Radiolabeling of an Anti-Carcinoembryonic Antigen Antibody Fab’ Fragment (CEA-Scan) with the Positron-emitting Radionuclide Tc-94m

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Abstract
The goal of this work was to test whether an antibody-based agent approved for use as a single-photon-emitting imaging agent when radiolabeled with technetium-99m could be labeled comparably with a positron-emitting nuclide, technetium-94m. “Instant kits” containing lyophilized NP-4 antibody Fab’ fragment of an ant carcinoembryonic antigen IgG (CEA-Scan) from the same manufactured lot were reconstituted with either Tc-99m or Tc-94m, as solutions of sodium pertechnetate in isotonic saline solution. Radioanalyses of the labeled Fab’ fragments by size-exclusion high-performance chromatography and TLC were carried out. Equivalent results were obtained for radioimmunoconjugates when each was analyzed with both methods. Facile incorporation of Tc-94m into tumor-targeting Fab’ antibody fragments will enable investigation of such agents for tumor-specific imaging using positron emission tomography.

Introduction
CEA-Scan (Arcitumomab) is composed of a MAb Fab’ fragment of NP-4 anti-CEA MAb lyophilized and compounded with a stannous reducing agent (1). Before use, it is reconstituted with 1 ml of readily available Tc-99m pertechnetate solution, usually in 0.9–1.1-GBq (25–30-mCi) single patient dose amounts. The components dissolve, and the stannous ion reduces the added pertechnetate, which then binds to hinge-region free thiol groups on the Fab’ fragment. The labeling is accomplished within 5 min and is quantitative, making the Tc-99m-Fab’ ready for immediate use with no further manipulation needed. Hence, the term “instant kit” has been applied to the product. The Tc-99m-Fab’ is used with planar imaging and single photon emission computed tomography. Similar agents can be prepared with any MAAb, making the system usable for targeting any disease state to which a MAb can be raised (2–6).

Recently, whole-body PET has been under active investigation as a superior imaging modality. In particular, fluorine-18 labeled 2-fluoro deoxyglucose has been used successfully in a number of cancers, as was recently comprehensively reviewed (7). F-18 has been coupled to MAb fragments in attempts to combine the advantages of MAb specificity and PET, but the preparative methods required are complicated because of the synthetic difficulties inherent in organofluorine chemistry (8–10). As an alternative, over a dozen other positron-emitting nuclides have been suggested for MAb-directed PET, and the relative advantages and disadvantages of candidate nuclides were also recently reviewed (11). Few of these nuclides have a half-life (t1/2) long enough for protracted labeling manipulations, yet short enough not to impart high radiation doses to patients.

Tc-94m (t1/2 = 52 min, 72% positron decay) is produced, as described previously, from irradiation of enriched 96% Mo-94, with the ground state isomer present at only 5.5% of the total activity (end of bombardment; Ref. 12). The nuclide can be produced in sufficient yields with small cyclotrons (Ep < 17 MeV) using the 94Mo(p,n)94mTc reaction. As the recommended early time point image acquisition times for Tc-99m-CEA-Scan are between 2 and 5 h postinjection, and with the “instant” labeling technology in place, the use of Tc-94m for MAb-directed PET imaging becomes an interesting possibility. In addition, use of Tc-94m-labeled Fab’ conjugates could be used to quantify in vivo uptake kinetics of the analogous Tc-99m-Fab’ conjugates using Tc-94m and PET. In this work, we set out to show that CEA-Scan could be labeled with Tc-94m and Tc-99m in the same simple manner.

Materials and Methods
Vials containing CEA-Scan were supplied by Immunomedics, Inc. (Morris Plains, NJ). Each vial contains a sterile, lyophilized formulation of 1.25 mg of NP-4 Fab’ together with 0.29 mg of stannous chloride. When reconstituted with 1 ml of Tc-99m-pertechnetate, the resulting solution has a pH of 5–7. Tc-99m-pertechnetate was obtained from an in-house Mo-99/ Tc-99m generator. Tc-94m was produced at the CV 28 compact cyclotron at the Forschungszentrum (Jülich, Germany). Tc-94m-pertechnetate was obtained by separation from cyclotron-irradiated Mo-94 trioxide (13, 14). Both nuclides were dissolved in isotonic saline, and both were used as 100 MBq/ml solutions. Quality control for radiochemical purity of pertechnetate was carried out by TLC using silica gel on aluminum plates (Merck, Darmstadt, Germany) using acetone as eluent.

For each nuclide, 1-ml fractions of pertechnetate were used to reconstitute the MAb vial, with the vial being inverted several times, yet short enough not to impart high radiation doses to patients.

References
2. To whom requests for reprints should be addressed, at Immunomedics, Inc., 300 American Road, Morris Plains, NJ 07950. Phone: (973) 605-1330, ext. 235; Fax: (973) 605-1103.
3. The abbreviations used are: MAb, monoclonal antibody; CEA, carcinoembryonic antigen; HPLC, high-performance liquid chromatography; PET, positron emission tomography.
Antibody Fragment Radiolabeling with Tc-94m

Fig. 1 UV (top) and radiomatic (bottom) analyses from HPLC of Tc-99m-CEA-Scan.

Fig. 2 UV (top) and radiomatic (bottom) analyses from HPLC of Tc-94m-CEA-Scan.

Fig. 3 Radioactivity analysis of TLC of Tc-99m-CEA-Scan.

Results and Discussion

Typical HPLC results using Tc-99m and Tc-94m are shown in Figs. 1 and 2, respectively. For each labeled protein, the UV traces (top) show two peaks at retention times of 8.6 ± 0.2 and 9.7 ± 0.2 min. By reference to column protein standards, these fractions elute with apparent molecular weights of 50,000 and 100,000–150,000 and correspond to NP-4-Fab’ and NP-4-F(ab')2, respectively. For each nuclide, the radioactivity traces are shown in the bottom panels of Figs. 1 and 2, and for each, the predominant species is the Tc-labeled Fab’ fragment. No pertechnetate (retention time, 14–15 min) remains. Typical TLC results are shown in Figs. 3 and 4. In this system, both Tc-99m-NP-4-Fab’ (Fig. 3) and Tc-94m-Fab’ (Fig. 4) remain at the origin, whereas free pertechnetate migrates with the solvent front. In both cases, no pertechnetate is present.

Labeling of these kit-formulated NP-4-Fab’ fragments are as straightforward for Tc-94m as for Tc-99m. Very high protein labeling yields, typically about 99%, were achieved after the 10-min reaction period. Our experience with the Tc-99m nuclide suggests that the incorporation reaction is quantitative within a few minutes after mixing, and 5 min is the recommended room temperature incubation time for Tc-99m-Fab’ preparation. Some further attention to the Tc-94m labeling process is warranted, though, because a substantially higher amount of NP-4-Fab’ has reoxidized to NP-4-F(ab')2 with Tc-94m (Fig. 1, top) than with Tc-99m (Fig. 2, top). This translates into a higher percentage of Tc-labeled F(ab')2 in the case of the Tc-94m (Fig. 1, bottom) than the Tc-99m (Fig. 2, bottom). Ideally, for the 52-min half-life Tc-94m nuclide, the percentage of the biologically longer-lived Tc-94m-F(ab')2 should be kept to a minimum. The most
likely explanation for the difference in Fab' to F(ab')2 ratios observed with the two nuclides is a different propensity toward reoxidation of the Fab' thiol groups under slightly differing labeling conditions, and this probably can be addressed by modifying handling procedures during the labeling process.

The Tc-94m nuclide has less than ideal physical properties for PET imaging, resulting in an intrinsic spatial resolution of 3.3 mm (11). Nevertheless, Tc-94m and PET should provide image resolution superior to that of the corresponding Tc-99m-labeled agent used with single photon emission computed tomography. Moreover, Tc-94m is not hampered by complex labeling chemistry requirements (as compared to F-18, Br-76, Zr-89, Cu-64 and others), due to an imaging procedure. Finally, radiation doses from multiday emissions (as compared to I-124, I-131, etc.) are short enough that patients will not be exposed to excessive radiation doses from multiday emissions (as compared to I-124, Zr-89, Cu-64 and others), due to an imaging procedure. Finally, labeling methods should be facile in the clinical environment to ensure widespread acceptance of a modality. Thus, Tc-94m-labeled Fab' fragments for PET may best fit the multidisciplinary demands that are placed on all prospective new imaging agents. The present work should pave the way for future studies on Tc-94m-Fab' moieties as prospective PET agents.

**References**


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