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Advancements in immunotherapy: a nanoparticle perspective

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Abstract

Immunotherapy is a cancer treatment option that works by helping the immune system fight cancer cells. The different immunotherapy treatments include checkpoint inhibitors, cytokine therapy, adoptive T cell therapy, antibody-based therapies, cancer vaccines and oncolytic viruses. In order for these immunotherapies to provide more effective results, nanoparticles are being developed to help improve drug targetability, delivery, and immune cell activation. The use of nanoparticles in immunotherapy reduces the limitations of immunotherapy, by creating tumor specific drug delivery systems to minimise side effects, increase the effectiveness of adoptive T cell therapy and CAR T-cell therapy, trigger trained immunity, allow drugs to be delivered deep into the tumor, and reduce the influence of the immunosuppressive tumour microenvironment. Formulation of immunotherapeutic drugs, ligands and/or antibodies in nanoparticles could further advance immunotherapy by increasing its effectiveness and targetability. It could function as a pan-tumoricidal therapy with the additional ability to ablate metastasized tumors. Nanoparticle associated immunotherapy could significanlty reduce off-target and side effects.

Keywords

Nanotechnology, Immunotherapy, Immuno-oncology, Cancer, Tumor, Nanoparticles, Carcinogenesis, Checkpoint inhibitors, Oncolytic virotherapy, CAR T cell therapy

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Introduction

Cancer is the second leading cause of death worldwide, with an estimated 19.3 million new cases yearly and almost 10 million cancer related deaths (1). It is the uncontrollable division of cells in the body that eventually spreads to other parts of the body. Cancer, or neoplasm may be caused by an accumulation of detrimental mutations in the genome. Single mutations are usually not sufficient to cause a cell to become cancerous. The root cause of these mutations is multifactorial, being dependent on a myriad of factors that involve genetics, exposure to carcinogens and environment conditions, among others (2). The treatment options for cancer are constantly developing and generally used in conjunction, such as with surgery to remove tumors, chemotherapy, radiation therapy, immunotherapy, hormone therapy etc (3). This literature review focuses on recent and emerging research on the implications of using nanoparticles in immunotherapy.

Cancer immunotherapy is a treatment option that helps the immune system fight cancer. Recently the use of immunotherapy to treat cancer patients has become widespread, since it not only treats primary tumors, but may also prevent metastasis and recurrence (4). However immunotherapy does have some limitations that limit its efficacy and safety. Nanoparticles present one avenue that may increase the efficacy and safety of immunotherapy and of immuno-oncologic drugs.

What prevents the immune system from recognizing tumor cells?

The immune system and cancer interact intimately, the type of interaction determines whether tumor growth and metastasis is inhibited or enhanced (5). Some cancers can express antigens that can be detected by immune cells; these include viral antigens if an oncogenic virus is the cancer catalyst, mutated host genes and over-expressed or abnormally expressed genes (6). Oncogenic viruses such as human papillomaviruses (HPV), epstein–barr virus (EBV) and human herpesvirus 8 are suspected carcinogens (7). The immune system can destroy cells presenting tumor antigens, however as cancers are genetically unstable and constantly mutating, they can suppress or confound biological and chemical signals that generate an immune response (5). Tumors can also down-regulate the histocompatibility complex molecules that are used by T-cells as recognition epitopes. In the immune system, CD8+ cytotoxic T cells and CD4+ helper T (Th)1 cells decrease cancer development through processes that involve production of interferon (IFN)-γ and cytotoxins. However, other factors such as chronic inflammation may override the effects of cytotoxins and IFN-γ, and in turn promote cancer development. For example, the risk of hepatocellular carcinoma, the most common type of liver cancer, has been shown to be correlated to the duration of inflammation caused by the hepatitis B and C viruses. Chronic inflammation also plays a critical role in the growth of colon and pancreatic cancers (5).

Tumors create an immunosuppressive tumor microenvironment (TME) which is populated with immunosuppressive cells such as regulatory T cells, myeloid-derived suppressor cells, tumor associated macrophages and associated with an increased expression of immunosuppressive

molecules such as programmed death – ligand 1 (PD-ligand 1) (8). A number of studies have shown that regulatory T cells that are derived from tumors have a significantly greater immunosuppressive activity than naturally occurring regulatory T cells (5). Regulatory T cells are a subpopulation of T cells that suppress immune response, and therefore are abundent in the TME, preventing the destruction of cancer cells by the immune system. Furthermore, the increased expression of molecules that act as immune system checkpoints, such as PD-1 and PD-L1, provide a favorable environment for the cancer to continue growing in its manufactured microenvironment (9).

Immunotherapy treatments:

There are different types of immunotherapeutic treatments available to cancer patients: checkpoint inhibitors, adoptive T cell therapy (ATC), chimeric antigen receptor (CAR T-cell) therapy, and oncolytic virotherapy. Checkpoint inhibitors work by inhibiting checkpoints on the immune system such as PD1, PDL1 and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) pathways that are usually activated by the cancer, to allow for the immune system to mount a response. Several checkpoint inhibitors are approved by the FDA; these include Atezolizumab, Avelumab, Cemiplimab and others that target the PD1, PDL1 or the CTLA-4 pathway. However, these immunotherapeutic drugs inhibit immune checkpoints nonspecifically throughout the body and can have side-effects that manifest as autoimmune diseases or aberrant cytokine expression (10).

Cytokine therapy represents another immunotherapy option that activates the body's immune system to fight cancer cells (11). Cytokines are molecular messengers of the immune system that enable cells of the immune system to communicate, and therefore they play an integral role in immune responses and inflammation. Cytokines and interleukins can be used to stimulate immune cells such as T cells and natural killer cells (12). Due to the central role cytokines play in modulating the activity of immune cells, cytokine therapy has the potential to cause off-target effects. Cytokine modulating therapeutic drugs have been approved that stimulate immune cells for patients with neuroblastoma, lymphoma, leukaemia, sarcoma and melanoma (11).

In adoptive T cell therapy, tumor-specific cytotoxic T cells are infused in the patient for recognising and killing tumor cells in the body. In this type of immunotherapy, T cells are harvested from the patient’s blood or tumor, are genetically engineered and then stimulated to expand in a lab so that when they are sufficiently expanded, they can be infused back into the patient's body in vastly increased numbers; of the order of a 100 billion cells (13). Some types of cancer such as advanced melanoma have been treated by ATC. A similar therapy is CAR T-cell therapy, where T cells are modified ex vivo and equipped with chimeric antigen receptors (CARs) that target cancer associated antigens. Natural killer cells and tumor infiltrating lymphocytes (TILs) can also be expanded ex vivo and infused into patients for similar effects (14).

Another type of immunotherapy is oncolytic virotherapy, which uses oncolytic viruses—

viruses that specifically attack cancer cells — to help patients fight cancer. Oncolytic viruses can kill cancer cells by virus-mediated cytotoxicity and a variety of other cytotoxic immune mechanisms. These viruses directly attack cancer cells, causing them to lyse and release cancer associated antigens in the body. These antigens are identified by the immune system, in turn leading to T cell activation. Some viruses, such as the reovirus, mumps virus, and moloney leukaemia virus, have a natural preference for cancer cells, whereas others such as measles, and herpes simplex virus can be engineered to make them cancer-specific. This approach is actively being explored and several clinical trials are underway (15).

Cancer vaccines represent another type of immunotherapy that can be used for prevention as well as for treatment. Viruses such as HPV can cause cervical cancer in patients. Therefore as a preventative measure, the HPV vaccine has been developed to prevent infection and/or subsequent carcinogenesis (16). Another preventative vaccine is the anti-hepatitis B virus (HBV) vaccine to prevent HBV-associated hepatocellular carcinoma. Bacillus calmette-guérin (BCG) and sipuleucel-T (Provenge®) are approved cancer vaccines. The BCG vaccine uses weakened bacteria to stimulate the immune system for treating early-stage bladder cancer. The sipuleucel-T vaccine is composed of the patient's own dendritic cells that are altered in the lab, to treat prostate cancer (15).

Antibody-based immunotherapies are also being investigated. In monoclonal antibody treatment, laboratory-produced molecules act as antibodies specific to the cancer. Monoclonal antibodies can help flag cancer cells for other immune cells to locate. They can kill cancer cells by perturbing the cell membrane. They can also prevent cell growth, angiogenesis, block immune system inhibitors or cause the cell to self-destruct (17). A type of monoclonal antibody is the antibody-drug conjugate, a targeted antibody altered to carry anti-cancer drugs, so that when it binds to the cancer cells, it delivers the drug at the tumor site. Such an approach of delivering chemotherapeutic drugs directly to tumors can reduce the side effects that are usually associated with chemotherapy (18). Yet another type of antibody is the bispecific T cell engager, which are prepared by combining two different antibodies so as to bind to two different targets. This helps T cells bind to the cancer cells and destroy them (19).

The Use of Nanotechnology in Immunotherapy:

The size of the nanoparticle and its surface properties play integral roles in their biodistribution and half-life. Nanoparticles that are larger than 7 nm in hydrodynamic diameter evade renal filtration and urinary excretion. Particles larger than 100 nm are rapidly eliminated from circulation by phagocytes of the reticulo-endothelial system (20). The surface properties of nanoparticles can be modified by the addition of polyethylene glycol or other polymers, resulting in prolonging the circulation time by inhibiting phagocytic uptake (21). This increase in circulation half-life can promote the recruitment and accumulation of the nanoparticles in tumors. On the other hand, some nanoparticle surface modifications can also inhibit tumor uptake. In order to overcome this limitation, active tumor

targeting approaches that involve the conjugation of tumor-specific ligands to the nanoparticle surface have been developed. Coating the nanoparticles with ligands for tumor receptors, such as trastuzumab (Herceptin®), folate or transferrin, improves the delivery and uptake of the particle by the tumor (17). However, only less than 5% of all nanoparticles accumulate at the desired site or elicit the desired response (22).

The zeta potential of the nanoparticle influences its biological activity, especially with respect to macrophage ingestion and the enhanced permeation and retention (EPR) effect. Positively charged nanoparticle exhibit a greater uptake by macrophages than those that are negatively charged. Therefore negatively charged nanoparticles are better able to evade macrophage ingestion and remain in circulation for a longer time. Most nanoparticles are eliminated from the bloodstream after administration due to perturbation of their structure by dilution with blood. For example, polymeric micelles may not be able to maintain their structural integrity due to rapid dilution and structural dissociation (17). Amphoteric molecules constituting the nanoparticle should possess a very low critial micellar concentration (CMC) or critial bilayer concentration (CBC) so that dilution of the order of 50 to 100 times with blood does not perturb the structural integrity of the nanoparticle. In this regard, lipid amphoteric molecules have some of the lowest CMCs' of the order of nM, which makes them suitable as nanocarrier composition molecules that maintain structural integrity upon dilution with blood. Discovering or inventing new biocompatible amphiphilic molecules with low CMC designed to be formulated into nanoparticles constitutes an as yet under-researched area that has the potential to significantly improve targetability and efficacy. Non-covalently attached ligands face similar problems of dissociation from the nanoparticle upon dilution (23).

Poly Lactic-co-Glycolic Acid (PLGA) microparticles provide protection to the nanoparticles until delivery to tumor sites. PLGA nanoparticles are FDA approved biodegradable molecules that improve the stability of peptide based drugs by encapsulating them. However, it is unclear if PLGA particles can be loaded with sufficient peptide-major histocompatibility complexes (pMHC), antiCD28 mAb, and IL-2 molecules to trigger substantial T-cell activation in-vivo (17). Nanoparticles are also being developed to aid drugs to be delivered through the blood-brain barrier, to treat brain cancer as well as other CNS disorders. This application represents a significant improvement, considering that current strategies are extremely limited to deliver drugs into the central nervous system through the blood-brain barrier. PLGA microparticles and polymers have been developed to encapsulate therapeutic drugs for brain tumors. These nanoparticles carry immunomodulatory drugs and can aid immunotherapy and chemotherapy (24).

Nanoparticles were engineered using parts of the bacillus calmette–guérin (BCG) vaccine, phospholipids, cholesterol and apolipoprotein A-1 and administered to mice and monkeys. The nanoparticles included peptidoglycan derivatives to enable the induction of trained immunity by activating the nucleotide-binding oligomerization domain-containing protein 2 (NOD2) receptor. The animals'

innate immune cells demonstrated hallmarks of trained immunity such as dividing quickly, rapidly responding to pathogenic threats and an increase in cytokine production (25).

Antigen-capturing nanoparticles (AC-NP) delivered antigen-presenting cells to improve the efficacy of αPD-1 treatment versus the treatment without AC-NP (12). The AC-NPs have been used to improve treatment responses to immunotherapy and induce the abscopal effect by capturing tumor-derived protein antigens that are released during radiotherapy and transporting them to antigen presenting cells, therefore significantly promoting cancer immunity (26). In recent studies, PLGA and maleimide-PEG AC-NPs substantially improved immunotherapy, partly by inducing the abscopal effect (17).

Nanoparticles are being developed to trigger trained immunity in the body. Trained immunity is the ability of the innate immune system to form innate immune memory so that it can elicit a stronger and faster response to a secondary exposure (27). Even though it was long believed that innate immunity lacks memory, new studies show that innate immune cells undergo metabolic and epigenetic rewiring. If done successfully this can stimulate the production of trained myeloid cells that can kill tumor cells even in the immunosuppressive tumor microenvironment (28).

The use of nanoparticles reduced Regulatory T cells and increased CD8+ T cells and myeloid cells in the immunosuppressive tumor microenvironment, in mice with melanoma. The nanoparticles were loaded with drugs that blocked immunosuppressive pathways. Nanoparticles loaded with a phosphoinositide-3-kinase (PI3K) inhibitor allowed the immune cells to overcome the immunosuppressive TME (29). The use of nanoparticles in conjunction with other immunotherapies can substantially slow the growth of cancer. Nanoparticles are used as vehicles for drug delivery to deliver imaging tags or cytotoxic drugs to tumors. These particles carry a myriad of molecules and therefore can be engineered to be soluble in biological fluids. Many of the nanoparticles that deliver drugs to tumors have reached clinical trials (17).

Nanoparticles also extend the circulation time of these drugs and promote their preferential accumulation in tumors, due to increased extravasation near the tumor vicinity via the EPR effect. Upon extravasation, the nanoparticles tend to accumulate because of impaired lymphatic drainage around tumor sites. This nanoparticle size facilitated passive drug delivery system minimises the systemic toxicity of chemotherapeutic drugs as compared to non-nanoparticle administered drugs (30). The efficacy of nanoparticles relies on the EPR effect, even though the EPR effect varies across the different tumor types due to differences in the vascular permeability and anatomy. Therefore, targeting based on EPR is not predictable or consistent especially when the cancer has metastasized (17).

Nanoparticles are also used alongside adoptive T cell therapy to reduce the influence of the immunosuppressive tumor microenvironment. Nanoparticles can be used to carry large quantities of interleukin-15 (IL-15), a super-agonist complex which can attach directly to T cell membranes. This ensures that IL-15 is released only in primary

tumor sites, and in turn also activates the T cells. The T cells that have been altered during the adoptive T cell therapy, have more viability and functioning because of the nanoparticles that attach onto it, increasing their effectiveness (31). The ATC treatment could be paired with nanoparticles that alter the TME , so that the T cells from the ATC treatment can be more effective. In order to improve ex vivo expansion of antigen-specific T cells in ATC, a nano polyethylene glycol (PEG) hydrogel platform was engineered to stimulate the T cells. This platform used gold nanoparticles that were conjugated with anti-CD3 antibodies, and integrin-activating peptides. Through the modification of ATC using nanoparticles and nanotechnology, T cell activation, proliferation, and memory were amplified, making the treatment more effective (32).

Nanoparticles are also being used to enhance CAR T-cell therapy, as a current limitation in CART-cell therapy is the immunosuppressive tumor microenvironment. Checkpoint inhibitors are used to blunt this tumor driven immunosuppression but these drugs can cause severe side effects. Therefore nanoparticles are being developed to deliver a combination of immunomodulatory drugs that can remove pro-tumor cells while simultaneously activating anti-tumor cells. One study administered liposomal nanoparticles carrying a PI3K inhibitor and coated with the tumor-targeting peptide iRGD. This improved the CAR T-cell therapy efficacy by allowing the CAR-T cells to target the solid tumor, and did so without generating systemic toxicity (33).

Therapeutic strategy	Particle engineering
Reduce macrophage uptake	Hydrophilic polymers Modulating nanoparticle shape and zeta potential Cellular hitchinking Increased rigidity by modifying composition
Immune cell targeting	Attaching ligands for T and B lymphocyte engagement
Deep tumor penetration	Cell penetrating amino acid sequences of attachment peptides Chemical modifications

Table1: Nanoparticle engineering to achieve desired therapeutic effects

Nanoparticles are also used alongside antibody-based immunotherapies to improve drug delivery, and to blunt the cytokine storm; a phenomenon that causes elevated levels of circulating cytokines due to immune hyperactivation. Antibody-based immunotherapies often trigger a series of cytokine releases and cause a cytokine storm

that is detrimental to patient health, due to the non-specific drug-delivery. However, there is room for improvement for these nanoparticles such as designing a nanoparticle that can avoid non-specific uptake in the macrophages of the reticuloendothelial system (34). Hydrophilic polymers are used to reduce macrophage uptake and promote longer circulation times. The nanoparticle shape also influences macrophage evasion. For example, mesoporous silica nanoparticles shaped in the form of long rods showed decreased excretion rates when compared to their counterparts that were engineered to be short rods. Another method to evade circulating macrophages is a process called cellular hitchhiking, where nanoparticles act as stowaways on the surface of non-immunogenic cells in order to keep circulating (35).

Another current limitation with immunotherapy is the inability to deliver the drug deep into a tumor. The rapid rate of tumor cell division results in the generation of highly vascularized regions at the periphery of a tumor, surrounding an avascular core (36). In order to address this limitation, peptide and chemical modifications to the nanoparticle surface have been designed to improve the penetration of the nanoparticle into the tumor. For example, polyarginine was used to enhance the permeation of lipid nanoparticles (35). Another limitation in nanoparticle immunotherapy is determining how to modulate nanoparticle biodistribution. To this end, the nanoparticle is commonly attached to a ligand or peptide that facilitates homing to a target (35).

Nanoparticles can also be used in vaccines for targeted delivery of immunomodulatory and immunostimulatory molecules. The nanoparticles can protect the antigen or adjuvant from the biological environment, increase the half-life, limit the systemic toxicity, promote delivery to antigen presenting cells (APC) and trigger the activation of tumor associated antigen specific T-cells (35). Antigens and adjuvants that are encapsulated in nanoparticles, show enhanced B and T cell activation when compared to their non-encapsulated counterparts (17).

Certain nanoparticles have inherent immunostimulatory properties so that antigens delivered by these particles can induce T and B cell responses without exogenously added adjuvants. Such antigen delivering nanoparticles induced potent T-cell and antibody responses against ova-expressing lymphoma and colon adenocarcinoma; thereby delaying tumor growth and increasing the survival time in animal studies. The size of the particle affects the biological activity, as small particles that are equal or less than 40 nm can readily reach lymph nodes and facilitate the uptake by dendritic cells (DC).The endocytosis of these nanoparticles by the DCs triggers the activation of danger-sensing pathways and promotes DC activation and maturation into immunogenic APCs (17).

Nanoparticles are also used to deliver non-specific DC stimuli to tumor-draining lymph nodes (TDLN). Tumor antigens are taken up by APCs and then presented to T-cells, when they drain into TDLN. Recent experiments have used nanocarriers of DC stimulatory molecules to promote the activation of TDLN

DCs (37). When a subcutaneous injection of nanoparticle-bound cytosine-phosphate guanine (CpG) oligonucleotides was administered, they were rapidly taken up by APCs and triggered the release of cytokines that activated effector CD4+ T-helper-type 1 cells. This resulted in a strong antitumor immune response, slowing the growth of the subcutaneous tumor. The size of these nanoparticles plays a role in efficacy as nanoparticles that were greater than 100 nm exhibited lower efficacy. A recent study showed that the delivery of OVA and CpG by 30 nm diameter micellar nanoparticles triggered an immune response that was significcantly greater than that induced by nanoparticles carrying only OVA. This indicates that the role of nanoparticle size varies as a function of the nanoparticle type. This may provide an avenue for further engineering.

Stimulating interferon genes (STING) pathways leads to immune cell activation and antitumor effects, which can be activated by a cytosolic double-stranded DNA. STING agonists show substantial antitumor activity but are unstable and highly polar compounds, which reduce their cellular uptake. Nanoparticles help improve the delivery of these agonists and have been shown to increase survival in murine models. Cyclical drug loaded nanoparticles that mimic double-stranded DNA can also stimulate STING (38). Mn^{2+} ions amplify STING agonist activity and therefore can aid in immunotherapy. Mn^{2+} was coordinated with cyclic dinucleotide STING agonists to create a nanoparticle for delivering amplified STING agonists to immune cells (39).

Modified viral vectors are used to deliver RNA to T-cells ex vivo in order to genetically modify T-cells, such as those for chimeric antigen receptors in CAR-T cell therapy. Advancements in nanotechnology allow nanoparticles to be used instead of these modified viral vectors. Through programming endogenous CD3+ or CD8+ T cells with lipid or polymer nanoparticles that are loaded with nucleic acids, T cells can be silenced and/or engineered to express specific antigen receptors. These nanoparticles can also aid in the manufacturing of CAR T cells. Antigen-presenting nanoparticles for mRNA delivery have been developed using pMHCI molecules to attach to T-cells (40).

Discussion

Immunotherapy has advanced significantly over the past decade, allowing cancer patients to receive better treatment options. However, immunotherapy treatments do not work for all cancers or patients, and sometimes cause severe side-effects. The use of nanotechnology in these treatments could allow for a targeted drug delivery system to minimise side effects, increase the effectiveness of checkpoint inhibition, antibodies and adoptive T Cell Therapy, and trigger adaptive immunity. The use of these approaches has improved therapeutic outcomes of cancer treatment when compared with similar non-particle based therapies. Despite advances in drug delivery using nanoparticles, only around 5% of all nanoparticles accumulate at the desired site or elicit the desired effect. This is currently being addressed by researchers by altering the chemical composition of the nanoparticles in order to increase efficacy and tagetability based on size, surface chemistry,

composition, zeta potential and administration methodology.

Therefore nanoparticle chemical structure, formulation and pharmacokinetics need to be further improved to increase drug accumulation in cancer tissue. If the nanoparticles are designed so as not to perturb vital biological processes, are not toxic to the body, can escape macrophage ingestion, deliver drugs deep into tumor tissue, cause immunoactivation and exhibit favorable biodistribution profiles, the efficacy of immunotherapy can be significantly improved. Nanotechnology has the potential to reduce the limitations of immunotherapy and to propel immunotherapy at the forefront of cancer treatment.

Conclusion

Immunotherapy works by activating the immune system, improving its function of recognizing tumor associated antigens, or by blocking tumor environment driven or generalized immunosuppressive processes. Remission and survival rates have significantly increased since its introduction into the therapeutic armementarium. However, the stream of research is still in its infancy and many questions, specifically with regard to the mechanism of action, remain unanswered. Nanotechnology may provide the impetus and the means to increase the efficacy, targetability, and safety of immunotherapeutic treatments.

Nanoparticles can be used to deliver immunotherapeutic drugs to (as yet inaccesible) tumor sites, improve pre-existing treatments such as adoptive T cell therapy, CAR T cell therapy and checkpoint inhibition, reduce off-target effects, reduce the detrimental influence of the tumor suppressive microenvironment and activate innate and adoptive immunity. Nanoparticle based immunotherapy has shown promising results in mammalian animal models and in early stage clinical trials. A synergy of nanotechnology and immunotherapy may provide significantly improved outcomes for cancer patients.

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References

1. Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: A Cancer Journal for Clinicians, 71(3), 209–249. https://doi.org/10.3322/caac.21660

2. Weinberg, R. A. (1996). How cancer arises. Scientific American, 275(3), 62-70.

3. What Is Cancer? (2017). Cancer.org. https://www.cancer.org/treatment/understanding-your-diagnosis/what-is-cancer.html

4. Immunotherapy for Melanoma. (2021). Cancer Research Institute. https://www.cancerresearch.org/en-us/immunotherapy/cancer-types/melanoma

5. Vinay, D. S., Ryan, E. P., Pawelec, G., Talib, W. H., Stagg, J., Elkord, E., Lichtor, T., Decker, W. K., Whelan, R. L., Kumara, H. M. C. S., Signori, E., Honoki, K., Georgakilas, A. G., Amin, A., Helferich, W. G., Boosani, C. S., Guha, G., Ciriolo, M. R., Chen, S., & Mohammed, S. I. (2015). Immune evasion in cancer: Mechanistic basis and therapeutic strategies. Seminars in Cancer Biology, 35, S185–S198. https://doi.org/10.1016/j.semcancer.2015.03.004

6. MacPherson, G., & Austyn, J. (2012). Exploring immunology: concepts and evidence. John Wiley & Sons.

7. Brouchet, L., Valmary, S., Dahan, M., Didier, A., Galateau-Salle, F., Brousset, P., & Degano, B. (2005). Detection of oncogenic virus genomes and gene products in lung carcinoma. British Journal of Cancer, 92(4), 743–746. https://doi.org/10.1038/sj.bjc.6602409

8. Ohue, Y., & Nishikawa, H. (2019). Regulatory T (Treg) cells in cancer: Can Treg cells be a new therapeutic target? Cancer Science, 110(7), 2080–2089. https://doi.org/10.1111/cas.14069

9. Takeuchi, Y., & Nishikawa, H. (2016). Roles of regulatory T cells in cancer immunity. International immunology, 28(8), 401–409. https://doi.org/10.1093/intimm/dxw025

10. Esfahani, K., Roudaia, L., Buhlaiga, N., Del Rincon, S. V., Papneja, N., & Miller, W. H. (2020). A Review of Cancer Immunotherapy: From the Past, to the Present, to the Future. Current Oncology, 27(12), 87–97. https://doi.org/10.3747/co.27.5223

11. Conlon, K. C., Miljkovic, M. D., & Waldmann, T. A. (2019). Cytokines in the Treatment of Cancer. Journal of Interferon & Cytokine Research, 39(1), 6–21. https://doi.org/10.1089/jir.2018.0019

12. Cytokines as Therapy | Center for Cancer Research. (2015). Cancer.gov. https://ccr.cancer.gov/news/landmarks/article/cytokines-as-therapy

13. Perica, K., Varela, J. C., Oelke, M., & Schneck, J. (2015). Adoptive T Cell Immunotherapy For Cancer. Rambam Maimonides Medical Journal, 6(1), e0004. https://doi.org/10.5041/rmmj.10179

14. Dai, H., Wang, Y., Lu, X., & Han, W. (2016). Chimeric Antigen Receptors Modified T-Cells for Cancer Therapy. Journal of the National Cancer Institute, 108(7), djv439. https://doi.org/10.1093/jnci/djv439

15. Russell, S. J., Peng, K.-W., & Bell, J. C. (2012). Oncolytic virotherapy. *Nature Biotechnology*, *30*(7), 658–670. https://doi.org/10.1038/nbt.2287

16. Skeate, J. G., Woodham, A. W., Einstein, M. H., Da Silva, D. M., & Kast, W. M. (2016). Current therapeutic vaccination and immunotherapy strategies for HPV-related diseases. Human vaccines & immunotherapeutics, 12(6), 1418–1429. https://doi.org/10.1080/21645515.2015.1136039

17. Kun Shao, Santiswarup Singha, Xavier Clemente-Casares, Sue Tsai, Yang Yang, and Pere Santamaria. (2015). ACS Publications. https://doi.org/10.1021/nn5062029

18. Schirrmacher, V. (2020). Cancer Vaccines and Oncolytic Viruses Exert Profoundly Lower Side Effects in Cancer Patients than Other Systemic Therapies: A Comparative Analysis. Biomedicines, 8(3), 61. https://doi.org/10.3390/biomedicines8030061

19. *Targeted Antibodies: mAbs, ADCs, BiTEs, and More*. (2014). Cancer Research Institute. https://www.cancerresearch.org/en-us/immunotherapy/treatment-types/targeted-antibodies

20. Davis, M. E., Chen, Z., & Shin, D. M. (2008). Nanoparticle therapeutics: an emerging treatment modality for cancer. Nature Reviews Drug Discovery, 7(9), 771–782. https://doi.org/10.1038/nrd2614

21. Gabizon, A., Catane, R., Uziely, B., Kaufman, B., Safra, T., Cohen, R., Martin, F., Huang, A., & Barenholz, Y. (1994). Prolonged Circulation Time and Enhanced Accumulation in Malignant Exudates of Doxorubicin Encapsulated in Polyethylene-glycol Coated Liposomes1. Cancer Research, 54(4), 987–992. https://aacrjournals.org/cancerres/article/54/4/987/500720/Prolonged-Circulation-Time-and-Enhanced

22. Perica, K., De León Medero, A., Durai, M., Chiu, Y. L., Bieler, J. G., Sibener, L., Niemöller, M., Assenmacher, M., Richter, A., Edidin, M., Oelke, M., & Schneck, J. (2014).Nanoscale artificial antigen presenting cells for T cell immunotherapy. Nanomedicine: Nanotechnology, Biology and Medicine, 10(1), 119–129. https://doi.org/10.1016/j.nano.2013.06.015

23. Wan, J., Kim, Y., Mulvihill, M. J., & Tokunaga, T. K. (2018). Dilution destabilizes engineered ligand-coated nanoparticles in aqueous suspensions. Environmental Toxicology and Chemistry, 37(5), 1301–1308. https://doi.org/10.1002/etc.4103

24. Teleanu, D., Chircov, C., Grumezescu, A., Volceanov, A., & Teleanu, R. (2018). Blood-Brain Delivery Methods Using Nanotechnology. Pharmaceutics, 10(4), 269. https://doi.org/10.3390/pharmaceutics10040269

25. Priem, B., van Leent, M., Teunissen, A., Sofias, A. M., Mourits, V. P., Willemsen, L., Klein, E. D., Oosterwijk, R. S., Meerwaldt, A. E., Munitz, J., Prévot, G., Vera Verschuur, A., Nauta, S. A., van Leeuwen, E. M., Fisher, E. L., de Jong, K., Zhao, Y., Toner, Y. C., Soultanidis, G., Calcagno, C., … Mulder, W. (2020). Trained Immunity-Promoting Nanobiologic Therapy Suppresses Tumor Growth and Potentiates Checkpoint Inhibition. Cell, 183(3), 786–801.e19. https://doi.org/10.1016/j.cell.2020.09.059

26. Min, Y., Roche, K. C., Tian, S., Eblan, M. J., McKinnon, K. P., Caster, J. M., Chai, S., Herring, L. E., Zhang, L., Zhang, T., DeSimone, J. M., Tepper, J. E., Vincent, B. G., Serody, J. S., & Wang, A. Z. (2017). Antigen-capturing nanoparticles improve the abscopal effect and cancer immunotherapy. Nature Nanotechnology, 12(9), 877–882. https://doi.org/10.1038/nnano.2017.113

27. Tercan, H., Riksen, N. P., Joosten, L. A. B., Netea, M. G., & Bekkering, S. (2020). Trained Immunity. Arteriosclerosis, Thrombosis, and Vascular Biology. https://doi.org/10.1161/atvbaha.120.314212

28. Priem, B., van Leent, M. M. T., Teunissen, A. J. P., Sofias, A. M., Mourits, V. P., Willemsen, L., Klein, E. D., Oosterwijk, R. S., Meerwaldt, A. E., Munitz, J., Prévot, G., Vera Verschuur, A., Nauta, S. A., van Leeuwen, E. M., Fisher, E. L., de Jong, K. A. M., Zhao, Y., Toner, Y. C., Soultanidis, G., & Calcagno, C. (2020). Trained Immunity-Promoting Nanobiologic Therapy Suppresses Tumor Growth and Potentiates Checkpoint Inhibition. Cell, 183(3), 786-801.e19. https://doi.org/10.1016/j.cell.2020.09.059

29. van Leent, M. M. T., Priem, B., Schrijver, D. P., de Dreu, A., Hofstraat, S. R. J., Zwolsman, R., Beldman, T. J., Netea, M. G., & Mulder, W. J. M. (2022). Regulating trained immunity with nanomedicine. *Nature Reviews Materials*, *7*(6), 465–481. https://doi.org/10.1038/s41578-021-00413-w

30. Yan, L., Shen, J., Wang, J., Yang, X., Dong, S., & Lu, S. (2020). Nanoparticle-Based Drug Delivery System: A Patient-Friendly Chemotherapy for Oncology. Dose-response : a publication of International Hormesis Society, 18(3), 1559325820936161. https://doi.org/10.1177/1559325820936161

31. Tang, L., Zheng, Y., Melo, M. B., Mabardi, L., Castaño, A. P., Xie, Y.-Q., Li, N., Kudchodkar, S. B., Wong, H. C., Jeng, E. K., Maus, M. V., & Irvine, D. J. (2018). Enhancing T cell therapy through TCR-signaling-responsive nanoparticle drug delivery. Nature Biotechnology, 36(8), 707–716. https://doi.org/10.1038/nbt.4181

32. Balakrishnan, P. B., & Sweeney, E. E. (2021). Nanoparticles for Enhanced Adoptive T Cell Therapies and Future Perspectives for CNS Tumors. Frontiers in Immunology, 12. https://doi.org/10.3389/fimmu.2021.600659

33. Zhang, F., Stephan, S. B., Ene, C. I., Smith, T. T., Holland, E. C., & Stephan, M. T. (2018). Nanoparticles that reshape the tumor milieu create a therapeutic window for effective T cell therapy in solid malignancies. Cancer Research, canres.0306.2018. https://doi.org/10.1158/0008-5472.can-18-0306

34. Ding, X., Sun, X., Cai, H., Wu, L., Liu, Y., Zhao, Y., Zhou, D., Yu, G., & Zhou, X. (2022). Engineering Macrophages via Nanotechnology and Genetic Manipulation for Cancer Therapy. Frontiers in Oncology, 11. https://doi.org/10.3389/fonc.2021.786913

35. Toy, R., & Roy, K. (2016). Engineering nanoparticles to overcome barriers to immunotherapy. Bioengineering & translational medicine, 1(1), 47-62.https://doi.org/10.1002/btm2.10005

36. Toy, R., Hayden, E., Camann, A., Berman, Z., Vicente, P., Tran, E., ... & Karathanasis, E. (2013). Multimodal in vivo imaging exposes the voyage of nanoparticles in tumor microcirculation. ACS nano, 7(4), 3118-3129. https://doi.org/10.1021/nn3053439

37. Munn, D. H., & Mellor, A. L. (2006). The tumor-draining lymph node as an immune-privileged site. Immunological Reviews, 213(1), 146–158. https://doi.org/10.1111/j.1600-065x.2006.00444.x

38. Mitchell, M. J., Billingsley, M. M., Haley, R. M., Wechsler, M. E., Peppas, N. A., & Langer, R. (2020). Engineering precision nanoparticles for drug delivery. Nature Reviews Drug Discovery, 20(2), 101–124. https://doi.org/10.1038/s41573-020-0090-8

39. Sun, X., Zhang, Y., Li, J., Park, K. S., Han, K., Zhou, X., Xu, Y., Nam, J., Xu, J., Shi, X., Wei, L., Lei, Y. L., & Moon, J. J. (2021). Amplifying STING activation by cyclic dinucleotide–manganese particles for local and systemic cancer metalloimmunotherapy. Nature Nanotechnology, 16(11), 1260–1270. https://doi.org/10.1038/s41565-021-00962-9

40. Su, F.-Y., Zhao, Q. H., Dahotre, S. N., Gamboa, L., Bawage, S. S., Silva Trenkle, A. D., Zamat, A., Phuengkham, H., Ahmed, R., Santangelo, P. J., & Kwong, G. A. (2022). In vivo mRNA delivery to virus-specific T cells by light-induced ligand exchange of MHC class I antigen-presenting nanoparticles. Science Advances, 8(8). https://doi.org/10.1126/sciadv.abm7950