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Chelated Metal Ions for Therapeutic and Diagnostic Applications

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Use of radiolabeled monoclonal antibodies (mAb's) that localize and bind to malignant cells continues to be an attractive mechanism for targeting and delivering either an imagable isotope, be it a γ-emitter or B+-emitter, or a particle emitter such as a B−-emitter or an a-emitter for therapeutic applications.

Mab's can be viewed as macromolecular targeting reagents that possess: a discrete chemical structure, sites for chemical modification, and can be made available in high purity and in workable amounts. For these applications, there are minimal criteria that must be met to insure success. The methods employed to radiolabel mAb's must not alter specificity nor alter the rate of catabolism. Finally, of paramount importance, the radiolabel must be securely linked to the mAb. Conjugation to mAb of a radio-metal complex via suitable bifunctional chelating agents provides a wide variety of possible half-lives and emission characteristics. The complex then must be adequately thermodynamically and kinetically stable to minimize release of the isotope *in vivo*.

Numerous recent reports of positive results from clinical trials involving the use of B−-emitting radiolabeled antibodies and the majority have used either 131I (t1/2 = 8.04 d) and 90Y (t1/2 = 64.1 h) for the treatment of either leukemias or lymphomas. These results validate the potential for this modality being a viable clinical therapy. Despite these results, the range of the B− particle is several millimeters and the majority of energy deposition does not occur immediately along the emission track. Cytotoxicity attributed to mAb's radiolabeled with 90Y has been attributed to result from "cross-fire" and not as a direct effect from the emission occurring at the cell surface. Therefore, B−-emitting radioisotopes have been proposed to be less useful for micrometastatic disease and thus might also contribute to normal tissue toxicity. An a-emitting radionuclide might be a better choice in this case due to very high cytotoxicity, a short path length emission, and immediate energy deposition that should minimize collateral cytotoxicity. Examples of metallic-emitters that have been studied are 212 Bi (t1/2 = 45 min) and 213 Bi (t1/2 = 45 min).

Bifunctional derivatives of diethylenetriamine pentaacetic acid (DTPA) developed for use with 90Y proved to form complexes with Bi(III) that were labile *in vivo*. Efforts to develop improved ligands for 90Y that incorporated a trans-cyclohexyl ring into the backbone of DTPA led to the family of CHX-DTPA ligands, which were found to meet the requisite criteria for use of the Bi(III) isotopes. Preclinical experiments confirmed both the stability of the CHX-DTPA ligands for the Bi(III) isotopes and the therapeutic applicability of these cc-emitting isotopes such that a phase I clinical trial was initiated to treat AML at Memorial Sloan-Kettering with an antiCD33 antibody using 213 Bi.

Objectives and Results

Many have expounded that use of 213 Bi might be limited by its half-life to treatment of circulatory malignancies. Despite this, we chose to investigate the possibility of using an engineered mAb, HuCC49CH2, which had been reported to target very rapidly while exhibiting efficient whole body clearance. An initial experiment performed was to determine a maximum tolerated dose (MTD) for the 213 Bi labeled immunoconjugate. As there was minimal information concerning the use of a combination of this mAb, 213Bi, and ip administration, four dose ranges were used in 30 mice that had been inoculated with an LS- I 74T tumor 10 days prior to treatment (50–200 mm3). Results ranged from no response (80–115 uCi), delayed tumor growth (149 uCi, 223–270 uCi), to significant regression of tumor growth with the high dose (316–450 uCi) with no overt toxicity observed in any animal. The experiment was repeated to determine the MTD, but also to verify that this response was valid and whether it could be expanded. To this end, mice were again inoculated with tumors in the flank (83.8 more or less of 31.5 mm3) and four different doses were again administered ip. All of the controls (n = 5) had to be terminated after 14 days due to tumor size. At

the lowest dose (n = 8), 250 uCi, there was one complete, long-term response, with all of the remaining not responding at all. Raising the injected dose to 500 uCi (n = 9) resulted in two long-term responses that exhibited no signs of tumor, three partial responses, i. e, tumor growth was adequately delayed so as to permit the animals to survive for up to 30 days, and the remaining animals not responding. Increasing the dose to 750 uCi (n = 10), provided five long-term tumor free responses, two partial responses, and again, three that did not respond. At the final dose of 1 mCi (n = 10), all responded, with two partial responses, with the remainder completely tumor free or exhibiting a very late, delayed slow re-growth of the tumor. No overt toxicity was observed and no deaths were attributable to radiation effects. Thus, while a MTD was not achieved, demonstration of a potentially effective therapy using a targeted a-emitter was gained.

In conjunction with this study, treatment of LNCaP tumors with mAb J591, which targets PSMAext was radiolabeled with 213Bi. This study also demonstrated that an effective response, i.e., doubling the median tumor-free life span of inoculated animals, was indeed possible, providing that the tumor was accessible and that the mAb could deliver a significant amount of the isotope within the physical half-life constraints. These results reinforce the extreme potential of the use of cc-emitters and that the perceived limitations to their use can and should be obviated by the development of better targeting methods and reagents.