# Development of radioimmunotherapy with astatine-211-counjugated antibodies

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Macromolecules and macroproteins hardly extravasate from blood vessels at a normal organ site due to their large molecular weight. By contrast, since the permeability of blood vessels at tumor sites is higher than that at normal organs, macromolecules and macroproteins can extravasate from tumor vessels. Moreover, due to the poor lymphatic drainage system at tumor sites, macromolecules and macroproteins are retained for a prolonged time after extravasation. As a result, macromolecules and macroproteines selectively and efficiently accumulate in tumors. This phenomenon is the enhanced permeability and retention (EPR) effect,[1] which is the rationale of drug delivery systems for cancer treatment. Antibodies such as immunoglobulin G (IgG), the molecular weight of which is approximately 150 kDa, accumulate in tumors via the EPR effect. In addition to the EPR effect, antigen-antibody interaction with target molecules expressed in tumors enhances the tumor accumulation of antibodies.[2]

We successfully produced several types of monoclonal antibodies (mAbs) that recognize target molecules highly or specifically expressed in tumors, and applied mAbs to drug carriers. The antibody-drug conjugate (ADC) is composed of an mAb that recognizes target molecules in tumors and low-molecular weight anticancer agents, and it is an example of armed antibodies. Previously, using our mAbs, we prepared ADCs and demonstrated that they showed potent antitumor effects in cancer xenograft models.[3–5]

Alpha particles are characterized by higher liner energy transfer (LET) and shorter range in tissue than other types of radiation, resulting in efficient deoxyribonucleic acid (DNA) double-strand breaks in accumulated cells with minor effects on adjacent cells. Accordingly, since the successful delivery of alpha emitters to tumor sites is expected to yield efficient antitumor effects with mild toxicity, we focus on the development of radioimmunotherapy (RIT) with an alpha-emitter-conjugated antibody.

In this study, we labeled antibodies with astatine-211, one of the most promising alpha emitters for cancer treatment, and evaluated the binding activities and *in vitro* cytocidal effects of the radioactive antibodies.

We synthesized astatine-211 in the $^{209}$Bi$(\alpha, 2n)^{211}$At reaction using the RIKEN Azimuthally Varying Field (AVF) cyclotron.

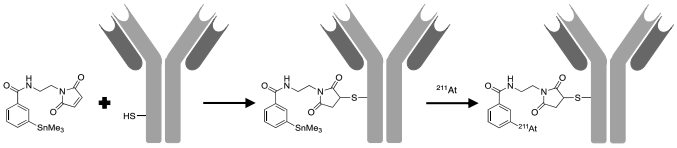


Fig. 1. Flowchart to prepare astatine-211-conjugated antibodies.

Figure 1 shows a flowchart to prepare astatine-211-conjugated antibodies. First, we reduced the interchain disulfide bonds of mAbs[4,5] and attached a trimethylstannyl benzoate linker to an mAb with reactive sulfhydryl groups via a thioether bond between the sulfhydryl and maleimide groups. Then, we labeled liker-antibody complexes with astatine-211 via a halogen-exchange reaction. Subsequently, the astatine-211-conjugated antibodies were purified away from unconjugated astatine-211 using a PD-10 desalting column (GE Healthcare Life Sciences, Chicago, IL, USA). To increase the labeling efficiency, we optimized the procedure to purify astatine-211 from bismuth and calculated the labeling rate by dividing the radioactivities of immunoconjugates by those of astatine-211 that was initially applied to the reaction solution (100 MBq); the labeling rate was approximately 60%. We successfully prepared astatine-211-conjugated antibodies using both mAbs newly developed by us and trastuzumab, a clinically available mAb for patients with breast or gastric cancer.

We evaluated the affinity of astatine-211-conjugated antibodies by flow cytometry. The radioactive antibodies bound to cancer cells depending on the expression level of target molecules on the cell membrane, and the specific affinity of immunoconjugates was comparable to the corresponding mAbs. As a result, astatine-211-conjugated antibodies exerted *in vitro* cytocidal effects on cancer cells depending on the expression of target molecules on the cell membrane.

In conclusion, we synthesized astatine-211 using the RIKEN AVF cyclotron and succeeded in constructing astatine-211-conjugated antibodies. The present data warrant further basic studies for future clinical development.

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