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Antibody Drug Conjugates of Near-Infrared Photoimmunotherapy (NIR-PIT) in Breast Cancers

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Abstract

Worldwide, the incidence rate of breast cancer is the highest in women. Approximately 2.3 million people were newly diagnosed and 0.685 million were dead of breast cancer in 2020, which continues to grow. Triple-negative breast cancer (TNBC) is the most aggressive breast cancer subtype with a higher risk of recurrence and metastasis, but disappointly, there are no effective and specific therapies clinically, especially for patients presenting with metastatic diseases. Therefore, it is urgent to develop a new type of cancer therapy for survival improvisation and adverse effects alleviation of breast cancers. Near-infrared photoimmunotherapy (NIR-PIT) is a newly developed, photochemistry-based cancer therapy. It was drive by an antibody–photoabsorber conjugate (APC) which is triggered by near-infrared light. The key part of APC is a cancer-targeting monoclonal antibody (mAb) that can bind to receptors or antigens on the surface of tumor cells. Because of this targeted conjugate accumulation, subsequent deployment of focal NIR-light results in functional damage on the targeted cell membranes without harming the immediately adjacent receptor-negative cells and evokes a kind of photochemical, speedy, and highly specific immunogenic cell death (ICD) of cancer cells with corresponding antigens. Subsequently, immature dendritic cells adjacent to dying cancer cells will become mature, further inducing a host-oriented anti-cancer immune response, complicatedly and comprehensively. Currently, NIR-PIT has progressed into phase 3 clinical trial for recurrent head and neck cancer. And preclinical studies have illustrated strong therapeutic efficacy of NIR-PIT targeting various molecular receptors overexpressed in breast cancer cells, including EGFR, HER2, CD44c, CD206, ICAM-1 and FAP-α. Thereby, NIR-PIT is in early trials, but appears to be a promising breast cancer therapy and moving into the future. Here, we present the specific advantages and discuss the most recent preclinical studies against several transmembrane proteins of NIR-PIT in breast cancers.

Keywords

breast cancer, near-infrared photoimmunotherapy (NIR-PIT), antibody–drug conjugate, mAb-IR700, cancer therapy

Abbreviations:

APC, antibody–photoabsorber conjugate; CSCs, cancer stem cells; DCs, dendritic cells; EGFR, epidermal growth factor receptor; EPR, enhanced permeability and retention; FAP-α, fibroblast activation protein alpha; HER2, EGFR type; ICAM-1, intercellular adhesion molecule-1; ICD, immunogenic cell death; IR700, IRDye700DX; mAb, monoclonal antibodies; MDSCs, myeloid-derived suppressor cells; NIR, near-infrared; PIT, photoimmunotherapy; PTT, photothermal therapy; PDT, photodynamic therapy; SUPR, super-enhanced permeability and retention; TNBC, triple-negative breast cancer

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Introduction

Breast cancer is the most common malignant cancer among women in China, occupying approximately 15% of new diagnosed cancers.[1,2] Compared to other subtypes, triple-negative breast cancer (ER−/PR−/HER2−, TNBC) shows unfriendly characters of poorer prognosis, poor survival rates and higher metastasis rates because of its limited molecular targets and primary or secondary drug chemotherapy resistance.[3–6] For surgeons, performing entire tumor resections often present a major burden, particularly when the tumors infiltrate vital organs and vasculature.[7] Radiotherapy and chemotherapy also have significant limitations that inevitably cause toxic effects to normal cells including immune cells, which go against patients' physical recovery and ultimately lead to overall debilitation.[8] Current cancer immunotherapies offer hope of overcoming challenges, which destruct cancer cells by activating cytotoxic immune cells to stimulate systemic immune responses and resist tumor metastasis and recurrence, rather than killing cancer cells straight. A therapy that utilizes the host immune system is theoretically possible. But excessive tumor burden may overwhelm the anti-cancer ability of the host immune system. Moreover, non-specific activation of the immune system can cause autoimmune-like damage to normal tissues.[9] Therefore, it is urgent for a novel and synergistic cancer treatment to breakthrough these therapeutic bottlenecks.

Photoimmunotherapy (PIT), which is a synergistic strategy associated phototherapy with immunotherapy against cancers, demonstrates favorable properties. Near-infrared photoimmunotherapy (NIR-PIT) is dependent on near-infrared (NIR) light, ensuring selectively kill cancer cells while causing no harm to normal tissue.[10] This molecularly targeted phototherapy is based on systemically injecting a conjugate of a near-infrared, water-soluble, silicon–phthalocyanine derivative, IRdye700DX (IR700), and a monoclonal antibody (mAb). Because monoclonal antibodies can selectively bind to antigens on cancer cell surfaces, then irradiating NIR light can drives photochemical transformations of the antibody–photoabsorber conjugate (APC) and eventually destruct the cell membrane and cause cell rupture (Figure 1).[11–14] Moreover, NIR-PIT can clearly differ from conventional photodynamic therapy (PDT) or photothermal therapy (PTT), which rely on cytotoxic singlet oxygen and hyperthermia, respectively. It mainly activates immunity response through the induction of immunogenic cell death (ICD) of cancer cells to play an anti-cancer role.[14,15] Results of the Phase 1/2 clinical trials of NIR-PIT targeting epidermal growth factor receptor (EGFR) against inoperable head and neck squamous cell cancer presents great potential. A global phase 3 clinical trial of that is ongoing. [16] Thus, NIR-PIT appears to be a novel cancer therapy with great potential and remains to be deeply explored. Here we present current evidence on NIR-PIT mechanisms of action and discuss the most recent preclinical studies of NIR-PIT against various effective molecular targets in breast cancers.

Insights into NIR-PIT Mechanism(s) of Action

Highly Specific Cell Killing

A superiority of NIR-PIT compared with other conventional cancer therapies is specificity and selectivity for killing cancer cells resulting from the nature of mAbs. Since the mAbs tends to bind cancer cells that overexpress the targeted cancer-associated antigens, near-infrared light activation at 690 nm choose to kill those cells rather than adjacent normal cells.[10] In addition, as a non-ionizing radiation, NIR light does not cause damage to DNA, and non-target cells suffer no toxic effects because its dosage does not obviously alter tissue temperature. Furthermore, by itself, IR700 is a water-soluble photoreactive dye and is not inherently phototoxic; therefore, unconjugated IR700 dissociated from APC is easily excreted in urine without residue.[10,17,18] This highly targeted cancer works unless combining the target-specific APC with the tumor-targeted exposure of NIR light. Actually, many patients have showed significant shrinks in tumor size in the clinical trials while experiencing few side effects.

Immunogenic Cell Death (ICD)

Highly selectively cancer cell death induced by NIR-PIT can evokes multiple tumor-specific immune response. Subsequent deployment of focal NIR-light is invitation to structural changes in APC and some danger signals of membrane destruction. Subsequently, local dendritic cells are initiated, and cancer-specific naïve T cells are stimulated to become mature and proliferate resulting in wider immune response. This process is known as ICD.[15,19–21] Moreover, NIR-PIT also rapidly releases various neoantigens which can be recognized and responded by newly primed $CD8^+$ T cells and enhances activation of the adaptive systemic immune response. In addition, the effect is not limited in the NIR-PIT treated tumors, a similar type of tumors located elsewhere in the body also stop growing, which means distant effects.

To further enhance the cancer-targeted NIR-PIT immune response, it is possible to combine conventional systemic immunotherapy. In animal models, the group that received a combination of NIR-PIT and an immune checkpoint inhibitor showed immediate tumor shrinkage and better curation within a few days. Moreover, it was not only presented in situ effects, distant responses such as metastasis also disappeared within days. And the failure in animals that experienced a complete response to re-vaccinate the tumor also suggested that these mice treated with NIR-PIT had acquired immunity against the initial tumor (Figure 2).[12,20] Simultaneously, NIR-PIT can take an effective control over immunosuppressive cells in the tumor microenvironment by targeting immunoregulatory cells including Treg and myeloid-derived suppressor cells (MDSCs), contributing to restore normal immune system function and overcome bottlenecks of conventional immunologic drugs including some immune checkpoint inhibitors. Therefore, the combination of NIR-PIT and several adjuvant immunotherapies may be a promising new form of NIR-PIT.

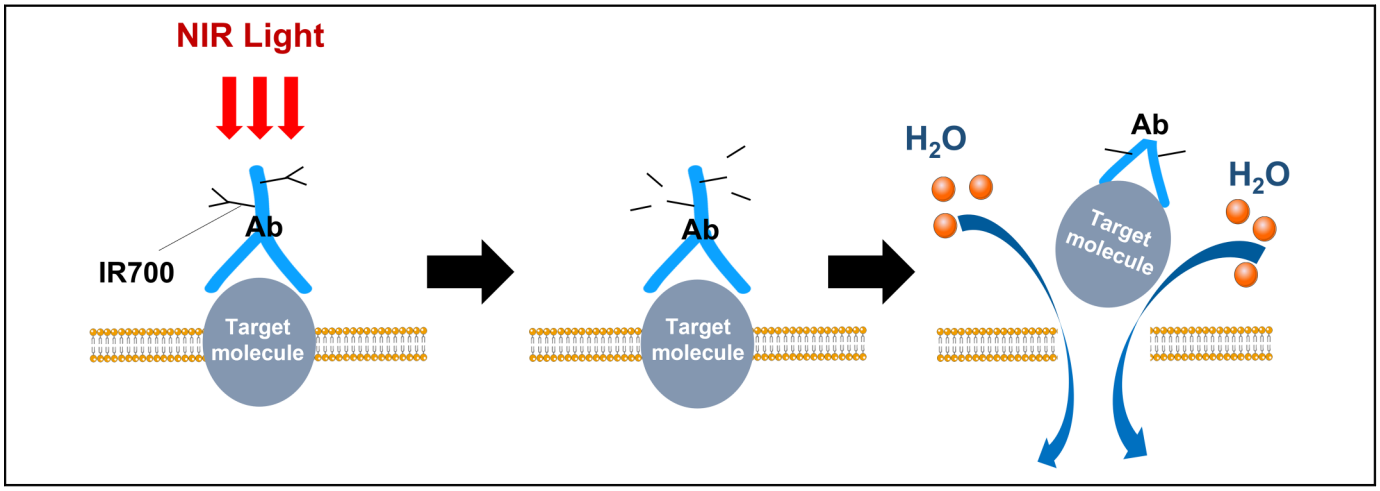

Figure 1. A scheme for demonstrating cellular cytotoxicity induced by NIR-PIT.

Super-Enhanced Permeability and Retention (SUPR) Effects

The instant effect on tumor vascularity is one of the unique advantages of NIR-PIT. Indeed, a majority of tumors exist vascular leakiness contributing to enhanced permeability and retention (EPR), but NIR-PIT result in a more significant enhancement in vascular permeability because of urgent and enormous cancer cell death adjacent to tumor vessels, a phenomenon named as the super-enhanced permeability and retention (SUPR).[22–25] SUFR after NIR-PIT lead to further enhancement of the delivery volume of multiple nano-sized or macromolecular drugs. The core cause is the space enlargement between the blood vessels and the remaining tumor tissues, where the perivascular cancer cells are triggered immediate necrosis and subsequently blood volume and velocity increases and decreases, respectively (Figure 3).

Therefore, a nano-sized anti-cancer agent combined with NIR-PIT could be more effective than either single therapy.[24,26] Other than special drugs, SUPR effects also permit enhanced delivery for other antibodies and APCs, whose intertumoral distribution is improved by enlarged leakage into the tumor bed after NIR-PIT.[27–29] A study utilizing FDA approved daunorubicin (DaunoXome) encapsulated with liposome and nanoparticle paclitaxel (nab-paclitaxel; Abraxane) bound with albumin-in mouse xenograft models of cancer, demonstrated that NIR-PIT cooperated with either drug showed greatest advantage.[30,31] Furthermore, uncaging reactions induced by NIR light could transmit bioactive compounds encapsuled in particles to any favorable site of the body, illustrating a remarkable chemical progress and prospect of ongoing study.[32,33] A research showed that at the absence of NIR, utilizing panitumumab-IR700 (pan-IR700) or CyEt-Panitumumab-Duocarmycin (CyEt-Pan-Duo), a kind of NIR-releasing compound, showed a higher efficiency and more homogeneous distribution in delivered dose because of the SUPR effect, compared with either NIR-PIT or NIR-release therapy alone, which potentially represents a novel combination to achieve stronger antitumor effects.[34]

The Preclinical Studies of NIR-PIT in Breast Cancers

Recently, several preclinical results have showed latent breakthroughs of NIR-PIT for the treatment of breast cancers, especially TNBC, one of the most malignant tumors in females, whose frequent occurrence of pleural metastasis further worsens patient prognosis.[17] The most representative studies are illustrated based on the involved body system, as described in Table 1.

EGFR ubiquitously regulates epithelial tissue development and homeostasis with a high overexpression in 70% of TNBCs, so anti-EGFR is an ideal target for NIR-PIT. In this aspect, a remarkable tumor suppression and a prolonged survival rate was presented in athymic nude mice suffering from EGFR-positive human TNBCs with a treatment of cetuximab-IR700 or panitumumab-IR700 and in combination with NIR-light.[17,34,35] CyEt-Panitumumab-Duocarmycin degree of labeling 4, CyEt-Pan-Duo, bonds tightly in EGFR receptor expressing in specific cancer cells and subsequently releases duocarmycin after NIR light inducement. Previous researches about NIR-PIT demonstrated enhanced microdistribution of CyEt-Pan-Duo and therapeutic responses on MDAMB468 tumors with EGFR-expression, illustrating the greatest therapeutic effect of the combination of NIR-PIT and NIR-release therapy.[34] Moreover, the comprehensive application of nanotherapy and NIR-PIT is appealing to researchers. A novel type of cetuximab-targeted nanoparticle, named as CTX-AuNR, on the condition of NIR irradiation, could fully exert the abilities of ROS generation and confrontation with hypoxia microenvironment in TAM-embedded BT-20 spheroids, and regulation of reprograming TAM to the anti-tumor M1 phenotype in TME, which brought an inspiration for strengthening therapeutic effects and overcoming conventional drug resistance to patients with EGFR-overexpressing and TAM infiltrated TNBC.[35] In addition, a study performed bioluminescence imaging depended on ATP in MDA-MB-468luc cell line with the expression of luciferase and EGFR, to assess the therapeutic effectiveness of different light power

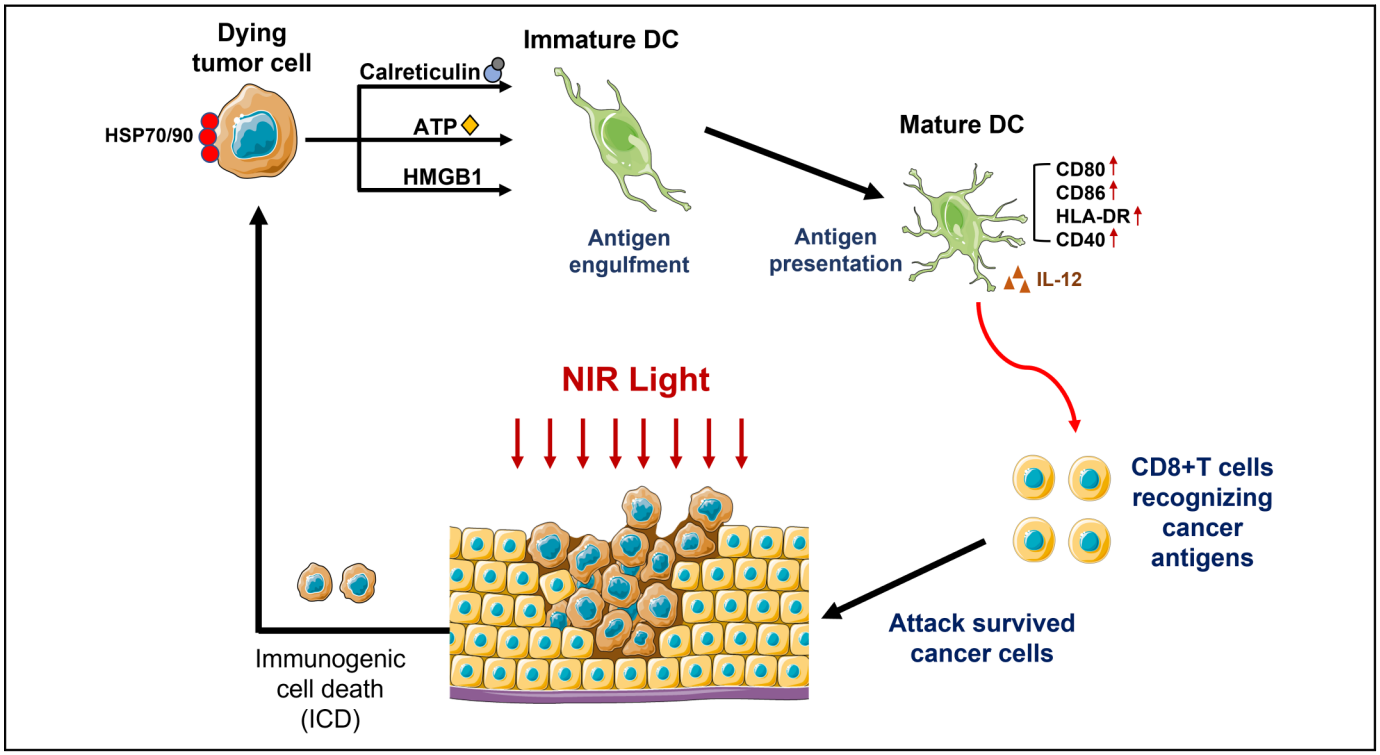

Figure 2. Schematic presentation of immune response after NIR-PIT.#

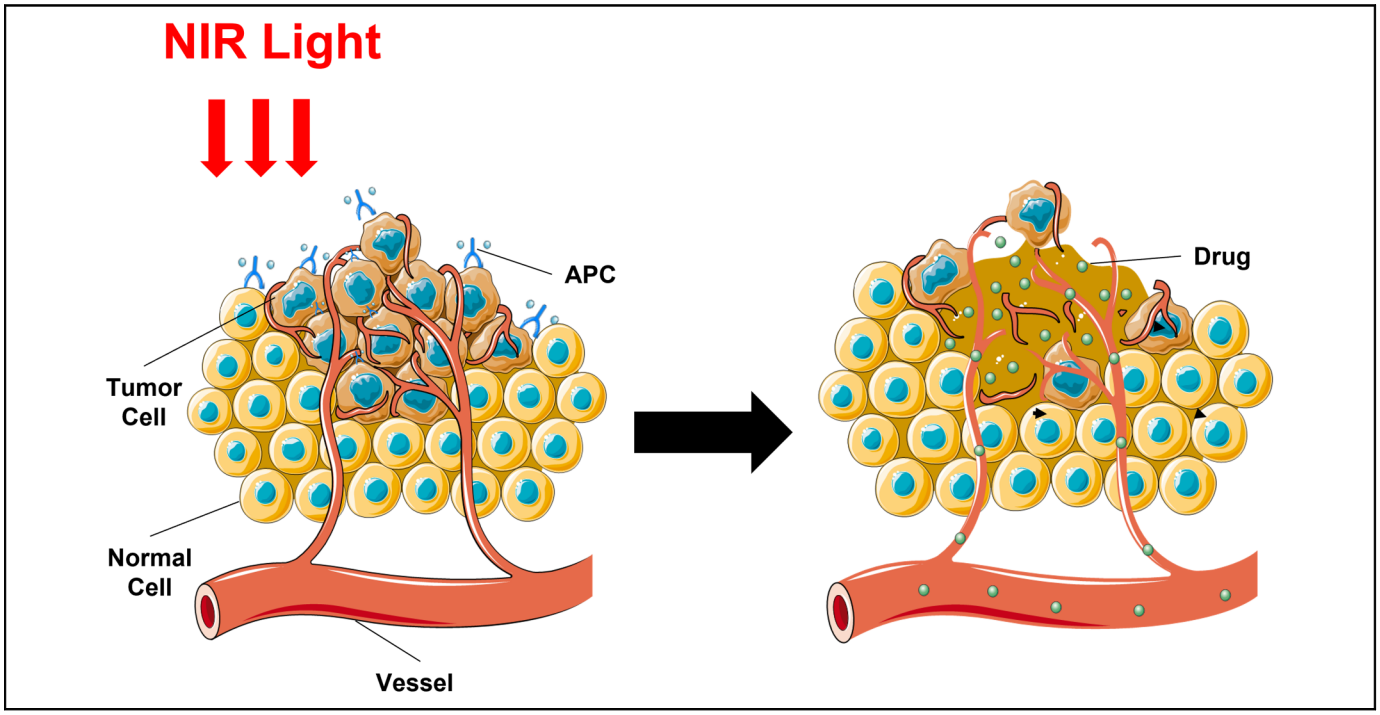

Figure 3. Schematic demonstration for mechanism of SUPR effects induced by NIR-PIT.#

and dose of PIT components. Results showed that increasing the dose of Pan-IR700 could offset lowering effects caused by the lessen light power to achieve the equal level of efficacy, which provide a method to reduce cytotoxicity as possible.[14]

Similarly, CD44, a famous cancer stem cells (CSCs) marker, is a non-kinase transmembrane glycoprotein to mediate uncontrollable cell growth and viciously cell migration, which has been generally acknowledged as a meaningful diagnostic and

Table 1. The Main Preclinical Studies Investigating the Potential Breakthroughs of NIR-PIT for Breast Cancers.

Antigen targeted	Conjugated	Cell lines used in *in vivo* experiments	Year
EGFR[17]	Anti-EGFR (Cetuximab) moAb	MDAMB231, MDAMB468	2015
EGFR[34]	Anti-EGFR (Panitumumab) moAb	MDA-MB468	2018
EGFR[35]	Anti-EGFR (Cetuximab) moAb	BT-20, MDA-MB453	2021
CD44[36]	Anti-CD44 moAb	MDA-MB-231, BT-474	2016
HER2[37]	Anti-HER2 (Trastuzumab) moAb	SKBR3, MDA-MB-231, and MCF7	2014
HER2[38]	Anti-HER2 (Trastuzumab) moAb	BALB/3T3 cells, HCC-1419 cells	2017
HER2[39]	HER2 Affibody	SK-BR3, BT474, MDA-MB361	2019
HER2[40]	Anti-HER2 (Trastuzumab) moAb and HER2 Affibody	SK-BR3, MDA-MB361, JIMT1	2021
CD206[41]	Anti-CD206 moAb	4T1	2016
ICAM-1[42]	Anti- ICAM-1 moAb	MDAMB468-luc, MDAMB231 cells	2022
FAP-α[43]	Anti-CD44 moAb (AF3715)	MDA-MB-231	2022

EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor-2; ICAM-1, intercellular adhesion molecule-1; FAP-α, fibroblast activation protein alpha.

prognostic biomarker.[44,45] And a study demonstrated that either moAbs or small protein mimetic affibodies conjugated to the phthalocyanine dye IRDye700DX could result in the selective injury of breast cancer cells and dramatic reduction of tumor growth when exposed under NIR-light at 690 nm.[36]

For patients suffering with breast cancers, the incidence of HER2 positivity have been reported ranging from 15% to 20%.[45] Nowadays, HER2 is an accepted therapeutic target of breast cancers and trastuzumab has been authorized for treating HER2-positive breast cancer by the FDA.[36] However, trastuzumab-dependent therapy has limitations in the clinical application. Firstly, HER2-expressing tumors only accounted for approximately one fourth of primary breast cancers, resulting in a small range application. Moreover, many patients with therapeutic response to trastuzumab at first inevitably develop drug resistance to cause disease progression. Therefore, a research used a adenoviral vector with deficiency in replication, bore a gene that encoded the HER2 extracellular domain (Ad-HER2-ECD). When synergized with trastuzumab-IR700, the vector could transduce HER2-ECD into HER2-negative human cancer cells and selectively killed HER2-negative cells in vitro to further broad the range of clinical application. With the help of viral transduction technology, the indication of antibody-oriented therapy will expand even to the target-negative patients, which represents a potential method to break the limitations of, and overcome resistance to, cancer therapy.[37] Moreover, the extent of damage to cell viability depends on NIR-light-intensity and HER2 affibody–IR700 conjugate concentration. The application of affibody molecules may provide unique opportunities in the clinical translation of NIR-PIT because of their rapid tumor clearance and excellent tissue penetration.[39] Currently, the combination of NIR-PIT using both HER2 Affibody–IR700Dye conjugate and trastuzumab–IR700Dye conjugate maximized the proportion of necrotic cell death in HER2-positive breast cancer cells and rarely killed HER2-negative breast cancer cells. In addition, it was also exciting that this combination of NIR-PIT exerted significantly therapeutic effects against cancer cells with low expression of HER2, trastuzumab-resistant cells (JIMT1), and brain metastatic cells of breast cancer (MDA-MB361).[40] These findings demonstrate that affibody-based PIT is a favorable alternative to moAb-based options, especially for patients with acquired resistance to other conventional anti-HER2 therapies. Due to limits in depth penetration of NIR light, trastuzumab emtansine (T-DM1), an antibody–drug conjugate composed with the monoclonal antibody trastuzumab bonded to the cytotoxic agent maytansinoid DM1, and IR700 plus NIR light treatment, was employed to trigger greater cytotoxic effects for HER2-expressing cells in vitro than T–IR700 plus NIR light treatment, which represents a novel NIR-PIT agent for treating large tumors that always cause failure in sufficient NIR light irradiation to activate the photoabsorber IR700.[38]

ICAM-1 is a kind of transmembrane proteins as well as a molecule of the Ig superfamily with a function of cell adhesion. TNBC possess higher ICAM-1 expression compared with other subtypes of breast cancers.[42,46–48] The application of ICAM-1-targeted NIR-PIT killed TNBC cells in a dose-dependent way in vitro. Furthermore, under the treatment of ICAM-1-targeted NIR-PIT, targeted cells showed early histological signs of cell injury, including widespread cytoplasmic degeneration. The inhabitation of subsequent tumor growth and improvement of survival rate also revealed the destruction of cancer cells caused by NIR-PIT.[42]

In the process of cancer development, cancer cells are infiltrated by myeloid cells, including tumor-associated macrophages (TAMs), which are recruited from the peripheral blood to the microenvironment of tumor in the shape of monocyte precursor form.[49] CD206, named as macrophage mannose receptor, is specifically expressed on the surface of non-inflammatory M2 macrophages on the condition of alternative activation.[50–52] A research has reported that a CD206-oriented PIT probe conjugating a monoclonal anti-CD206 antibody with IRDye700, could not only significantly suppress the growth of tumor subcutaneously injected with 4T1 cancer cells resistant to sorafenib, but prevent the metastasis to lungs under NIR exposure, suggesting the potential role of TAM-targeted PIT in the treatment of tumors with congenital or acquaint resistance to conventional drugs.[41]

Fibroblast activation protein alpha (FAP-α) is a crucial target mainly expressed by cancer-associated fibroblasts (CAFs), exerting the protumorigenic and immune suppressive functions. Compared with adjacent normal tissue, invasive breast cancer tissue had the higher proportion of FAP-α + CAFs. And when co-cultured with cancer cells, FAP-α expression in fibroblasts was further improved. Therefore, a research tried to conjugate AF3715, a kind of antibody with high binding affinity to FAP-α, and the phthalocyanine dye IR700, to achieve a complex, FAP-α-IR700. Subsequently, its effectiveness in specifically reducing FAP-α expressing cell populations with PIT was estimated, with results showing that FAP-α-IR700-PIT effectively inhibited tumor growth, which provide a new promising strategy to eliminate FAP-α + CAFs.[43]

Limits and Future Perspectives for NIR-PIT

NIR-PIT shows great superiority over conventional cancer treatments. However, some limitations of NIR-PIT remain to be settled. Firstly, NIR-light penetration may restrict the application of NIR-PIT, especially in large tumor sizes, which retard the intraoperative clearance of minimal residual focus or lymph node metastases. Therefore, NIR light exposure through intestines into depth may be an effective method, which also requires the helps of fibreoptical diffusers to realize.[53,54] The combinational employment of external and interstitial exposure of NIR light can be readily accessible to clinical practice and realize a more stable and homogeneous light distribution. Secondly, initiatively occurring cancers are not always expressing a single tumor-specific antigen, suggesting the efficiency of simultaneously targeting various antigens. In the current studies, a cocktail of APCs against different tumor antigens could greatly solve this dilemma.[55] Thirdly, most studies in vivo have been performed in immunodeficient mice, most commonly athymic nude mice, which still exist a certain gap between clinical researches and animal xenografts. Therefore, what require substantial further exploration are that how host immune cells and systemic antitumor immunity activated by NIR-PIT exactly contribute to the clearance of distant metastases and the prevention of tumor relapse in details. Lastly, despite of the ambiguity of the most suitable treatment domains of NIR-PIT for different types of tumors, it is crucial factors that the expressional level of targeted molecule on the tumor cell surface or distribution of immune cells with antitumorigenic or pro-tumorigenic effects in TME, in order to select ideal patients and combinations of NIR-PIT. However, further investigation remains to be explored to identify the best possible combination strategy with NIR-PIT in different tumor types.

Conclusion

Breast cancer is the most common malignant cancer among women both in China and worldwide. For nonmetastatic breast cancer, the main goals of therapy are to clear tumor from the breast and regional lymph nodes as thoroughly as possible, and prevent metastatic recurrence. However, for patients suffering from metastatic breast cancers, therapeutic goals are prolonging life and symptom palliation.[4] NIR-PIT is a currently novel type of molecular antigen-oriented and photochemistry-based cancer treatment with the ability of selectively killing tumor cells and highly enhancing the host immune response, including promoting maturation of DCs and initiating cytotoxic T cells to interplay with cancer-related antigens released from destroyed cancer cells. In addition, the systemic antitumor immunity initiated by NIR-PIT contributes to the elimination of distant metastases and the prevention of tumor recurrences. When employed in combination with immunostimulatory therapies, NIR-PIT enable exert more extensive anti-cancer effects and offer a chance to estimate the potential applicability of follow-on or concomitant treatments by both fluorescent imaging and particularly immediate quantitative assessment on antigen release and immune cell activation induced by NIR-PIT. Thus, NIR-PIT is a potential therapeutic approach to breast cancers, indicating a bright development prospect of NIR-PIT.

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Contributors

YC and YX wrote the manuscript. YS and YL prepared paper materials. JH designed and produced the figures. XL and JJ outlined and supervised and edited the draft and approved of the final version to be submitted. All authors approved the final version of the manuscript.

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