Intra-tumor analysis of trastuzumab distribution by PID staining, breakthrough method with high visibility and single cell quantification

**Background and Objectives**

- **Background:** In the development of drugs, measurement of drug concentration in target tissue has been thought to be crucial for predicting its efficacy and safety. Insufficient drug exposure increases the risk of resistance and recurrence. However, it is presumed that antibody drugs including trastuzumab may distribute unevenly within tumor tissue due to their large molecule size and complex tumor microenvironment. Moreover, immature methodology made it difficult to observe the distribution of these drugs in tumor and the causal relationship between drug delivery and tumor resistance mechanism is still unknown.

- **Objectives:**
  - To develop imaging methodology to visualize and quantify the intact molecule in tumor
  - To perform pharmacokinetics (PK) analysis in tumor and compare with conventional blood PK analysis.
  - To evaluate the factors affected to antibody drug distribution in solid tumor

**Methods and Workflow**

- **Pharmacokinetics (PK) study of trastuzumab in animal model**
  - For preparation of cell line derived xenograft (CDX), human breast cancer cell lines BT474 (HER2 positive) and MDA-MB231 (HER2 negative) were subcutaneously implanted in SCID mice. Trastuzumab (10 mg/kg) was administrated intraperitoneally. Tumor and other organs were collected at several time points.
  - **Imaging PK by PID staining**
    - As with immunohistochemistry, trastuzumab in tumor serial section was recognized by anti-trastuzumab antibody (HCA177; Bio-Rad, Hercules, CA, bdistyred by Konica minolta Inc., Tokyo, Japan) and labeled by newly developed fluorescent nanoparticles tagged Phosphorus-Integrated Dots (OTD) which have 30,000 times the intensity of conventional dyes (K. Gimpel, et al, Sci Rep, 7(17309), 2017)

**I. Macro and Micro imaging in tumor**

- **Macro Imaging**
  - PID staining enabled to evaluate "Macro imaging" of whole tumor (mm) and "Micro imaging" of single cell resolution (1 µm).
  - Simultaneous staining with hematoxylin made it possible to ascertain drug distribution with local tissue morphology such as stroma, blood vessel and necrosis site.

- **Micro Imaging**
  - Obtained fluorescence image by PID staining and counterstaining using a microscope.
  - Reproject the position of the origin of the cell in the tissue.
  - The drugs within 13 µm from them are assigned to the cell, and the rest is discarded.
  - (22 µm - distance from each cell nucleus to the cell membrane)

**II. Novel concept of drug quantification**

- To quantify antibody drugs in tumor, we have proposed a new concept.
  - # of drugs bound to single cell

**III. Digitization of drug delivery in whole tumor**

- It was necessary to digitize drug delivery in entire tumor to investigate individual differences, comparison with multiple specimen, and effect of concurrent medication.
  - Obtain 25 to 30 images in the maximal cleavage plane of the tumor
  - The number of drugs bond per single cell are calculated in each of them.
  - Frequency distribution is depicted by histogram.

**IV. Intra-tumor macro imaging PK study of trastuzumab**

- A. In BT474 CDX (HER2 positive), heterogeneous trastuzumab distribution regardless of homogeneous HER2 expression.
- B. In MDA-MB231 CDX (HER2 negative), less trastuzumab was distributed in stroma cell area.
- C. Plasma concentration of both CDX models was not different at each time point.
- D. Intra-tumor trastuzumab distribution in BT474 CDX was significantly higher than that in MDA-MB231 CDX.

**V. Micro imaging of region specific distribution**

- Region specific analysis reveals trastuzumab was less distributed in stroma and infiltrated lymphocyte and much accumulated at necrosis compared to viable tumor.

**Conclusions and Future direction**

- We successfully analyze intra-tumor PK of intact trastuzumab using the PID staining method in CDX model. HER2 expression has a great influence on delivery and behavior of trastuzumab.
- Compared to conventional LC-MS/MS method, the advantage of this method is selective quantification of interested region.
- Extrapolative prediction of the intra-tumor PK using plasma PK is difficult at this point, further analysis is required.
- Seek the factors regulate heterogeneous delivery of trastuzumab, and the solutions for homogeneous drug exposure.
- CDX may not reflect patient tumor, patient derived xenograft (PDX) animal model is also needed for clinical application.
- We explore the impact of drug delivery on cancer treatment by multi-faced approach.