Near infrared photoimmunotherapy in the treatment of pleural disseminated NSCLC: preclinical experience

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Online Data Supplement
**Supplementary Videos**

**Supplementary video S1.**
**Time-lapse Imaging of NIR-PIT in vitro.**

Time-lapse sequential images shows morphologic changes of the cells and rapid membrane damage detected by PI staining after NIR light irradiation in cells treated with tra-IR700 (for 25 min observation in total). Flashing light is NIR light irradiation (2 J/cm²).

- **Anterior half:** DIC time-lapse image of Calu3-luc-GFP cell treated by NIR- PIT.
- **Posterior half:** Fluorescence of PI time-lapse image of Calu3-luc-GFP cell treated by NIR-PIT.

**Supplementary video S2.**
**Fluorescence thoracoscopic imaging for characterization of the pleural disseminated NSCLC model.**

Fluorescence thoracoscopic imaging shows pleural metastases in the thoracic cavity and the colocalization of GFP and IR700 fluorescence. 100 µg of APC was intravenously injected 1 day before the imaging.

- **Anterior half:** GFP fluorescence thoracoscopy imaging of pleural metastases.
- **Posterior half:** IR700 fluorescence thoracoscopy imaging of pleural metastases.

**Supplementary video S3, S4.**
**Fluorescence thoracoscopic imaging after NIR-PIT**

Fluorescence thoracoscopic imaging shows intrathoracic tumors and colocalization of GFP and IR700 fluorescence. 100 µg of APC was intravenously injected 1 day before the imaging.

- **S3 Anterior half:** GFP fluorescence thoracoscopy imaging of control tumors (received only APC but no NIR-light).
- **S3 Posterior half:** IR700 fluorescence thoracoscopy imaging of pleural tumors (received only APC but no light).
- **S4 Anterior half:** GFP fluorescence thoracoscopy imaging after NIR-PIT.
- **S4 Posterior half:** IR700 fluorescence thoracoscopy imaging after NIR-PIT.
Supplementary Figure S1.

Specific targeted necrotic cell death was observed after NIR-PIT *in vitro*. Calu3-luc-GFP cells were co-cultured with 3T3-RFP (non-HER expressing) cells. They were treated with tra-IR700 and observed (before and after irradiation of NIR light). Target specific necrotic cell death was observed upon excitation with NIR light (after 30 min) on Calu3-luc-GFP cell but not 3T3-RFP cells. *Calu3-luc-GFP cell, Bar = 25 µm.*
Supplementary Figure S2.

Blocking of *in vitro* NIR-PIT effect with excess trastuzumab. *In vitro* NIR-PIT was evaluated by FACS using PI staining. Calu3-luc-GFP cells were incubated with or without the addition of 1,000 µg/mL trastuzumab for 1 hr, then added 10 µg/mL Tra-IR700 and incubated for 6 hr. 1 hr after NIR irradiation, FACS analysis with PI staining was performed (n = 4, *p < 0.0001, **p = ns, Student’s t test). *In vitro* NIR-PIT was blocked with excess trastuzumab.
**Supplemental Figure S3.**

*In vivo* IR700 fluorescence imaging in response to repeated NIR-PIT. *In vivo* IR700 fluorescence imaging in response to repeated NIR-PIT. IR700 fluorescence decreased after NIR-PIT in both models (A, flank model, B. pleural disseminated model).