Supplemental data S1

Plasma extracellular binding domain (EBD) testing kit differences

Noting that the plasma EBD concentrations obtained on this patient population was much lower than values in prior publications, additional validation studies were performed. For the ^{111}In -CHX-A"-DTPA trastuzumab patient study, the HER2 ELISA kit from Millipore Sigma (St. Louis, MO, USA) was used on patient plasma samples. This kit uses a monoclonal mouse capture antibody derived from a mouse myeloma cell line NS0-derived recombinant human ErbB2 (aa23-652) with a goat polyclonal detection antibody derived from the same immunogen. To validate the accuracy of the methods and our results, we obtained both fresh frozen human plasma and serum samples from three breast cancer patients participating on the HER2 dendritic cell vaccine trial (NCT01730118). Our assay detected the following plasma and serum concentrations, respectively: (1050pg/mL, 1651pg/mL, 1619pg/mL) and (179pg/mL, 135pg/mL, and 709pg/ml). Such concentrations approximated the average plasma (~1600pg/mL) and serum (~256pg/mL) concentrations obtained by Sigma, and the company confirmed our assay performed well (personal correspondence between Dr. TM Sissung and Millipore Sigma Technical Services on 3/16, 2018). While the results from the present assay are different from previously published concentrations, this observation is most-likely caused by differences between the antibodies used in the Sigma ELISA and the Wilex assay from Martell Diagnostic Laboratories (Roseville, MN, USA; formerly Wilex Inc, Cambridge, MA, USA) (Banys-Paluchowski M, *et al.* (2017) Sci Reports, 7: 17307) and that we utilized plasma whereas the Wilex assay requires serum."

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