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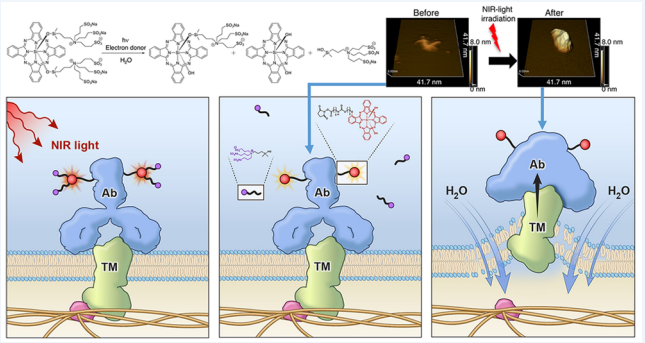
Near-Infrared Photoimmunotherapy of Cancer

Hisataka Kobayashi* and Peter L. Choyke

Molecular Imaging Program, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Building 10, Room B3B69, MSC1088, Bethesda, Maryland 20892-1088, United States

Web-Enhanced Feature

CONSPECTUS: This Account is the first comprehensive review article on the newly developed, photochemistry-based cancer therapy near-infrared (NIR) photoimmunotherapy (PIT). NIR-PIT is a molecularly targeted phototherapy for cancer that is based on injecting a conjugate of a near-infrared, water-soluble, silicon-phthalocyanine derivative, IRdye700DX (IR700), and a monoclonal antibody (mAb) that targets an expressed antigen on the cancer cell surface. Subsequent local exposure to NIR light turns on this photochemical "death" switch, resulting in the rapid and highly selective immunogenic cell death (ICD) of targeted cancer cells. ICD occurs as early as 1 min after exposure to NIR light and results in irreversible morphologic changes only in target-expressing cells based on the newly discovered photoinduced ligand release reaction that induces physical changes on conjugated antibody/antigen complex resulting in functional damage on cell membrane. Meanwhile, immediately adjacent receptor-negative cells are totally unharmed. Because of its highly targeted nature, NIR-PIT carries few side effects and healing is rapid. Evaluation of the tumor microenvironment reveals that ICD induced by NIR-PIT results in rapid maturation of immature dendritic cells adjacent to dying cancer cells initiating a host anticancer immune response, resulting in repriming of polyclonal $CD8^+$T cells against various released cancer antigens, which amplifies the therapeutic effect of NIR-PIT. NIR-PIT can target and treat virtually any cell surface antigens including cancer stem cell markers, that is, CD44 and CD133. A first-in-human phase 1/2 clinical trial of NIR-PIT using cetuximab-IR700 (RM1929) targeting EGFR in inoperable recurrent head and neck cancer patients successfully concluded in 2017 and led to "fast tracking" by the FDA and a phase 3 trial (https://clinicaltrials.gov/ct2/show/NCT03769506) that is currently underway in 3 countries in Asia, US/Canada, and 4 countries in EU. The next step for NIR-PIT is to further exploit the immune response. Preclinical research in animals with intact immune systems has shown that NIT-PIT targeting of immunosuppressor cells within the tumor, such as regulatory T-cells, can further enhance tumor-cell-selective systemic host-immunity leading to significant responses in distant metastatic tumors, which are not treated with light. By combining cancer-targeting NIR-PIT and immune-activating NIR-PIT or other cancer immunotherapies, NIR-PIT of a local tumor, could lead to responses in distant metastases and may also inhibit recurrences due to activation of systemic anticancer immunity and long-term immune memory without the systemic autoimmune adverse effects often associated with immune checkpoint inhibitors. Furthermore, NIR-PIT also enhances nanodrug delivery into tumors up to 24-fold superior to untreated tumors with conventional EPR effects by intensively damaging cancer cells behind tumor vessels. We conclude by describing future advances in this novel photochemical cancer therapy that are likely to further enhance the efficacy of NIR-PIT.



1. BACKGROUND

Three major cancer therapies; surgery, radiation, and chemotherapy, have been the traditional mainstays of oncology treatment for over a half century. Each method aims to reduce cancer burden while minimizing side effects. However, each treatment is well-known to cause substantial damage to normal cells, including immune cells, which becomes counterproductive to recovery and ultimately contributes to the overall debilitation of the patient. A new approach, cancer immunotherapy, seeks to use T-cell activating cytokines, immune-checkpoint inhibitors, depletion of regulatory T-cells (Tregs), and cell-based therapies to selectively control tumor growth. These methods have proven effective in some patients despite substantial side effects. However, the current cancer immunotherapies do not directly instigate cancer cell death but, rather, kill cancer cells by activating cytotoxic immune cells.[1,2] Large cancer burdens may overwhelm the host immune system's ability to fight the cancer. Meanwhile, nonspecific off-target activation of the immune system can cause autoimmune-like damage to normal tissue. Theoretically, a therapy that selectively kills cancer cells while activating the local host immune response would be ideal.

One such approach is near-infrared photoimmunotherapy (NIR-PIT).[3] NIR-PIT differs from conventional cancer therapies in its selectivity for killing cancer cells while

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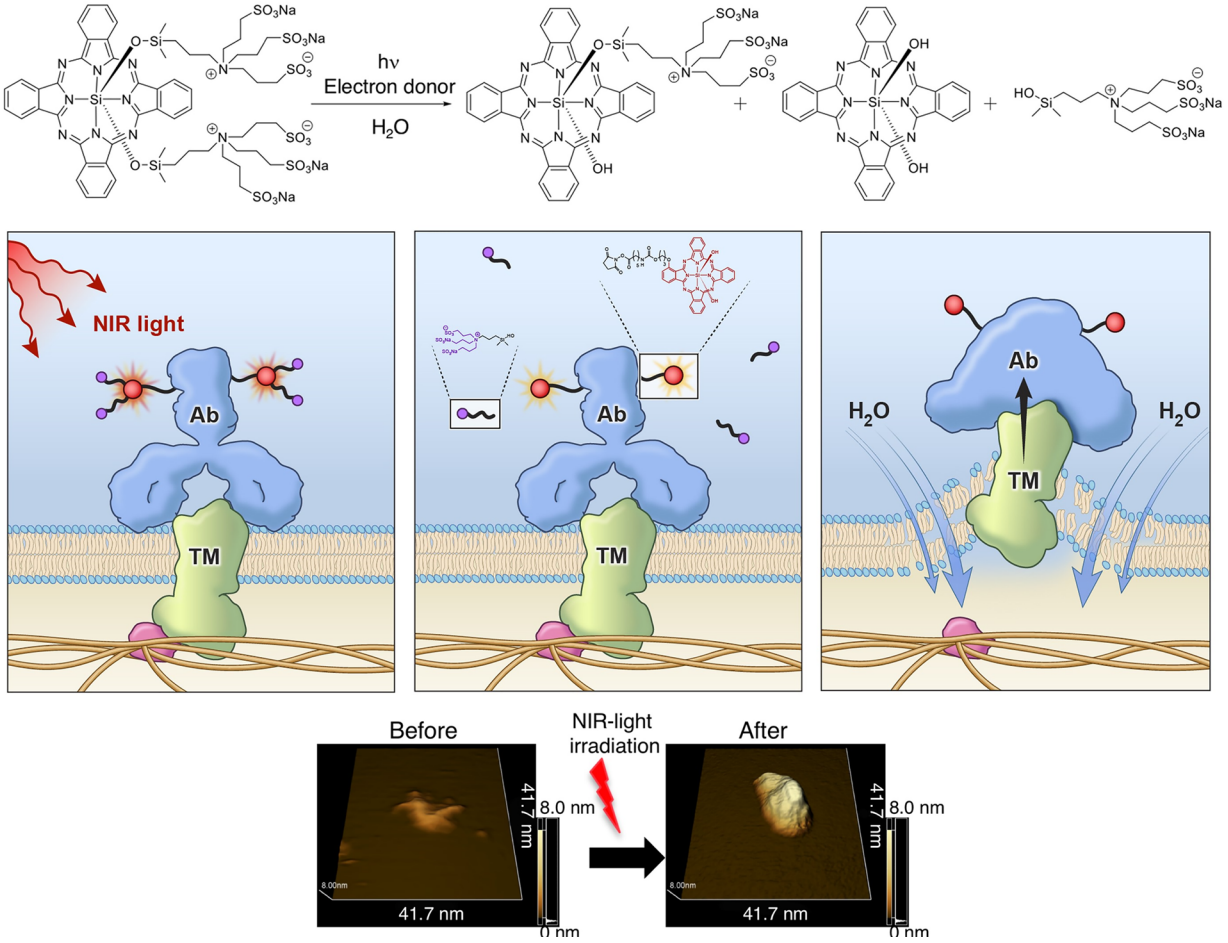

Figure 1. Scheme for chemistry basis of NIR-PIT (top), physical changes conjugated proteins (middle), and single antibody molecule imaging before and after NIR-PIT (bottom).

activating the host antitumor immune response. A first-in-human phase 1/2 clinical trial of NIR-PIT using cetuximab-IR700 (RM1929) targeting EGFR in patients with inoperable head and neck squamous cell cancer successfully concluded in late 2017. A "fast-tracked" global phase 3 clinical trial began in 2019 (https://clinicaltrials.gov/ct2/show/NCT03769506). Early results suggest NIR-PIT is superior to existing second and third line therapies for recurrent head and neck cancers. Thus, NIR-PIT appears to be a promising new form of cancer therapy.

NIR-PIT is based on the injection of a conjugate of an antibody, which binds a cell surface marker on the cancer, and a photoactivating chemical (APC). A major feature of NIR-PIT compared to other approaches is its specificity for cancer. The specificity derives from targeting by the monoclonal antibody (mAb). Cell killing is initiated by excitation of the antibody-bound photoactivating chemical, IRDye700DX (IR700), with near-infrared light at 690 nm. NIR light is nonionizing, causes no damage to DNA, is harmless to normal cells and penetrates a few centimeters into the tissue. Since the APC binds predominantly to cancer cells that overexpress the targeted cancer-associated antigens, light activation results in selective cancer cell killing while not harming adjacent normal cells including tumor infiltrating immune cells. Furthermore, by itself, IR700 is a water-soluble photo dye with no phototoxic or biotoxic properties of its own; therefore, unbound IR700 that dissociates from the APC is safe and is readily excreted in urine. The combination of the target-specific APC and the limited exposure of light to the tumor results in a highly targeted cancer therapy with minimal to no damage to normal tissues. This theory has been borne out in early phase 1/2 clinical trial results.

Importantly, unlike other traditional therapies, the highly specific cancer cell death induced by NIR-PIT does not compromise host immunity against cancer but even activates multiclonal tumor-specific immune response. In fact, the rapid nature of the cell death associated with NIR-PIT makes it highly immunogenic. NIR-PIT rapidly releases cancer-specific antigens and membrane damage danger signals which induce activation of local dendritic cells, which prime and educate cancer-specific naïve T cells leading to proliferation and cell-mediated cancer cell killing. This process is known as immunogenic cell death (ICD) and NIR-PIT is perhaps the best example of this mechanism of inducing host immunity. Therefore, NIR-PIT could overcome problems of conventional antibody-based therapy, including inhomogeneous or insufficient delivery of antibodies or ADC and tumor heterogeneity because NIR-PIT induced multiclonal immune response could eliminate surviving cancer cells after NIR-PIT, even if insufficient APCs bind to cancer cells because of inhomogeneous expression of target antigens or uneven delivery or insufficient dosage. Additionally, since NIR-PIT does not have limitation of repeated treatments, multiple NIR-PITs could also help overcome these problems.

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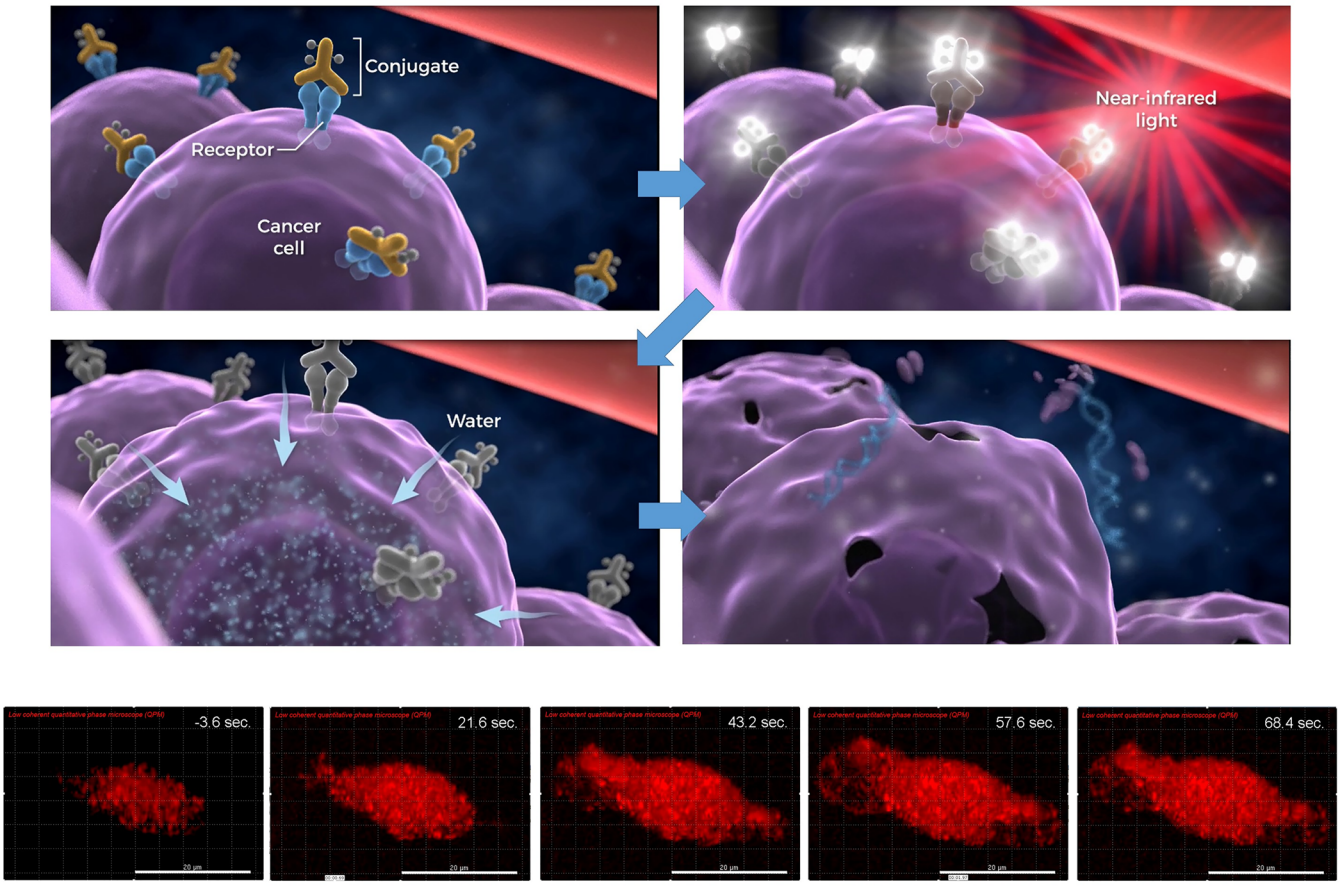

Figure 2. Scheme and serial microscopic images for cellular cytotoxicity induced by NIR-PIT (see Video).

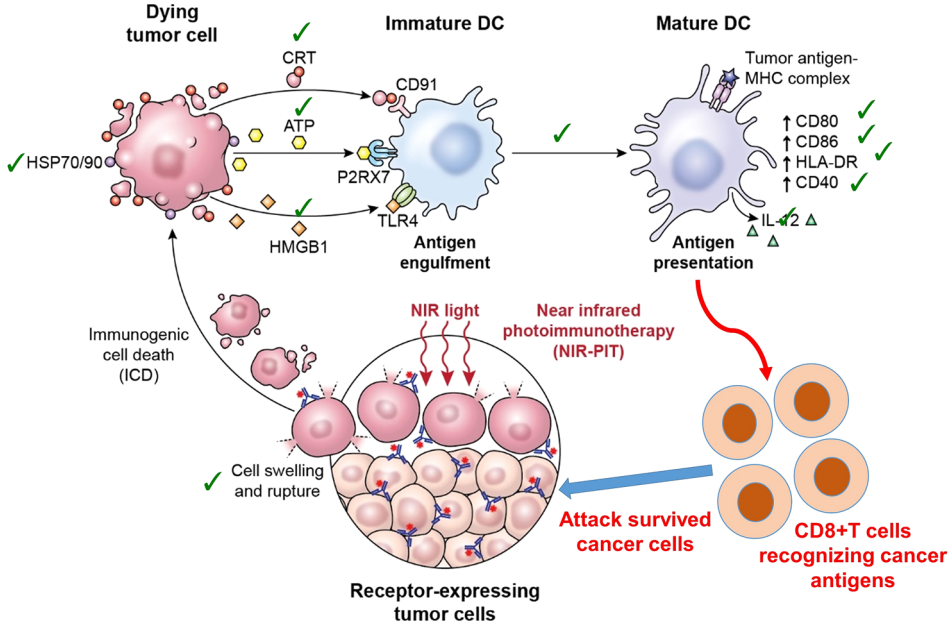

Figure 3. Biology of immunogenic cell death induced by NIR-PIT that leads to enhance antitumor host immunity against treated cancer cells.

2. MECHANISM OF CYTOTOXICITY

The molecular mechanisms of cell death caused by NIR-PIT have recently been elucidated.[4] Upon exposure to NIR, photoinduced chemical changes to the IR700 molecule itself and on the APC were identified. Under hypoxic or electron donor-rich conditions, which are common in NIR-PIT-treated tumor beds, IR700 undergoes photochemical ligand reactions that release the hydrophilic side chains of IR700 and cause the remaining molecule to become very hydrophobic (Figure 1).

This chemical change leads to the formation of a Z-stack multimer of silicon-phthalocyanine IR700 rings or water-insoluble aggregates of APCs or APC-antigen complexes

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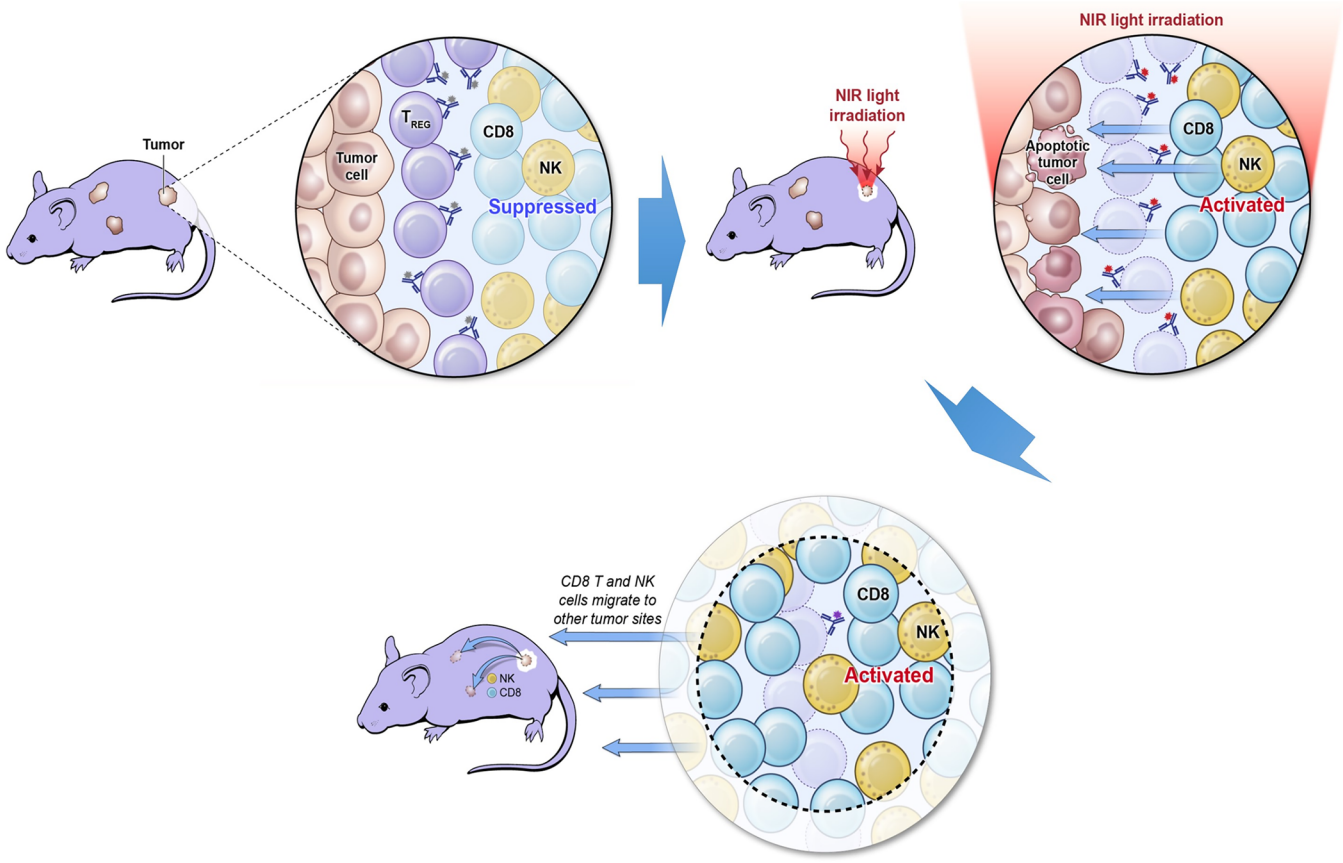

Figure 4. Selective depletion of regulatory T-cell (Treg) by NIR-PIT induced systemic antitumor host immunity.

leading to quenching of IR700 fluorescence. The photochemical ligand release reaction leads to physicochemical changes within the APC-antigen complex, which reduces cell membrane integrity because of damage to transmembrane target proteins.

The mechanism of cellular cytotoxicity underlying NIR-PIT was further investigated with three-dimensional dynamic live cell microscopy, radioactive and fluorescent probes, and biological markers.[5] For instance, three-dimensional dynamic low coherence quantitative phase microscopy (3D-QPM) was used to depict changes on the cellular membrane while dual-plane inverted selective plane illumination microscopy (diSPIM) was used for depicting the release of cellular contents immediately following NIR-PIT. The 3D-QPM imaging showed that cells initially swell by approximately 3-fold as water flows into the cell following damage to the cell membrane.(Figure 2).

Rapid swelling causes large tears in the membrane allowing the release of intracytoplasmic contents into the extracellular space. Observations made using diSPIM in cells expressing cytoplasmic green fluorescent protein (GFP) revealed that the GFP was confined within the cell during swelling but was quickly dispersed once the cell membrane ruptured at which time the cell volume abruptly decreased. Cell bursting was not prevented by considerable amounts of NaN_3, a singlet oxygen quencher, or when temperatures were set to 4 °C. However, when cells were placed in a hyperosmotic buffer with 50 mM dextran cell swelling was inhibited. Although organic and macromolecular fluorescent dyes were excluded from the during cell swelling, $H_2{}^{15}O$ readily entered cells immediately after NIR-PIT. The chemical and physical damage, which induced rapid swelling of the cell and disruption of the cell membrane are characteristic of ICD. NIR-PIT caused rapid activation of stress markers including heat shock proteins 70 and 90, dying signals such as calreticulin, ATP and HMGB1, which promote maturation of immature dendritic cells, followed by initiation of a host immune response against released antigens from dying cancer cells (Figure 3).

To investigate whether oxidative changes of lipid molecules caused by reactive oxygen species might account for the weakening of the cellular membrane lipid bilayer, mass spectroscopy was employed. Phosphatidylcholine was analyzed in cells before and after NIR-PIT. The results showed that 16–1 phosphatidyl choline, a major component of the lipid membrane, showed minimal oxidation to a hyper-oxide lipid. The amount was so minute—less than 1 ppm—even after exposure to NIR light in 100-fold excess, that it was unlikely to be responsible for the membrane disruption.[4]

3. MECHANISM OF IMMUNE ACTIVATION

NIR-PIT results in ICD that promotes maturation of immature dendritic cells in the immediate microenvironment of the cancer cell.[5] After cancer cell-targeted NIR-PIT, newly primed CD8+T cells reacted to a larger repertoire of cancer antigens compared with CD8+T cells before NIR-PIT, and proliferated in treated tumor beds.[6] Therefore, anticancer host immunity was strengthened after cancer-cell targeted NIR-PIT largely because of the re-education and subsequent proliferation of CD8+T cells. While cancer targeted NIR-PIT itself may not immediately kill all the cancer cells in a tumor, the host immune response appears to kill a high percentage of the remaining cells, at least in some instances. Thus, NIR-PIT can

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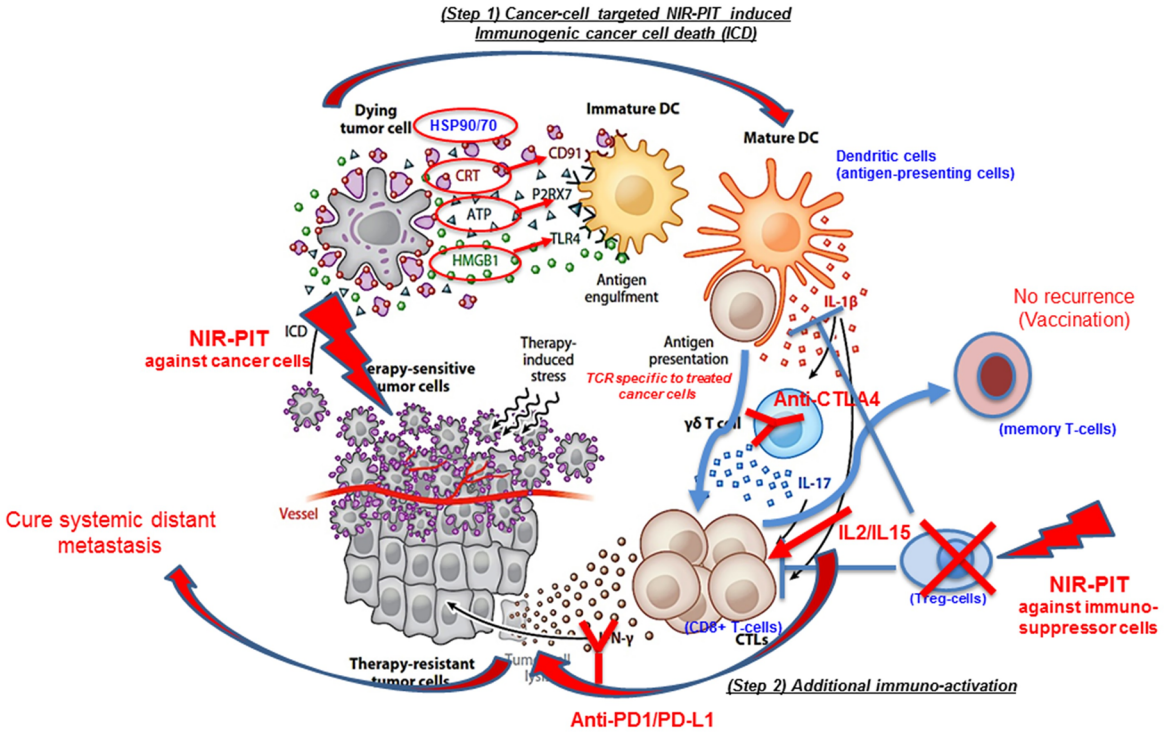

Figure 5. Combination of cancer-target NIR-PIT and immune-target NIR-PIT activates systemic antitumor host immunity for treating distant metastasis and induces immune memory for avoiding recurrence.

lead to complete eradication of the tumor after only one or two treatments. The original testing of NIR-PIT was in immune deficient animals so the full extent of the immune response was not realized until first-in-human trials resulted in better than expected results and was subsequently confirmed in immune competent animal models.

Conventional cancer immunotherapy includes the use of T-cell activating type 1 cytokines, such as IL-2 and IL-15, immune-checkpoint inhibitors, such as anti-CTLA4 or anti-PD1/PDL1 antibodies, and depletion of immune-suppressor cells such as the negative regulatory T-cell (Treg) or the myeloid derived suppressor cell (MDSC). These therapies operate on the principle of activating pre-existing CD8+T cells not only in tumor beds but also other parts of the body. Therefore, they suffer from off-target effects sometimes mimicking autoimmune diseases. Unlike these therapies, NIR-PIT locally enhances host immunity without systemic side effects. Moreover, by selectively eliminating immune-suppressor cells in local tumor beds using immune-suppressor cell-targeting antibodies against CD25 or CCR4 for Treg cells and CXCR2 for MDSC, one could further enhance host immunity.

Local Treg cell depletion with Treg-targeted NIR-PIT against CD25 is highly effective in syngeneic mouse models.[7] CD8+T and NK cells in treated tumor beds were fully activated within a few hours after depletion of Tregs with NIR-PIT. Interestingly, this Treg targeted NIR-PIT also had an effect on nontreated tumors even though the treatment was directed at only one targeted lesion, an example of the "abscopal" effect. (Figure 4)

Direct antitumor NIR-PIT could be combined with conventional systemic cancer immunotherapy, including immune-checkpoint inhibitors (CPI), to increase its effectiveness by further activating CD8+T cells after NIR-PIT. Although this strategy appears effective in animal models, it could result in unwanted side effects caused by the CPI.[6] Using a combination of NIR-PIT and a CPI, tumors started shrinking immediately and disappeared several days after treatment. Once tumors were eliminated with the combination therapy, the animal's immune system rejected any attempts to reinoculate the tumor in the same mouse suggesting that these mice had gained immunity against the initial tumor (Figure 5).

4. APPLICATIONS OF NIR-PIT

NIR-PIT can be applied to any cancer with overexpressed target membrane proteins for which there is a suitable monoclonal antibody. NIR-PIT has been successfully performed with APCs targeting EGFR, HER2, PSMA,[8] CEA,[9] GPC3,[10] mesothelin,[11] CD25,[12] CD20,[13] PD-L1,[14] CD44,[15] CD133, Laminine33, and MUC1 in vivo and in vitro. Special note is made of NIR-PIT directed at CD44[16] and CD133,[17] which are considered markers of cancer stem cells in breast cancer and glioblastoma, respectively, Tumor regrowth was greatly suppressed after CD44 or CD133-targeted NIR-PIT. Additionally, mouse models of tumors located in the xenograft flank, peritoneally,[18] pleurally,[19] and solitary[20] or miliary[21] lung metastasis, orthotopic cancers in athymic and immunocompetent mice,[22,23] and spontaneous lung cancer in transgenic mice[24] were also successfully treated with NIR-PIT. Since 690 nm light can penetrate and treat cancers around 1 cm from the surface or the light source, deeply seated tumors were also treated with interstitial NIR light exposure using fibro-optical diffusers inserted through catheter needles[25] or endoscopes,[26] techniques that could be readily adapted to clinical practice.

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5. IMAGING EVALUATION OF NIR-PIT THERAPEUTIC EFFECTS

There is no immediate change in tumor size after NIR-PIT. When possible, direct observation demonstrates that the tumor turns a whitish color, but it is not always possible to directly observe a treated tumor. Therefore, the therapeutic effects of NIR-PIT can be monitored with several different imaging modalities. Light released after activating IR700 can be detected with fluorescence cameras and fluorescence disappears after NIR-PIT because of the formation of dimers or oligomers of phthalocyanine cores or precipitation of conjugated proteins after both ligands detach following the photochemical ligand release reaction. Therefore, decreased IR700 emission on fluorescence imaging after NIR PIT could be an indicator that the photoinduced ligand release reaction has occurred indicating adequate delivery of light at that site.[4] This, however, may not indicate treatment success. Because of the near-immediate cell death, imaging methods such as 18F-fluorodeoxyglucose positron emission tomography (18F-FDG-PET) could be a rapid response marker of treatment success. (Figure 6)[27]

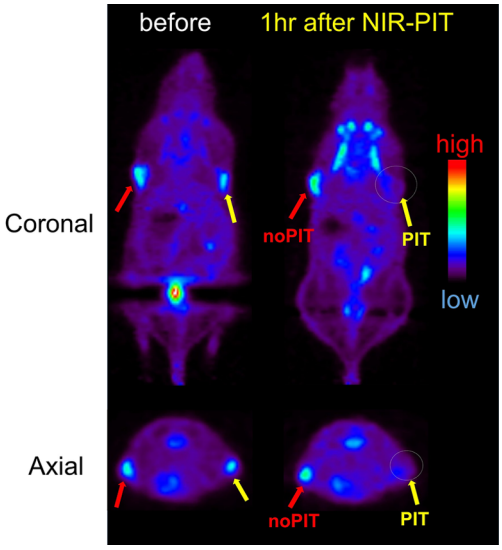

Figure 6. Immediate decrease of glucose metabolism after NIR-PIT is depicted by ^{18}F-FDG PET.

This could be seen much earlier than actual physical changes in the tumor can be measured. Additionally, there are two advanced imaging technologies; fluorescence lifetime imaging and bioluminescence imaging, which can evaluate acute NIR-PIT treatment but these are limited to preclinical studies. By detecting a shortened fluorescence lifetime of IR700, acute necrotic/immunogenic cell death can be inferred.[28] By depicting release and hydrolysis of ATP from dying cells with necrotic/immunogenic cell death, bioluminescence imaging also works as a good experimental tool for monitoring acute NIR PIT effects in luciferase expressing tumors in mouse models.[22]

6. SUPERENHANCED UPTAKE AND RETENTION (SUPR) FOLLOWING NIR-PIT

Another unique feature of NIR-PIT is its immediate effect on blood drug delivery. While some degree of enhanced permeability and retention (EPR) is present in most tumors due to vascular leakiness, following NIR-PIT one can observe marked increases in permeability and leakage from vessels, especially for macromolecules. This has been termed the superenhanced permeability and retention (SUPR) effect to draw a distinction with EPR.[29] Whereas EPR only allows for a modest delivery of nanosized therapeutic agents and similar compounds, SUPR following NIR-PIT results in dramatically enhanced leakage by a factor of up to 24-fold. By inducing immediate necrosis in the perivascular cancer cells, a space forms between the vessels and the remaining tumor, allowing the vessel to enlarge, while increasing blood volume and decreasing blood velocity. (Figure 7)

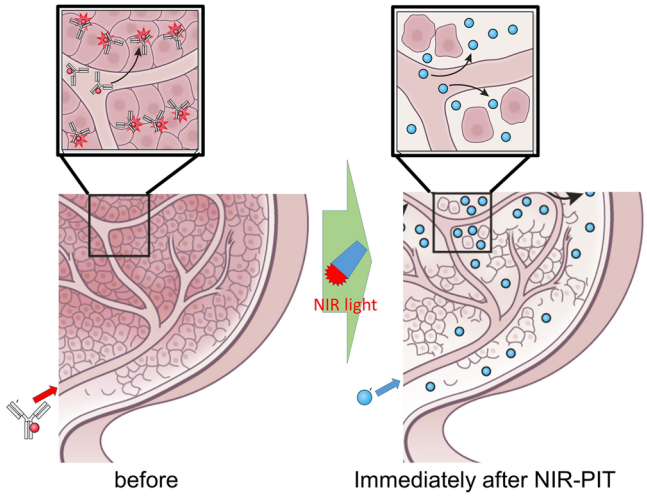

Figure 7. Mechanism of superenhanced permeability and retention (SUPR) effects induced by NIR-PIT

Consequently, there is improved delivery of nanosized therapeutic agents into the treated tissue where they can remain and be effective for several days. Therefore, a combination of NIR-PIT and nanosized anticancer agents could be more effective than either of the therapies alone and this could be another mechanism by which any residual tumor following NIR-PIT treatments could be eliminated. In a study employing FDA approved liposome-encapsulated daunorubucin (DaunoXome)[29] and nanoparticle albumin-bound paclitaxel (nab-palitaxel; Abraxane)[30] in mouse xenograft models of cancer, NIR-PIT in combination with either drug had significantly better therapeutic effects than with either therapy alone. SUPR effects also allow for enhanced delivery of other antibodies and APCs with increased leakage into tumor beds after initial NIR-PIT treatments.[31−33] Mouse xenograft cancer models showed that multiple applications of light following single or multiple doses of APC slowed regrowth and increased progression free survival. Antibody-drug conjugates (ADCs), such as photoactivatable drug release systems, could also be incorporated in the series of treatments by (1) performing NIR-PIT and inducing SUPR effects and, then, (2) delivering ADCs through the SUPR effect and exposing the tumor site to a second dose of NIR light.[34] Low molecular weight anticancer agents that bind to proteins also behave similarly to nanosized agents, making them applicable to increased delivery through the SUPR effect.

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7. NIR-PIT TREATMENT OF CIRCULATING TUMOR CELLS (CTCs)

Circulating tumor cells (CTCs) are thought to be one mechanism by which tumors can metastasize. CTCs circulate in the vasculature until they successfully graft in sites that permit the cell to recruit other normal stromal cells crucial to the development of the tumor microenvironment, as well as other CTCs. While CTCs are circulating, they are known to harbor characteristic cell surface markers that could readily be targeted with specific APCs. Continuous NIR illumination of surface vessels, such as at the wrist or neck, performed with light sources from bracelets or necklaces could be used to periodically reduce CTCs. Reduced CTC levels by themselves are associated with prolonged survivals and reduced risk of metastases. Thus, NIR-PIT directed at CTCs could be a means of prolonging progression free survival.

8. NIR-PIT IN TISSUE ENGINEERING

The new field of tissue engineering allows stem cells to be placed on specific scaffolds to grow new organs or heal wounds. Unfortunately, during the growth of these cells, teratomas may develop rendering the graft useless. Such teratomas have characteristic cell surface markers that are amenable to the development of APCs. NIR-PIT could be used to eliminate teratomas without damaging the remainder of the 2D or 3D-graft and thus save the graft from being discarded.[35,36] This could improve throughput and lower costs associated with tissue regeneration.

9. SUMMARY

Cancer-targeted NIR-PIT has great potential to become a widely applicable cancer therapy. NIR-PIT decreases the number of cancer cells and enhances host immune response in a highly selective manner reducing side effects. When combined with immune-activation therapies, NIR-PIT, not only treats the local tumor, but also reduces or eliminates systemic metastasis and prevents recurrence in some animal models. Since the effects of NIR-PIT improve when host immunity is intact, NIR-PIT may eventually become a first line cancer therapy while other existing therapies, such as radiation and chemotherapy, that damage the immune system may be relegated to secondary and tertiary lines of therapy.

■ ASSOCIATED CONTENT

Web-Enhanced Feature

Video showing serial microscopic images for cellular cytotoxicity.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: 240-858-3069. Fax: 240-402-3191. E-mail: Kobayash@mail.nih.gov.

ORCID

Hisataka Kobayashi: 0000-0003-1019-4112

Notes

The authors declare no competing financial interest.

Biographies

Hisataka Kobayashi is the Chief scientist in the Molecular Imaging Program at the National Cancer Institute of the National Institutes of Health in Bethesda, Maryland. Dr. Kobayashi was awarded his M.D. and Ph.D. (Immunology/Medicine) by the Kyoto University in Japan. He joined as a postdoctoral fellow the Nuclear Medicine Department at the Clinical Center of the National Institutes of Health in 1995 and moved to his current position in the Molecular Imaging Program at NCI in 2004. His interest is in developing novel molecular imaging agents and technologies especially for targeting cancers.

Peter L. Choyke is the Director of the Molecular Imaging Program at the Center for Cancer Research of the National Cancer Institute in Bethesda, Maryland. Dr. Choyke is a graduate of Jefferson Medical School and received training in Diagnostic Radiology at Yale University and the University of Pennsylvania. He joined the Diagnostic Radiology Department at the Clinical Center of the National Institutes of Health in 1988 and formed the Molecular Imaging Program at NCI in 2004. His interest is in accelerating the treatment of cancer by using novel molecular imaging agents which target specific features of cancers.

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■ REFERENCES

(1) Decker, W. K.; da Silva, R. F.; Sanabria, M. H.; Angelo, L. S.; Guimarães, F.; Burt, B. M.; Kheradmand, F.; Paust, S. Cancer Immunotherapy: Historical Perspective of a Clinical Revolution and Emerging Preclinical Animal Models. *Front. Immunol.* **2017**, *8*, 829.

(2) Sathyanarayanan, V.; Neelapu, S. S. Cancer immunotherapy: Strategies for personalization and combinatorial approaches. *Mol. Oncol.* **2015**, *9*, 2043−2053.

(3) Mitsunaga, M.; Ogawa, M.; Kosaka, N.; Rosenblum, L. T.; Choyke, P. L.; Kobayashi, H. Cancer cell-selective in vivo near infrared photoimmunotherapy targeting specific membrane molecules. *Nat. Med.* **2011**, *17*, 1685−1691.

(4) Sato, K.; Ando, K.; Okuyama, S.; Moriguchi, S.; Ogura, T.; Totoki, S.; Hanaoka, H.; Nagaya, T.; Kokawa, R.; Takakura, H.; Nishimura, M.; Hasegawa, Y.; Choyke, P. L.; Ogawa, M.; Kobayashi, H. Photoinduced Ligand Release from a Silicon Phthalocyanine Dye Conjugated with Monoclonal Antibodies: A Mechanism of Cancer Cell Cytotoxicity after Near-Infrared Photoimmunotherapy. *ACS Cent. Sci.* **2018**, *4*, 1559−1569.

(5) Ogawa, M.; Tomita, Y.; Nakamura, Y.; Lee, M. J.; Lee, S.; Tomita, S.; Nagaya, T.; Sato, K.; Yamauchi, T.; Iwai, H.; Kumar, A.; Haystead, T.; Shroff, H.; Choyke, P. L.; Trepel, J. B.; Kobayashi, H. Immunogenic cancer cell death selectively induced by near infrared photoimmunotherapy initiates host tumor immunity. *Oncotarget* **2017**, *8*, 10425−10436.

(6) Nagaya, T.; Friedman, J.; Maruoka, Y.; Ogata, F.; Okuyama, S.; Clavijo, P. E.; Choyke, P. L.; Allen, C.; Kobayashi, H. Host Immunity Following Near-Infrared Photoimmunotherapy Is Enhanced with PD-1 Checkpoint Blockade to Eradicate Established Antigenic Tumors. *Cancer Immunol. Res.* **2019**, *7*, 401−413.

(7) Sato, K.; Sato, N.; Xu, B.; Nakamura, Y.; Nagaya, T.; Choyke, P. L.; Hasegawa, Y.; Kobayashi, H. Spatially selective depletion of tumor-associated regulatory T cells with near-infrared photoimmunotherapy. *Sci. Transl. Med.* **2016**, *8*, 352ra110.

(8) Nagaya, T.; Nakamura, Y.; Okuyama, S.; Ogata, F.; Maruoka, Y.; Choyke, P. L.; Kobayashi, H. Near-Infrared Photoimmunotherapy Targeting Prostate Cancer with Prostate-Specific Membrane Antigen (PSMA) Antibody. *Mol. Cancer Res.* **2017**, *15*, 1153−1162.

(9) Maawy, A. A.; Hiroshima, Y.; Zhang, Y.; Heim, R.; Makings, L.; Garcia-Guzman, M.; Luiken, G. A.; Kobayashi, H.; Hoffman, R. M.; Bouvet, M. Near infra-red photoimmunotherapy with anti-CEA-

DOI: 10.1021/acs.accounts.9b00273
Acc. Chem. Res. 2019, 52, 2332−2339

IR700 results in extensive tumor lysis and a significant decrease in tumor burden in orthotopic mouse models of pancreatic cancer. *PLoS One* **2015**, *10*, No. e0121989.
(10) Hanaoka, H.; Nagaya, T.; Sato, K.; Nakamura, Y.; Watanabe, R.; Harada, T.; Gao, W.; Feng, M.; Phung, Y.; Kim, I.; Paik, C. H.; Choyke, P. L.; Ho, M.; Kobayashi, H. Glypican-3 targeted human heavy chain antibody as a drug carrier for hepatocellular carcinoma therapy. *Mol. Pharmaceutics* **2015**, *12*, 2151−2157.
(11) Nagaya, T.; Nakamura, Y.; Sato, K.; Zhang, Y. F.; Ni, M.; Choyke, P. L.; Ho, M.; Kobayashi, H. Near infrared photoimmunotherapy with an anti-mesothelin antibody. *Oncotarget* **2016**, *7*, 23361−23369.
(12) Nakajima, T.; Sano, K.; Choyke, P. L.; Kobayashi, H. Improving the efficacy of Photoimmunotherapy (PIT) using a cocktail of antibody conjugates in a multiple antigen tumor model. *Theranostics* **2013**, *3*, 357−365.
(13) Nagaya, T.; Nakamura, Y.; Sato, K.; Harada, T.; Choyke, P. L.; Kobayashi, H. Near infrared photoimmunotherapy of B-cell lymphoma. *Mol. Oncol.* **2016**, *10*, 1404−1414.
(14) Nagaya, T.; Nakamura, Y.; Sato, K.; Harada, T.; Choyke, P. L.; Hodge, J. W.; Schlom, J.; Kobayashi, H. Near infrared photoimmunotherapy with avelumab, an anti-programmed death-ligand 1 (PD-L1) antibody. *Oncotarget* **2017**, *8*, 8807−8817.
(15) Nagaya, T.; Nakamura, Y.; Okuyama, S.; Ogata, F.; Maruoka, Y.; Choyke, P. L.; Allen, C.; Kobayashi, H. Syngeneic Mouse Models of Oral Cancer Are Effectively Targeted by Anti-CD44-Based NIR-PIT. *Mol. Cancer Res.* **2017**, *15*, 1667−1677.
(16) Jin, J.; Krishnamachary, B.; Mironchik, Y.; Kobayashi, H.; Bhujwalla, Z. M. Phototheranostics of CD44-positive cell populations in triple negative breast cancer. *Sci. Rep.* **2016**, *6*, 27871.
(17) Jing, H.; Weidensteiner, C.; Reichardt, W.; Gaedicke, S.; Zhu, X.; Grosu, A. L.; Kobayashi, H.; Niedermann, G. Imaging and Selective Elimination of Glioblastoma Stem Cells with Theranostic Near-Infrared-Labeled CD133-Specific Antibodies. *Theranostics* **2016**, *6*, 862−874.
(18) Sato, K.; Choyke, P. L.; Kobayashi, H. Photoimmunotherapy of gastric cancer peritoneal carcinomatosis in a mouse model. *PLoS One* **2014**, *9*, No. e113276.
(19) Sato, K.; Nagaya, T.; Choyke, P. L.; Kobayashi, H. Near infrared photoimmunotherapy in the treatment of pleural disseminated NSCLC: preclinical experience. *Theranostics* **2015**, *5*, 698−709.
(20) Sato, K.; Nagaya, T.; Mitsunaga, M.; Choyke, P. L.; Kobayashi, H. Near infrared photoimmunotherapy for lung metastases. *Cancer Lett.* **2015**, *365*, 112−121.
(21) Sato, K.; Nagaya, T.; Nakamura, Y.; Harada, T.; Choyke, P. L.; Kobayashi, H. Near infrared photoimmunotherapy prevents lung cancer metastases in a murine model. *Oncotarget* **2015**, *6*, 19747−19758.
(22) Mitsunaga, M.; Nakajima, T.; Sano, K.; Kramer-Marek, G.; Choyke, P. L.; Kobayashi, H. Immediate in vivo target-specific cancer cell death after near infrared photoimmunotherapy. *BMC Cancer* **2012**, *12*, 345.
(23) Sano, K.; Mitsunaga, M.; Nakajima, T.; Choyke, P. L.; Kobayashi, H. In vivo breast cancer characterization imaging using two monoclonal antibodies activatably labeled with near infrared fluorophores. *Breast Cancer Res.* **2012**, *14*, R61.
(24) Nakamura, Y.; Ohler, Z. W.; Householder, D.; Nagaya, T.; Sato, K.; Okuyama, S.; Ogata, F.; Daar, D.; Hoa, T.; Choyke, P. L.; Kobayashi, H. Near Infrared Photoimmunotherapy in a Transgenic Mouse Model of Spontaneous Epidermal Growth Factor Receptor (EGFR)-expressing Lung Cancer. *Mol. Cancer Ther.* **2017**, *16*, 408−414.
(25) Okuyama, S.; Nagaya, T.; Sato, K.; Ogata, F.; Maruoka, Y.; Choyke, P. L.; Kobayashi, H. Interstitial near-infrared photoimmunotherapy: effective treatment areas and light doses needed for use with fiber optic diffusers. *Oncotarget* **2018**, *9*, 11159−11169.
(26) Nagaya, T.; Okuyama, S.; Ogata, F.; Maruoka, Y.; Choyke, P. L.; Kobayashi, H. Endoscopic near infrared photoimmunotherapy using a fiber optic diffuser for peritoneal dissemination of gastric cancer. *Cancer Sci.* **2018**, *109*, 1902−1908.
(27) Sano, K.; Mitsunaga, M.; Nakajima, T.; Choyke, P. L.; Kobayashi, H. Acute Cytotoxic Effects of Photoimmunotherapy Assessed by 18F-FDG PET. *J. Nucl. Med.* **2013**, *54*, 770−775.
(28) Nakajima, T.; Sano, K.; Mitsunaga, M.; Choyke, P. L.; Kobayashi, H. Real-time monitoring of in vivo acute necrotic cancer cell death induced by near infrared photoimmunotherapy using fluorescence lifetime imaging. *Cancer Res.* **2012**, *72*, 4622−4628.
(29) Sano, K.; Nakajima, T.; Choyke, P. L.; Kobayashi, H. Markedly enhanced permeability and retention effects induced by photoimmunotherapy of tumors. *ACS Nano* **2013**, *7*, 717−724.
(30) Hanaoka, H.; Nakajima, T.; Sato, K.; Watanabe, R.; Phung, Y.; Gao, W.; Harada, T.; Kim, I.; Paik, C. H.; Choyke, P. L.; Ho, M.; Kobayashi, H. Photoimmunotherapy of hepatocellular carcinoma-targeting Glypican-3 combined with nanosized albumin-bound paclitaxel. *Nanomedicine (London, U. K.)* **2015**, *10*, 1139−1147.
(31) Liang, C. P.; Nakajima, T.; Watanabe, R.; Sato, K.; Choyke, P. L.; Chen, Y.; Kobayashi, H. Real-time monitoring of hemodynamic changes in tumor vessels during photoimmunotherapy using optical coherence tomography. *J. Biomed. Opt.* **2014**, *19*, 098004.
(32) Tang, Q.; Nagaya, T.; Liu, Y.; Lin, J.; Sato, K.; Kobayashi, H.; Chen, Y. Real-time monitoring of microdistribution of antibody-photon absorber conjugates during photoimmunotherapy in vivo. *J. Controlled Release* **2017**, *260*, 154−163.
(33) Tang, Q.; Nagaya, T.; Liu, Y.; Horng, H.; Lin, J.; Sato, K.; Kobayashi, H.; Chen, Y. 3D mesoscopic fluorescence tomography for imaging micro-distribution of antibody-photon absorber conjugates during near infrared photoimmunotherapy in vivo. *J. Controlled Release* **2018**, *279*, 171−180.
(34) Nagaya, T.; Gorka, A. P.; Nani, R. R.; Okuyama, S.; Ogata, F.; Maruoka, Y.; Choyke, P. L.; Schnermann, M. J.; Kobayashi, H. Molecularly Targeted Cancer Combination Therapy with Near-Infrared Photoimmunotherapy and Near-Infrared Photorelease with Duocarmycin-Antibody Conjugate. *Mol. Cancer Ther.* **2018**, *17*, 661−670.
(35) Sato, K.; Nakajima, T.; Choyke, P. L.; Kobayashi, H. Selective cell elimination in vitro and in vivo from tissues and tumors using antibodies conjugated with a near infrared phthalocyanine. *RSC Adv.* **2015**, *5*, 25105−25114.
(36) Sato, K.; Choyke, P. L.; Hisataka, K. Selective Cell Elimination from Mixed 3D Culture Using a Near Infrared Photoimmunotherapy Technique. *J. Visualized Exp.* **2016**, No. 109, No. e53633.

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