic outcomes by making "cold" non-immunoresponsive tumors and metastases "hot" and immune-responsive. Three different approaches to nano-immunotherapy are available, in which nanomedicines are used to target cancer cells, the tumor immune microenvironment, and the peripheral immune system. Cancer cells are typically targeted with nanomedicines that induce immunogenic cell death, releasing tumor antigens and molecular patterns that indicate danger, such as calreticulin, high mobility group box 1, and adenosine triphosphate. By promoting the generation of CD8+ cytotoxic T cells, adjuvants alert antigen-presenting cells to take up, process, and present the former. As well as inhibiting immunosuppressive cells, such as M1-like tumor-associated macrophages, nanomedicines targeting the tumor immune microenvironment also inhibit the expression of immunosuppressive molecules, such as transforming growth factor beta, which potentiate cancer immunotherapy. Nanomedicines can also be used in the tumor immune microenvironment to promote the activity of antigen-presenting cells and cytotoxic T cells. By engineering and strengthening peripheral effector immune cell populations, nanomedicines targeting the peripheral immune system aim to enhance antigen presentation and cytotoxic T cell production in secondary lymphoid organs, such as lymph nodes and spleens, thus promoting immunity against cancer. Despite the fact that most immunomodulatory nanomedicines are still in preclinical development, early clinical trials have shown promising results. The right nanomedicine formulation must be combined with the right immunotherapy in the right patient in order to ensure efficient translation of nano-immunotherapy constructs and concepts. Nano-biomarker identification is currently underway, as are some immuno-biomarker initiatives, such as Immunoscore and Cancer Immunograms, which are partially established. This combination of protocols will enable the identification and use of individualized and improved nanomedicine-based treatments to boost the performance of cancer immunotherapy by capturing individual nano-immuno-statuses.

Keywords: Nanomedicine; Cancer; Immunotherapy

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Introduction

Cancer immunotherapy

Clinical cancer treatment is being radically reshaped by immunotherapy. More than a century after the first immunomodulatory therapeutics (such as Coley's toxins) were described, immunotherapy has just recently become widely accepted as an anticancer treatment modality. It achieved unprecedented results in a number of cases, including complete regression of advanced-stage (metastasized) cancers and long-term disease-free survival, in several cancer types, including malignant melanoma and lung cancer [1-3].

As a result of recent advances in cancer biology and anticancer immunity, this immunotherapeutic revolution has benefited greatly. James Allison and Tasuku Honjo were awarded the Nobel Prize in Physiology or Medicine for "the discovery of cancer therapy by inhibiting negative immune regulation." In particular, the Nobel prize was awarded for the discovery of immune checkpoints (cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and programmed death/ligand 1 (PD-1/PD-L1)), which have been used to develop anticancer antibodies that target these checkpoints. The use of chimeric antigen receptor (CAR) T-cell therapies, which have mainly been used for treating hematological malignancies, has also been proven to be effective cancer immunotherapy, in addition to immune checkpoint inhibitors, which are mostly used for solid tumors. The Food and Drug Administration (FDA) approved eight new anticancer drugs between 2014 and 2018, six for PD-1/L1 blockade and two for CAR T-cell therapy [4]. In addition, recent advances in the development and testing of cancer vaccines, particularly those based on neoantigen delivery, have demonstrated the potential of this immunotherapeutic approach.

A cancer-immunity cycle illustrates the principle(s) of cancer immunotherapy. The cycle begins when tumor antigens are released, which are taken up, processed, and presented to naive T cells by antigen-presenting cells (APCs) [5]. In this way, cytotoxic T cells are generated that are capable of specifically recognizing and killing cancer cells. Antigens and co-stimulation signals are released by lysed cancer cells to promote another round of immune reaction. Cancer immunotherapy

targets a number of negative feedback immune regulatory pathways that tumors can disrupt, disrupting essential elements of the cancer-immunity cycle (Figure 1) [6].

Immunotherapy has achieved substantial clinical success, but so far it has only been effective in a relatively small number of patients. Multiple strategies are currently being used to address this shortcoming, including the use of biomarkers to differentiate between patients with "cold" non-immunoresponsive lesions versus those with "hot" immunoresponsive tumors and metastases. Moreover, as will be discussed below, a growing number of nano-immunotherapies are currently being explored in combination immunotherapy studies [7-8].

Cancer nanomedicine

Medical nanotechnology is referred to as nanomedicine. While this also involves the development of nano-sized materials and methods for the *ex vivo* diagnosis and staging of diseases, the term nanomedicine is traditionally used to refer to 1 to 100 nm-sized drug delivery systems that, after intravenous injection, travel throughout the body to selectively accumulate in pathological regions, which are intended to elicit pharmacological effects specifically there, while avoiding drug accumulation and drug actions elsewhere [9-11].

Two main mechanisms are typically used for nanomedicine-based tumor targeting, passive targeting and active targeting. Matsumura and Maeda, as well as Jain and colleagues, discovered the Enhanced Permeability and Retention effect three decades ago. By decorating nanoparticles with targeting ligands, such as antibodies or peptides, active targeting recognizes receptors overexpressed at the pathological site [3, 12]. The advantages and disadvantages of each strategy can be categorized according to their overall targeting efficiency, specific cell delivery, formulation complexity, and translational potential, for example. Recent reviews, discussions, and debates have focused on these issues. Figure 2 provides a general overview of the biological processes the NCs participate in once administered *in vivo*, as well as their clinical implications. It is important to note, however, that the fate and therapeutic outcome of NCs depend heavily on their specific chemical composition and other specific structural characteristics, such as their surface properties [13].

Additionally, to killing cancer cells directly, nanomedicines can modulate immune responses against malignancies. A nanomedicine can accomplish this by targeting cancer cells to cause immunogenic cell death; targeting immune cells, such as macrophages, dendritic cells, and T cells, or immunosuppressive pathways in tumor immune microenvironments; and targeting peripheral immune systems, such as lymph nodes and spleens. More and more evidence suggest that nanomedicines can enhance antitumor immunity and synergize with established immunotherapies to improve response rates and survival times [14, 15].

Nanomedicine and immunotherapy together

Nanomedicine and immunotherapy have gained growing attention in the last few years. The steady increase in papers reporting on nanoparticles for drug delivery since the turn of the millennium demonstrates the high level of activity in nanomedicine. Since 2011, when the first immune checkpoint inhibitor was approved by the FDA, immunotherapy research has seen a significant increase [16]. The number of publications on nano-immunotherapy also experienced strong growth in 2011, and it has been expanding exponentially since 2013. As this number grows, it reflects the high hopes associated with the use of nanomedicine formulations in cancer immunotherapy for personalizing and improving results [17-19].

This account describes three main types of cancer nano-immunotherapy, which are further sub-grouped according to their targets: cancer cells, tumor immune microenvironment, and peripheral immune system. Only a few papers discuss hematological malignancies (primarily via targeting the peripheral immune system) among these three immunomodulatory approaches. A growing number of nanomedicines are exploring these three strategies. Nano-immunotherapy needs to be improved in order to take full advantage of nanomedicine's immunomodulatory potential in clinical immuno-oncology. Hence, we propose protocols that may help to individualize and improve nano-immunotherapy by integrating lessons learned from both nanomedicine and immune-oncology (such as the use of biomarkers for stratification). To ensure effective cancer nano-immunotherapy, nano-immuno-biomarkers should be considered systematically and systematically [3, 20]. It is necessary to assess immune biomarkers in order to thoroughly characterize the immune status of individual patients (and, in particular, their tumors and metastases). Nano-immunotherapy combination regimens for individual patients will be designed based on this information.

Nanomedicines for Cancer Immunotherapy

Typically, cancer nanomedicine aims to improve chemotherapeutic drug delivery to tumors and metastases, in order to kill cancer cells directly. Recent years have seen an increase in the use of nanomedicines to enhance immune response against cancer as well as to synergize with clinically established immunotherapies [21]. There are three main directions in which nanomedicines are being explored, including targeting cancer cells, targeting tumor immune microenvironments, and targeting peripheral immune systems.

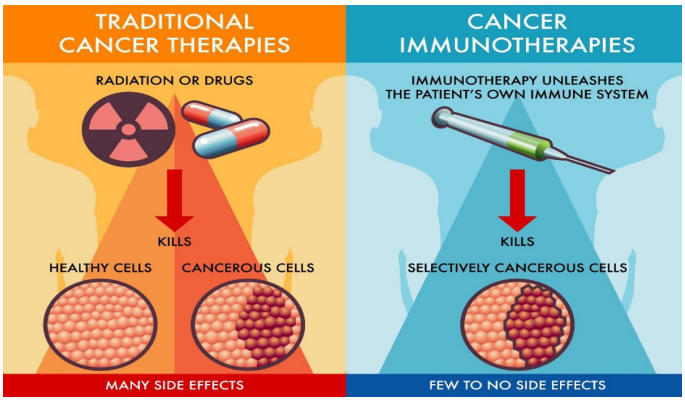

Figure 1: Traditional cancer therapies and cancer immunotherapies [6].

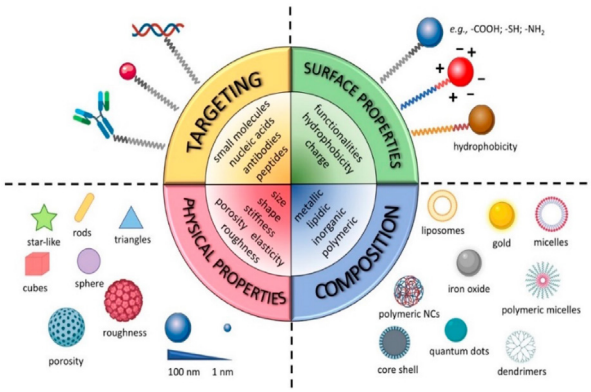

Figure 2: Physical and chemical properties of nanocarriers.

Developing immunotherapies that target the tumor immune microenvironment (TIME)

The use of nanomedicines that modulate TIME is another important strategy for promoting the efficacy of anticancer immunotherapy. As exemplified, immunosuppressive pathways and mediators are frequently upregulated in TIME. It is evident that tumors are being infiltrated by immunosuppressive cells, such as tumor-associated macrophages (TAM) and myeloid-derived suppressor cells (MDSC), as well as soluble inhibitors, such as indoleamine 2,3-dioxygenase (IDO) and transforming growth factor beta (TGF-β). To overcome local tumor immunosuppression mediated by TAM, MDSC and/or soluble inhibitors, nanomedicines modulating the TIME accumulate in tumors via passive/active targeting mechanisms [22]. Inhibiting immunosuppression in the TIME increases infiltration, proliferation, maturation, survival, and/or activity of effector immune cells, such as cytotoxic T cells, thereby improving immunotherapy outcomes [23].

Tumors contain a large population of TAMs. In malignant lesions, TAM is polarized by cells with an M2-like phenotype, which is "pro-tumor", hindering the infiltration of effector T cells, and preventing effective anti-PD-1 treatment. A superparamagnetic iron oxide nanoparticle formulation FDA-approved for the treatment of iron deficiency anemia, ferumoxytol, was shown to convert M2-like TAM into M1-like TAM by Daldrup-Link and colleagues. Ferumoxytol inhibits primary and metastatic liver and lung tumor growth by altering macrophage polarization after systemic injection [24]. Similarly, cyclodextrin nanoparticles efficiently targeted a small molecule toll-like receptor 7/8 agonist to macrophages in the TIME, thereby improving the efficacy of checkpoint-inhibitory immunotherapy. As an *in situ* forming hydrogel during tumor surgery, calcium carbonate nanoparticles functionalized with anti-CD47 antibodies, which reacted with protons in the TIME, causing the pH to increase and macrophages to polarize toward an M1 phenotype upon local application. To block tumor cells' "don't eat me" signals, anti-CD47 antibodies were incorporated. In combination, these two effects enhanced macrophage endocytosis of tumor cells and improved the outcome of checkpoint blockade therapy. Leukocytes in tumors are primarily macrophages and neutrophils. Using plant virus nanoparticles, enhanced tumor infiltration of CD11b+Ly6G+ activated neutrophils, while reducing CD11b-Ly6G+ quiescent neutrophils, resulting in efficient immunotherapy for metastatic cancer [25, 26].

Additionally, nanomedicines can target immunosuppressive molecules in TIME, such as IDO and TGF-β. Kynurenine, a potent metabolite that suppresses T cells, is generated by IDO by converting tryptophan into kynurenine. (Pre-)clinical trials have extensively investigated small molecule IDO inhibitors [27]. Nanomedicine formulations are increasingly incorporating them, aiming to modulate TIME to improve immunotherapeutic outcomes. Photodynamic therapy (PDT), as well as radiotherapy, are synergistic with nanomedicines loaded with IDO inhibitors. In a mouse model of pancreatic ductal adenocarcinoma, An IDO inhibitor with the ICD inducer oxaliplatin [3, 28]. A small molecule IDO inhibitor was combined with a peptide blocking PD-L1 in peptide-based nanoparticles that allowed release of payloads in mild acidic environments. Combining nanomedicines inhibited melanoma growth in mice by activating anticancer immunity. TGF-β reduces immunotherapy's success by inhibiting tumor checkpoints. In line with this notion, enclosed a TGF-β inhibitor in liposomes targeting T cells. A nanomedicine-mediated TGF-inhibition in mice bearing B16F10 melanoma significantly activated T cells both *in vitro* and *ex vivo*, and controlled tumors and metastases [29]. Co-loaded a TGF-β inhibitor and interleukin-2 in nanoparticles and were able to alleviate immunosuppression and augment T cell proliferation. A TGF-β siRNA-containing nano-formulation, which achieved ~50% knockdown of TGF-β expression in tumors and which synergized with cancer vaccination, as exemplified by significantly more cytotoxic T cell infiltration and significantly enhanced tumor growth inhibition [30-34].

Nanomedicines that modulate immune cells, including macrophages and cytotoxic T cells, can be applied to TIME to directly enhance their function. By modifying polystyrene nanoparticles with anti-HER2 antibodies and calreticulin, a multivalent bi-specific nano-bioconjugate engage (mBiNE). Calreticulin served as an "eat me" signal to trigger macrophage endocytosis of HER2-overexpressing tumor cells when combined with mBiNE. In mice bearing HER2high EO771/E2 tumors, mBiNE significantly increased macrophage and T cell infiltration, cytokine production, and therapeutic efficacy after intertumoral injection. IL-2 and a 4-1BB ligand were used in liposomes to activate effector T cells in the TIME [35-37]. As a result of the double-drug-loaded liposomes, more effector T cells infiltrated the tumor, which led to cytokine production and granzyme expression, which improved antitumor immunotherapy. Endosome-disrupting polymerases deliver an intracellular STING agonist that does not cross the cell membrane in its native form. In addition to improving anticancer immunity, these polymerases enhanced checkpoint blockade therapy's efficiency [38].

TIME can be modulated by nanomedicines in two different ways to potentiate anticancer immunity, e.g., by reducing immunosuppression and by promoting immunoactivation. Synergy between these two strategies and clinically established immunotherapeutics can be achieved, for example through immune checkpoint inhibitor antibodies [39]. A promising future avenue for boosting local and systemic antitumor immunity could be targeting immune cells with nanomedicines (rather than directly targeting cancer cells), since nanomaterials typically interact strongly with immune cells in the tumor microenvironment, especially TAMs.

Using the peripheral immune system as a target

Increasing attention is being paid to nanomedicines targeting immune compartments located outside of tumors (referred to as peripheral immune systems) [40]. As antigen is presented in lymph nodes (LNs) and spleens, the peripheral immune system plays an essential role in cancer immunity. It is common for the peripheral immune system to be impaired during the onset and progression of cancer. Through potentiating antigen presentation and engineering T cells, immunomodulatory nanomedicines can restore the peripheral immune system's functions [41].

APCs are able to take and process nanoparticulate antigens more efficiently than small molecules and soluble vaccines when nanomedicines are injected intradermally or subcutaneously [42]. The nano-vaccines produced an anticancer immune response upon local injection and were efficiently drained into the LNs [43]. The toll-like receptor 7/8 agonist imidazoquinolinone was chemically entrapped in pH-sensitive nanogels before injection into LNs. Through modifying CpG and antigens with lipid moieties which bind to endogenous albumin, LNs can also be targeted after systemic injection [44]. In addition to improving vaccination and immunotherapy efficiency, nano-vaccines generally enhance adjuvant tolerability in vaccination approaches.

As a result of nanomedicines, antitumor vaccination can also be induced without the use of conventional adjuvants. PLGA nanoparticles, for instance, were used by Wang and colleagues [40] to improve antigen

presentation by DCs, which then drained to LNs to deliver tumor antigens to DCs. A bilateral tumor model was used to elicit strong immunity in both tumors, which synergized with anti-PD-1 antibodies after one tumor was irradiated to release antigens and injected with nanoparticles. In LNs, Gao and colleagues delivered antigens using nanoparticles based on pH-sensitive PEG-b-polymethacrylate polymers [45-47]. As a result of activating the STING pathway, antigen-loaded nanoparticles induced a stronger vaccination than free antigens combined with conventional adjuvants (e.g., polyinosinic:polycytidylic acid (pol(I:C)). Antigen-encoding mRNAs were formulated as part of a lipoplex that targeted APCs in the spleen when intravenously injected. Multiple clinical trials are currently investigating this nano-vaccine, which achieved strong immunity and generated *de novo* cytotoxic T cells.

It has also been demonstrated that nanomedicines can replace APCs by directly generating cytotoxic T cells instead of triggering them to present antigens to naive T cells. For synthetic APCs to form an immune synaptic connection with naive T cells, nanomaterials with sufficient flexibility and multivalency are crucial [48]. Synthetic APCs from poly(isocyanopeptide) modified with 3-5 anti-CD3 antibodies per 150-200 nm polymer chain induced by the expression of CD69, an early T cell activation marker, and IFN-γ. APCs were synthesized using clusters of iron nanoparticles coated with leukocyte membranes containing peptides loaded with major histocompatibility complex I and anti-CD28 antibodies. In tumor-bearing mice, these synthetic APCs effectively stimulated and activated cytotoxic T cells, and they inhibited tumor growth when administered with T cells [49-53].

Immunomodulatory nanomedicines can target circulating T cells. According to Irvine and colleagues [41], liposomes loaded with IL-15 super agonist and IL-21 improved T cell persistence and homing to the LNs and spleen. Tang et al. [24] created nanogels that were crosslinked via disulfide bonds and triggered IL-15 superagonist release when T cell receptor signaling was activated. Stephan et al.'s [23] chimeric antigen receptor (CAR) nano-formulation strengthens T cells in addition to strengthening T cells [54]. By injecting CAR T cells intravenously into leukemia-bearing mice, some of the laborious procedures for manufacturing CAR T cells can be bypassed.

To enhance the viability and activity of T cells, nanomedicines target the peripheral immune system to potentiate antigen presentation and cytotoxic T cell production. In addition, T cells can be engineered to recognize and kill cancer cells. A lipoplex formulation containing antigen-encoding mRNAs is already undergoing clinical trials, both as monotherapy and in combination with established immunotherapies [3, 55]. It is clear from the progress made to date that combining nanomedicine with immunotherapy can substantially improve the outcome of cancer immunotherapy in patients, both for nanomedicines targeting the peripheral immune system, as well as for nanomedicines targeting cancer cells and the tumor immune microenvironment.

Identifying and targeting cancer cells

It is possible to induce immunogenic cell death (ICD) using nanomedicines. An important trigger and enhancer of anticancer immunity, ICD is a specific type of cell death that releases tumor antigens and danger-associated molecular patterns. Radiotherapy, PDT/photothermal therapy, and other physical stimuli can induce ICD, along with certain types of chemotherapeutics (e.g., doxorubicin, oxaliplatin, cyclophosphamide). Calreticulin (CRT) and adenosine triphosphate (ATP) are released into the extracellular environment during ICD, two classic features. Tumor antigens are taken up and processed by APCs, resulting in the generation of cytotoxic T cells, which migrate to tumors and eradicate them [56]. ICD-inducing nanomedicines, such as doxorubicin-loaded liposomes, are increasingly used along with immune checkpoint blockade therapies. ICD-inducing nanomedicines, such as doxorubicin-loaded liposomes, are useful for improving the efficacy of immune checkpoint blocking therapies. Rios-Doria and colleagues published an exemplary study in which Doxil was combined with several clinically relevant immunotherapies, including anti-PD-1, anti-PD-L1 and anti-CTLA4 antibodies as well as tumor necrosis factor receptor agonists. A significant difference was shown between immunocompetent and immunodeficient mice when Doxil was used for immunopotentiation. In addition, Doxil significantly improved the efficacy of immunotherapy by promoting CD8+ T cell proliferation (via ICD). The immunopotentiation produced by Doxil was significantly stronger than that produced by free doxorubicin at the same dose. In a similar study, oxaliplatin-loaded PLGA nanoparticles induced ICD more effectively and activated the immune system more potently than free oxaliplatin. Chemotherapeutic drugs are more efficiently targeted to cancer cells via nanomedicine formulations, and also lymphotoxicity is avoided, which is potently induced by free chemotherapeutic drugs [57-59].

Aside from improving the delivery of standard chemotherapeutics, nanomedicines have also been used to improve PDT and radiotherapy agents' immunotherapeutic potential. PDT was to immunoactivate inorganic nanoparticles loaded with pyrolipid. Tumor necrosis factor receptor alpha, interleukin 6 and interferon gamma levels were increased following nano-PDT treatment. Additionally, CD4+ and CD8+ T cell infiltration into tumors was significantly improved [60]. As a result of the efficient induction of anticancer immunity in the nano-PDT-treated primary tumor, the abscopal effect was further enhanced, enhancing the efficacy of anti-PD-1 therapy in distant lesions. Nanoparticle-based PDT has already been combined with nanoparticle-based ICD induction, as shown by nano formulations of oxaliplatin and pyrolipid, and of doxorubicin and chlorine e6. A nice example of nano-radio-immunotherapy [61-63]. In orthotopic gliomas in rats, the authors used lipid nanoparticles loaded with rhenium-188 for internal radiotherapy, with the added benefit of retaining radioisotopes in tumors after local administration. In addition to increasing cytokines and immune cells infiltrating tumors, radiotherapy nanoparticles increased levels of circulating cytokines.

ICD is typically induced by immunomodulatory nanomedicines to potentiate the cancer-immunity cycle by targeting cancer cells. Nanomedicines also improve antitumor immunity by reducing systemic (lymphocyte) toxicity, which also enhances immunotherapy results. To induce systemic immunity, nanomedicines need not be administered systemically: via the abscopal effect, locally injected or locally activated nanoparticles can induce systemic immunity. It is also possible to potentiate immunotherapy by other (nano-)chemotherapy effects other than inducing ICD. For example, paclitaxel enhances DC maturation and function, and doxorubicin decreases myeloid-derived suppressor cells in tumors. In the next few years, a significant number of nanomedicine formulations (especially those that induce ICD) will be tested in combination with established immunotherapeutics (Table 1) [64].

Nano-immunotherapy: achieving individualized and improved results

An increasing number of pre-clinical studies are demonstrating the effectiveness of nanomedicine and immunotherapy. The important thing is to explore only rationally designed nano-immunotherapy combinations; formulating anti-PD-(L)1 antibodies in nanomedicines,

Table 1: Genomic, transcriptomic, and proteomic datasets for cancer research that are publicly available.

Project name	Sample source	Phenotype	Data types	Sample size
The Cancer Genome Atlas (TCGA)	Tissue derived	Adult tumor and adjacent normal	WES, RNA-seq, other	>10 000
Clinical Proteomic Tumor Analysis Consortium (CPTAC)	Tissue derived	Adult tumor and adjacent normal	MS proteomics, WGS, WES, RNA-seq, other	~3000 (ongoing)
Cancer Cell Line Encyclopedia (CCLE)	Cell lines	Adult and pediatric tumor	WGS, WES, RNA-seq, other	~1000
International Cancer Genome Consortium (ICGC)	Tissue derived	Adult tumor	WGS, WES, RNA-seq, other	>24 000 donors (86 cancer projects)
Therapeutically Applicable Research to Generate Effective Treatments (TARGET)	Tissue derived	Pediatric tumor	WGS, WES, RNA-seq, other	~1700
St. Jude Pediatric Cancer Genome Project (PCGP)	Tissue derived	Pediatric tumor	WGS, WES, RNA-seq	~2000
Human Tumor Atlas Network (HTAN)	Tissue derived	Adult and pediatric tumor	WES, bulk and single-cell RNA-seq, other	Ongoing
Genotype-Tissue Expression (GTEx)	Tissue derived and cell lines	Adult normal	WGS, RNA-seq, other	>17 000 (>900 individuals)

for example, will probably not add much value. The clinical translation of both anticancer nanomedicines and immunotherapeutics will be crucial to ensuring progress in nano-immunotherapy [65-68]. There is growing evidence that molecularly targeted small molecule anticancer drugs, as well as nanotherapeutics and immunotherapeutics, only work well in certain subpopulations of cancer patients. This has resulted in the need for strategies for stratifying patients in clinical trials and in practice. Nano-immunotherapy can be implemented rapidly and efficiently with such strategies.

Antitumor vaccination can also be induced using nanomedicines without using conventional adjuvants. PLGA nanoparticles, for example, have been used to enhance antigen presentation by DCs through the adsorption of tumor antigens and delivery of the antigens to LNs. The nanoparticles were injected into one tumor and irradiated to release antigens, resulting in strong immunity in both tumors, which synergized with anti-PD-1 antibodies. APCs in LNs were delivered antigens using nanoparticles based on pH-sensitive PEG-b-polymethacrylate polymers [69,70]. Nanoparticles containing antigens induced stronger vaccination than free antigens combined with conventional adjuvants (e.g., poly(I:C)), possibly by activating the STING pathway. Through intravenous injection of a lipoplex containing antigen-encoding mRNAs, Sahin and colleagues targeted APCs in the spleen. Multiple clinical trials are currently being conducted with this nano-vaccine, which has demonstrated strong immunization and *de novo* cytotoxic T cell generation [71].

It has also been found that nanomedicines can replace APCs by directly generating cytotoxic T cells rather than stimulating their presentation to naive T cells [72]. An immune synapse needs to be formed between naive T cells and such synthetic APCs require nanomaterials that are flexible and multivalent. In an experiment, induced the expression of the early T cell activation marker CD69 and the production of IFN- by modifying poly (isocyano peptide) with 3-5 anti-CD3 antibodies per 150 - 200 nm of polymer chain in synthetic APCs. Based on iron nanoparticle clusters coated with leukocyte membranes bearing peptide-loaded major histocompatibility complex I and anti-CD28 antibodies as co-stimulatory ligands, synthesized APCs [73, 74]. In tumor-bearing mice, these synthetic APCs stimulated and activated cytotoxic T cells, and they inhibited tumor growth effectively.

Using nanomedicines that modulate immune function, circulating T cells can be targeted. To improve T cell persistence and homing to LNs and spleen, liposomes loaded with the cytokines IL-15 super agonist and IL-21. Using disulfide bonds, cytokine-based nanogels were crosslinked via disulfide bonds to release IL-15 super agonist in response to T cell receptor signaling. In addition to strengthening T cells, Stephan et al developed a nano formulation for transfusing CAR genes [3, 75, 76]. By injecting systemically generated CAR T cells into leukemia-bearing mice, some of the laborious procedures involved in CAR T cell manufacture can be bypassed.

In addition to potentiating antigen presentation and cytotoxic T cell generation, nanomedicines enhance T cell viability and activity. T cells can also be engineered to recognize and kill cancer cells [77]. In monotherapy settings as well as in conjunction with established immunotherapeutics, one of these formulations, i.e., antigen-encoding mRNAs within a lipoplex, has already entered clinical trials. As a result of the progress made so far for nanomedicines targeting the peripheral immune system, as well as nanomedicines targeting cancer cells and the TIME, it is clear that combining nanomedicine with immunotherapy can significantly improve the outcome of cancer immunotherapy [78].

A good therapeutic outcome requires (pre-)selecting the right patients in immunotherapy. PD-L1 expression in tumors, tumor mutational burden, and microsatellite instability have already been studied in this way. However, these biomarkers alone are only moderately effective at predicting patient response to immunotherapy. Immunoscores and cancer immunograms have been developed to improve the predictability of immune biomarkers [79]. Using cancer immunograms, researchers can identify biomarkers to guide and improve immunotherapy interventions aimed at interacting between cancer and the immune system in individual patients. Using Immunoscore, we are able to predict the clinical outcome of colorectal cancer based on the location, type, density, and function of immune cells. This systematic histopathological analysis of the immune contexture in human tumors has gradually led to the establishment of the Immunoscore for the classification of malignant tumors, a tool that not only predicts standard survival outcomes, but also helps to achieve a better response to cancer immunotherapy [80].

Combination immunotherapy is guided by the Immunoscore, which was recently updated. T cells are present in tumors in four different phenotypes: absent, altered-excluded, altered-immunosuppressed, and optimal. Combination immunotherapy should be considered for the first three phenotypes. Likewise, different strategies for nanomedicine-based enhancement of cancer immunotherapy should be developed in each of these situations.

Several clinical situations can be envisioned to illustrate biomarker-guided nano-immunotherapy. Using ICD-inducing nanomedicines, for example, can be used to kill tumor cells and trigger the cancer-immune cycle if a patient presents with an immunosuppressed tumor phenotype. Anti-PD-1/L1 immunotherapy could be well combined with this type of nano-therapy. When tumors have high

levels of cytotoxic T cells infiltrating, but also a high level of M2-like macrophages (which suppress anti-tumor immunity and anti-PD-1/L1), nano-immunotherapy may involve macrophage-modulating nanomedicines, such as ferumoxytol, as well as nanoparticles loaded with agonists of toll-like receptors. Third, we can consider a patient with altered-immunosuppressed tumors that have a low mutational burden and a low level of preexisting antitumor immunity [81, 82]. Since ICD cannot overcome issues relating to low numbers of clonal mutations in this situation, nanomedicines that induce ICD may not be very useful. Nano-vaccines containing neoepitopes, on the other hand, may be a good and rational choice, since they can trigger the production and activity of cytotoxic T cells.

It is necessary to reduce T cell exclusion in patients with altered-excluded tumor phenotypes. This can be achieved, for example, by downregulating TGF-49, resulting in less fibrotic tumors and allowing T cells to penetrate tumors more efficiently. Nanomedicines that actively promote extracellular matrix degradation can also be considered, such as tumor-targeted collagenase or angiotensin receptor blockers that inhibit collagen I synthesis. Nanomedicines loaded with siRNA targeting vascular endothelium growth factor may be useful for tumors with poor T cell infiltration due to excessive angiogenesis [83, 84]. The nano-co-treatments may improve T cell infiltration through processes such as vascular normalization and enhanced perfusion, thereby enhancing the effectiveness of immunotherapeutics such as checkpoint inhibitors.

A relatively small number of preclinical reports have explored biomarker-guided nano-immunotherapy. The immuno-biomarkers associated with 4T1 mouse breast cancer and B16F10 mouse melanoma were analyzed in two studies. These two models were characterized by high expression levels of the immunosuppressive molecules IDO and TGF-β, respectively. When tumor-bearing mice were treated with nanomedicine formulations loaded with an IDO inhibitor and with siRNA downregulating TGF-β, respectively, the outcome of anti-PD-1 and nano-vaccine-based cancer immunotherapy could be strongly enhanced. These examples illustrate that initially identifying (via biomarkers) and subsequently inhibiting/targeting (using nanomedicines) specific immunosuppressive pathways which are overly active in specific tumor models - and ultimately in individual patients - could be extremely beneficial for individualizing and improving nano-immunotherapy combination regimens [85].

In combination, nanomedicines can be used to improve anti-cancer immunotherapy efficacy through a variety of systems and strategies. A number of significant advances have been made at the preclinical level in recent years, and promising early clinical proofs of concept have been demonstrated. In order to ensure optimal therapeutic outcomes in nano-immunotherapy, biomarkers must be developed to identify which immunosuppressive or immunoactive cells or pathways must be targeted [86, 87]. The insights will guide the design and development of immuno-modulatory nanomedicines, facilitate the clinical translation of nanomedicines in general, and - most importantly - contribute to the development of better cancer treatments.

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None.

Conflict of Interest

None.

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