

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

Kazuhide Sato, Tadanobu Nagaya,  
Peter L. Choyke, and Hisataka Kobayashi

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/cm<sup>2</sup>).

**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. 100µg 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.

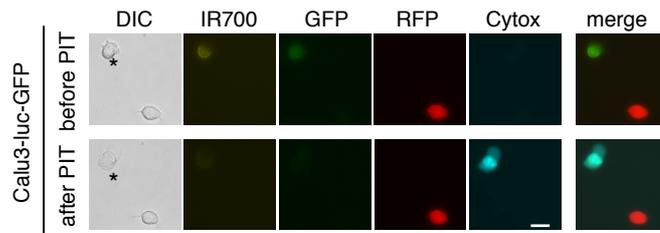
**S3 Anterior half:** GFP fluorescence thoracoscopy imaging of control tumors (received only APC but no NIR-light).

**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.

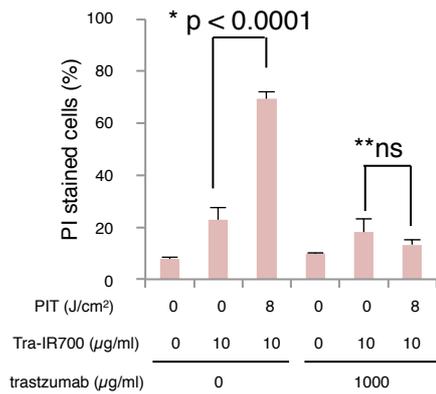
## Supplementary Figure S1



### 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  $\mu\text{m}$ .

## Supplementary Figure S2



### Supplementary Figure S2.

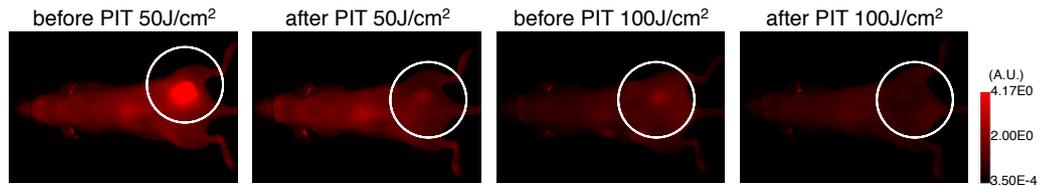
#### Blocking of *in vitro* NIR-PIT effect with excess trastuzumab.

*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 6hr. 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

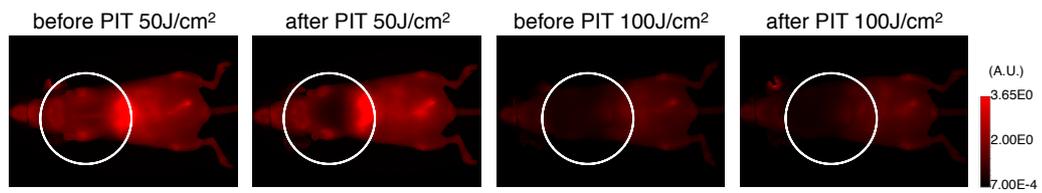
A

Calu3-luc-GFP flank tumor model



B

Calu3-luc-GFP pleurally disseminated model



### Supplementary 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).