Novel high-sensitive drug detecting, PID method reveals intra-tumor pharmacokinetics of trastuzumab and factors affect to its remarkable heterogeneity

Ryosuke Matsukane1,3, Mitsuhiro Hayashi1, Masaru Takahashi2, Mayu Ouchi1, Hiroaki Aikawa1, Hisatake Okada2, Satohiro Masuda3, Akinobu Hamada1

1Division of Molecular Pharmacology, National Cancer Center Research Institute, Tokyo, Japan, 2Product Development Division, Bio-Healthcare Business Unit, Healthcare Business Headquarters, Konica Minolta, Inc., Tokyo, Japan, 3Department of Clinical Pharmacology and Biopharmaceutics, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan

(Background)
Detection and precise quantification of monoclonal antibody (mAb) is still insufficient in tissues and it causes ambiguity of pharmacokinetics. How heterogeneous drug delivery affects tumor regression or resistance is also unclear. We focused on novel fluorescent nanoparticles called PIDs (phosphor integrated dots). PIDs have 30,000 times the intensity of conventional dye and provide us high visibility and novel quantification concept at single cell resolution. (Sci Rep, 7 (1):7509, 2017) We applied this material to investigate intra-tumor pharmacokinetics of mAb.

(Method)
Human epidermal growth factor receptor 2 (HER2) positive human breast cancer cell line BT474 and HER2 negative MDA-MB231 was subcutaneously implanted in SCID/Beige. Trastuzumab (10 mg/kg), clinically approved anti-HER2 mAb, was administrated intraperitoneally. After preparing frozen sections, trastuzumab in tissues was recognized by anti-trastuzumab antibody and labeled by PIDs as with immunohistochemistry. Quantification was performed in obtained image using PID analyzer and this novel method was validated by conventional LC-MS/MS analysis.

(Results)
The amount of trastuzumab in BT474 tumor was significantly larger than that in MDA-MB231 although there was no difference in plasma concentration in both model. In BT474, heterogeneous distribution was observed at 8, 24 and 72 hours after administration regardless of homogenous HER2 expression. Moreover, correlation analysis suggested that stroma distribution strongly affected to those drug delivery into tumor. In the steady state, the distribution became almost uniform and tumor regression rate was well correlated with the amount of drugs inside tumor. We also investigated distribution to heart because trastuzumab sometimes caused cardiotoxicity in practical situation. 5 weeks after weekly administration, while morphological damages was not observed, trastuzumab significantly penetrated to throughout of heart.

(Conclusion)
Trastuzumab was visualized and quantified precisely in tumor and other tissues. Further analysis is required to extrapolate how mAb behaves in tissues but PID method must be valuable in early stage of drug development.