

Pretargeted Radioimmunotherapy of Ovarian Cancer with ^{225}Ac and an Internalizing Antibody

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The field of nuclear medicine and theranostics has never been as vibrant as it is today (1–3). Over the last decade, largely because of the approval of several new radiopharmaceuticals by the U.S. Food and Drug Administration and the European Medicines Agency for diagnosis and treatment of various types of cancer, a large number of startup companies have been formed around the globe. Numerous research laboratories have devoted significant resources and effort to the development of novel radiopharmaceuticals that can be translated into the clinic for cancer patient management as well. For diagnostic purposes, the most common radioisotopes used in the clinic are still $^{99\text{m}}\text{Tc}$, ^{18}F , ^{11}C , and ^{68}Ga , although there are an increasing number of studies using radioisotopes such as ^{64}Cu and ^{89}Zr . For therapeutic purposes, that is, radioligand therapy or targeted radionuclide therapy, there are, generally speaking, 3 types of radionuclides that can be used: α -emitting radioisotopes (e.g., ^{223}Ra , ^{225}Ac , ^{211}At , ^{213}Bi , and ^{212}Pb), β -emitting radioisotopes (e.g., ^{131}I , ^{177}Lu , ^{90}Y , ^{67}Cu , and ^{47}Sc), and Auger-emitting radioisotopes (e.g., $^{123/125}\text{I}$, ^{67}Ga , $^{99\text{m}}\text{Tc}$, ^{111}In , and ^{201}Tl). This is a decade of unprecedented excitement and high expectations for novel radiopharmaceuticals.

Some literature also refers to radioligand therapy and targeted radionuclide therapy as a *radionuclide–drug conjugate*, which is obviously based on the commercial successes of antibody–drug conjugates. However, we argue that *radionuclide–drug conjugate* is not a scientifically accurate term because the radionuclide itself is the drug that causes cell killing, not, as the name *radionuclide–drug conjugate* suggests, the antibody or other ligand.

When we compare the 3 relevant types of radionuclides, that is, α -emitters, β -emitters, and Auger-emitters, there are advantages and disadvantages to each choice, and the right choice may be dependent on a variety of factors, such as the targeting ligand, the size of the tumor, the cost, and the risk-to-benefit ratio, to name just a few. β -emitters (e.g., ^{177}Lu , ^{90}Y , and ^{131}I) have been extensively used in the clinical setting (with some radiopharmaceuticals approved or soon to be approved for clinical use); to be effective, Auger-emitters often require precise targeting of the cancer cell nucleus, a requirement that can be challenging to achieve in high efficiency; α -emitters are much more effective in cell killing at a

short distance ($<100\text{ }\mu\text{m}$) but may have significant toxicity if not targeted properly to the tumor tissue. Such toxicity may be exacerbated by the fact that the strong recoil of an α -emission will cause the radioisotope to detach from the chelator, and the daughter radionuclides may accumulate substantially in other organs such as the kidneys and potentially cause dose-limiting toxicity. This possibility has been one of the major concerns and challenges regarding the clinical use of ^{225}Ac , as it has 4 α -decays. Therefore, researchers have been investigating various strategies to reduce the potential toxicity of ^{225}Ac and its daughter radionuclides, such as kidney protection, pretargeting, or the use of nanomaterials to trap ^{225}Ac and its daughters.

In this issue of *The Journal of Nuclear Medicine*, Chung et al. reported the use of ^{225}Ac for human epidermal growth factor receptor-2 (HER2)-targeted treatment of small-volume ovarian peritoneal carcinomatosis (OPC) in a mouse model (4). The approach adopted in this comprehensive study was pretargeted radioimmunotherapy (PRIT), which may need some elaboration. The 3 components used for HER2-targeted PRIT were an anti-HER2/anti-DOTA IgG-single-chain variable fragment (scFv) bispecific antibody (BsAb), a clearing agent (CA; DOTA(Y)-conjugated poly-*N*-acetylgalactosamine glycodendron), and a radiohaptent that is either ^{225}Ac -Pr (“Pr” denotes *Proteus*-DOTA; for therapy) or ^{111}In -Pr (for imaging and dosimetry estimation). Pr represents the radiohaptent precursor (molecular weight $\sim 1,350\text{ Da}$), which consists of 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid (DO3A, a radio-metal chelator), separated by a tetraethylene glycol (PEG₄) linker to a ^{175}Lu complex of 2-benzyl-DOTA (5).

The BsAb (molecular weight $\sim 210\text{ kDa}$) was produced in Chinese hamster ovary cells and purified by protein A affinity chromatography, as the investigators previously reported in 2018 (6). It can bind to HER2 on the ovarian cancer cells for tumor targeting, as well as provide a handle for binding to the radiohaptent for imaging (with ^{111}In) or therapy (with ^{225}Ac). Since the BsAb circulates for a long time in mice (potentially even longer in humans), the CA (molecular weight $\sim 9\text{ kDa}$) was designed, optimized, and used to rapidly remove the circulating BsAb. This occurs by forming a complex via the DOTA(Y) moiety, which can subsequently be cleared via liver asialoglycoprotein receptor recognition and catabolism (7). After the circulating BsAb is cleared, the radiohaptent (i.e., ^{225}Ac -Pr) was injected, accumulated rapidly in the tumor tissue, and caused cancer cell killing. As a small molecule, ^{225}Ac -Pr clears rapidly from the mouse body, minimizing the potential toxic side effects caused by the daughter

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radionuclides of ^{225}Ac (e.g., ^{221}Fr , ^{217}At , ^{213}Bi , ^{213}Po , and ^{209}Bi), which will not be inside DO3A or Pr anymore because of the recoil of the initial α -decay.

Tremendous effort has been devoted to pretargeting over the last several decades for imaging or therapy of cancer. Excellent review articles are available on this topic (8,9). Generally, and for most of the literature reports, noninternalizing antibodies were used for pretargeting, as the antibody needs to be accessible by the subsequently injected radiolabeled small molecule. Interestingly, in the study of Chung et al. (4), the antibody used for HER2 (over-expressed in a significant percentage of ovarian cancer) was an internalizing antibody, trastuzumab. According to the experimental findings investigating the internalization kinetics of anti-HER2 BsAb-pretargeted ^{111}In -Pr, the average ^{111}In internalization over the 48-h period was about 60%, much higher than the control non-internalizing antibody for GPA33 (~15%). It was concluded via cellular dosimetry calculations that such a PRIT system could deliver about a 5 times greater absorbed dose to the cell nucleus than a noninternalizing system, desirable for better therapeutic efficacy. Future optimization of radiohaptens delivery with an even higher percentage of internalization may further increase the absorbed dose delivered to the tumor tissue.

It is worth pointing out that since trastuzumab is an internalizing antibody, it was estimated that the mean surface-bound ^{131}I -BsAb at 24 h (which is the interval between injections of BsAb and the CA) was less than 10% (i.e., most of the injected BsAb was not accessible for $^{225}\text{Ac}/^{111}\text{In}$ -Pr). Since the model used in this study is small-volume OPC, the BsAb (0.25 mg/1.19 nmol, as determined by the previous study (6)) was injected intraperitoneally. Such an injection will likely lead to faster HER2 binding and tumor accumulation than intravenously injected BsAb. Therefore, the 24-h interval could potentially be shortened for perhaps a higher percentage of BsAb on the tumor cell surface for $^{225}\text{Ac}/^{111}\text{In}$ -Pr binding or a higher percentage of internalization for the latter. That being said, whether such an intraperitoneal injection will be applicable to the clinical situation of PRIT of OPC remains to be determined.

The CA used in this study deserves some attention. The commonly used CA is a 500-kDa dextran-DOTA hapten conjugate, which can bind to the anti-DOTA(M)-scFv domain of the BsAb in circulation, which then is removed from the blood via the reticulo-endothelial system. Since a 500-kDa CA is too large to extravasate efficiently, it does not bind significantly to tumor-associated BsAb. In addition, the polydispersity of this 500-kDa CA (poor reproducibility for compliance with current good manufacturing practices), as well as enzymatic degradation of the CA, hampers its potential clinical translation. Therefore, a DOTA-dendron CA consisting of 16 terminal α -thio-*N*-acetylgalactosamine residues was synthesized and systematically investigated in a recent *in vivo* study (7). It was concluded that this new CA could be used for enhanced blood clearance of BsAb, with the optimal conditions being intravenous injection of a BsAb dose of 0.25 mg (1.19 nmol), use of a 24-h interval between BsAb and intravenous injection of the CA (25 μg ; 2.76 nmol), and use of a 4-h interval between injections of the CA and a ^{177}Lu -based radiohaptens. The same dosing regimen was adopted in this work for ^{225}Ac -based PRIT, except that intraperitoneal (instead of intravenous) injections were used for the BsAb and $^{225}\text{Ac}/^{111}\text{In}$ -Pr. Interestingly, the CA was still injected intravenously. It is unclear whether the

CA is still necessary in this case, as most of the $^{225}\text{Ac}/^{111}\text{In}$ -Pr may not even enter the bloodstream. More detailed investigation may be needed in the future, regarding timing interval, dose of each agent, the use of CA, etc., to give the best protocol for PRIT of OPC.

The chemical structure of $^{225}\text{Ac}/^{111}\text{In}$ -Pr may also warrant some discussion. The C8.2.5 scFv used for DOTA binding in the BsAb was developed more than a decade ago and exhibited notable metal specificity (10). It has picomolar affinity for DOTA complexes of lutetium and yttrium and nanomolar affinity for indium and gallium chelates, which were shown to give very different tumor uptake values for *in vivo* pretargeting when different radiohaptens were used (5). Therefore, although the direct use of ^{177}Lu -DOTA-Bn worked quite well for pretargeting, tumor uptake of ^{225}Ac -DOTA-Bn was much lower and far from satisfactory. Hence, Pr was synthesized (5): p-SCN-Bn-DOTA was loaded with nonradioactive ^{175}Lu to yield a p-SCN-Bn-DOTA- ^{175}Lu complex, which was then coupled to NH_2 -PEG₄-DO3A to yield the Pr used in this work (4). The ^{175}Lu -DOTA-Bn portion of the Pr confers picomolar affinity to the anti-DOTA C8.2.5 scFv moiety of the BsAb, whereas the DO3A can be efficiently labeled with either ^{225}Ac or ^{111}In , or certain other radiometals if needed.

It was concluded that all treatments were well tolerated, whether they were performed with 1-cycle or 2-cycle anti-HER2-PRIT (4). More importantly, both treatments were highly effective, evidenced by serial bioluminescence imaging of the OPC tumor burden, as well as statistically significant extension of survival for the anti-HER2-PRIT groups. When analyzing statistical values of bioluminescence imaging, one must keep in mind both that intraperitoneal tumor growth is highly heterogeneous and that the bioluminescence imaging signal does not correlate linearly with the overall tumor burden. Survival data may be a better indication of therapeutic efficacy in this study, which was indeed quite impressive.

We applaud the authors for performing comprehensive histologic analyses and toxicity studies of the mice undergoing PRIT (most of the data were presented in the supplemental material (4)), which is usually done for clinical studies but rarely seen in pre-clinical research. No significant weight loss of the mice was observed after PRIT. On the basis of histopathology, the only finding of organ injury was minimal-to-mild renal tubular degeneration. However, this did not affect the renal function based on serum blood urea nitrogen and creatinine data. Lastly, all hematologic parameters were within normal limits for treated mice. All these promising results suggested little to no acute or chronic toxicity for PRIT, and further dose escalation is possible if needed in the future.

In conclusion, this study reported a promising approach to treating HER2-expressing OPC in small-animal models. Since there is no effective treatment for this devastating disease in the clinic, this PRIT strategy may fulfill an unmet urgent clinical need in the future, upon further optimization and clinical translation. Since the C8.2.5 scFv used for DOTA binding has picomolar affinity for DOTA complexes of yttrium (10), the $^{86}\text{Y}/^{90}\text{Y}$ theranostic radioisotope pair should certainly be evaluated in the future; such a pair not only would enable true cancer theranostics with the same chemical entity (using different isotopes of the same element Y) but also would offer better imaging characteristics via PET than ^{111}In -based SPECT. We look forward to more exciting follow-up

studies in the PRIT space, which holds tremendous potential for revolutionizing cancer patient management.

DISCLOSURE

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