Balance and modulation of immunoediting for cancer treatment using synergistic nano-photo-immuno effects

Abstract: Nanotechnology, photonics, and immunotherapy are far-reaching technologies with the potential to revolutionize the field of cancer diagnostics and therapeutics. While each technology has limitations in cancer treatment, they can be synergized to exert profound impact on the balance and modulation of immunoediting in tumor microenvironment (TME) and in the entire host immune system. We provide our perspectives on how nano-photo-immuno interactions can be used as an effective therapy, particularly when combined with other treatment modalities, such as checkpoint immune therapy, chemotherapy, and TME modulation, to provide a long-term, tumor-specific immunity against tumor metastasis and tumor recurrence.

Keywords: immunoediting; immunotherapy; metastatic cancers; nanotechnology; photonics; tumor microenvironment.

The concept of cancer immunotherapy is not new, but the idea of using specific immune cell types to combat cancer is a modern concept. William Coley, a surgeon in 19th century New York City, is credited with founding cancer immunotherapy. Deeply affected by the loss of a young cancer patient, Coley did a thorough investigation of case studies in cancer patients and discovered a link between bacterial infection and cancer regression. More than a hundred years ago, in a bold attempt to provide cancer patients with some form of treatment regimen, Dr. Coley developed a cocktail of Gram-negative and Gram-positive heat-killed bacteria known as the “Coley Toxin”. He directly injected this mixture into the tumor to initiate a high fever over a period of several days and noticed that the solid tumors would become softer, change color, sometime ulcerate, and ultimately undergo necrosis, resulting in cancer regression. With this simple treatment, he had remarkable success, noting several cases in which some patients were cured while in others there was initial success on the treated tumors despite later recurrence in other locations [1, 2]. All of this was done without the knowledge that the immune system was being triggered to kill the tumor.

Despite Coley’s initial, limited success in working towards a cancer vaccine, little progress was achieved following his death for several decades. This was largely attributed to the fact that the immune system remained largely unstudied until the late 1960s, when T cells were discovered by Jacques Miller [3], followed by the characterization of the antigen presenting cell, dendritic cells, by Ralph Steinman [4, 5]. Despite these seminal discoveries, little attention was given to the concept of cancer immunotherapy, likely owing to the belief that tumors are “self” tissues and are unlikely to stimulate an immune response, despite tumor cells undergoing random mutations. It was not until 1998 when Schreiber and colleagues presented concrete evidence of immune surveillance [6, 7] and expounded upon the immune surveillance hypothesis posited independently by Sir MacFarlane Burnet and Lewis Thomas in the 1950s [8] that cancer immunotherapy really became a topic worth investigating.

To date there are five different classes of cancer immunotherapies, many of which have been around for decades [9]. These include immunomodulators, antibody targeted cell elimination, vaccines, oncolytic viruses, and cell-based immunotherapies [9-12]. The first cell-based immunotherapy to treat hematologic cancers was performed at the University of Minnesota in 1970s in the form of bone marrow transplantation [13]. This was followed by the approval of the first immunomodulator, interferon alpha (IFNa), for leukemia treatment in 1986 [14, 15]. Nearly 80 years after Coley’s seminal work using bacteria to stimulate antitumor immunity, the bacillus Calmette-
Guerin (BCG) tuberculosis vaccine was being used for bladder cancer treatment in the mid-1970s [15, 16]. In 1997, antibodies against the B cell surface marker CD20 were approved for lymphoma treatment, giving rise to antibody-based targeted therapeutics [17]. Lastly, in 2015 the oncolytic virus T-Vec was approved to treat advanced lymphoma patients [18, 19].

Due to the success of these therapies in prolonging patient survival, cancer immunotherapy has recently become accepted as a main-stream therapy, particularly when the journal Science named cancer immunotherapy the “Breakthrough of the Year” in 2013 [20] and, subsequently, when the 2018 Nobel Prize in Physiology or Medicine was awarded to the pioneers of the “discovery of cancer therapy by inhibition of negative immune regulation” [21]. Specifically, leading to the epitome of cancer immunotherapy is the discovery of immune regulating ligands coined immune “checkpoints”. To maintain immune homeostasis at steady-state or following an infection, the immune system has evolved ways to turn off or tune down the immune response through these immune checkpoints. There are many cell surface checkpoint molecules expressed on the surface of activated CD4 and CD8 T cells, but the most famous are cytotoxic T lymphocyte associated protein 4 (CTLA-4) and programmed cell death 1 (PD-1) [22, 23]. These molecules can be highly expressed on tumor infiltrating T cells, and actively prevent their tumoricidal functions. The major focus of immune checkpoint therapy (ICT) is to block these surface checkpoint proteins on immune cells to prevent their immunosuppressive signaling.

Another major function of ICT is to block checkpoint proteins, such as programmed cell death ligand 1 (PD-L1), on tumor surfaces. PD-L1 binds to PD-1 to prevent T cell mediated tumor killing, in response to interferon gamma (IFNγ) [23, 24]. Blocking PD-L1 interactions with PD-1 prevents the inhibitory signal from binding and tuning down the T cell response.

While the immunotherapies, particularly ICT, seek to eliminate tumors by releasing the brakes on T cells that specifically recognize tumor cells, they have failed to achieve satisfactory outcomes in clinical studies thus far [25, 26]. To understand the current limitations of ICT we must fully understand the tumor microenvironment (TME) and its interplay with the entire immune system. Immune cells, tumor cells, the extracellular matrix (EM), TME metabolites, and cancer associated fibroblasts (CAFs) are intimately interconnected and can influence each other [27–30]. This concept is illustrated in Shreiber’s hypothesis of “Cancer Immunoediting” which is an immune-centric hypothesis to describe the dual role of the immune system in inhibiting and promoting tumor progression [31]. Cancer immunoediting is divided into three sequential phases known as the three E’s: elimination, equilibrium, and escape [32, 33]. During the elimination phase, which expounds upon the initial immune surveillance hypothesis, the innate and adaptive immune systems work in concert to detect and destroy tumors, preventing uncontrolled tumor growth. The equilibrium phase suggests that the adaptive immune system, which is capable of recognizing specific tumor antigens, inadvertently selects tumor cells capable of surviving the initial immune attack/recognition. In this phase the immune cells co-exist with the tumor cells, which remain dormant, creating an equilibrium between the two camps. During the escape phase, these dormant tumor cells become active either by actively preventing immune recognition/destruction, or by establishing an immunosuppressive TME that actively prevents tumor killing and enhances tumor growth [32–34].

The hypothesis of cancer immunoediting is nicely illustrated in human cancer responses to cancer treatment, particularly ICT. Patients receiving ICT that do not achieve long-term benefit can be divided into three categories: primary resistance, adaptive resistance, and acquired resistance [35–37]. The primary resisters do not respond to immunotherapy due to both tumor intrinsic and tumor extrinsic factors in TME. The adaptive resisters have tumor immune recognition, but the tumor actively adapts to circumvent immune attack, effectively creating an arms race between the immune system and the TME. The acquired resisters initially respond well to ICT, but then relapse after a period of time despite continuous therapy. All three types of resistance to ICT have a close connection with TME.

Characterizing the evolvement of the TME following ICT and other immunotherapies, as well as common tumor resistance mechanisms can guide us in the development of combinational therapies to overcome therapeutic resistance. The TME has multiple complementing immunosuppressive components. If one component is removed, the remaining components compensate for that loss. These compensatory mechanisms limit the success of cell-targeted therapy in solid tumors. Therefore, the key is to design a “smart” targeted therapy that can tackle the compensatory mechanisms that exist within the TME. One promising approach is an ablative therapy combining photonics, nanotechnology and immunotherapy, adaptable specifically to the targeted tumor.

Photonics and nanotechnology were arguably two of the most far-reaching scientific and technological
advances in the 20th century. Lasers have opened new doors in diagnostics and therapeutics. Particularly in cancer treatment, photothermal therapy (PTT) and photodynamic therapy (PDT) have become major tools with impressive outcomes [38–42]. In addition to the direct tumor cell destruction via photothermal interactions through PTT and photochemical interactions through PDT, phototherapy also induces immunogenic cell death, leading to the release of tumor associated antigens (TAAs), tumor specific antigens (TSAs), and danger associated molecular patterns (DAMPs) [43, 44]. Additionally, targeted ablation has another important function: it can disrupt the established TME, potentiating immune cell infiltration and tumor killing. However, the impacts on the TME by phototherapies alone have achieved limited success in treating cancers, particularly metastatic cancers.

By using phototherapy alone for cancer treatment, we tend to lose sight that the tumor is self-tissue, to which the adaptive immune system is selected not to respond. While tumor antigens are immunogenic, because TSAs can generate T cell responses capable of eliminating tumors, we hypothesize that these immunogenic TSAs are far less abundant than “self” antigens. To generate an effective systemic response to the TSAs, addition of a strong immune stimulant is required. This is evidenced by the additive, often synergistic, effect observed when PTT or PDT is combined with an immunostimulant, resulting in enhanced elimination of treated primary tumors and untreated metastases [45–47]. However, in many cases, this combination can enhance animal survival but not eliminate the targeted tumor [48–50]. While the reasons for this incomplete elimination of the tumors have not been widely explored in detail, the tumor intrinsic and extrinsic factors that contribute to resistance to immunotherapy treatment in humans likely play a dominant role.

Nanoparticles have been used for years in PTT and PDT [48, 51, 52]. They have been used as light-absorbers for PTT, photosensitizers for PDT, and/or as carriers of phototherapy agents specifically to target tumor cells. Recently, however, more investigators have been taking advantage of the dynamic nature of nanoparticles and using them as a multipurpose tool to deliver TME modulators, chemotherapeutic drugs, and/or immune stimulants simultaneously and specifically to tumors [53, 54]. This delivery system reduces off-target effects, improves pharmacokinetics, and limits side effects imposed by systemic treatments. While nanoparticles loaded with tumoricidal cargos have achieved certain success, they have not resulted in high curative potential when not combined with ablation [55–57].

Nanotechnology-based ablative immunotherapies can be a targeted, multifaceted approach to overcome such limitations. Immune-ablation combined with nanoparticles can help overcome tumor intrinsic and extrinsic factors. We term this combinatorial approach nano- ablative immunotherapy (NAIT). The hypothesis behind NAIT is that the nanoparticles augment phototherapy for tumor ablation to disrupt TME homeostasis, releasing TAAs, TSAs, DAMPs, and stimulating recruitment of new immune cells to the TME. Adding the immune stimulation following ablation enhances the immune response to TSAs and ensures that the recruited immune cells in the treated tumor polarize into an inflammatory phenotype. Therefore, nanoparticles ensure the specificity of the tumor ablation and the specific release of the immune stimulants. In addition, the nanoparticles should also release cargos that directly target the tumor cells and/or the TME to prevent the remaining tumor cells from adapting and re-establishing an immunosuppressive TME.

Specifically, the locally administered nanoparticles, TME modulators, chemotherapeutics, and/or immunostimulants, either before and/or after photoablation, could effectively remodel the immunosuppressive TME, hence successfully modulating the co-evolvement between tumor cells and immune cells in the TME. The concept of NAIT is depicted in Figure 1. As shown in Figure 1, the optimal approach is to start with delivery of a nanoparticles that carry different therapeutic agents, particularly immunological stimulants, chemotherapeutics, and TME modulators, to the target tumor. The phototherapy-potentiated tumor ablation will destroy tumors and induce immunogenic tumor cell death to recruit immune cells. Any residual tumor/stromal cells left over from ablation will then be targeted by the chemotherapeutics, while the TME modulators help prevent the reestablishment of an immunosuppressive TME. The nanoparticles carried immunostimulant, when combined with the released tumor antigens and DAMPs, amplifies the proinflammatory activation of immune cells and antigen-presenting cells (APCs), such as dendritic cells (DCs). The matured APCs can then present the tumor antigens to the T cells in tumor-draining lymph nodes (TDLNs) for T cell activation and proliferation. Finally, these T cells, primed against the treated tumors, can enter the TME and eliminate the residual tumors at the treatment site and the untreated distal metastases. A similar process can also occur in the spleen, which is where T cells also develop and are critical for the effectiveness of immunotherapies [58, 59].
In scenario depicted in Figure 1, the TME modulators and the chemotherapeutics play a much more significant role in the untreated metastases, allowing for recruitment of inflammatory cells into the TME through chemotherapy induced ICD of the tumor cells. Furthermore, the TME modulators will help suppress the immunosuppressive functions of metabolites and cytokines within the TME, enhancing the antitumor function of the recruited antitumor immune cells. The immune stimulant will further recruit inflammatory immune cells to enter the metastatic TME and help prolong/enhance their tumor killing activity to eventually eliminate the tumor or keep it from growing. One important fact is that each tumor is unique, and the TME is ever evolving and adapting to prevent immune mediated killing. Additionally, TMEs of the metastases can be different from TMEs of the primary tumors. The nanoparticles used in NAIT can potentially overcome these issues as they are customizable and can be combined with a variety of chemotherapeutics, TME modulators, anti-inflammatory cytokines and metabolite inhibitors, and/or immune modulators to enhance the elimination of the untreated metastases, as shown in Figure 2.

In summary, the combination of nanotechnology, photonics, and immunotherapy possesses great potential in cancer treatment. It can balance the 3 Es of the immunoediting process by positively shaping the TME and the entire immune system. It also primes the immune system for other therapies, particularly ICT. The future studies...
Figure 2: Illustration of the addition of nanoparticles loaded with chemotherapeutics, immune stimulants, and TME modulators, as well as their effects on the untreated metastatic tumors.

A. The chemotherapeutics result in immunogenic cell death and the release of tumor antigens and DAMPs, while the immune stimulants enhance the antitumor immune responses generated by the tumor antigens and DAMPs. The TME modulators help sustain tumor killing and prevent and/or slow the reestablishment of the immunosuppressive TME.

B. Without the addition of nanoparticles loaded with the therapeutic cargos, the immune response to the metastases is limited since the TME is not altered apart from a few CTLs. CTL, cytotoxic lymphocyte; TAM, tumor associated macrophages; Treg, T regulatory cell; DC, dendritic cell; NK, natural killer cell; CAF, cancer associated fibroblast; NKT, natural killer T cell.
should aim at taking full advantage of developing optimal combinatorial approaches using these technologies for clinical applications to treat cancer.

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**References**


