Practical synthesis of ^{211}At-labeled immunoconjugate by double click method for α-emission cancer radiotherapeutics†

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In this paper, a facile synthesis of an ^{211}At-labeled immunoconjugate that is used as an α-emission molecular targeting therapy is described. We synthesized a tetrazine probe modified with closo-decaborate(2-), which is a prosthetic group that forms a bioavailable stable complex with ^{211}At. Our one-pot three-component double-click labeling method,[1)] which consists of RIKEN click[2–4)] and tetrazine ligation,[5)] was utilized to introduce the decaborate to HSA (human serum albumin) or trastuzumab (anti-HER2 antibody) using decaborate-tetrazine **1** and TCO (*trans*-cyclooctene)-aldehyde **2** without reducing the antibody binding affinity, as shown in Fig. 1. The average number of molecules attached to HSA was determined as 2 decaborate moieties (a **1** + **2** molecule underwent a 1,065 MW increase) by the MALDI-TOF mass spectroscopic analysis in comparison to the intact HSA molecular weight.

Next, the astatination of decaborate-trastuzumab was conducted by treating solutions of decaborate-trastuzumab with Na[^{211}At] in the presence of chloramine T as an oxidant over 5 min at room temperature. As shown in Fig. 2, the labeling was performed using 1 μM decaborate-trastuzumab in 0.05% PBS-T and Na[^{211}At], 75 MBq, in PBS to furnish ^{211}At-labeled trastuzumab with a specific activity of 1.7 MBq/μg in 49% RCY. The potential loss of antigen recognition activity in the ^{211}At-labeled trastuzumab with a high specific activity was assessed by measuring the dissociation constant K_d of the obtained ^{211}At-labeled trastuzumab. This value was found to be 1.0 nM, indicating no impairment to the affinity. Reacting 0.1 μM decaborate-trastuzumab with Na[^{211}At], 104 MBq, in PBS provided ^{211}At-labeled trastuzumab in 30% RCY with a very high specific activity of 15 MBq/μg.

An intratumor injection of 6.3 μg of the ^{211}At-labeled trastuzumab with 1.4 MBq in BALB/c nude mice implanted with HER2-expressing epidermoid cancer cells yielded effective suppression of tumor growth, as shown in Fig. 3. Our work provides one of the most practical ^{211}At-labeling methods to develop molecular cancer radiotherapeutics.

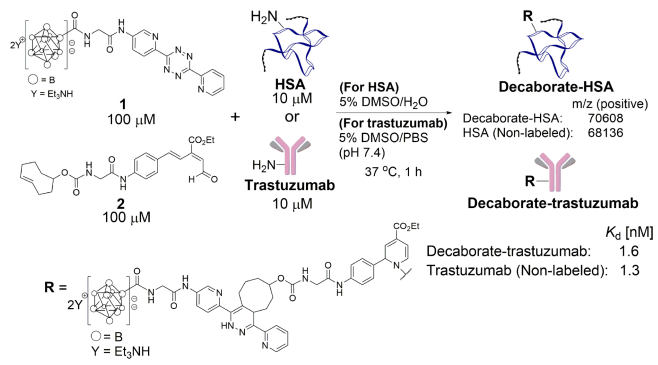

Fig. 1. Preparation of decaborate-HSA/trastuzumab via the one-pot three-component double-click labeling method. Dissociation constants (K_d) of the decaborate-trastuzumab measured by the QCM method.

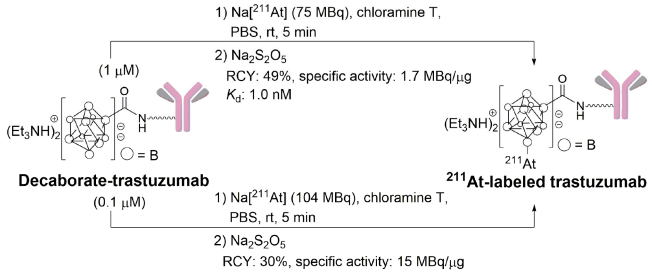

Fig. 2. Radiolabeling of decaborate-trastuzumab. RCY (Radiochemical yield) was obtained from the radioactivity of the purified radiolabeled product against the added Na[^{211}At].

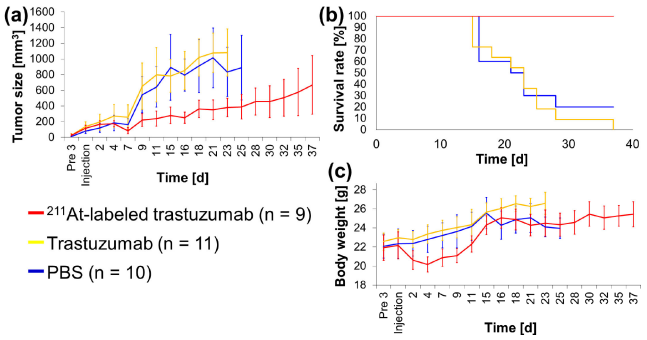

Fig. 3. Therapeutic efficacies of α-emitting ^{211}At-labeled trastuzumab, trastuzumab, or PBS after intratumor injection.

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