Theranostic near infrared photoimmunotherapy.

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Three modes of cancer therapy, surgery, radiation and chemotherapy, have been central to modern oncologic therapy. Molecular targeted cancer therapies have been introduced to target specific pathways, while minimizing side effects but have had limited success except in several notable cases. Here, we employ an activatable hydrophilic photosensitizer based on a near infrared (NIR) phthalocyanine dye, IRdye700DX (IR700), which is covalently conjugated to one of several humanized monoclonal antibodies (MAb) targeting cancer-specific cell-surface molecules. When exposed NIR light, the conjugates visualize cancer cells with low dose of NIR light and induce highly selective cell death in vivo with therapeutic dose of NIR light, a process termed "near infrared photo-immunotherapy" (NIR-PIT) (1). **(Fig. 1)**

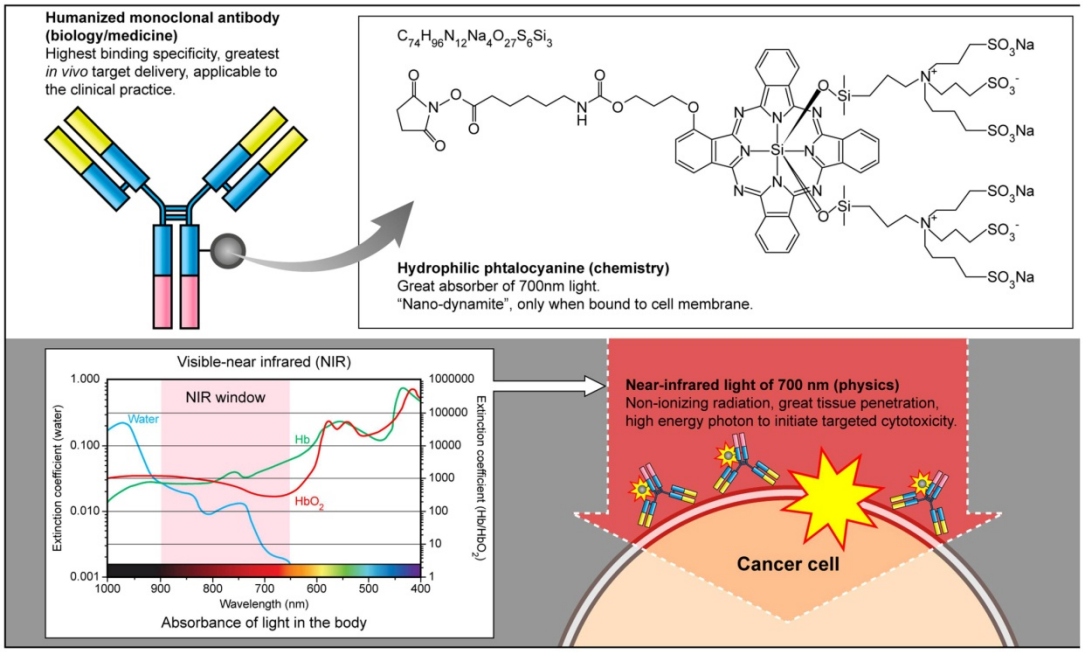

Fig. 1. Mechanism of NIR photo-immunotherapy, its physical and chemical basis.

Antibody-photosensitizer conjugates (APC: MAb conjugated with IR700) bound target-specific cell death could be induced within 1 minute of exposure to NIR light and resulted in cellular swelling, bleb formation, and rupture of vesicles indicating necrotic cell death. No phototoxicity was observed in co-cultured receptor-negative cells after

incubation with APC, even when APC was not removed from the medium during light exposure. Greater than 90% of cancer cell death in vivo was demonstrated with bioluminescence imaging and ^{18}F-FDG PET(2, 3). Thereafter, greater than 90% tumor shrinkage was observed in vivo within 3 days of the NIR irradiation, with no apparent side effects, only in target tumors with NIR light exposure, and more than 80% of mice showed tumor-free survival for more than a year with an optimized regimen(4). IR700 fluorescence produced by the APC permitted guidance of light delivery and allowed for monitoring after therapy. Disappearance of IR700 fluorescence indicates sufficient exposure of NIR light for inducing maximum therapeutic NIR-PIT effects. (Fig. 2) Furthermore, immediately after NIR-PIT, more than 20-fold superior delivery and retention of nano-sized particles (SUPR effect) was induced in NIR-PIT treated tumors compared with non-treated tumors with conventional enhanced permeability and retention (EPR) effects(5). (Fig. 3) Therefore, NIR-PIT combined with nano-sized cancer agents showed synergic therapeutic effects(6).

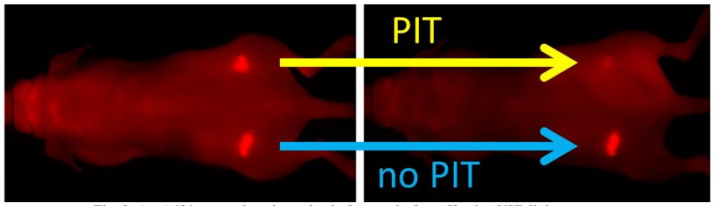

Fig. 2 An A431 tumor bearing mice before and after effective NIR light exposure. NIR was exposed based on IR700 fluorescence guidance. After effective PIT, IR700 fluorescence nearly disappeared.

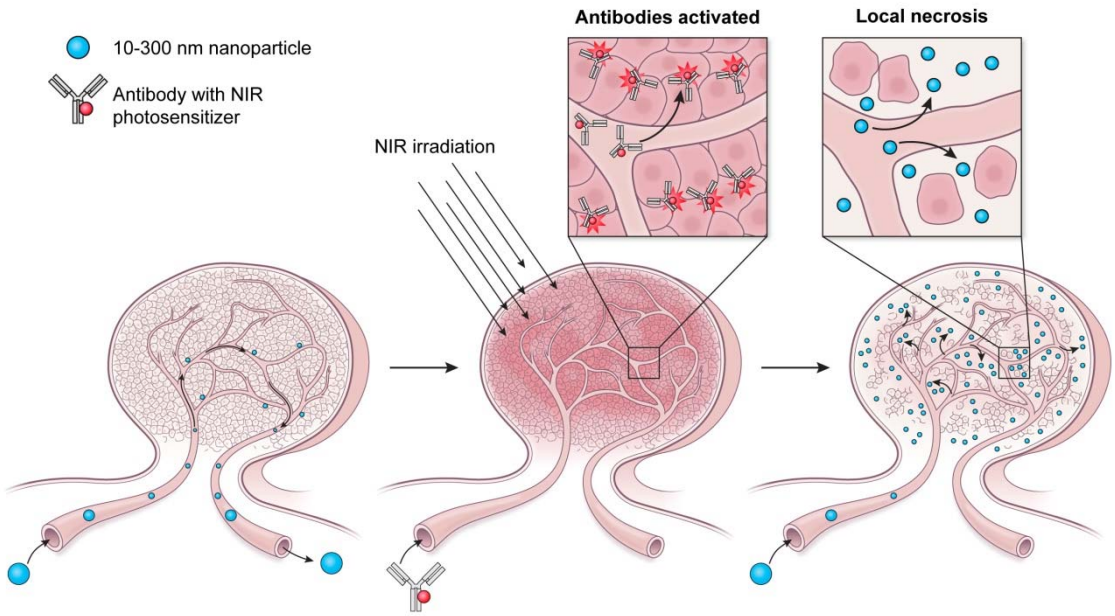

Fig. 3. Mechanism of NIR-PIT induced super-enhanced permeability and retention (SUPR) effect.

The MAb-IR700 NIR-PIT was most effective, when conjugates were bound to the cell

membrane, but showed no phototoxicity, when unbound, suggesting a novel mechanism for NIR-PIT compared with conventional photodynamic therapies. Successful pre-clinical NIR-PIT studies have been performed or are on-going against more than 20 different molecular targets expressing on different cancers including EGFR, HER2, PSMA, CD25, GPC3, mesothelin, CD133, CD44, CEA, laminin322, and more. Now the first-in-human clinical trial of NIR-PIT against head and neck cancer patients targeting EGFR is under preparation for starting by employing the cetximab-IR700 photo-immunoconjugate in early 2015. In conclusion, theranostic NIR fluorescence image-guided, target-selective NIR-PIT based on MAb-IR700 cell membrane binding enables selective treatment of cancer with no apparent side effects to normal cells or surrounding tissue.

References:

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