International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2046-2056www.ijprajournal.com ISSN: 2249-7781

Mini Review: Car-T Cells with Iron-Oxide Nanoparticles Used in Cancer Treatment

K. Vijayasri,* K. Vijaya Lakshmi, P.D.S.S. Prakash, MulpuriRuthvija Chowdary

Malla Reddy College of Pharmacy (Affiliated to Osmania University), Maissammaguda, Kompally (post), Secunderabad 500100

Submitted: 05-02-2023 Accepted: 20-02-2023

ABSTRACT:
Cancer immunotherapy is a novel approach for cancer treatment which boosts immune system significantly as compared to chemotherapeutics or radiation. Cell migration is an integral process in a therapeutic immune response, and the ability to track and image the migration of immune cells **in-vivo** allows for better characterization of the disease and monitoring of the therapeutic outcomes. In recent decades, the advent of immune-based therapies, most notably Chimeric Antigen Receptors -T cell therapy has revolutionized cancer treatment which is a potent strategy for eliminating tumors by harnessing the immune system. This therapy is approved by the USFDA for the treatment of B-cell acute lymphoblastic leukemia which showed promising results of numerous studies indicating CAR-T cell therapy in treating blood cancers. The nanoparticles are used in cancer treatment for targeted drug release which diminishes the toxicity. In this context, iron oxide nanoparticles are promising candidates in immunotherapy as they are biocompatible in nature, have flexible surface chemistry and display magnetic properties that may be used in contrast-enhanced MRI. Complex and suppressing tumor microenvironment, tumor antigen heterogeneity, cell trafficking, CAR-T cell exhaustion, and reduced cytotoxicity in the tumor site limit the applicability of CAR-T cell therapy and highlights the requiring to improve the performance of this treatment. Currently more than 300 adoptive T-cell therapy trials are under investigation. Despite the enthusiasm, application of CAR T-cell therapy to solid tumours has had little success, although positive outcomes are increasingly being reported for these diseases. Strategies to improve CAR T-cell therapy responses, particularly for solid tumours, by combining CAR T-cell therapy with radiotherapy through the use of careful monitoring and non-invasive imaging. In this mini review, CAR T-cells with Iron oxide nanoparticles were used in cancer immunotherapy, developments in labeling and tracking various immune cells using Iron-oxide nanoparticles and their effectiveness as MRI contrast agents, different cancer treatment methods in combination with CAR-T cell therapy and their therapeutic outcomes, which can be a helpful perspective for improving cancer treatment in the near future.

KEY WORDS: CAR-T cell, Anti-cancer agents, Iron oxide nanoparticles, Immunotherapeutic drugs.

I. INTRODUCTION:

Chimeric Antigen Receptors with T-cells (CAR-T) were first proposed in the late 1980s, these synthetic antigen receptors were not tested clinically as a cancer therapy until 2006. The initial results in adult patients were not promising due to poor persistence of genetically-engineered lymphocyte (1). In the present trends there are many cellular immunotherapies such as CAR-T cells therapy, in which T-cells expressing antibody-based CAR targeting tumor antigen, is an effective therapy against different types of hematological malignancies and solid cancers (2). CARs are unique receptors that are designed to target a specific tumor antigen to functionally reprogram T-lymphocytes. As T-lymphocytes are genetically engineered to express these artificial receptors to target cancer cells, which may be termed as immunotherapy/gene therapy/cancer therapy (3).

Immunotherapy also called as biotherapy as the immune system in the body is naturally capable of detecting pathogens and cancerous cells. In recent years, it has emerged as an important branch for treatment of similar types of diseases but its protective mechanism may differ. Certain

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2046-2056www.ijprajournal.com ISSN: 2249-7781

immunotherapies boost the immune system, whereas others directly target the cancer cells. Each treatment type has its advantages and disadvantages depending on the disease type. Another approach involves the administration of immune components, such as synthetic, modified immune proteins that are genetically engineered to target tumor antigens. CAR-T cells treatment has achieved success in treating hematopoietic malignancies. The principal cells of humoral and cellular immune response are the lymphocytes, which specifically recognize and respond to antigens to produce antibodies or directly kill infected cells. The ability of these cells to take up nanoparticles (NPs) is generally considered as lower than that Zhu and co-workers, who exposed mice intratracheally with 4µg or 20µg of Iron-oxide nanoparticles (IONPs) and reported that NP-induced exosomes were responsible as signaling mediators for this Th1 immune activation. According to the World Health Organization (WHO), cancer is the second most important cause of death in Europe. Due to its manifold manifestations, it is not possible to treat all patients according to a uniform scheme. However, all solid tumors have one thing in common: independent of the tumor's molecular subgroup and the treatment protocol, the immune status of the tumor, especially the amount of Tumor Infiltrating Lymphocytes (TILs), is important for the patient's clinical outcome – the higher the number of TILs, the better the outcome. For this reason it seems desirable to increase the number of TILs.

The cytotoxicity induced through particle-mediated or iron-mediated oxidative stress IONPs may be recognized by the immune system and can, thus, induce different immunological effects (4). IONPs are generally considered as safe and non-toxic in vivo, however, such particles can have side effects such as local pain, hypotension, hypersensitivity, anaphylactic shock, vasodilatation and paraesthesia which were the reasons for withdrawal of the earlier clinical applied dextran/carboxydextran-coated IONP-formulations (Feridex®/Endorem®, Resovist®/Cliavist®, Sinerem®/Combidex®). A still approved formulation is Ferumoxytol (Feraheme®, Rienso® in the EU) which is primarily used as iron therapy and has been shown to be tolerable in high doses up to 510 mg per injection. However, there is also evidence of risks of hypersensitivity reactions and also the longterm effects seem to be not fully evaluated. Besides cytotoxicity induced through particle-mediated or iron mediated oxidative stress, IONPs may be recognized by the immune system and can, thus, induce different immunological effects. These immune effects are – just like the aforementioned cellular and molecular effects – highly dependent on the particle characteristics. Size, shape, surface charge and particle coating have been described previously to greatly influence the immune effects of IONPs. On the one hand, this is explained by the fact that these parameters also influence the IONPs biodistribution and toxicokinetic profile, on the other hand, certain coatings have been shown to induce certain immune effects. For example, polyethyleneimine (PEI)-coated IONPs have been shown to enhance polarization of human dendritic cells (DCs), while dextran-coated IONPs have been shown to suppress the proliferation activity of T-lymphocytes.

One way to accumulate T-cells in the tumor area is to make them magnetizable and attract them with an external magnetic field (5). Magnetization can be achieved by Superparamagnetic Iron oxide Nanoparticles (SPIONs) which can be bound to the cells' surface or internalized into the cells. For this study, SPIONs with different coatings were synthesized and incubated with immortalized mouse T lymphocytes. SPIONsstabilized with lauric acid (LA) coated in situ or afterwards showed high toxicity. Addition of an albumin layer increased the biocompatibility but reduced cellular uptake. To increase the cellular uptake the albumin coated particles were aminated, leading to both higher uptake and toxicity, dependent on the degree of amination. In the presence of an externally applied magnetic field, T cells loaded with selected types and amounts of SPIONs were guidable. With this promising pilot study we already can demonstrate that it is possible to attract SPIONs bearing T cells by an external magnet. To sum up, biocompatibility and uptake of SPIONs by T cells are opposing events. Thus, for the functionalization of T cells with SPIONs the balance between uptake and toxicity must be evaluated carefully.

Efforts are now underway to evaluate the efficacy of CAR T-cell therapy in solid tumors. However, a key limitation in the advancement of T-cell therapy is the lack of information on the biodistribution of the T cells in patients. Therefore, there is a need for the identification of translatable

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methods for tracking the therapeutic T cells noninvasively. We describe the mechanical labeling of CAR T cells with an FDA-approved iron oxide nanoparticle to demonstrate the noninvasive and multimodal imaging of the CAR T cells. This method may be utilized for monitoring T cells in clinical trials. Metastatic osteosarcoma has a poor prognosis with a 2-yrs, event-free survival rate of ~15 to 20%, highlighting the need for the advancement of efficacious therapeutics.

However, clinical trials with CAR T cells in solid tumors have encountered significant challenges and have not yet demonstrated convincing evidence of efficacy for a large number of patients. A major bottleneck for the success of CAR T-cell therapy is our inability to monitor the accumulation of the CAR T cells in the tumor with clinical-imaging techniques. To address this, we developed a clinically translatable approach for labeling CAR T cells with Iron oxide nanoparticles, which enabled the noninvasive detection of the iron-labeled T cells with magnetic resonance imaging (MRI), photoacoustic imaging (PAT), and magnetic particle imaging (MPI). Using a custom-made microfluidics device for T-cell labeling by mechanoporation, we achieved significant nanoparticle uptake in the CAR T cells, while preserving T-cell proliferation, viability, and function. Multimodal MRI, PAT, and MPI demonstrated homing of the T cells to osteosarcomas and off-target sites in animals administered with T-cells labeled with the iron oxide nanoparticles, while T cells were not visualized in animals infused with unlabeled cells. This study details the successful labeling of CAR T cells with ferumoxytol, thereby paving the way for monitoring CAR T cells in solid tumors.

Recently cancer has been identified as leading cause of mortality worldwide. Developed several conventional treatments and cytotoxic immunotherapies and released into the market. Considering the complex behavior of tumors, the involvement of numerous genetic, cellular factors involved in tumorigenesis and metastasis (there is a need to develop a promising immunotherapy that targets tumors at both the cellular and genetic levels). CAR-T cell therapy has emerged as a novel therapeutic in which T cells derived from patient blood are engineered in vitro to express artificial receptors targeted to a specific tumor antigen. Recently, they had identified the tumor antigens without the involvement of the Major Histocompatibility Complex. The use of this therapy was successful in the last few years, with a reduction in remission rates of up to 80% for hematologic cancer, particularly for acute lymphoblastic leukemia (ALL) and non Hodgkin lymphomas, such as large B cell lymphoma. Recently, antiCD19 CAR therapy, or UCART19, has been shown to be efficacious in treating relapsed/refractory hematologic cancer. CD20 and CD22 cell surface tumor antigens, found in the majority of leukemias and lymphomas which were considered as potential targets by pharmaceutical companies and research organizations, and trials have been ongoing in this direction. Although this therapeutic regimen is currently confined to treating hematologic cancer, the increasing involvement of several auxiliary techniques, such as (bispecific CAR, Tan CAR, inhibitory CAR, combined antigens, the clustered regularly interspaced short palindromic repeats gene editing tool and nanoparticle delivery), may substantially improve its overall anticancer effects. CAR-T cell therapy has the potential to offer a rapid and safer treatment regimen to treat nonsolid tumors and solid tumors.

Cancer has been one of the most significant causes of mortality, worldwide. Cancer immunotherapy has recently emerged as a competent, cancer-fighting clinical strategy. Nevertheless, due to the difficulty of treatments, costs, and off-target adverse effects, the implementation of cancer immunotherapy described by the Antigen-Presenting Cell (APC) vaccine and Chimeric Antigen Receptor T-cell therapy ex-vivo in large clinical trials have been limited. Nowadays, the nanoparticles theranostic system as a promising target-based modality provides new opportunities to improve cancer immunotherapy difficulties and reduce their adverse effects. Meanwhile, the appropriate engineering of nanoparticles taking into consideration, nanoparticle characteristics (size, shape, and surface features) as well as the use of these physicochemical properties for suitable biological interactions, provides new possibilities for the application of nanoparticles in cancer immunotherapy. In this review article, we focus on the latest state-of-the-art nanoparticle-based antigen/adjuvant delivery vehicle strategies to professional APCs and engineering specific T lymphocyte required for improving the efficiency of tumor-specific immunotherapy.

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Cancer immunity cycle:

While discussing the specific molecular and cellular pathways that were exploited in cancer immunotherapy, the response of the immune system to cancer is important in and in turn, however these processes can be enhanced through the use of IONPs. The cancer immunity cycle explains the interaction between the immune system as well as the tumor cells. However, specific pathways and biomarkers vary amongdifferent cancer types, the initial step in the cancer immune response begins with the release of cancer antigens by cancer cells. Antigens are, by definition, substances (usually proteins or carbohydrates) that elicit an immune response from their host, and are classified according to their source. Although cancer cells are endogenous to the body, cancer antigens are classified as neoantigens, Cancer antigens can be either tumor-specific antigens, present only in tumor cells, or tumor-associated antigens, which are aberrantly expressed in tumor cells but also found in normal cells and therefore, can induce central immune tolerance. These antigens are captured by antigen-presenting cells (dendritic cells). The DCs process and present these antigens onto their surface with Major Histocompatibility Complex class I or II molecules and migrate to lymph nodes. T cells residing in lymph nodes can be used to recognize the antigens present on DCs through T-cell receptors. This interaction subsequently leads to priming. As well as activation of T cells which are then able to migrate away from the lymph node, finally recognize the antigen on tumor cells (11-13).

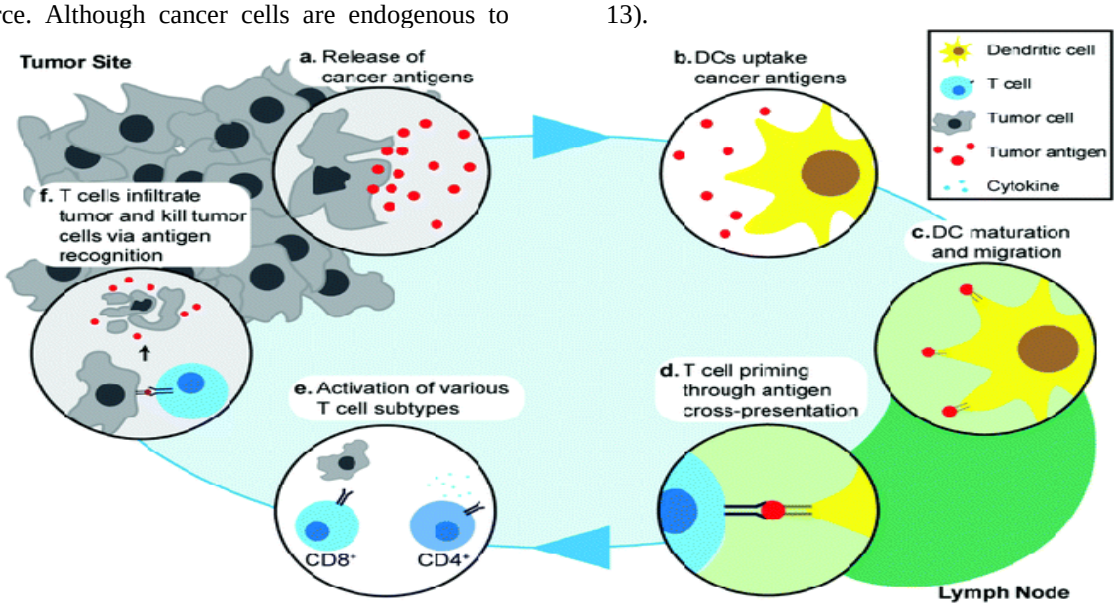

Figure.1 Schematic representation of the immune system's response to cancer.

(a) Tumor cells release tumor antigens as they die.
(b) These tumor antigens are recognized by antigen-presenting cells such as dendritic cells.
(c) Dendritic cells undergo maturation and present the processed antigen on their surface with the major histocompatibility complex. Matured dendritic cells migrate to lymph nodes.
(d) Dendritic cells present antigens to T cells resident in the lymph node, and T cells can recognize the antigens using T cell receptors. Upon this recognition, T cells are activated into cytotoxic T lymphocytes.
(e) The activated T cells then migrate to the tumor site. Various subtypes of T cells are involved in immune response. CD8+ cells are directly involved in killing tumor cells, whereas CD4+ cells release cytokines to regulate the immune response.
(f) Activated T cells recognize tumor cells and induce apoptosis, upon which more tumor antigens are released, beginning the cycle again.

Different types of T cells play different roles in cancer immunotherapy:

$CD8^+$ T-cells/Cytotoxic T-lymphocytes (CTLs): These are capable of directly recognizing antigens and kill tumor cells.

$CD4^+$ T-cells/Helper T-cells: These play an indirect role by regulating the immune response

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through release of cytokines that can activate and signal other immune cells including CTLS. Activated T-cells migrate and infiltrate tumors, and an increased presence of T cells in the tumor microenvironment has been associated with improved prognosis in various cancer types. The release of cancer antigens upon tumor cell death restarts the cancer immunity cycle again—by addition to the main cancer immunity cycle, there is also involved of other types of immune cells with response to cancer, such as natural killer (NK) cells that can identify, kill tumor cells by identifying oncogenic transformations, macrophages in the tumor environment that can regulate the inflammatory response and recruitment of other immune cells.**T-cells:**These are a type of lymphocytic cells that originate from the thymus and reside in lymph nodes and are differentiated into various types in cases where there is an interact between cancer cells and APCs. The evaluation of therapeutic efficacy is mainly dependent on the observation of tumor reduction post-treatment, visualization of T-cell migration could reveal the T-cells activity and T-cells therapeutic activity (23,24).Recently, IONP's-based dual modality (MRI/fluorescence) cellular imaging probe has been developed which consists of an Ironoxide core-coated with PEG conjugated with fluorescent dyes. These IONPs do not alter the cellular function of human murine T-cells, effective labels for T-cell tracking in-vivo in animal models (25). The labelled T-cells were injected intravenously and migration of T-cells to transplanted allograft heart and lung as a result of immune rejection was observed in the regions of hypointense MRI signal in both allograft heart and lung for 24 hrs and 48 hrs after administration of IONPs, corresponding to increased presence of T cells in the organs as part of an acute rejection of the transplants. Similar results were being observed in other method that utilized IONPs conjugated with Rhodamine-B as dual MRI/fluorescence probes. However, the non-phagocytic nature of T-cells is a barrier to overcome for efficient labeling of T-cells. Internalization of IONPs by T-cells was observed to be an order of magnitude lower than that of monocytes and other phagocytic immune cells. Electroporation and surface modification with Human Immunodeficiency Virus-1 (HIV-1) transactivator peptides were used to improve the cellular uptake of IONPs by T-cells via transfection was being explored (26,27). Transfection agents aid the crossing of the cell membrane and can be used to facilitate the internalization of IONPs for cell tracking applications. IONPs and transfection agents were mixed to form complexes, which were incubated with T cells extracted from Lewis rats. The transfection agents tested include lipofectamine, poly-L-lysine, polyethyleneimine (PEI), and FuGene6 (commercially available lipidic multicomponent transfection agent). The labeling efficiency was analyzed through magnetic separation and X-ray fluorescence spectroscopy. IONP:PEI complexes, yielded the highest labeling efficiency (60%), followed by poly-L-lysine and lipofectamine, and FuGene6. The trend in the labeling efficiency was correlated with the IONP:PEIs zeta potential as PEI had the highest zeta potential. IONP:PEI was also shown to induce the greatest cytotoxicity, indicating a trade-off between labeling efficiency and cytotoxicity. Advances in T-cell tracking leads to efficacy of T cell-based cancer immunotherapy such as CAR-T cell therapy could be assessed through correlation of T-cell migration and antitumor activity of the T cells (14-17,28).

Applications of IONPs for cancer immunotherapy:

Nanoparticles hold promise as a crucial component in the advancement of immunotherapy, through their capacity to selectively engage immune cells. The interplay between nanoparticles and immune cells encompasses the activation of Antigen Presenting Cells (APCs), the triggering of T cell activation, and the alteration of macrophage function.

The adaptable nature of the surface chemistry of IONPsfacilitates the conveying of immunogenic molecules, adjuvants, and therapeutic agents to immune cells, and the conjugation of immunogenic molecules onto the IONPs confers protection against degradation in the living organism (18,19).

The operation of cancer vaccines is centered around inducing the immune system's response to cancer by administering tumor antigens, which are subsequently internalized by DCs. This process leads to the activation of T cells, which are directed to specifically target tumor cells expressing the associated antigen. The efficacy of IONP-based cancer vaccines is further enhanced by their unique physical and chemical properties, such as biocompatibility, biodegradability, and surface functionalization capacity, which allow for targeted

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Volume 8, Issue 1 Jan-Feb 2023, pp: 2046-2056www.ijprajournal.com ISSN: 2249-7781

delivery and sustained release of immunogenic molecules. Furthermore, the magnetic properties of IONPs have also been used in adoptive cell therapy and T-cell enrichment, and magnetization of cytotoxic T-cells and NK cells allows guided delivery of these cells to specific tumor sites.

The alteration of the polarization state of Tumor Associated Macrophages (TAMs) within the tumoral microenvironment into pro-inflammatory macrophages via IONPs can induce an anti-tumoral immune response. Photo thermal therapy leverages the iron oxide cores of IONPs to both trigger immune responses in tumors and deliver immune modulatory agents to reinforce the immune response (19).

Additionally, IONPs can serve as a platform for delivering checkpoint inhibitory molecules in the context of immune checkpoint blockade therapy. This will showcases the numerous strategies employed using IONPs to booster the activity and responsiveness of immune cells to fight against cancer.

DC-based cancer vaccines:

In the context of cancer immunology, Antigen Presenting Cells (APCs) internalize cancer antigens, undergo antigen processing, and present the processed peptides on their surface via Major Histocompatibility Complex (MHC) class-I or class-II molecules. The presence of the antigen on the APC surface elicits recognition by native T cells, which then differentiate into cytotoxic T lymphocytes (CTLs) or helper T cells capable of executing the immune response within the organism. The regulation of antigen uptake and migration of APCs can prove pivotal in augmenting the host immune response to cancer (20). Dendritic Cells (DCs), a subclass of APCs, exhibit the capacity to internalize and process various types of cancer antigens, and activate native T-cells into CTLs following migration to lymph nodes. As a key driver of the immune response against tumors, DCs have garnered significant attention as a target for the development of cancer vaccines.

Formulations of cancer vaccines utilizing Iron Oxide Nanoparticles (IONPs) have demonstrated efficacy in improving antitumor immunity by facilitating efficient delivery of antigens to Antigen Presenting Cells (APCs) through enhancement of antigen solubility and bioavailability. A study utilizing IONPs-OVA nanocomposites resulted in a significant improvement in DC stimulation and reduction in tumor burden in-vivo. The treatment was characterized by elevated expression of pro-inflammatory cytokines including Interleukin-6 (IL-6), Tumor Necrosis Factor-α (TNF-α), and Interferon-γ (IFN-γ) in DC2. A comparison of murine DCs treated with IONPs-OVA nanocomposites versus those treated with free OVA revealed a marked difference in cytokine expression.

To evaluate the immunotherapeutic potential of IONP-OVA in-vivo, mice bearing subcutaneous tumors were administered with saline, free OVA, and free IONP. The group treated with IONP-OVA displayed a dramatic reduction in tumor burden, whereas no tumor growth inhibition was observed in the group treated with free OVA. Similar findings were reported in other studies that employed IONPs-OVA complexes. The potential use of nanoparticle-based formulations as prophylactic vaccines against solid tumors and metastasis was explored through the stimulation of macrophages. A study was conducted using mice that were administered with different formulations, including phosphate-buffered saline (PBS), free OVA, IONPs, and two formulations of IONP-OVA with varying ratios of IONP to OVA near lymph nodes. One week after administration, the mice were injected with B16-OVA melanoma cells. The results showed that the administration of IONP-OVA complexes effectively prevented tumor growth, while free OVA was unable to do so. The improved in vivo antitumor immune response was attributed to the protection of cancer antigens from intracellular degradation and inactivation.

The study also demonstrated the immune stimulatory capability of the IONP-based vaccines through increased expression of Tumor Necrosis Factor-alpha (TNF-α), which was indicative of macrophage stimulation and acceleration of tumor immunotherapy. While the immune stimulatory effect of free IONPs on tumor reduction was observed in some experiments, this trend was not consistently observed in other studies, indicating that IONPs may act as potential as adjuvants, but further investigation is required.

The potential underlying mechanism for the observed immunomodulatory effect of IONPs is the release of signaling molecules by macrophages upon interaction with the particles. Antigen delivery to DCs can be accomplished via RNA transfection, which encodes for a specific

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tumor antigen. The utilization of cationic liposomes as a vector for encapsulating IONPs and therapeutic mRNA (IO-RNA-NP) allows for the use of IONPs as MRI contrast agents in tracking DC migration in-vivo. Improved transfection efficiency is achieved with the incorporation of IONPs into the liposomal vector, as the cationic lipid molecules interact with the cell membrane via electrostatic interactions and the lipid nature of the vector facilitates membrane fusion. This leads to an increase in DC activation and function.

Compared to traditional RNA transfection via electroporation, which utilizes an electric field to enhance cell membrane permeability, transfection through IO-RNA-NP results in high secretion of co-stimulatory molecules that amplify T cell activation signals and IFN-α, which is crucial for inducing an antitumor immune response. Intradermal injection of DCs transfected through IO-RNA-NP or electroporation into mice bearing subcutaneous B16F10-OVA tumors was performed to evaluate the efficacy of the transfection methods (20).

The IO-RNA-NP administration group displayed a substantial suppression of tumor growth compared to the group receiving treatment through electroporation. Additionally, the migration of DCs loaded with IO-RNA-NP was monitored utilizing Magnetic Resonance Imaging (MRI), with the MRI intensity resulting from the IONPs in the lymph nodes evaluated as a biomarker of antitumoral response. The subjects were divided into groups based on the observed MRI intensity on day 2 post-treatment, and the correlation between survival outcomes and initial DC migration was established. The MRI-predicted responders, characterized by DC migration in the top 75 percentile on day 2, showed a 39% increase in median survival in comparison to those with DC migration in the bottom 25th percentile when the tumor cells and vaccines were administered simultaneously. Similar results were obtained in models with established tumors. The design of an efficacious cancer vaccine necessitates the precise selection of the tumor antigen to effectively activate CTLs and the attainment of an optimal level of antigen concentration in DCs for cross-presentation to prime T cells (11). Cancer vaccines based on IONPs have demonstrated the capability to activate DCs by delivering tumor antigens in a highly efficient manner, while protecting free antigens from degradation, as well as providing a means of monitoring DC migration to assess the therapeutic response of the vaccine.

Magnetic field-assisted cell migration:

T cells are differentiated from other types of lymphocytes by the presence of a T-cell receptor on their surface, which allows them to recognize specific antigens and undergo maturation into various T cell subsets. The efficacy of immunotherapy as an antitumor treatment is dependent upon the activation of cytotoxic T cells (CTLs) and their ability to effectively target and eliminate cancer cells(9).To enhance the presence of CTLs at tumor sites, T cells can be functionalized with Iron oxide nanoparticles (IONPs) and directed towards the tumor using an external magnetic field. This magnetic guidance of T cells may reduce systemic side effects and achieve therapeutic success with a reduced number of T cells.

Previous studies have indicated that IONP-labeled T cells can be directed along an external magnetic field in-vitro and in-vivo experiments have explored magnetic field-guided migration of IONP-labeled T cells (7-9). The observation of T cell retention at the popliteal lymph node after application of a magnetic field has shown an increased retention of CD4+ and CD8+ cells labeled with IONPs compared to T cells without IONPs (21, 22).

Activation of tumor associated macrophages:

Macrophages, a type of immune cell, play a critical role in immune defense mechanisms. They are responsible for eliminating foreign materials and cellular debris through phagocytosis and regulating inflammatory responses by secreting cytokines (29). In the context of tumor microenvironments, tumor-associated macrophages (TAMs) are known to be polarized into either pro-inflammatory M1 or cell proliferation-promoting M2 states by anti-inflammatory cytokines. Such polarization contributes to the progression of tumors by promoting tumor growth and angiogenesis. By reprogramming TAMs to the M1 state, it becomes possible to induce apoptosis of tumor cells within the tumor microenvironment, thereby addressing the challenge of tumor penetration by M1 macrophages outside the tumor. As a result, activation of macrophages, also known as macrophage reprogramming, has become a

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subject of significant interest in cancer immunotherapy research.

IONPs have been demonstrated to have significant potential as an innovative cancer immunotherapy. They can effectively deliver various biomolecules such as OVA and Toll-like receptor agonists, which can trigger the polarization of macrophages and help suppress tumor growth. The metabolic degradation of IONPs results in increased iron content in macrophages, which in turn, stimulates their polarization to the pro-inflammatory M1 state (30). The iron levels within macrophages can regulate their polarization; high expression of ferritin within M1 macrophages helps store iron inside cells, while high expression of ferroportin in M2 macrophages transports iron out of cells. The administration of liposomal IONPs followed by lysosomal degradation releases iron, which is then ingested by macrophages, leading to their M1 polarization.

In experimental studies, the administration of IONPs in mice after the injection of cancer cells resulted in a significant suppression of tumor growth compared to control groups. The potential of IONPs to address metastasis has also been demonstrated in studies where their administration prior to the injection of cancer cells led to tumors that were six times smaller in size than the control. Furthermore, the use of porous hollow IONPs to deliver 3-methyladenine, an inhibitor of phosphoinositide 3-kinase (PI3K) γ, has shown promise in promoting an immune response and repolarizing TAMs to M1-type macrophages (30).The system demonstrated its effectiveness in suppressing tumor growth by inhibiting the expression of PI3K γ and upregulating the production of NF-κB p65, a key protein in the immune response-promoting complex NF-κB. The simultaneous polarization of macrophages by IONPs and the addition of 3-methyladenine showed a synergistic effect in suppressing tumor growth in mouse models. Additionally, a study explored the artificial reprogramming of macrophages using hyaluronic acid-coated IONPs to enhance the effects of macrophage polarization. The results showed that when RAW 264.7 mouse macrophages were incubated with IONPs and then transplanted into 4T1 tumor-bearing mice, they were able to specifically target and kill cancer cells, polarize resident tumor-associated macrophages (TAMs) to M1-type macrophages, amplify the anticancer effect, and remain resistant to cytokines in the tumor microenvironment that would otherwise suppress pro-inflammatory macrophages. In a comparison of different treatments in BALB/c mice with subcutaneously inoculated 4T1 tumors, transplantation of IONP-programmed macrophages resulted in significantly greater tumor growth suppression compared to treatment with macrophages or IONPs alone. The magnetic guidance enabled by IONP labeling further enhanced the tumor growth suppression by allowing for chemotaxis of macrophages along the cytokine gradient. These results highlight the potential of IONPs as delivery vehicles for immunostimulatory molecules and their innate ability to induce M1 macrophage polarization through iron release upon degradation. Macrophage activation is a crucial step in the anticancer immune response as it can amplify the therapeutic effects of other immune cells involved in cancer immunity. Further investigation into the concurrent administration of IONP-based macrophages and other therapeutic modalities would further demonstrate the utility of IONPs in cancer immunotherapy.

Photothermal therapy and magnetic hyperthermia therapy:

The synergistic combination of various therapeutic strategies has been a topic of significant interest in cancer therapy. Conventional cancer treatments, such as chemotherapy and radiotherapy, are often utilized together to enhance their therapeutic efficacy. Similarly, the application of iron oxide nanoparticles (IONPs) in cancer immunotherapy can be combined with other cancer therapies to induce a more potent antitumoral response. Photothermal therapy (PTT) is a technique that involves converting light, typically in the infrared spectrum, into heat to destroy target tissue. Nanoparticles, including IONPs, can amplify this effect, confining the ablation to a localized area and minimizing harm to healthy tissue. Moreover, local hyperthermia can stimulate an immune response, promoting the recruitment of immune cells and the development of anticancer immune responses (10, 11). Gold nanoparticles have been utilized in PTT clinical trials as photosensitizers, but IONPs provide a safer and biocompatible alternative to gold nanoparticles in PTT and have shown promising therapeutic outcomes in preclinical animal models (26).A nanoagent was developed that combined immune stimulation and magnetic responsiveness to target

International Journal of Pharmaceutical Research and Applications
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tumors using MRI and ultrasound imaging while concurrently enhancing photothermal effects. The nanoagent was designed with the incorporation of cytosine-phosphate-guanine (CpG) oligodeoxynucleotides as potent immunostimulatory adjuvants for delivery to the tumor site. CpG motifs are prevalent in bacterial DNA and are known to induce potent immunogenic responses. In vitro evaluation was performed by treating 4T1 breast cancer cells with IONPs either incorporating or lacking CpG, free CpG, or saline,and culturing bone marrow-derived dendritic cells (DCs) on the lower chamber of a transwell system. The immunostimulatory effects were evaluated through DC maturation, as measured by the upregulation of typical co-stimulatory molecules. The treatment with free CpG led to a greater extent of DC maturation compared to CpG-incorporated IONPs. However, laser irradiation significantly accelerated DC maturation, demonstrating the immunostimulatory effects of photothermal therapy (PTT) mediated by IONPs. In the in vivo treatment model, mice inoculated with 4T1 tumors on each flank were treated with combinations of IONPs, CpG, infrared laser, and an external magnetic field. The primary tumor was treated while the untreated tumormodeled metastatic behaviour. The treatment of the primary tumor with IONPs, laser, and magnetic field greatly suppressedtumor growth, with or without CpG incorporation.

The results showed that treatment with the antibody-conjugated IONPs resulted in a greater tumor growth suppression and enhanced survival rate compared to the administration of free individual antibodies. Furthermore, the efficacy of targeting multiple checkpoint pathways through the use of IONPs was investigated by treating mice with nanoparticles conjugated with both anti-PD-L1 and anti-4-1BB antibodies. The results indicated that the administration of nanoparticles with both antibodies resulted in a significant extension of survival and reduction of tumor size compared to the treatment with nanoparticles conjugated with only one type of antibody. These findings demonstrate the potential of targeting multiple checkpoint pathways for cancer immunotherapy and highlight the utility of IONPs as multifunctional delivery systems for checkpoint inhibition.

II. CONCLUSION

The utilization of iron oxide nanoparticles (IONPs) presents a novel avenue for enhancing immunotherapeutic approaches to cancer treatment. The ability to adjust the size of the IONPs through synthesis parameters and surface chemistry affords a means of modifying not only the inherent properties of the nanoparticles, but also their interactions with immune cells, without inducing substantial changes to the activity or viability of these cells. This property enables the tracking of IONP-labeled immune cells using imaging modalities such as magnetic resonance imaging (MRI) that take advantage of the distinct characteristics of the iron oxide core. The migration and localization of immune cells to lymph nodes and tumors are critical for inducing antitumor responses, and IONPs provide a non-invasive method for tracking transplanted cells to evaluate therapeutic outcomes.

Furthermore, IONPs have been applied to cancer vaccines, where they enable antigens to be more efficiently recognized by dendritic cells (DCs) by protecting them from degradation in circulation, resulting in increased antigen cross-presentation to prime T cells for attacking tumor cells. IONPs have also been used to increase the migration and retention of immune cells that mediate tumor cell death. The targeted delivery of T-cells and natural killer (NK) cells incorporating IONPs to tumors through an external magnetic field has demonstrated increased presence of these cells at the tumor site and resultant reduction in tumor size through immunogenic activity.

IONPs have also been shown to suppress tumor growth by reprogramming and polarizing macrophages into a pro-inflammatory state. Additionally, IONP-based systems have been developed to induce local hyperthermia through near-infrared irradiation or by using an alternating magnetic field to ablate tumors, as well as to trigger immune responses by delivering immunostimulants to the tumor site. The efficacy of checkpoint blockade therapy was improved by IONPs by facilitating the delivery and monitoring of inhibitory molecules.

The widespread clinical adoption of cancer immunotherapy is hindered by several challenges. The current state of cancer immunotherapy is associated with systemic side effects and limited efficacy against solid tumors. This is in part due to the difficulty of penetrating

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 1 Jan-Feb 2023, pp: 2046-2056www.ijprajournal.com ISSN: 2249-7781

the abnormal extracellular matrix and the immune-suppressive microenvironment of these tumors.

Additionally, the high cost of immunotherapeutic drugs is a significant economic barrier. For instance, Sipuleucel-T, an FDA-approved immunostimulant for prostate cancer, costs $93,000 for three injections and has a median overall survival benefit of 4.1 months. The advent of novel cancer immunotherapy drugs undergoing clinical trials presents an opportunity for IONPs to enhance the efficiency and safety of these treatments. As a potent delivery vehicle, IONPs can more effectively transport immunotherapeutic agents to their intended targets while protecting them from degradation in the extracellular matrix, potentially reducing the required dose and alleviating some of the financial burden.

Moreover, the adoptive cell therapy/ technique holds the possibility of endowing immune cells with the capability to diffuse through the tumor more efficiently and evade the adverse impact of the immune-suppressive microenvironment surrounding the tumor. The non-phagocytic nature of T cells presents a challenge in labeling and tracking them with imaging probes, but the studies reviewed here demonstrate the capability of IONPs to label and magnetize T cells in a highly efficient manner, thereby enabling their responsiveness to external magnetic fields. This opens up the possibility of using IONPs in concert with novel T cell-based immunotherapeutic approaches, such as CAR T cell therapy, to monitor and guide the migration of T cells in vivo. Additionally, the evidence suggests that IONP-based vaccines can evoke therapeutic effects that can potentially target metastatic cells, thereby providing an avenue for the development of more potent vaccine designs that can effectively regulate and monitor the activation and migration of T cells aimed at metastasis. The advancements in the field of NK-based immunotherapy, such as the development of engineered CAR NK cells and the use of allogeneic and autologous NK cells, have augmented their anti-tumor activity, thereby presenting an opportunity for the integration of IONPs with these novel approaches. A comprehensive study of the interaction between biomaterials and host tissue is indispensable in evaluating the biocompatibility of the material and elevating its therapeutic effect. Although some studies have analyzed the interaction between IONPs and immune cells, a comprehensive overview of the role of IONPs in the immune response and immune cell activity could accentuate their potential as a cancer immunotherapeutic agent. The clinical applications of FDA-approved IONPs formulations have already exhibited their safety and biocompatibility, which surpasses other metal-based nanoparticle systems approved for clinical use. This advantage provides a basis for the utilization of IONPs to enhance therapeutic outcomes as further advancements are made in the field of cancer immunotherapy.

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