



# Recent progress of astatine-211 in endoradiotherapy: Great advances from fundamental properties to targeted radiopharmaceuticals

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## ABSTRACT

Astatine-211 ( $^{211}\text{At}$ ,  $t_{1/2} = 7.21\text{ h}$ ) emitting two  $\alpha$  particles with energies of 5.87 and 7.45 MeV, can lead to a high linear energy transfer ( $\text{LET} = 98.84\text{ keV}/\mu\text{m}$ ) and short tissue range ( $50\sim 90\mu\text{m}$ ). Since the 1950s,  $^{211}\text{At}$  had stepped into endoradiotherapy and has always been regarded as one of the most promising  $\alpha$ -emitters for targeted-alpha therapy (TAT) in various malignancies. In the past two decades,  $^{211}\text{At}$  related radiopharmaceuticals have achieved great progress in the studies of basic physicochemical properties of astatine,  $^{211}\text{At}$  labeling strategies, preclinical and clinical studies, producing profound effects in nuclear medicine. This work will give a panorama of  $^{211}\text{At}$ -related researches in the recent 20 years, which will cover both the fundamental insights of  $^{211}\text{At}$  radiochemistry and applied labeling compounds. It can provide some important hints for the studies of TAT and other radiopharmaceuticals applied in tumor radiotherapy.

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## 1. Introduction

Recently, targeted-alpha therapy (TAT) has drawn much attention both in preclinical and clinical researches. The LET of  $\alpha$  particles is  $\sim 100\text{ keV}/\mu\text{m}$ , which is about 500 times higher than that of widely-used  $\beta^-$  particles. High LET value enables  $\alpha$  particles to more effectively trigger DNA double-strand breaks (DSBs), thus inducing cell cycle arrest and leading to mitotic cell death, apoptosis or necrosis [1,2]. Meanwhile, the path length of  $\alpha$  particles ( $50\sim 100\mu\text{m}$ ) is equivalent to the distance of  $5\sim 10$  cells, which can reduce the irradiation to surrounding healthy cells and unnecessary damage to normal tissues and organs [3]. These excellent properties make TAT show great advantages in the treatment of minimal residual tumors after primary treatment including surgery, external radiotherapy and chemotherapy.

There are over 2000 known radionuclides, and about 400 are  $\alpha$ -emitting. Ideal  $\alpha$ -emitters for TAT should have several basic characteristics [4]: (1) suitable half-lives for radiolabeling, purification and transportation, (2) contain no long-lived toxic decay daughters, (3) and there are available production methods for clinical applications. These criteria will screen all candidates to no more than ten, including  $^{149}\text{Tb}$ ,  $^{211}\text{At}$ ,  $^{212/213}\text{Bi}$ ,  $^{223/224}\text{Ra}$ ,  $^{225}\text{Ac}$ ,  $^{226/227}\text{Th}$  and  $^{230}\text{U}$  (Table 1) [5,6]. Among all these available  $\alpha$  nuclides,  $^{211}\text{At}$

has unique advantages in terms of nuclear properties (Fig. 1). The half-life of  $^{211}\text{At}$  ( $t_{1/2} = 7.21\text{ h}$ ) is long enough for multi-step radiolabeling and is also compatible with plenty of carriers, such as small molecules, peptides and antibody/fragments [7].  $^{211}\text{At}$  is regarded as a 100%  $\alpha$ -emitter with two  $\alpha$  particles with energies of 5.87 and 7.45 MeV. Despite its potential uptake by bone, liver and kidney, the toxicity of  $^{207}\text{Bi}$  can be negligible [8]: 370 MBq of  $^{211}\text{At}$ , the currently highest dose administered to a human, only leads to about 3.7 kBq of  $^{207}\text{Bi}$ . There is a chemical conversion of astatine to polonium which will cause the radionuclide to detach from the carriers, but the  $^{211}\text{Po}$  daughter is not affected by nuclear recoil since it comes from  $^{211}\text{At}$  by electron capture (EC). Even it can immediately escape from the cell surface and diffuse freely,  $^{211}\text{Po}$  with a short half-life of 0.52 s tend to decay to  $^{207}\text{Pb}$  within two cell diameters beyond the original cell surface [9]. In contrast, when  $^{211}\text{At}$  decays into  $^{211}\text{Po}$  by EC, the characteristic X-rays of 77–92 keV can be used for measuring  $^{211}\text{At}$  by  $\gamma$ -detectors in laboratory or clinical centers, which are also available for imaging by planar or SPECT scanners [10–13]. Additionally,  $^{211}\text{At}$  can be produced by bombarding natural Bi with  $\alpha$  particles ( $\geq 20\text{ MeV}$ ) on a cyclotron, which makes it more available than most medical  $\alpha$ -emitters.

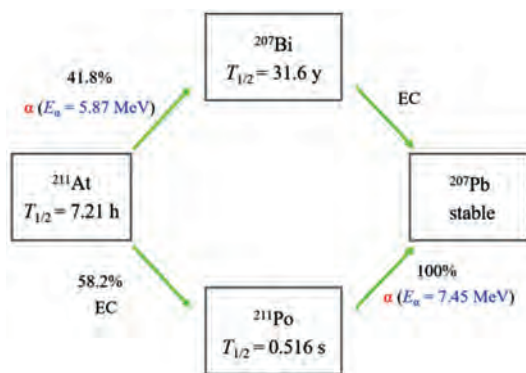
Actually, in 1950, Hamilton *et al.* [14] found that  $^{211}\text{At}$  could induce extreme degree of radiation injury of the thyroid gland in the rat without apparent involvement of the adjacent parathyroid gland, demonstrating that this  $\alpha$ -emitter might be of essen-

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**Table 1**  
Several  $\alpha$ -emitters suitable for TAT [5,6].

| Nuclide           | $T_{1/2}$ | $E_{\alpha}$ (MeV) | Production                                                                                              |
|-------------------|-----------|--------------------|---------------------------------------------------------------------------------------------------------|
| $^{149}\text{Tb}$ | 4.12 h    | 3.97               | $^{152}\text{Gd}(p, 4n)^{149}\text{Tb}$                                                                 |
| $^{211}\text{At}$ | 7.21 h    | 5.87/7.45          | $^{209}\text{Bi}(\alpha, 2n)^{211}\text{At}$                                                            |
| $^{212}\text{Bi}$ | 10.6 h    | 6.08               | $^{224}\text{Ra}$ decay                                                                                 |
| $^{213}\text{Bi}$ | 45.6 m    | 5.88               | $^{225}\text{Ac}$ decay                                                                                 |
| $^{223}\text{Ra}$ | 11.4 d    | 5.87/22.4          | $^{227}\text{Th}$ – $^{223}\text{Ra}$ generator                                                         |
| $^{224}\text{Ra}$ | 3.63 d    | 5.69/21.0          | $^{228}\text{Th}$ – $^{224}\text{Ra}$ generator                                                         |
| $^{225}\text{Ac}$ | 9.92 d    | 5.83/26.7          | $^{232}\text{Th}(p, x)^{225}\text{Ac}$                                                                  |
|                   |           |                    | $^{226}\text{Ra}(p, 2n)^{225}\text{Ac}$                                                                 |
| $^{226}\text{Th}$ | 30.6 m    | 6.34               | $^{230}\text{U}$ – $^{226}\text{Th}$ generator                                                          |
| $^{227}\text{Th}$ | 18.7 d    | 6.04               | $^{227}\text{Ac}$ decay                                                                                 |
| $^{230}\text{U}$  | 20.2 d    | 5.89               | $^{230}\text{Pa}$ decay/<br>$^{231}\text{Pa}(p, 2n)^{230}\text{U}/^{231}\text{Pa}(d, 3n)^{230}\text{U}$ |

**Fig. 1.** Decay diagram of  $^{211}\text{At}$ .

tial therapeutic application in hyperthyroidism. Since then,  $^{211}\text{At}$  had stepped into endoradiotherapy and kept drawing more and more attention in nuclear medicine. Especially,  $^{211}\text{At}$ -related radiopharmaceuticals have made great progress since 2000. It has been demonstrated that  $^{211}\text{At}$  is one of the most promising  $\alpha$ -nuclides for TAT and corresponding radiopharmaceuticals have widely-clinical prospects. Although several breakthroughs about  $^{211}\text{At}$ -labelled pharmaceuticals have been involved in some of the latest comprehensive reviews focused on introducing TAT or endoradiotherapy [15,16], there is still a lack of specific work illustrating the new developments about  $^{211}\text{At}$ -based radiotherapy over the past decade, as the latest one can be dated to 2013 [8]. Considering newly emerging advances in  $^{211}\text{At}$ -based medicines should have profound effect in TAT, especially those involving the fundamental properties and clinical trials, we believe that it is very important to retrospect recent progresses of  $^{211}\text{At}$  again.

In this review, we will introduce the progress of  $^{211}\text{At}$  about the basic properties of astatine, the separation methods of  $^{211}\text{At}$ , the brief strategies for  $^{211}\text{At}$  labeling and the preclinical and clinical trials about related targeted pharmaceuticals. It will mainly cover most of the work from 2000, since ~70% of the publications involving  $^{211}\text{At}$  are located in 2000–2021, when astatine/astatine- $^{211}\text{At}$  is typed as the topic to search articles through *web of science* without other limitations (on February 28, 2022). In addition, despite of considerable amount of the work involving  $^{211}\text{At}$  in 1940–2000 focused on the nuclear properties of astatine, there is already a review from Zalutsky *et al.* [7] in 2000 specializing on introducing  $^{211}\text{At}$ -labelled radiopharmaceuticals. The goals of this work include: (1) providing some general introduction of  $^{211}\text{At}$  to the newcomers in TAT, so that they can recognize both the prospects and challenges of  $^{211}\text{At}$ -related radiopharmaceuticals; (2) systematically concluding the progress of  $^{211}\text{At}$ -based radiotherapy in recent years for researchers who are engaging in radiochemistry and nuclear medicine; (3) summarizing some prob-

**Table 2**  
The nuclear properties of  $^{191}\text{--}^{224}\text{At}$ .

| Mass | $t_{1/2}$ | Decay                    | Mass | $t_{1/2}$ | Decay                    |
|------|-----------|--------------------------|------|-----------|--------------------------|
| 191  | 1.7 ms    | $\alpha$                 | 208  | 1.63 h    | $\alpha$ , EC, $\beta^+$ |
| 192  | 88 ms     | $\alpha$                 | 209  | 5.42 h    | $\alpha$ , EC, $\beta^+$ |
| 193  | 28 ms     | $\alpha$                 | 210  | 8.1 h     | $\alpha$ , EC, $\beta^+$ |
| 194  | 40 ms     | $\alpha$                 | 211  | 7.21 h    | $\alpha$ , EC            |
| 195  | 0.29 s    | $\alpha$                 | 212  | 0.314 s   | $\alpha$ , EC, $\beta^+$ |
| 196  | 0.388 s   | $\alpha$ , EC, $\beta^+$ | 213  | 125 ns    | $\alpha$                 |
| 197  | 0.388 s   | $\alpha$ , EC, $\beta^+$ | 214  | 558 ns    | $\alpha$                 |
| 198  | 4.2 s     | $\alpha$ , EC, $\beta^+$ | 215  | 0.10 ms   | $\alpha$                 |
| 199  | 7.03 s    | $\alpha$ , EC, $\beta^+$ | 216  | 0.30 ms   | $\alpha$                 |
| 200  | 43 s      | $\alpha$ , EC, $\beta^+$ | 217  | 32.6 ms   | $\alpha$ , $\beta^-$     |
| 201  | 1.4 m     | $\alpha$ , EC, $\beta^+$ | 218  | 1.5 s     | $\alpha$ , $\beta^-$     |
| 202  | 3.1 m     | $\alpha$ , EC, $\beta^+$ | 219  | 56 s      | $\alpha$ , $\beta^-$     |
| 203  | 7.4 m     | $\alpha$ , EC, $\beta^+$ | 220  | 3.71 m    | $\alpha$ , $\beta^-$     |
| 204  | 9.1 m     | $\alpha$ , EC, $\beta^+$ | 221  | 2.3 m     | $\beta^-$                |
| 205  | 26.9 m    | $\alpha$ , EC, $\beta^+$ | 222  | 54 s      | $\beta^-$                |
| 206  | 30.6 m    | $\alpha$ , EC, $\beta^+$ | 223  | 50 s      | $\beta^-$                |
| 207  | 1.81 h    | $\alpha$ , EC, $\beta^+$ | 224  | 1.3 m     | $\beta^-$                |

lems before clinical translation of  $^{211}\text{At}$ -labelled compounds, and comprehensively evaluate the application potential of  $^{211}\text{At}$ . We believe that this work is not only helpful to the development of  $^{211}\text{At}$ -labelled radiopharmaceuticals, but can also provide some important hints for endoradiotherapy researches based on other  $\alpha$ -emitters.

## 2. Basic properties of astatine

### 2.1. Isotopes of astatine

Astatine ( $Z=85$ ), located at the seventh group of the periodic table, was discovered in 1940 by Corson *et al.* [17]. It was obtained by bombarding natural Bi target with 32 MeV of  $\alpha$  particles and was initially named as “eka-iodine”, due to its high similarity to iodine. In 1947, it was formally identified as astatine, from the ancient Greek “astatos” which means instability [18]. Astatine is often considered one of the rarest of all elements, and the total amount of natural astatine at any given time range is estimated from a few hundred milligrams to 30 g. Among all isotopes of astatine,  $^{214}\text{--}^{219}\text{At}$  occurs naturally in the decay chains of transuranium elements, and the most abundant one is  $^{217}\text{At}$  ( $t_{1/2}=32.6$  ms) decayed from  $^{237}\text{Np}$  [19]. Other isotopes of astatine are artificially synthesized, and  $^{210}\text{At}$  has the longest half-life of 8.1 h. The isotopes of astatine with the mass lighter than 217 decay by pure  $\alpha$ -emission or by a mixture of  $\alpha$ - and EC/ $\beta^+$ -emissions,  $^{217}\text{--}^{220}\text{At}$  undergo both  $\alpha$ - and  $\beta^-$ -emissions, while astatine isotopes with mass  $> 220$  decays by pure  $\beta^-$ -emission (Table 2).

Although  $^{207}\text{--}^{210}\text{At}$  with suitable half-lives ( $>1$  h) can emit  $\alpha$ ,  $\beta^+$  as well as  $\gamma$  rays, therefore show possible application in nuclear medicine, the prospect of these radionuclides is severely clouded by some other factors. For  $^{207/208}\text{At}$ , there is no suitable way for producing medically-required amount of such radionuclides. It is previously-reported that  $^{209}\text{At}$  might be suitable for SPECT imaging in nude mice [20], but the production of  $^{209}\text{At}$  requires high-energy protons (480 MeV) to bombard uranium carbide targets [21]. The production of  $^{210}\text{At}$  is not an serious issue and it was ever used to replace  $^{211}\text{At}$  as  $\gamma$ -emitter to quantify the *in vivo* distribution of astatine [22], but its daughter  $^{210}\text{Po}$  ( $t_{1/2}=138.38$  d) is extremely toxic, making this nuclide almost have no prospect in biomedicine. Similarly, the  $\alpha$ -emitters  $^{208/209}\text{Po}$  ( $t_{1/2}=2.90$  and 124 y) in the decay chains of  $^{208/209}\text{At}$  also limit their medical practice: once entering the organism, they may also cause long-term unnecessary internal irradiation and corresponding biotoxicity. As a result, only  $^{211}\text{At}$  has currently been

employed in radiopharmaceuticals and been receiving more and more attention.

## 2.2. Physicochemical properties of At

As pointed out by Kozimor *et al.* [23], At should be one of the least understood elements in the periodic table. The most important reason is that, even for  $^{211}\text{At}$  with relatively long half-life and nontoxic decay daughters, it can be safely produced and operated with the amount limited to be 37 kBq to 4 GBq, roughly equivalent to  $4.8 \times 10^{-13}$  g to  $5.2 \times 10^{-8}$  g (rarely reaches the upper limit). Such quantities are beyond the detection limits of the most sensitive mass spectrometry, not to mention NMR, EPR, UV-vis and X-ray spectroscopy. Besides, for most chemical reactions,  $^{211}\text{At}$  currently ranges from  $10^{-13}$  g to  $10^{-9}$  g, and may even be smaller than the amount of trace organic species and metals in solvents. Thus, these intrinsic trace impurities may interfere during related reaction even catalyze unexpected reaction pathways.

According to periodic law, it seems feasible to use I as an analogue of At in fundamental study. However, this strategy must be cautiously taken since At is semi-metallic [24–26], while I is absolutely non-metallic. There are also theoretical studies showing that  $\text{Al}_{13}$  cluster and At possess similar chemical reactivity [27], especially in electron transfer process. Meanwhile, both of them can form stable compounds with isobutylbenzene. Obviously, this kind of analogy at atomic levels is difficult to be extrapolated to macroscopic system. For the most widely-used  $^{211}\text{At}$ , 1 MBq equals just 0.06 pmol atoms, making it statistically unlikely to exist as  $^{211}\text{At}_2$ . Up to now, the color of astatine is not even known and is speculated to be black based on the fact that halogens from fluorine to iodine get darker [28]. The melting and boiling points of At are estimated as 302 and 337 °C, respectively [4]. On silica, the adsorption enthalpies of At,  $\text{AtO}_2$  and  $\text{HAtO}$  determined by Serov *et al.* [29] are  $124 \pm 5$ ,  $80 \pm 5$  and  $47 \pm 5$  kJ/mol, while the sublimation enthalpies are  $148 \pm 25$ ,  $106 \pm 30$  and  $56 \pm 30$  kJ/mol, respectively. On the gold surface, the adsorption enthalpies of At and  $\text{AtO}_2$  were determined to be  $147 \pm 15$  and  $124 \pm 5$  kJ/mol, respectively.

The behavior of At in different solutions is also complicated.  $^{211}\text{At}$  is stable in chloroform and loses no activity even when the solvent is completely evaporated. However, the species distribution in chloroform is very variable. At can react with chlorine and other oxygen-containing radiolysis products derived from chloroform, producing a variety of species in a dose-dependent manner [30]. It has been demonstrated that the volatilization of free  $^{211}\text{At}$  is an heterogeneous process and 2.3 times higher than that of  $\text{Na}^{211}\text{At}$  even in confined space [31]. This may be tightly related to the various oxidation states of At (–1, +1, +3, +5, +7) [32], which also lead to multiple species in aqueous solution, e.g.,  $\text{At}^-$ ,  $\text{At}(0)$ ,  $\text{AtO}^-$ ,  $\text{AtO}^+$ ,  $\text{AtO}_2^-$ ,  $\text{AtO}_3^-$  and  $\text{AtO}_4^-$ . However, for a long time, the specific species of At lacked relevant experimental data to support. Recently, Guo *et al.* [33] successfully measured the absolute movement velocity of At monovalent anion in acidic reducing condition to be  $-8.26 \pm 0.59 \times 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ , which was highly coherent with theoretical calculation and directly proved the existence of  $\text{At}^-$ . Champion *et al.* [34] confirmed that  $\text{At}(0)$  cannot exist in aqueous solution, while  $\text{At}(-\text{I})$ ,  $\text{At}(-\text{I})$  and  $\text{At}(-\text{III})$  should belong to  $\text{At}^-$ ,  $\text{At}^+$  and  $\text{AtO}^+$  in the range of pH 1–2, with the standard redox potentials of  $\text{At}^+/\text{At}^-$  and  $\text{AtO}^+/\text{At}^+$  pairs being 0.36 and 0.74 (0.01 V vs. NHE), respectively. It has also been demonstrated that the main species of At at pH 11 is  $\text{AtO}(\text{OH})_2^-$  but not  $\text{At}^-$  [35]. Meanwhile,  $\text{AtO}^+$  is able to form a neutral substance  $\text{AtO}(\text{OH})$  mainly through the reaction of  $\text{AtO}^+ + \text{H}_2\text{O}(\text{l}) \rightleftharpoons \text{AtO}(\text{OH})(\text{aq}) + \text{H}^+$  when increasing solution pH, and the equilibrium constant was  $10^{-1.9}$  [36].  $\text{At}^+$  can form stable ternary complexes  $\text{IAtBr}^-$  in aqueous solution, with an equilibrium constant of  $10^{7.5 \pm 0.2}$  [37]. Nishinaka *et al.* [38] speculated that At can also exist as  $\text{AtO}_3^-$  and

$\text{AtO}_4^-$  in the presence of strong oxidizer (such as  $\text{KIO}_4$ ). However, this result was based on the comparison of  $R_f$  values between At and I species ( $\text{IO}_3^-$  and  $\text{IO}_4^-$ ) using thin layer chromatography (TLC) methods, which still needs more direct evidences.

As a typical intermolecular interaction, halogen bond is formed by attraction between a halogen atom X from a molecule or a molecular segment R-X (R is usually more electronegative than X or is X itself) and another atom from R-X or a different molecule B. In a typical case, B is usually a Lewis base, and R can be another halogen atom or an inorganic/organic residue [39]. Loosely, halogen bonds occur when there are attractive interaction between an electrophilic region associated with a halogen atom in one molecular entity and a nucleophile region in another or the same molecular entity [40]. Guo *et al.* [41,42] demonstrated that  $\text{AtI}$  can interact with various Lewis bases through halogen bond, and it is a stronger halogen bond donor than  $\text{I}_2$ . They also found that tributylphosphine oxide ( $\text{Bu}_3\text{PO}$ ) and  $\text{AtI}$  can form either 1:1 or 2:1 complex [43]. The former has the currently strongest halogen bond, and the formation constant is as high as  $10^{4.24 \pm 0.35}$ . For the 2:1 complex, a  $\text{Bu}_3\text{PO}$  molecule is first linked to  $\text{AtI}$  by halogen bond, while another one participated in the formation of the terpolymer by forming hydrogen bond with the organic entity.

The metallic property of At has encouraged researchers to look for suitable chelating agents which can form stable At-centered coordination complexes. A study performed by Bassal *et al.* [44] involving more than 20 model ligands indicated that those contain S and O donors exhibit higher affinity for  $\text{AtO}^+$ . However, density functional theory (DFT) calculations showed that S or N atoms are more likely to interact with the O atom in  $\text{AtO}^+$  rather than directly coordinating with At atom. It also had been demonstrated that both  $\text{At}^+$  and  $\text{AtO}^+$  can form 1:1 and 1:2 complexes with  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{SCN}^-$  and thioalix[4]arene tetrasulfonate [45,46]. But there is still a lack of adequate evidence for the coordination between At and these selected ligands and the formations of corresponding complexes were more likely driven by ionic interactions. In fact, how to obtain enough experimental data to confirm the interaction between astatine and donor atoms may be the key and difficult point of relevant research.

In recent years, computational chemistry which is not dependent on the availability of astatine, has become one of the important means to investigate At chemistry. For a lot of species including  $\text{At}^-$ ,  $\text{AtX}$  ( $\text{X} = \text{H}/\text{H}^+$ , F-Ts),  $\text{AtF}_3$ ,  $\text{AtO}^+$ ,  $\text{HOAt}$ ,  $\text{CH}_3\text{At}$  and  $\text{Y}_3\text{C-At}$  ( $\text{Y} = \text{F}$  to At), etc., the electronic property [47–54], bonding mechanism [55–59], solvation mode [60–64], halogen bond [65–71], molecular configuration [72–76] and adsorption energy [77] have been well studied. For organic astatine compounds, related researches mainly focus on the inherent mechanism of C-At bond [78–80] as well as the aromaticity of astatine-substituted borazine ( $\text{B}_3\text{N}_3\text{At}_6$ ) and benzene ( $\text{C}_6\text{At}_6$ ) [81,82]. It should be noted that for a heavy element like At, spin-orbit coupling and relativistic effects have very important effects on the theoretical calculations, so one must pay enough attention when talking about related bonding process. Meanwhile, for a certain characteristic property, different calculation methods may have huge difference in the final results. For example, for the first ionization energy and electron affinity of At, the calculated values by different methods vary in the range of 8.965–10.004 and 2.110–2.423 eV, respectively [47–49]. Therefore, before experimental evidence is obtained, the accuracy of theoretical calculation will lack reliable calibration. Fortunately, by using laser ionization spectroscopy, the first ionization energy of At was determined by Rothe *et al.* [83], which is 9.31751(8) eV, while the electron affinity was identified as 2.41578(7) eV by Leimbach *et al.* [84]. The determination of both basic data of At is of great significance. Firstly, it can offer important references which will prompt the computational researches involving At chemistry. Secondly, these data are helpful to recognize the reaction mecha-

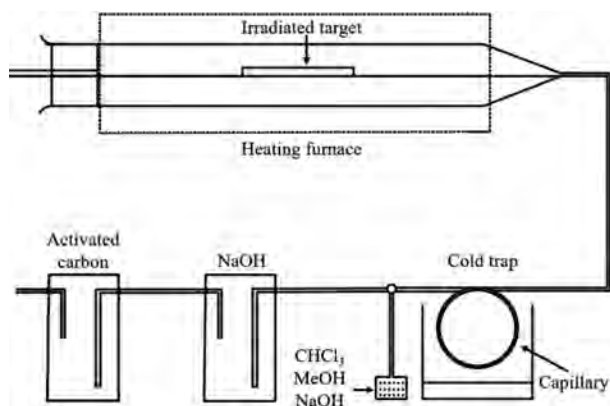


Fig. 2. A simplified distillation apparatus for separating  $^{211}\text{At}$ .

nism, especially for  $^{211}\text{At}$  labeling and deastatination. Thirdly, the development of At study can facilitate the fundamental research on some other heavy elements like Ts.

### 2.3. Production of $^{211}\text{At}$

The production of radionuclide  $^{211}\text{At}$  can be achieved by two approaches,  $^{211}\text{Rn}$ - $^{211}\text{At}$  generator and  $^{209}\text{Bi}(\alpha, 2n)^{211}\text{At}$  reaction on accelerator.  $^{211}\text{Rn}$  has a longer half-life ( $t_{1/2} = 14.6\text{ h}$ ), allowing  $^{211}\text{At}$  to be supplied over medium and long distances. Even so, an great obstacle is that it uses  $42/60\text{ MeV}$  of  $^6\text{Li}$  to bombard natural Bi target for obtaining  $^{211}\text{Rn}$  through  $^{209}\text{Bi}(^6\text{Li}, 4n)^{211}\text{Rn}$  or  $^{209}\text{Bi}(^7\text{Li}, 5n)^{211}\text{Rn}$  reaction, which has strict requirements on accelerators with high energy of Li ions. Additionally,  $^{206}\text{Po}$ ,  $^{210}\text{At}$  and  $^{210}\text{Po}$  exist in the decay chain of  $^{210}\text{Rn}$  [from  $^{209}\text{Bi}(^7\text{Li}, 6n)^{210}\text{Rn}$  reaction] may contaminate  $^{211}\text{At}$  products, which is a big challenge to separation technology. Therefore, the production of  $^{211}\text{At}$  by  $^{209}\text{Bi}(\alpha, 2n)^{211}\text{At}$  reaction on accelerator is still the first choice at present. It is easier to achieve and has a high yield ( $16.3\text{ to }41\text{ MBq } \mu\text{A}^{-1}\text{ h}^{-1}$ ). One thing should be paid attention to is that the  $\alpha$  beam energy must be strictly controlled below  $29.0\text{ MeV}$  (usually  $28.0\sim 29.5\text{ MeV}$ ) to avoid/minimize the amount of  $^{210}\text{At}$  from  $^{209}\text{Bi}(\alpha, 3n)^{210}\text{At}$  reaction (nonnegligible,  $>30\text{ MeV}$ ). For current status in the production of  $^{211}\text{At}$ , as well as how to break the logistical borders between the academic and commercial networks in the development of  $^{211}\text{At}$ -labelled pharmaceuticals, one can turn to the latest review given by Feng *et al.* [85].

Generally, the most common separation of  $^{211}\text{At}$  is achieved by high-temperature distillation. The basic principle is that the boiling point of At is  $335\text{ }^\circ\text{C}$ , while that of Bi is as high as  $1564\text{ }^\circ\text{C}$ . Therefore, the separation of  $^{211}\text{At}$  from irradiated targets can be achieved by controlling the heating temperature. In a typical separation (Fig. 2), the irradiated Bi target is placed in a furnace connected to a stable carrier stream, and is heated rapidly to  $650\sim 800\text{ }^\circ\text{C}$  to make  $^{211}\text{At}$  sublimated. Gaseous  $^{211}\text{At}$  will be carried out by  $\text{N}_2$ , flow into the cold trap linked with negative pressure, condense and accumulate in the capillary. In the end, different solvents such as chloroform, methanol or NaOH are used to wash the capillary to obtain  $^{211}\text{At}$  products. The whole separation process can be controlled within 30 min. The  $^{211}\text{At}$  obtained by distillation is nearly carrier-free and there is no need to dissolve the irradiated target before separation. Hence, distillation is still the preferred approach used in the separation of  $^{211}\text{At}$ . However, the recovery of  $^{211}\text{At}$  in this approach is not stable and may vary in the range of  $51\%\sim 83\%$  [85].

In recent years, researchers also began to pay more attention to wet separation of  $^{211}\text{At}$  again. Burns *et al.* [86] demonstrated that 3-octanone and methyl isobutyl ketone can effec-

tively extract  $^{211}\text{At}$  from nitric acid solution with Bi highly retained in water. Furthermore, by loading 3-octanone onto Amberchrom@CG300M resin, they found that the resulting impregnation resin can achieve high-performance separation of  $151.7$  and  $362.6\text{ MBq}$  of  $^{211}\text{At}$  [87]. Specifically, the irradiated Bi target was first dissolved by  $4\sim 6\text{ mol/L HNO}_3$ , and the obtained solution was then loaded onto the impregnation resin column. The Bi substrate and other impurity nuclides were removed by using  $5.9\text{ mol/L HNO}_3$ , while  $^{211}\text{At}$  was retained on the column with a loss of  $<1\%$ . Using ethanol as eluent, more than  $90\%$  of  $^{211}\text{At}$  was washed from the resin, with the total volume lower than  $1\text{ mL}$ . Woen *et al.* [23] proposed to use commercial pre-filter resin ( $100\sim 150\text{ }\mu\text{m}$ ) to recover  $^{211}\text{At}$  from the irradiated Bi target. Different concentrations of HCl and  $\text{NH}_2\text{OH}\cdot\text{HCl}$  were used to remove Bi matrix and other impurity nuclides.  $^{211}\text{At}$  retained on the column was eluted by  $2\text{ mol/L NaOH}$ , with a recovery of  $51\%$ . Watanabe *et al.* [88] also tried to separate  $^{211}\text{At}$  using anionic resin and found that  $^{211}\text{At}$  would stay on the anionic resin when  $8\text{ mol/L HCl}$  and  $\text{HNO}_3$  rinsed the column, of which  $75.3\%\sim 79.1\%$  would be eluted by different concentrations of NaOH or NaOH-EtOH. For all the above-mentioned wet extractions, the separation time was in the range of  $20\sim 90\text{ min}$ , which is quite comparative to that of distillation.

There are also some reports of automatic extraction devices based on liquid-liquid or solid-liquid extraction [89,90]. However, the specific activity of obtained products was not high enough, which was not conducive to  $^{211}\text{At}$  labeling. Overall, the wet separation avoids using high-temperature devices, so there is no need to worry about the volatility of  $^{211}\text{At}$ , and the operation is easier to achieve automation for large-scale production. However, target dissolution is more time-consuming, and the possible existence of residual Bi and impurity nuclides may influence the quality of  $^{211}\text{At}$  product. For impregnation resin, the extractant may detach from the stationary and become impurity in the final product, whether this would influence  $^{211}\text{At}$  radiolabeling still needs to be checked. Distillation and wet separation should complement with each other, and both are still under continuous development, especially the emergence of automatic separation device. How to improve the separation efficiency of  $^{211}\text{At}$  will be one of the keys to promote the basic research and related radiopharmaceuticals.

## 3. $^{211}\text{At}$ -based radiopharmaceuticals

### 3.1. Labeling strategy of $^{211}\text{At}$

Usually, the radioastatination of various carriers can be achieved through either nucleophilic or electrophilic reactions. Unlike radioiodine,  $^{211}\text{At}$  is difficult to bind directly to proteins or peptides to form stable radiolabeled compounds. Therefore, a bi-functional coupling agent which can bind with both the targeting carrier and  $^{211}\text{At}$  is always needed. DFT calculations show that the stability of C-At bond is closely related to the chemical environment of At, which is also consistent with the change of C-I bond [78,79]. Briefly, C-At bond gradually becomes more stable in the order of in alky  $<$  alkenyl  $<$  aryl compounds, i.e.,  $\text{sp}^2$  C-At bond is stronger than  $\text{sp}^3$  one and higher conjugation is helpful for stabilizing C-At bond. Interestingly, B-At and C-At bonds exhibit completely opposite polarities: At in C-At bond is positive-charged, while it is negative-charged in B-At bond. This can well explain why C-At is more vulnerable to nucleophiles. Related *in vivo* experiments also showed that  $^{211}\text{At}$ -labelled compounds linked by borane derivatives are more stable than those linked by aromatic ones [91–95], but the former may influence the targeting and circulation of the carriers, making corresponding radiopharmaceuticals show long-time retention in blood, kidney or liver [96–99]. Therefore, the potential biotoxicity requires further systematic evaluation before its final application in  $^{211}\text{At}$  labeling.

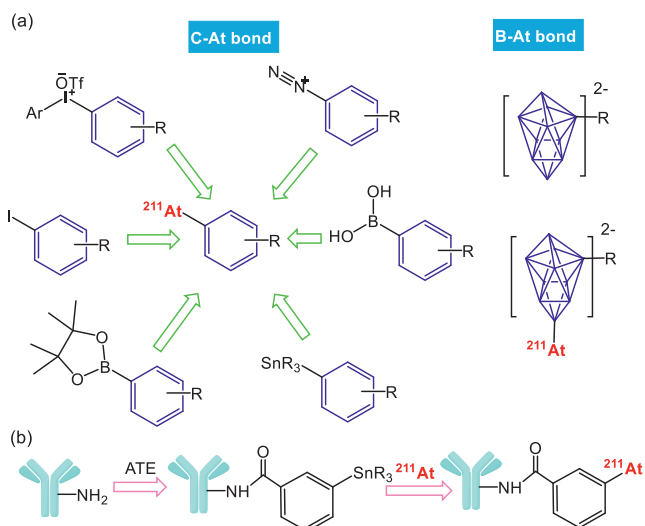


**Fig. 3.** (a) Different bifunctional coupling agent used for  $^{211}\text{At}$  labeling and (b) the most common radiolabeling strategies in current radioastatinated compounds. The blue cartoons present targeting vectors.

Common bifunctional coupling agents involving both C-At and B-At bonds for  $^{211}\text{At}$  labeling are shown in Fig. 3a, and more details about related radioastatination reactions have been described in other work [100]. Some nontypical approaches, including coordination of calixarene [101]/metal-organic complex [102–105], click-chemical self-assembly [106], nucleophilic substitution using boric acid/borate ester [107–109] and nano-coprecipitation [110,111], are also alternative paths for  $^{211}\text{At}$  labeling. However, due to the lack of reliable results *in vivo*, whether they can work in  $^{211}\text{At}$ -labelled pharmaceuticals also needs further verification. Currently, one-step  $^{211}\text{At}$  labeling based on aryl-trialkylstannane ester (ATE) derivatives is still the most widely-used strategy. In a typical process, ATE derivatives are first conjugated to the targeting vectors *via* amide reaction, resulting in corresponding precursors for halodestannylation (Fig. 3b) [112]. Recent studies have shown that the monoclonal antibodies (mAbs) with alkyltin-based prosthetic groups obtained in this method are highly stable at  $-20\sim 4\text{ }^{\circ}\text{C}$  for over 3 months in PBS (pH 7.4), which are still available for effective radioastatination [113].

In general, the instability of  $^{211}\text{At}$ -labelled compounds is mainly attributed to the break of C-At bond. Theoretically, diminishing the electrical deficiency of At is an alternative way to consolidate the C-At bond. For example, incorporating *N*-methyl or methylene between the amide bond and aromatic ring can significantly elevate the stability of corresponding labelled compounds [114,115]. The basic principle is that the amide bond directly located on the benzene might increase the electrical deficiency of At, which is unfavorable for the stability of C-At bond. Of course, the bifunctional coupling agent and pharmacophore can also be linked by other approaches rather than amide bonds, *e.g.*, reducing the disulfide bonds in proteins to sulfhydryl (-SH), which are then bonded to cycloolefin-containing bifunctional coupling agent through addition reaction [116]. Although related radiolabeling compounds are less prone to occur deastatination [117], size exclusion high-performance liquid chromatography (SE-HPLC) analysis indicated that reductants can cause partial cleavage of related proteins, diminishing its purity and immune-activity [118]. It is worth mentioning that  $^{211}\text{At}$  can also be directly labelled on RGD peptides through the tributyltin pre-incorporated into the vectors [119]. Corresponding labelled compounds keep high tumor targeting, suggesting their potential in  $^{211}\text{At}$ -based TAT.

The introduction of corresponding substituents beside At is another way for improving the stability of labeling compounds. However, Talanov *et al.* [120] found that directly incorporating electron-donating groups such as methoxyl or methanethiol at the *ortho*-position of alkyltin tends to reduce the radiochemical yield without enhancing the stability of corresponding labelled compounds. One possible reason is the introduction of substituting groups may increase the steric hindrance for halodestannylation. On the contrary, guanidyl at *ortho*- or *meta*-position can significantly improve the radiochemical yield and the stability of  $^{211}\text{At}$ -labelled compounds [121–124]. Obviously, guanidyl with longer chain structure is favorable to reduce the electrical deficiency of At while avoiding extra steric hindrances in astatination. In addition, when conjugated with pyridine instead of benzene derivatives, the stability of corresponding  $^{211}\text{At}$  labelled compounds also become lower [94], which may be due to the electron-drawing effect of N atom.

It should be noted that the  $^{211}\text{At}$  labeling rate may decrease when the radioactivity increases from pre-experimentally to clinically required amount. One of the main reasons is that high doses of radionuclides can produce significant radiation effects on the whole system through two approaches, *i.e.*, the solvent-induced consumption of bifunctional coupling agent and the redistribution of  $^{211}\text{At}$  species. According to Pozzi *et al.* [125], when chloroform was used as the reaction medium, ATE derivatives would undergo 50% degradation even at doses below 500 Gy. It was attributed to that chloroform tends to produce electrophilic chlorine radical under irradiation, which can consume ATE derivatives and reduce the labeling rate of  $^{211}\text{At}$  onto the organic precursors [126]. In methanol and benzene, more than 85% of the labeling precursors still remain stable even at the dose of 3500 Gy. The analysis results of HPLC showed that over 90% of  $^{211}\text{At}$  exist as a single species in methanol when the dose was less than 1000 Gy. In this situation, the halodestannylation can easily be achieved. However, when the dose reaches 3000 Gy, At mainly exists in the form of  $\text{At}^-$ , which cannot be effectively involved in electrophilic reaction to achieve radiolabeling [127]. In order to address the conjugate of high activity  $^{211}\text{At}$  onto targeting carriers, the most practical method is adding oxidants (such as *N*-succinimide or *N*-iodosuccinimide) in the reaction system, to inhibit the reduction of  $^{211}\text{At}$  species [127–129]. Besides, it is often necessary to perform large-scale  $^{211}\text{At}$  radiolabeling before clinical trials, to verify the stability and reliability of the designed labeling strategies [130–133].

Actually, due to the limited availability of  $^{211}\text{At}$ , labeling chemistry in a new system is generally carried out based on radioiodine ( $^{125}/^{131}\text{I}$ ) before being applied to radioastatination [134–137]. Typically, radioiodinated compounds are more stable *in vivo*, which is benefit from the better stability of C-I bond [138]. As a result, compounds labelled with radioiodine and radioastatine may differ in biodistribution. For instance, Wilbur *et al.* [139] found that the retention time of  $^{211}\text{At}$  labelled compounds in kidney was significantly longer than that of  $^{125}\text{I}$ -based conjugates, even the same targeting vectors were employed. Hence, it is always necessary to re-examine and compare some vital items, such as biodistribution and biotoxicity between radioiodinated and radioastatinated radiopharmaceuticals.

### 3.2. $^{211}\text{At}$ -based compounds in preclinical study

#### 3.2.1. $\text{Na}^{211}\text{At}$

$\text{Na}^+/\text{I}^-$  symporter (NIS) is an integral plasma membrane glycoprotein most commonly associated with the thyroid gland that mediates active transport of  $\text{I}^-$  to thyroid follicular cells [140]. Because iodine is an important component of triiodothyronine and tetraiodothyronine, thyroid function and its systemic consequences depend on adequate uptake of  $\text{I}^-$ . In addition to thyroid cells, NIS is also expressed in other tissues, including salivary glands, gastric

mucosa, and lactating mammary glands. The ability of NIS to accumulate  $I^-$  has therefore provided an important basis for targeted imaging and treatment of thyroid-associated diseases with radioiodine (mainly  $Na^{131}I$ ), which has achieved great clinical success.

The successful medical application of  $Na^{131}I$  naturally encouraged researchers to employ  $Na^{211}At$  in NIS-expressing tumors [141]. First of all, there is a very high similarity between these two halogens, so, theoretically, NIS can also mediate  $^{211}At^-$  transport to thyroid or other related tumors [142–145]. Secondly, dosimetry studies had shown that  $^{211}At$  had the highest biological effect in thyroid gland when compared with  $^{60}Co$ ,  $^{99m}Tc$ ,  $^{123}I$ ,  $^{125}I$  and  $^{131}I$  [146–151], resulting in significantly increased gene up-regulation [152–155]. Thirdly, the overall irradiation of the eyes caused by  $^{211}At$  was nearly 5 times lower than that from  $^{131}I$  irradiation [156]. Unfortunately, although the growth inhibit effect of  $Na^{211}At$  in NIS-expressing tumor models could be satisfactory [157,158], some studies also indicated that the non-specific uptake of  $Na^{211}At$  in several important organs including stomach, salivary gland, lungs and spleen was significantly higher than that of  $Na^{125/131}I$ , making the mean absorbed doses nonnegligible and may cause corresponding toxicity to organism [159–162].

Recently, Watabe *et al.* [10] found that one of the main reasons for this kind of biodistribution of free  $^{211}At$  might be that astatine in aqueous solutions tends to exist in a plenitude of species including  $At^+$ ,  $AtO^-$ ,  $At(OH)_2^-$ ,  $AtO_2^-$ ,  $AtO(OH)_2^-$ ,  $AtO^+$  in addition to  $At^-$ , thus reducing NIS mediated targeting. Therefore, if  $^{211}At$  could be controlled in a single state, better therapeutic effect might be expected. Fortunately, it was found that the uptake of  $Na^{211}At$  dispersed in citric acid solution by thyroid cancer cells were significantly increased. Correspondingly, more obvious tumor inhibition was observed in a dose-dependent manner. However, toxicity tests in both normal and tumor-bearing mice showed that 1.0 MBq of  $Na^{211}At$  would cause weight loss, bone marrow growth inhibition, and pathological changes in subjects [163,164]. Unfortunately, the application of  $Na^{211}At$  in NIS-expressing tumors was overshadowed again.

### 3.2.2. $^{211}At$ labelled small molecules

Small molecules tend to show rapid tumor accumulation and normal tissue clearance, which is well matched with the half-life of  $^{211}At$ . Considering that  $\alpha$  particles can cause relatively limited cell damage due to short path length, small molecule with higher penetration may have some advantages in tumor therapy. Metaiodobenzylguanidine (MIBG) is a functional analogue of neurotransmitter norepinephrine and is able to bind with sympathetic medulla tissue by norepinephrine transporter (NET). NET is upregulated in many neuroendocrine tumors, which enables  $^{131}I$ -labelled MIBG ( $[^{131}I]MIBG$ ) derivatives to be versatile in the treatment of neuroblastoma, pheochromocytoma and paraganglioma [165]. It is not hard to image,  $^{211}At$ -labelled benzylguanidine ( $[^{211}At]MABG$ ) derivatives are expected to show better performance in the treatment of NET-associated tumors [166–173]. Boyd *et al.* [174] illustrated that the inhibition of growth cancer cells transfected with methyladrenalin transporter (NAT) gene by  $[^{211}At]MABG$  was much higher than that of  $[^{131}I]MIBG$ . However,  $[^{131}I]MIBG$  could result in bystander effect in solid tumor, which is less obvious for  $\alpha$ -emitter predominated endoradiotherapy [175]. Recently, Ohshima *et al.* [176] found that  $[^{211}At]MABG$  could show significant anti-tumor effect in pheochromocytoma murine models. The tumor volume in the control group increased by  $509.2\% \pm 169.1\%$ , while those in the  $[^{211}At]MABG$  administrated groups (0.56 MBq) was  $9.6\% \pm 5.5\%$  at 21 d post-injection. Although higher injection doses of  $[^{211}At]MABG$  (1.1–3.3 MBq) would cause transient weight loss and leukopenia to subjects, but all mice regained weight at 28 d post-injection. However, the toxicity was irreversible when the dose was further higher than 4.4 MBq [177].

In order to maintain abnormal growth, several types of amino acid transporters are over expressed in tumor cells. This has facilitated the uptake and transportation of various amino acids in tumor cells, even crossing the blood-brain barrier. Therefore, various  $^{211}At$ -labelled amino acid derivatives have become one hot topic in TAT. Meyer *et al.* [178] found that both  $[^{211}At]$ -L-phenylalanine could specific bind to glioma cells. Anti-tumor studies in glioblastoma showed that both radioastatinated phenylalanine compounds could effectively inhibit glioma tumor growth and prolong the survival rate of murine models [179,180]. Ohshima *et al.* [181] demonstrated that  $[^{211}At]$ -L-phenylalanine also showed the ability to suppress the tumor growth and prolong the survival of murine ovarian cancer models. Xie *et al.* [182] found that 4- $^{211}At$ -astato-N-[4-(6-(isopropylamino)pyridine-4-yl)-1,3-thiazol-2-yl]-N-methylbenzamide ( $^{211}At$ -AITM) targeting ectopic metabolic glutamate receptors had good therapeutic effect in melanoma-bearing mice without obvious biotoxicity. In addition, Kaneda-Nakashima *et al.* [183] also demonstrated that  $^{211}At$ -labelled  $\alpha$ -methyl-L-tyrosine ( $^{211}At$ -AAMT) could double prolong the survival of pancreatic cancer models and effectively reduce the lung nodules in B16F10 metastasis models.

Makvandi *et al.* [184] conjugated  $^{211}At$  to 1-(4-iodophenyl)-8,9-dihydro-2,7,9a-triazabenzoc[d]azulen-6(7H)-one, obtaining a radioastatinated compound  $[^{211}At]MM4$  which could inhibit tumor growth in murine neuroblastoma model. The median survival was increased from 66 days in the control group to 87 days in the treatment group received a twice-fractionated injection of 370 kBq of  $[^{211}At]MM4$ . However, when a single injection of higher dose (1480 kBq) was administrated, significant weight loss was observed in murine models. When  $[^{211}At]MM4$  was used in combination with an immune checkpoint inhibitor, anti-programmed cell death protein 1 (anti-PD-1) [185], tumor growth was found to be 100% suppressed. The mechanism for the synergistic effect of radiotherapy and immunotherapy should be that the former can induce inflammation by causing DSBs inside cancer cells, and stimulate the organism to re-produce immune response. This is favorable to destroy the inhibitory effect of tumor cells on immune system, achieving efficient tumor treatment.

Kiess *et al.* [186] labelled  $^{211}At$  to a small molecule inhibitor which can target prostate specific membrane antigen (PSMA), and obtained the radioastatinated (2S)-2-(3-(1-carboxy-5-(4- $^{211}At$ -astatobenzamido)pentyl)ureido)pentanedioic acid. The  $^{211}At$ -labelled PSMA inhibitor was able to effectively inhibit tumor growth and prolong the survival of murine micro-metastatic models, but the uptake of radioactivity in thyroid and stomach was high, while the damage to the proximal renal tubules were also observed during the treatment. Different strategies had been adopted to address these two issues, including prolonging the chain linking pharmacophore and coupling agent, introducing guanidyl group at *ortho*-position of  $^{211}At$ , or changing the benzyl ring to pyridine [187]. As a result, another four  $^{211}At$ -labelled PSMA inhibitors were prepared, among which  $[^{211}At]GV-620$  had exhibit the highest *in vivo* stability and shortest renal retention time. Mease *et al.* [188] also proposed that conjugating  $^{211}At$  to high-performance  $^{177}Lu$ -labelled pharmaceuticals might be another alternative approach to prepare new effective radiopharmaceuticals with potential in TAT. A series of  $^{211}At$ -labelled compounds ( $^{177}Lu$  was replaced by  $^{211}At$ ) targeting PSMA were prepared, of which  $^{211}At$ -3-Lu had shown excellent targeting ability, pharmacokinetic characteristics, good antitumor effect and almost negligible biotoxicity.

Recently, our group found that fibroblast activation protein inhibitors (FAPIs) featured with rapid metabolism and short retention in tumor might be more favorable targeting ligands for  $^{211}At$  with relatively short half-life [135].  $^{211}At$ -labelled FAPI-04 was synthesized for the first time with an ideal radiochemical yield and radiochemical purity [189]. It was demonstrated that tumor growth

was dramatically slowed by  $^{211}\text{At}$ -FAP1-04 in a dose-dependent manner: glioma-bearing mice received 0.18, 0.37 and 0.55 MBq of  $^{211}\text{At}$ -FAP1-04 showed a reduced tumor volume of approximately 55%, 70% and 76% on the 15<sup>th</sup> day, respectively. The median survival in  $^{211}\text{At}$ -FAP1-04 group (24.0, 30.0 and 33.0 days for 0.18, 0.37 and 0.55 MBq of  $^{211}\text{At}$ -FAP1-04, respectively) was significantly improved compared to that in saline group (16.5 days), causing no significant changes in body weight or toxicity in the kidney, liver, stomach and thyroid over the experimental period.

### 3.2.3. $^{211}\text{At}$ -labelled peptides

Peptides are compounds of  $\alpha$ -amino acids linked by peptide bonds, which are intermediate products of protein hydrolysis. The advantages of radiolabeled peptides include relatively simple chemical synthesis, available radiolabeling, rapid clearance from circulation, faster penetration, more uniform tissue distribution, and lower immunogenicity [190]. Therefore, in recent years,  $^{211}\text{At}$ -labelled peptides have become active in tumor therapy. Vaidyanathan *et al.* [191–193] was the first to realize  $^{211}\text{At}$  labeling on octreotide derivatives and found that somatostatin receptor expressing tumor cells had certain uptake and endocytosis of the radioastatinated octreotide ( $^{211}\text{At}$ -octreotide) derivatives. Zhao *et al.* [194] found that  $^{211}\text{At}$ -octreotide exhibited observable higher uptake in lung, spleen, stomach and intestines than in other tissues. Through histological examination,  $^{211}\text{At}$ -octreotide demonstrated much more lethal effect than control groups (PBS, octreotide and free  $^{211}\text{At}$ ).

Aoki *et al.* [195] proposed to conjugate  $^{211}\text{At}$  onto bombesin derivatives and performed the therapeutic effect of corresponding radiopharmaceuticals in prostate tumor models. It indicated that the prepared [ $^{211}\text{At}$ ]AB-3 had the highest labeling rate ( $28.2\% \pm 2.4\%$ ) when polyethylene glycol (PEG) chain was introduced between the bombesin scaffold and the labeling motif. [ $^{211}\text{At}$ ]AB-3 retained good affinity towards cancer cells and could be highly endocytosed, demonstrating that the whole radiochemistry process had no damage on the targeting of bombesin. However, endocytosed [ $^{211}\text{At}$ ]AB-3 tended to occur efflux over time and the uptake by tumor was as low as  $2.56 \pm 0.24\%$  ID/g, possibly originating from its unsatisfactory stability.

Our group [196,197] labelled  $^{211}\text{At}$  onto a small molecule fusion peptide, VP2, and obtained a radiopharmaceutical [ $^{211}\text{At}$ ]At-SPC-VP2 targeting vasoactive intestinal peptide. It suggested that [ $^{211}\text{At}$ ]At-SPC-VP2 could effectively bind to a variety of tumor cells, including SW620 (colon cancer), A549 (human lung cancer), 4T1 (mouse breast cancer) and 7721 (human liver cancer). *In vivo* treatment experiments showed that [ $^{211}\text{At}$ ]At-SPC-VP2 had high stability and could produce better anti-tumor effect than both non-target nuclide and small molecule peptide. However, significant radioactivity was also observed in the stomach, which may be related to the deastatination of the labelled compound *in vivo*.

### 3.2.4. $^{211}\text{At}$ -labelled antibodies

Recently, radioimmunotherapy (RIT) based on antibody has received increasing attention in preclinical and clinical researches [198]. Naturally, conjugating  $^{211}\text{At}$  to intact antibodies or related fragments to obtain astatinated pharmaceuticals with excellent anti-tumor effect is also one of the hotspots in TAT. Our group [199] found that  $^{211}\text{At}$ -labelled mAb 3H11 and Fab fragments had obvious cytotoxic effect on human gastric cancer cells and  $^{211}\text{At}$ -labelled Fab fragments showed higher tumor uptake ( $9.48\sim 8.42\%$  ID/g) than  $^{211}\text{At}$ -labelled mAb ( $\sim 4.0\%$  ID/g). However, both radiopharmaceuticals had shown inadequate *in vivo* stability. When  $^{211}\text{At}$  was labelled onto insulin through pyridine derivatives, the obtained [ $^{211}\text{At}$ ]At-SPC-insulin showed good *in vitro* stability and the purity of the radiolabeled compound was still higher than 95% for 24 h [200]. The radioactivity was mainly accumulated in

liver, with the uptake of  $4.29\%$  ID/g at 30 min post-injection. However, thyroid, stomach, lungs and spleen also showed noticeable radioactivity uptake.

Kennel *et al.* [201] proposed to employ  $^{211}\text{At}$ -labelled mAb 201B to treat BALB/C mice bearing approximately 100 EMT-6 lung tumor clones (approximately 2000 cells). They found that 185 kBq of  $^{211}\text{At}$ -labelled 201B could extend survival rate of subject mice, and an injection of 370 kBq ( $25\sim 40\text{ Gy}$ ) of radiolabeled mAb was enough to eradicate all lung tumors, but mice received 740 kBq of  $^{211}\text{At}$ -labelled 201B occurred pulmonary fibrosis at 3–4 months post-injection. Akabani *et al.* [202] evaluated the efficacy of combination of  $^{211}\text{At}$ - and  $^{213}\text{Bi}$ -labelled 201B in lung tumor models by two-dimensional histological images and activity distribution at microscopic level. The results showed that radiolabeled mAbs were effective for the treatment of animals with small tumors, and the predicted therapeutic effect was consistent with experimental results. However, for large-size tumors, the therapeutic effect of radiopharmaceuticals was very limited.

Persson *et al.* [203] found that  $^{211}\text{At}$ -trastuzumab possessed a highly specific binding ability to breast cancer cells ( $K_D = 1.8 \pm 0.3\text{ nmol/L}$ ) and 74 cell-associated decay of  $^{211}\text{At}$  /cells could produce a cell survival fraction as low as  $(4.5 \pm 0.8) \times 10^{-4}$ . Akabani *et al.* [204] pointed out that the relative biological effect of  $^{211}\text{At}$ -trastuzumab was twice higher than that of external irradiation therapy, and it was able to inhibit the proliferation of HER2-positive tumor cells. Furthermore, the therapeutic effect of  $^{211}\text{At}$ -trastuzumab on carcinomatous meningitis was verified in murine models [205]. It had been proved that the median survival of tumor-bearing mice increased from 21 days in saline group to 45 and 48 days after intracranial administration of 1.22 and 2.44 MBq of  $^{211}\text{At}$ -trastuzumab, respectively. When animals were inoculated with a lower tumor burden and received 1.70 and 3.40 MBq of  $^{211}\text{At}$ -trastuzumab, the median survival was further extended to 68 and 92 days. An evaluation of radiotherapy efficacy of  $^{211}\text{At}$ -trastuzumab in ovarian cancer models given by Palm *et al.* [206] further indicated that 500  $\mu\text{g}$  cold trastuzumab combined with 400 kBq of  $^{211}\text{At}$ -trastuzumab could achieve tumor ablation with no obvious biotoxicity occurring during the treatment.

Kodaira *et al.* [207,208] found that the anti-tumor effects of  $^{211}\text{At}$ -trastuzumab on peritoneal metastasis of gastric cancer were significantly dependent on the injection method. The uptake of  $^{211}\text{At}$ -trastuzumab by tumor was more than  $60\%$  ID/g by intraperitoneal injection, while it decreased to  $18\%$  ID/g via vein injection. Two of the six mice received 1.0 MBq of the radiopharmaceuticals achieved tumor ablation, while the growth of the tumor was significantly inhibited in other three models. Kodaira *et al.* [209] confirmed that intravenously-injected  $^{211}\text{At}$ -trastuzumab was able to significantly accumulate in liver tumor tissues, and the absorbed dose was in the range of  $1\sim 7\text{ Gy}$  (peak at 2 Gy). Subsequently,  $^{211}\text{At}$ -trastuzumab was demonstrated to have significant therapeutic effect on liver metastasis from a primary gastric cancer, and it could effectively prolong the survival of tumor-bearing mice without causing serious toxicity to murine models [210]. Cederkrantz *et al.* [211] found that intraperitoneally-injected  $^{211}\text{At}$ -trastuzumab could result in an absorbed dose of  $0\sim 50\text{ Gy}$  for peritoneum in subjects, and a 34-week follow-up study indicated that the limiting dose should be  $30\sim 50\text{ Gy}$ .

Andersson *et al.* [212,213] pointed out that intraperitoneal injection of  $485\sim 555\text{ kBq}$  of  $^{211}\text{At}$ -MOv18 mAb could extend the median survival of ovarian cancer xenografted mice from 138 to 213 days, and 33% of the models were apparently tumor-free and possibly cured at 7 months. These positive results should prompt the application of  $^{211}\text{At}$ -MOv18 in the therapy of ovarian cancer. However, in the past decades, the most widely-studied  $^{211}\text{At}$  labelled compounds for treating ovarian cancer was based on MX35 F(ab')<sub>2</sub>, a murine mAb fragment targeting Le<sup>y</sup> antigen. Bäck *et al.*

[214] proved that the uptake of  $^{211}\text{At}$ -labelled MX35 F(ab')<sub>2</sub> by human ovarian tumor was 14 %ID/g at 7 h post-injection, while the ratio of tumor to blood uptake was 6.2 at 40 h post-injection. Elgqvist *et al.* [215,216] systematically evaluated the RIT efficacy of  $^{211}\text{At}$ -labelled MX35 F(ab')<sub>2</sub> in ovarian cancer models and found that tumor-free fraction (TFF) for mice bearing 4-week tumor were 25%, 22%, 50% and 61% after injected with 25, 50, 100 and 200 kBq of the radiopharmaceuticals, respectively. The minimum required activity of  $^{211}\text{At}$ -labelled MX35 F(ab')<sub>2</sub> for the treatment of ovarian cancer models was suggested to be 100 kBq, since TFF was higher than 50% when further increasing the dosage. A series of researches suggested that a variety of factors, including initial tumor volume [217–219], injection method [220,221] and specific activity of radiopharmaceuticals [222,223] had important impacts on the therapeutic effect of  $^{211}\text{At}$ -labelled MX35 F(ab')<sub>2</sub> ovarian cancer models. Briefly, high specific activity of  $^{211}\text{At}$ -labelled MX35 F(ab')<sub>2</sub> tends to exhibit better therapeutic effect on small-size tumors, and biotoxicity could be obviously diminished when fractionated-injection is adopted.

The therapeutic effect of  $^{213}\text{Bi}$  and  $^{211}\text{At}$ -labelled MX35 in 2-week ovarian cancer models showed that TFFs were 0.60 and 0.90 for  $\sim 2.7$  MBq of  $^{213}\text{Bi}$ -labelled mAb and  $\sim 0.44$  MBq of  $^{211}\text{At}$ -labelled mAb, respectively [224], indicating that  $^{211}\text{At}$  had the advantage of achieving better therapeutic effect. Chouin *et al.* [225] quantified the activity of  $^{211}\text{At}$ -labelled MX35 F(ab')<sub>2</sub> in micro-metastatic ovarian cancer models and found that high uptake and good retention of radiopharmaceuticals mainly existed on the surface of tumor cells, and 1.0 MBq of labelled compounds could make the tumor cells receive a dose of at least 12 Gy. For larger micro-metastatic tumors, activity uptake per cell was lower, probably due to the limited penetration of mAb. However, the average absorbed dose of tumor cells was still higher than 30 Gy due to the presence of crossfire. Bäck *et al.* [226] thus conducted a study of long-term renal toxicity of  $^{211}\text{At}$ -labelled MX35 F(ab')<sub>2</sub> in ovarian cancer model, and suggested that an average absorbed renal dose of  $\sim 10$  Gy was acceptable and kidney would not be the primary dose-limiting organ.

In fact, as a murine mAb, MX35 may induce immune response in humans, limiting the possibility of subsequent fractional therapy trials. Therefore, Lindegren *et al.* [227] investigated the differences in cell affinity and targeting specificity between  $^{211}\text{At}$ -labelled humanized mAb ( $^{211}\text{At}$ -Rembab200) and  $^{211}\text{At}$ -labelled MX35. They found that both radiopharmaceuticals possessed high similarity in terms of tumor antigen and cell binding, confirming that  $^{211}\text{At}$ -Rembab200 also had potential in the treatment of ovarian cancer. Palm *et al.* [228] also radioastatinated humanized farletuzumab ( $^{211}\text{At}$ -farletuzumab) targeting  $\alpha$ -folic acid receptor, for the treatment of abdominal metastatic ovarian cancer. In murine disseminated ovarian cancer models, they demonstrated that  $^{211}\text{At}$ -farletuzumab was able to inhibit the tumor growth more effectively than  $^{211}\text{At}$ -labelled MX35. This might be due to that farletuzumab has a weaker affinity to ovarian cancer cells than MX35, allowing corresponding labelled mAb to penetrate the tumor more effectively.

$^{211}\text{At}$ -labelled mAbs have also received much attention in TAT for various blood diseases [229]. Wesley *et al.* [230] found 16.65 MBq/kg of  $^{212}\text{Bi}$ - and 2.22 MBq/kg of  $^{211}\text{At}$ -labelled humanized mAb anti-Tac ( $^{211}\text{At}$ -HAT) could prolong the survival of cynomolgus cardiac allograft models to  $14.0 \pm 1.3$  and  $26.7 \pm 2.4$  days, respectively ( $8.2 \pm 0.5$  days in the control group), indicating that  $^{211}\text{At}$  behaved better in tumor inhibition. A radioastatinated anti-CD25 mAb,  $^{211}\text{At}$ -labelled 7G7/B6, had also shown potential for leukemia treatment and 555 kBq of  $^{211}\text{At}$ -labelled 7G7/B6 could significantly prolong the mean survival time of karpas299 leukemia models from 24 to  $>76$  days, with 5 of the 15 mice living more

than 5 months [231,232]. Moreover, a combination therapy in T-cell white blood models using  $^{211}\text{At}$ -labelled 7G7/B6 and cold daclizumab made 91% of subjects live as long as 94 days, which was 36% and 50% in  $^{211}\text{At}$ -labelled 7G7/B6 and cold daclizumab groups [233]. Nakamae *et al.* [234] found that  $^{211}\text{At}$ -B10-30F11 targeting CD45 was high-performance in generating bone marrow suppression, since all mice administrated with 0.74 or 1.85 MBq of radiopharmaceuticals had lethal myeloablation. Orozco *et al.* [235] demonstrated that  $^{211}\text{At}$ -B10-30F11 could significantly prolong the median survival time of leukemia murine models in a dose-dependent manner: the median survival time was 123, 101, 61 and 37 days for subjects received 888, 740, 444 and 0 kBq of  $^{211}\text{At}$ -labelled 30F11, respectively.

In canine model, Chen *et al.* [236–238] also demonstrated that seven of the eight models would exhibit long-term donor monocyte chimerism (19% to 58%) when pre-treated with 5.735–23.125 MBq/kg of  $^{211}\text{At}$ -CA12.10C12 before hematopoietic cell transplantation (HCT) followed by short-term immunosuppression of cyclosporine and mycophenolate. At doses below 14.985 MBq/kg, the dogs exhibited transient hepatotoxicity and subsequently autogenous recovery, without nephrotoxicity observed at the end of an 18–53 weeks follow-up. It has been demonstrated that a cell dose exceeding  $1 \times 10^6$  cells/kg in combination with non-myeloablative doses (14.80–18.13 MBq/kg) of  $^{211}\text{At}$ -CA12.10C12 could sustain the engraftment level in dog models after autologous HCT. A subsequent therapeutic study in canine models demonstrated 6.956–14.319 MBq/kg of  $^{211}\text{At}$ -CA12.10C12 in combination with 9.2 Gy total body irradiation (TBI) appeared to be more effective to overcome graft rejection in red cell disorders and other nonmalignant conditions treated with allogeneic transplantation [239].

Petrich *et al.* [240] found that  $^{211}\text{At}$ -AntiCD33 had significantly higher specific binding ability to leukemia cells and could effectively inhibit their proliferation. *In vivo* study on murine models showed that  $^{211}\text{At}$ -AntiCD33 possessed a characteristic biodistribution consistent with non-specific antibody retention in the reticuloendothelial system [241]. The largest proportion of radioactivity was observed in blood and blood-rich tissues, which was still over 85% at 21 h post-injection. During a follow-up of 6 months, no significant toxicity occurred in all treatment group. O'Steen *et al.* [242] carried out the treatment of invasive multiple myeloma based on  $^{211}\text{At}$ -OKT10-B10 and found that the radiopharmaceutical could effectively inhibit tumor growth and double the survival time of the model mice ( $>150$  days). Gouard *et al.* [243] synthesized  $^{211}\text{At}$ -labelled 9E7.4 and confirmed its therapeutic effect in multiple myeloma model: the survival rate at 150 d was 65% in the presence of 740 kBq of related radiopharmaceuticals.

Nestor *et al.* [244,245] conducted the evaluation of  $^{211}\text{At}$ -labelled U36 chimeric mAb on human neck squamous cell carcinoma (HNSCC) and found that the prepared radiopharmaceutical had high specific binding and endocytosis ability to cancer cells. The survival rate of each cell was estimate to be 10% after 50 decays, and 200 kBq of the radionuclides could prevent 90% of the tumor-bearing mice appearing tumor proliferation. Robinson *et al.* [246] carried out the treatment of human breast tumors using  $^{211}\text{At}$ -labelled SAPS C6.5, a bispecific antibody targeting HER2. It demonstrated that 0.740 and 1.665 MBq of  $^{211}\text{At}$ -labelled SAPS C6.5 extended the survival time of tumor-bearing mice by 30 and 57 days, respectively, and the higher activity could occur 60% tumor ablation after 1 year.

Turtoi *et al.* [247,248] found that human lymphocytes could show high sensitivity of gene expression and rapid regulation after exposure to  $^{211}\text{At}$ . Green *et al.* [249] compared the anti-tumor efficacy of  $^{211}\text{At}$ -labelled 1F5 mAb ( $^{211}\text{At}$ 1F5-B10) in large-size lymphoid xenograft and minimal residual disease (MRD) models. They

found that [ $^{211}\text{At}$ ]1F5-B10 had a moderate effect on solid tumors and mice received 1.776 MBq of the radiopharmaceuticals lived only 2–3 times longer than those in the control group. In contrast, 70% of the MRD models received 555 kBq of [ $^{211}\text{At}$ ]1F5-B10 could achieve complete tumor elimination. Bäck *et al.* [250] confirmed that two-fractionated of 1.5 and 1.9 MBq of  $^{211}\text{At}$ -labelled anti-PSCA A11 minibody ( $^{211}\text{At}$ -A11) targeting prostate stem cell antigen could inhibit tumor growth by nearly 85%. Eriksson *et al.* [251] demonstrated that 2.5 or 5.0 MBq of  $^{211}\text{At}$ -labelled BR96 (chimeric mouse/human IgG1 mAb) could facilitate half of the murine colon cancer models to achieve tumor ablation, when the initial tumor diameters were ~10 mm. Although RIT induced transient weight loss and bone marrow toxicity, the overall survival of the mice was significantly better than that of the control group.

Li *et al.* [252] tried to conjugate  $^{211}\text{At}$  to major histocompatibility complex CLASS I chain associated proteins A and B (MICA/B) and found the obtained  $^{211}\text{At}$ -anti-MICA/B Ab had high binding ability to human osteosarcoma cells SaOS2 and U2OS, of which 1.0 MBq could effectively inhibit the growth of HCT116 p53 $^{-/-}$ -positive tumor. They also compared the anticancer efficacy of  $^{211}\text{At}$ - and  $^{90}\text{Y}$ -labelled anti-frizzled Homolog 10 (FZD10) in synovial sarcoma models [253]. The results indicated that 1.85 MBq of the prepared  $^{211}\text{At}$ -labelled OTSA101 suppressed tumor growth immediately after injection, whereas this effect required several days in the case of  $^{90}\text{Y}$ -labelled OTSA101. The combination of  $\alpha$ - and  $\beta$ -emitters based radiopharmaceuticals was supposed to be more conducive in RIT. However, a study performance by Eriksson *et al.* [254] indicated that administrating 5 or 10 MBq/kg of  $^{211}\text{At}$ -labelled BR9 at 25 d post-injection of 400 MBq/kg of  $^{177}\text{Lu}$ -labelled BR96 had no effect on reducing the proportion of colon carcinoma bearing mice developing metastases. On the contrary, obvious bone marrow toxicity was observed in the treatment group. These results were discouraging, and at least proved that sequential RIT alone could not achieve synergistic effects.

For  $^{211}\text{At}$ -based RIT, how to enhance the stability of  $^{211}\text{At}$ -labelled mAbs also drew much attention, and an available approach might be introducing adjuvants into the media. Sundberg *et al.* [255] demonstrated that using lysosomal weak bases could improve the retention time of radiolabeled epidermal growth factor ( $^{211}\text{At}$ -EGF) in tumor cells. Takashima *et al.* [256] proposed to dissolve  $^{211}\text{At}$ -anti-TF mAb in sodium ascorbate (SA) and found that 1.2% SA could significantly enhance the cellular binding of  $^{211}\text{At}$ -anti-TF mAb from  $2.67\% \pm 0.40\%$  to  $17.52\% \pm 3.58\%$ . Compared with 1 MBq of  $^{211}\text{At}$ -anti-TF mAb in PBS, those containing 1.2% SA could further prolong the median survival of gastric cancer models from 30 to 34 days. These results showed that reductive adjuvants might effectively improve the stability of astatinated compounds, resulting in better therapeutic effects.

Many preclinical studies have shown that mAb-based TAT can achieve tumor ablation when the maximum tumor radius was in the range of 0.1–0.2 mm, while it has little effect on tumors with larger size, which might be due to the limited penetration ability of mAbs. Hence, pre-targeting radioimmunotherapy (PRIT) has become attractive in TAT. A pre-targeting therapy can be divided into two steps: in the first step, the pre-targeting molecule (mainly mAbs) is injected into the organism. The second step is activation step, that is, after the targeting precursor has highly enriched at the tumor site, the effector molecule with good penetration ability is injected, which will be fixed by coupling with the pre-targeting molecule. Pre-targeting location can make the radionuclides better target the tumor, minimizing the biotoxicity of radionuclides to normal tissues. Wilbur *et al.* [257] carried out a preliminary study of  $^{213}\text{Bi}$ - and  $^{211}\text{At}$ -labelled streptavidin for mAb pre-targeting. However, the effector molecules labelled with  $^{211}\text{At}$  were not suitable for PRIT due to their poor stability or low

labeling rate. Frost *et al.* [258,259] proposed to employ avidin-conjugated MX35 (Avidin-MX35) as the pre-targeting molecule and  $^{211}\text{At}$ -labelled biotinylated poly-L-lysine ( $^{211}\text{At}$ -B-PL<sub>SUC</sub>) as the effector molecule to achieve PRIT. In micro-metastatic ovarian cancer models, they found that PRIT exhibited no significant advantage in final anti-tumor effect over common RIT [260]. Navarro *et al.* [261] adopted five different click-chemical reaction strategies to conjugate the pre-targeting and effector molecules (polypeptides). Although BCN/TZ-IEDDA and DIBAC/N3-SPAAC showed obvious kinetic advantages, relevant *in vivo* experimental results are still needed.

Several *in vitro* studies also have shown that [262–265], multiple  $^{211}\text{At}$ -labelled mAbs (cocktails) or single  $^{211}\text{At}$ -labelled mAbs in combination with unlabeled mAbs could inhibit tumor growth more effectively than single administration. However, due to the lack of relevant *in vivo* evaluations of anti-tumor effect, or even *in vivo* distribution study, whether these strategies can work in the treatment of solid or metastatic tumors still needs to be verified. Actually, even for  $^{211}\text{At}$ -labelled mAbs or related fragments of which the stability and biodistribution in tumor-bearing mice had been performed [266–273], the practical anti-tumor and long-term biotoxicity also need to be verified.

### 3.2.5. $^{211}\text{At}$ -labelled nanomaterials

The application of nanotechnology in human clinical practice is known as nanomedicine and offers a powerful tool with high potential and multiple applications. For  $^{211}\text{At}$ -based endoradiotherapy, there are also some attempts based on nanomaterials. Kucka *et al.* [274] achieved  $^{211}\text{At}$  radiolabeling onto PEGylated silver-containing nanoparticles, and found that, in the absence of reductant, the radiochemical yields could be over 94%. Moreover, the obtained  $^{211}\text{At}$ -labelled nanoparticles exhibit high stability even when treated with a large surplus of competitive chloride ions. Hartman *et al.* [275] also performed  $^{211}\text{At}$  labeling on ultra-short single-wall carbon nanotubes (US-tube) in the presence of chloramine-T/*N*-chlorosuccinimide with radiochemical yields of 60.7% and 55.4% in water and methanol, respectively. The prepared  $^{211}\text{At}$ -labelled US-tube showed good stability, with the retention of  $^{211}\text{At}$  on the nanotube kept as 72%–85% and 85%–93% after washed with PBS and serum twice, respectively.

Dziawer *et al.* [276] carried out the labeling of  $^{211}\text{At}$  on gold nanoparticles (AuNPs) and performed the preliminary biotoxicity study of related radiolabeled nanomaterials. Specifically, neuropeptide fragment P(5–11) targeting the nerve type 1 receptor was first conjugated to 5–15 nm AuNPs, of which halogenation were achieved by subsequently adsorbing  $^{211}\text{At}^-$ . The prepared  $^{211}\text{At}$ -AuNP-S-PEG-SP(5–11) had good stability and could inhibit the growth of glioma cells. Sporer *et al.* [277] found that mixing  $^{211}\text{At}$  and 25–50 nm AuNPs before PEGylation could effectively achieve the  $^{211}\text{At}$  labeling onto the gold-based inorganic vector with a radiochemical yield of 99%. *In vivo* studies indicated that intravenously-injected  $^{211}\text{At}$ -labelled AuNP showed low uptake in the stomach and thyroid, high uptake in liver and spleen with long-time blood circulation.

Kato *et al.* [278] conjugated  $^{211}\text{At}$  with AuNP-S-mPEG ranging from 5 nm to 120 nm AuNPs, and confirmed that the obtained radiolabeling nanomedicines could significantly inhibit the growth of glioma and prostate cancer cells. Furthermore,  $^{211}\text{At}$ -AuNP-S-mPEG could well be located inside the xenograft after intratumoral injection. The tumor inhibition of  $^{211}\text{At}$ -AuNP-S-mPEG was significantly related to the particle sizes, and 5 nm  $^{211}\text{At}$ -AuNP-S-mPEG showed the best therapeutic effect, with no significant increase of the tumor size at 40 d post-injection. This might be that the smaller size of the nanomedicine was better favorable to penetrate to the center of the tumor. Liu *et al.* [279] also achieved  $^{211}\text{At}$  labeling of

gold nano-stars (GNS) with high radiochemical yield (>95%). The prepared  $^{211}\text{At}$ -labelled GNS had shown excellent *in vitro* and *in vivo* stability and could well inhibit tumor growth on glioblastoma multiforme (GBM) models. During a 14-day follow-up, the average tumor volume in the control group increased rapidly from  $82\text{ mm}^3$  to  $1456\text{ mm}^3$  while that in the treatment group received  $1.11\text{ MBq}$  of  $^{211}\text{At}$ -labelled GNS increased slowly from  $78\text{ mm}^3$  to  $189\text{ mm}^3$ . These results suggested that  $^{211}\text{At}$ -labelled GNS had great potential in the treatment of GBM.

Shi *et al.* [280] synthesized a semiconductor polymer nanopore (SPN) which was further modified with glucose-dependent insulin-stimulating polypeptide (GIP). After radioastatination, a new nanomedicine  $^{211}\text{At}$ -MeATE-SPN-GIP was obtained, of which  $1.1\text{ MBq}$  could significantly inhibit tumor growth in pancreatic cancer murine models without weight loss, renal and thyroid function disorder occurring. Biodistribution showed that the radioactivity in the stomach was high, suggesting that the stability of  $^{211}\text{At}$ -MeATE-SPN-GIP should be further enhanced. In addition, like all other nanomaterials, the uptake of intraperitoneally-injected  $^{211}\text{At}$ -MeATE-SPN-GIP by liver, spleen, and lungs was still significant, which made the long-term toxicity to these organs need to be verified.

Compared with small molecules, peptides and mAbs,  $^{211}\text{At}$ -labelled nanoparticles are not well studied. There are many factors for this, including the inconsistency of half-lives between  $^{211}\text{At}$  and the nanoparticles, the poor stability of nanoparticles, the uptake of nanomedicines by the reticuloendothelial system, and the long-term toxicity of nanoparticles, etc. Of course, some of the problems, such as the interception by reticuloendothelial system is an inherent issue for all nanomedicines, but not featured by  $^{211}\text{At}$ -labelled nanoparticles. An alternative solution to this problem, at least for  $^{211}\text{At}$ -labelled nanoparticles, is adopting local administration in tumor endoradiotherapy, with an attempt to reduce the metabolism of nanomedicine *in vivo*.

### 3.3. Clinical study of $^{211}\text{At}$ -based radiopharmaceuticals

Up to now, there are several clinical trials involving  $^{211}\text{At}$ -labelled pharmaceuticals, which were driven by previous positive results of preclinical results. Akabani *et al.* [281] found that  $^{211}\text{At}$ -labelled ch81C6 mAb could specifically accumulate in GBM tissue and the radioactivity was approximately 5 times higher than that in normal brain tissue. As a result, a lower survival probability of GBM cells than normal brain endothelial cells had been observed. Subsequently, a clinical study was carried out [282], in which 18 patients with recurrent brain tumors were injected with  $71\sim 347\text{ MBq}$  of  $^{211}\text{At}$ -labelled ch81C6 through a surgically created resection cavity (SCRC) and subsequently subjected to remedial chemotherapy. It had been demonstrated that a total of  $96.7\% \pm 3.6\%$  of  $^{211}\text{At}$  decayed in SCRC, and the mean blood pool percentage injection dose was  $\leq 0.3$ . Six patients developed grade 2 neurotoxicity within 6 weeks of  $^{211}\text{At}$ -labelled ch81C6 administration, which eventually disappeared completely in all but one patient. There was no grade 3 or higher toxicity observed in all patients, and no one required repeated surgery due to radionecrosis. Median survival was 54, 52, and 116 weeks for different patients with GBM, anaplastic astrocytoma and oligodendroglioma, respectively.

Andersson *et al.* [283] conducted a phase I clinical study with  $^{211}\text{At}$ -labelled MX35 F(ab')<sub>2</sub> in 9 patients with ovarian cancer. All subjects were received  $22.4\sim 101\text{ MBq/L}$  of  $^{211}\text{At}$ -labelled MX35 F(ab')<sub>2</sub> via peritoneal catheters. It indicated that the radiopharmaceuticals in the peritoneal fluid decreased to 50% initial activity concentration at 24 h, while those in serum increased to 6% at 45 h. Radioactivity in the thyroid would increase to 127% at 20 h without blocking, which was <20% with blocking. The cumulative uri-

nary excretion was  $40\text{ kBq}/(\text{MBq/L})$  at 24 h, and the estimated absorbed doses were  $15.6 \pm 1.0$ ,  $0.14 \pm 0.04$ ,  $0.77 \pm 0.19$ ,  $24.7 \pm 11.1$  and  $1.4 \pm 1.6\text{ mGy MBq}^{-1}\text{ L}^{-1}$  for peritoneum, red bone marrow, bladder wall, unblocked thyroid and blocked thyroid, respectively. It has been demonstrated that bladder, thyroid, and kidney had the highest contribution to estimated absorbed doses, while lungs, stomach, and bladder shared the largest doses when tissue-weight factor was taken into account [284]. The organ equivalent dose was 10% lower than the estimated tolerance dose when  $100\text{ MBq/L}$  of  $^{211}\text{At}$ -labelled MX35 F(ab')<sub>2</sub> was administrated.

Hallqvist *et al.* [285] performed a phase I clinical study involving 12 patients with recurrent ovarian epithelial carcinoma who had developed secondary or complete resistance to chemotherapeutic agents. They found that when the dose of  $^{211}\text{At}$ -labelled MX35 F(ab')<sub>2</sub> was up to  $215\text{ MBq}$ , no significant biotoxicity was observed during the treatment. Actually, most of the low-level biotoxicity was not directly related to endoradiotherapy itself. Four patients survived longer than 6 years, and one did not have tumor recurrence. Median overall survival was 35 months, with 1, 2, 5 and 10-year survival rates of 100%, 83%, 50% and 25%, respectively. Dosimetry studies showed that lower specific activity was associated with lower single cell dose, while in microtumors, higher specific activity might lead to lower central dose. No obvious radiotoxicity was observed during a 12-year follow-up, and none of the subjects showed any signs of decreased tolerance to recurrent therapy.

Based on the positive effects of  $^{211}\text{At}$ -labelled mAbs in blood disease models, Li *et al.* [133] recently announced an impending clinical study (NCT03128034) for the treatment of advanced hematological malignancies. Meanwhile, O'Steen *et al.* [242] also planned to apply  $^{211}\text{At}$ -OKT10-B10 in a clinical trial of treating invasive multiple myeloma. Absolutely, all these positive results will promote the clinical application of  $^{211}\text{At}$ -based radiopharmaceuticals.

## 4. Challenges and prospects of $^{211}\text{At}$ -labelled pharmaceuticals

As summarized in Table 3,  $^{211}\text{At}$ -based radiopharmaceuticals have received increasing attention from 2000. However, the development of  $^{211}\text{At}$ -labelled compounds is facing several challenges:

- (1) Production of  $^{211}\text{At}$ . Although some accelerators are currently capable of producing GBq-scale  $^{211}\text{At}$  (up to  $9\text{ GBq}$ ) [85], there are currently no more than 30 in the world available for producing  $^{211}\text{At}$ . Therefore, it is predictable that availability of  $^{211}\text{At}$  will still be a problem in the future. Since the cost of a large accelerator is often high, the intervention of governments may be an important way to solve the problem of  $^{211}\text{At}$  production and supply. Encouragingly, in several parts of the world, including the United States, the European Union and Japan, government agencies are taking concrete actions to build, upgrade or retrofit accelerators to increase the capacity of  $^{211}\text{At}$  production [85].
- (2) Basic chemistry of At. Compared to other  $\alpha$ -emitters, the recognition about the fundamental properties of  $^{211}\text{At}$  is limited. At present, many fundamental researches involving At chemistry are still focused on the properties of electron configuration, species states and halogen bonds in simple systems, those based on  $^{211}\text{At}$  labelled compounds are very rare. The exact mechanism of halogenated and deastanization of  $^{211}\text{At}$ , the specific denaturation path of radioastatinated compounds *in vivo*, and the law of C–At bond stability, etc., all lack thorough researches. Fortunately, the development of both computational chemistry and experimental methods have provided powerful tool to explore these is-

**Table 3**Preclinical investigations and clinical trials of  $^{211}\text{At}$ -radiopharmaceuticals in 2000–2022.

| Cancer types               | Radiopharmaceuticals                                                                                                                                                                                                                                       | Refs.                                     |
|----------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------|
| Preclinical investigations |                                                                                                                                                                                                                                                            |                                           |
| Thyroid cancer             | $\text{Na}^{211}\text{At}$                                                                                                                                                                                                                                 | [10,157,163,164]                          |
| Prostate cancer            | $\text{Na}^{211}\text{At}$ , $^{211}\text{At}$ -AAMT, $^{211}\text{At}$ [AB-3, $^{211}\text{At}$ -A11, $^{211}\text{At}$ -labelled PSMA inhibitor, $^{211}\text{At}$ -AuNP-S-mPEG                                                                          | [158,183,186–188,195,250,278]             |
| Pheochromocytoma           | $^{211}\text{At}$ [MABG                                                                                                                                                                                                                                    | [176,177]                                 |
| Melanoma                   | $^{211}\text{At}$ -AITM, $^{211}\text{At}$ -AAMT                                                                                                                                                                                                           | [182,183]                                 |
| Neuroblastoma              | $^{211}\text{At}$ [MM4                                                                                                                                                                                                                                     | [184,185]                                 |
| Pancreatic cancer          | $^{211}\text{At}$ -AAMT, $^{211}\text{At}$ -MeATE-SPN-GIP                                                                                                                                                                                                  | [183,280]                                 |
| Gastric cancer             | $^{211}\text{At}$ -labelled 3H11, $^{211}\text{At}$ -anti-TF mAb, $^{211}\text{At}$ -trastuzumab                                                                                                                                                           | [199,207–210,256]                         |
| Breast cancer              | $^{211}\text{At}$ [MABG, $^{211}\text{At}$ -trastuzumab, cocktails of $^{211}\text{At}$ -mAbs, $^{211}\text{At}$ -labelled SAPS C6.5                                                                                                                       | [203–205,246,265,270]                     |
| Lung cancer                | $^{211}\text{At}$ -octreotide, $^{211}\text{At}$ [At-SPC-VP2, $^{211}\text{At}$ -labelled 201B                                                                                                                                                             | [191–194,196,197,201,202]                 |
| Leukemia                   | $^{211}\text{At}$ -labelled 7G7/B6, $^{211}\text{At}$ -B10-30F11, $^{211}\text{At}$ -AntiCD33, $^{211}\text{At}$ -CXCR4 mAb, $^{211}\text{At}$ -labelled HAT                                                                                               | [230–235,240,241,272]                     |
| HCT                        | $^{211}\text{At}$ -CA12.10C12                                                                                                                                                                                                                              | [236–239]                                 |
| HNSCC                      | $^{211}\text{At}$ -labelled U36                                                                                                                                                                                                                            | [244,245,262]                             |
| Lymphoma                   | $^{211}\text{At}$ [1F5-B10, $^{211}\text{At}$ -labelled HAT                                                                                                                                                                                                | [229, 247–249]                            |
| Colon cancer               | $^{211}\text{At}$ -labelled BR96, $^{211}\text{At}$ -huA33                                                                                                                                                                                                 | [251,254,266,268]                         |
| Osteosarcoma               | $^{211}\text{At}$ -anti-MICA/B Ab                                                                                                                                                                                                                          | [252]                                     |
| Synovial sarcoma           | $^{211}\text{At}$ -labelled OTSA101, $^{211}\text{At}$ -scFv(L19)                                                                                                                                                                                          | [253,267]                                 |
| Epidermoid cancer          | $^{211}\text{At}$ [MABG, $^{211}\text{At}$ -EGF                                                                                                                                                                                                            | [255,264,269,273]                         |
| Mesothelin-positive cancer | $^{211}\text{At}$ -ET210-6, $^{211}\text{At}$ -ET210-28                                                                                                                                                                                                    | [271]                                     |
| Multiple myeloma           | $^{211}\text{At}$ -OKT10-B10, $^{211}\text{At}$ -labelled 9E7.4                                                                                                                                                                                            | [242,243]                                 |
| Glioblastoma               | $^{211}\text{At}$ [L-phenylalanine, $^{211}\text{At}$ -EGF, $^{211}\text{At}$ -labelled ch81C6, $^{211}\text{At}$ -FAPI-04, $^{211}\text{At}$ -AuNP-S-PEG-SP(5-11), $^{211}\text{At}$ -AuNP-S-mPEG, $^{211}\text{At}$ -labelled GNS                        | [135,178–180,189,263,264,276,278,279,281] |
| Clinical trials            |                                                                                                                                                                                                                                                            |                                           |
| Ovarian cancer             | $^{211}\text{At}$ [L-phenylalanine, $^{211}\text{At}$ -MOv18, $^{211}\text{At}$ -trastuzumab, $^{211}\text{At}$ -labelled MX35 F(ab') <sub>2</sub> , $^{211}\text{At}$ -Rembab200, $^{211}\text{At}$ -farletuzumab, $^{211}\text{At}$ -B-PL <sub>SUC</sub> | [181,206,211–228,258–260]                 |
| Multiple myeloma           | $^{211}\text{At}$ -OKT10-B10                                                                                                                                                                                                                               | [133]                                     |
| Glioblastoma               | $^{211}\text{At}$ -labelled ch81C6                                                                                                                                                                                                                         | [282]                                     |
| Ovarian cancer             | $^{211}\text{At}$ -labelled MX35 F(ab') <sub>2</sub>                                                                                                                                                                                                       | [283–285]                                 |

sues, which will result in more great progress in the near future.

- (3)  $^{211}\text{At}$  labeling strategies. Although there are a lot of strategies for labeling  $^{211}\text{At}$ , low reproducibility of radiochemical yield and poor stability of labelled compounds are still one of the important problems restricting the development of  $^{211}\text{At}$  in TAT. How to find the effective approaches for conjugating  $^{211}\text{At}$  and designed vectors, or how to find a more high-performance bifunctional coupling agent, will still be one of the key topics in future. Inspiringly, the nucleophilic substitution reactions based on aryl borate [107] and aryl iodobenzene [286] provide important references for the development of  $^{211}\text{At}$  labeling and deserve to be tried in more studies. The developments of computational chemistry can also offer important guidance for the design and synthesis of new bifunctional coupling agents.
- (4) Ideal targeting carrier. Because  $^{211}\text{At}$  has a relatively short half-life, small molecules with shorter biological half-lives, including amino acids, inhibitors, peptides and so on, often seem to match  $^{211}\text{At}$  better. However, after radioastatination, their specific targeting, stability and retention time at tumor site are not satisfactory. As for mAbs, they can show higher stability and retention time in tumor. However, their long biological half-lives are difficult to match the physical half-life of  $^{211}\text{At}$ . In this situation, mAb fragments

seem more favorable for  $^{211}\text{At}$ -based radiopharmaceuticals. To avoid long-time *in vivo* circulation and the uptake by normal organs and tissues, local administration of  $^{211}\text{At}$ -labelled mAbs/fragments might also be available to achieve region-targeted radiotherapy. The selection of appropriate tumor type should be another option. As least,  $^{211}\text{At}$ -labelled mAbs might be conducive to treat blood diseases due to their long-time circulation. Clearly, micro-metastatic tumors with a smaller size also seems to receive better therapeutic effect than solid ones.

## 5. Conclusion

In the past decades,  $^{211}\text{At}$ -based radiopharmaceuticals have made great progress in basic physicochemical properties, labeling strategies, preclinical and clinical studies. Accurate determination of the first ionization energy and electron affinity can not only promote the recognition of At element itself, but also provide important reference standard for the development of computational chemistry. Clarification of At species in aqueous solution, especially in oxidation conditions, is of great significance for the experimental development of  $^{211}\text{At}$  radiochemistry. New labeling strategies will give more and more choices for conjugating  $^{211}\text{At}$  onto the targeting vector.  $^{211}\text{At}$ -labelled radiopharmaceuticals have become more and more active in nuclear medicine, depending on the

emergence of novel targeting vectors. Although there are still several challenges in the development of  $^{211}\text{At}$ -related radiopharmaceuticals, the great successes in preclinical and clinical researches have already lighted up the prospect of  $^{211}\text{At}$  in practical tumor radiotherapy.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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