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RADIOIODINATION, QUALITY ASSESSMENT, IN VITRO AND IN VIVO STABILITY OF IODINE-125 NOFETUMOMAB

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SUMMARY

Iodine-125 radiolabeled monoclonal antibody (MAb) preparations used for radioimmunoguided surgery should be prepared in a manner to insure a stable, efficacious and safe product is used in clinical studies. A number of MAbs have been radioiodinated and used for radioimmunoguided surgery procedures including B72.3 and CC49. Individual MAbs and their fragments differ in their physico-chemical response to conditions of radiolabeling, effects of radiolysis, storage and general handling conditions. A radiolabeling and stability study was completed to evaluate the radiochemical purity and immunoreactivity of NR-LU-10 Fab (nofetumomab) radiolabeled with I-125 at three different specific activities using the IODO-GEN® method. Ratios of 2:1, 1:1, 0.4:1 and 0.2:1(mCi/mg) were used to determine the effects of increasing specific activity on the *in vitro* stability in a range of potential patient dosages. The radiochemical purity and immunoreactivity of each lot of radiolabeled nofetumomab were determined on days 0, 8, 15, 22, 29 and 36 after radiolabeling using standard tests of instant thin layer chromatography (ITLC), high performance liquid chromatography (HPLC) and whole cell binding assay of immunoreactivity. These standard tests indicated the radiochemical purity on the day of radioiodination was high for all specific activity levels (98-99% for ITLC and >98% for HPLC). Preparations at all specific activities showed a slight decrease in radiochemical purity over time with the amount of unbound I-125 increasing or remaining unchanged. The immunoreactivity net bound showed excellent binding with values ranging from 65-75%. The data indicate I-125 nofetumomab undergoes in vitro physico-chemical changes over time. However, the changes in radio-chemical purity and immunoreactivity were minimal and should prove to be clinically insignificant.

Key Words: nofetumomab, radioiodination, iodine-125, monoclonal antibody

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INTRODUCTION

Radioimmunoguided surgery involves the pre-operative administration of a radiolabeled compound which binds to disease tissue and allows detection during surgery with a gamma-detector probe (1-3). Originally developed at The Ohio State University and used in patients with colorectal, prostate, skeletal, melanoma and breast cancer, radioimmunoguided surgery has been shown in clinical trials to be safe and useful to the surgeon in evaluating the extent of disease during surgery (4-13). Most experience with radioimmunoguided surgery, which combines the use of an intraoperative probe detector with a radiolabeled compound for localizing disease tissue, has been in patients with colon and rectal carcinoma who were infused with murine monoclonal antibodies that recognize a tumor associated glycoprotein called TAG-72 (14-23). Although promising results were obtained in breast cancer patients with the first generation antibody to TAG-72, Mab B72.3, suboptimal localization rates with high numbers of false positive uptake in normal breast tissue and axillary nodes was seen (5,8). In addition, to allow for blood clearance and optimum localization, the time between infusion of the drug and surgery was greater than 3 weeks. This led to anxiety in the patients enrolled in the studies. These studies showed a need for a radioimmunopharmaceutical that provided greater tumor-to-background tissue ratios and reached this optimum disease-site uptake more quickly allowing breast cancer patients to have radioimmunoguided surgery as soon after diagnosis as possible. The development of fragments of monoclonal antibodies led to a compound with identical immunoreactivity to the whole, intact Mab but with more favorable pharmacokinetics. This provided the potential to eliminate the 3-4 week time period between infusion of the radiolabeled compound and surgery. In addition, because Mab fragments are smaller molecules, they have many advantages that may enhance the use of radioimmunoguided surgery including faster clearance of the blood pool background radioactivity, improved tumor penetration, improved targeting of antigen sites, less non-specific localization by normal tissue and less immunogenicity allowing multiple infusions.

NR-LU-10 Fab (NeoRx Corporation, Seattle, WA) is a murine IgG2b monoclonal antibody that recognizes a 40 kD glycoprotein expressed by most carcinomas of epithelial origin (24,25). The hybridoma cell line was developed by immunizing BALB/c

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mice with human small cell lung carcinoma cells that had been xenografted into nude mice. The monoclonal antibody produced was subjected to papain digestion to yield the NR-LU-10 Fab fragments. This antibody fragment reacts with small cell lung cancer, non-small cell lung cancer, colon, breast, liver, ovary, pancreas, kidney and prostate carcinomas. Clinical studies of the NR-LU-10 Fab fragment radiolabeled with the diagnostic radionuclide technetium-99m have proven its value in staging of lung cancer (26-28). A kit for the preparation of technetium Tc 99m nofetumomab merpentan (Verluma™, Du Pont Pharma, Billerica, MA) is commercially available for use in the nuclear medicine imaging of small cell lung cancer (29).

MATERIALS AND METHODS

Nofetumomab was radiolabeled with iodine-125 using a modification of the IODO-GEN® method. The reaction vessels used were 20-ml liquid scintillation vials. To prepare the reaction vessels, a solution of the IODO-GEN® in HPLC grade chloroform (1mg/ml) was mixed and aliquots of 500μl pipetted into the reaction vessels to provide 500 micrograms of IODO-GEN® per vial. The chloroform was completely evaporated away using a stream of dry inert gas (nitrogen) in a chemical fume hood. During the evaporation process, the vials were gently rolled in order to uniformly distribute the IODO-GEN® up on the sides of the vial as the chloroform evaporated. The vials were sealed using Parafilm®, placed in a beaker, and stored in an aluminum foil wrapped dessicator to keep out light until use.

The resin column was previously sanitized with 100ml of 1.0N sodium hydroxide. The solution was pulled through the column and allowed to remain on the column 12-20 hours. It was then neutralized with 1 column volume of phosphate buffered saline, 1 column volume of 1N HCL and another column volume of phosphate buffered saline added again to obtain neutral pH 7.0. The pH of the column eluate was verified with pH paper.

Radioiodination of nofetumomab with iodine-125 was completed by first placing the desired amount of protein into the reaction vessel. The amount of protein was determined in order to prepare the different specific activities of 2mCi/mg, 2mCi/5mg, 1mCi/mg and 1mCi/5mg. This step was followed by addition of radioactive iodine-125 as

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sodium iodide. In each case, the reaction was allowed to proceed for 10 minutes with occasional swirling by hand. During this reaction time period, the reaction vessel was assayed in the dose calibrator to determine the amount of radioactive iodine-125 transferred to the reaction vessel. The reaction vial was incubated at normal room temperature (20-25°C). The reaction was stopped by removal of the bulk mixture from the reaction vessel. All radioiodinations were completed in triplicate in order to have three sample vials for each specific activity preparation at each time measurement. A total of 15 vials (5 vials for sampling prepared in triplicate) for each specific activity level were prepared.

The radiolabeled protein was withdrawn from the reaction mixture and placed on a sanitized Bio-Rad AG 1-X8(Cl^-) resin disposable prefilled chromatography column to remove any free iodine-125 from the radiolabeled protein. Approximately 5ml of phosphate buffered saline was added to the column to elute all the radiolabeled protein from the column. The elution mixture was collected in a 30ml sterile and pyrogen-free vial.

The radioactive concentration was determined by averaging radioactive measurements of two 100μl aliquots. These samples were pooled to determine

nofetumomab protein concentration using Lowery's protein assay. The radioactive value was used to calculate the total volume of HSA and phosphate buffered saline to add to the radiolabeled protein in order to obtain the desired radioactive dosage in the vials (2mCi or 1mCi). The appropriate amount of HSA and phosphate buffered saline were added to the 30ml vial containing the radiolabeled protein to obtain the final vials. The vials were stored at 2-8°C.

Quality assessment was completed on each of the four different specific activity preparations within twenty-four hours of radiolabeling and on the same lots of product on days 8, 15, 22, 29 and 36 after radioiodination. The vials were stored in the refrigerator until the day of quality assessment testing. Included in the quality assessment tests were instant thin layer chromatography (ITLC-SG strips in 85% methanol solvent) and high performance liquid chromatography (HPLC) all for determination of radiochemical purity of the prepared products. Immunoreactivity determinations were completed using a live cell binding assay involving the LS-180 cell

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line with a cell viability >95% and at least 10^8 cells/ml (30). In addition, sterility and endotoxin level were completed on the day of radioiodination and on day 36 after radioiodination.

Clinical grade material typically uses a volume of 4ml for injection of the antibody. Considering an approximate 3000ml plasma volume in the adult patient, this is a dilution of 1:750. Using a 1:10 dilution should enhance any in vivo degradation of the radiolabeled protein. Two dilutions of each of the three radiolabeled lots from the four different specific activity preparations were made. The first dilution included 0.1ml of the radiolabeled iodine-125 nofetumomab diluted 10x in sterile PBS and stored at 37°C. The second dilution included 0.1ml of the radiolabeled I-125 nofetumomab diluted 10x in fresh, pooled human serum and stored at 37°C. Quality assessment was completed on the two stored samples in the same manner as the original preparations. Each assessment, one in pooled human serum and one in sterile PBS, was run in triplicate for each of the four specific activity levels. The dilution in PBS served as a negative control. Quality assessment was completed using HPLC and ITLC for radiochemical purity analysis at Day 0, Day 1, Day 2 and Day 7 after inoculation into PBS or pooled human serum. Immunoreactivity was completed at Day 0 and on Day 7 after inoculation into PBS or pooled human serum.

RESULTS

Radioiodination of the nofetumomab at four different specific activities indicated excellent incorporation of the iodine-125 onto the protein. Radiochemical purity results indicated >97% of the iodine-125 bound by ITLC and >98% of the iodine-125 bound by HPLC. Immunoreactivity of the final product was also excellent with values >70% using the live cell assay.

Storage of the final product under refrigeration for a period of 36 days did not lead to a significant decrease in any of these values as shown in Tables 1-4.

Radiochemical purity remained high with values >97% for ITLC and >96% for HPLC assay procedures, respectively. Immunoreactivity showed only a slight decrease in value, but was maintained at levels >66% which meets the acceptance criteria.

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Table 1. Nofetumomab Radiolabeled with I-125 • 2mCi/5mg

	Time	Immunoreactivity Net Bound	ITLC	HPLC		
				Aggregates	Bound	Free I-125
In Vitro	Day 0	-	98.6 ± 0.1	1.1 ± 0.1	98.5 ± 0.4	0.4 ± 0.3
	Day 1	70.76± 1.1	-	-	-	-
	Day 8	67.7 ± 3.5	98.0 ± 0.4	0.6 ± 0.1	97.9 ± 0.3	1.4 ± 0.2
	Day 15	70.8 ± 1.7	98.3 ± 0.6	0.6 ± 0.1	97.9 ± 0.1	1.5 ± 0.1
	Day 22	69.1 ± 1.6	97.8 ± 0.5	0.6 ± 0.04	97.8 ± 0.1	1.7 ± 0.2
	Day 29	68.0 ± 1.9	97.5 ± 0.4	0.6 ± 0.1	97.3 ± 0.3	2.1 ± 0.3
	Day 36	66.1 ± 0.5	97.9 ± 0.6	0.8 ± 0.1	97.5 ± 0.2	1.7 ± 0.1
In Vivo PBS Control	Day 0	70.3 ± 2.7	98.4 ± 0.9	0.9 ± 0.1	98.3 ± 0.4	0.8 ± 0.3
	Day 1	-	97.9 ± 0.7	0.6 ± 0.02	99.1 ± 0.3	0.4 ± 0.2
	Day 2	-	98.7 ± 0.6	0.3 ± 0.1	99.2 ± 0.1	0.4 ± 0.1
	Day 7	65.8 ± 1.1	98.1 ± 1.3	0.3 ± 0.1	98.9 ± 0.1	0.8 ± 0.1
In Vivo Pooled Human Serum	Day 0	72.5 ± 1.9	97.4 ± 1.8	0.4 ± 0.3	99.0 ± 0.3	0.6 ± 0.1
	Day 1	-	98.6 ± 1.1	0.9 ± 0.7	98.6 ± 0.7	0.4 ± 0.1
	Day 2	-	98.2 ± 0.4	1.1 ± 0.7	98.6 ± 0.8	0.4 ± 0.1
	Day 7	69.8 ± 0.9	98.2 ± 1.2	1.9 ± 0.4	96.3 ± 1.3	1.8 ± 0.8

Table 2. Nofetumomab Radiolabeled with I-125 • 2mCi/1mg

	Time	Immunoreactivity Net Bound	ITLC	HPLC		
				Aggregates	Bound	Free I-125
In Vitro	Day 0	-	97.9 ± 0.6	1.6 ± 0.2	98.2 ± 0.2	0.2 ± 0.05
	Day 1	71.8 ± 1.4	-	-	-	-
	Day 8	67.6 ± 1.9	98.0 ± 0.3	1.2 ± 0.2	97.5 ± 0.2	1.3 ± 0.02
	Day 15	74.1 ± 0.7	97.5 ± 0.3	1.2 ± 0.04	97.9 ± 0.2	0.9 ± 0.2
	Day 22	70.5 ± 0.5	97.2 ± 1.1	1.1 ± 0.03	97.1 ± 0.1	1.8 ± 0.2
	Day 29	68.9 ± 1.6	96.8 ± 0.8	1.2 ± 0.3	96.5 ± 0.3	2.3 ± 0.1
	Day 36	69.2 ± 1.1	97.2 ± 0.4	1.2 ± 0.1	96.2 ± 0.1	2.6 ± 0.1
In Vivo PBS Control	Day 0	74.2 ± 2.0	95.3 ± 1.2	1.2 ± 0.2	98.5 ± 0.4	0.3 ± 0.2
	Day 1	-	95.7 ± 0.9	0.7 ± 0.1	98.1 ± 0.7	1.1 ± 0.6
	Day 2	-	98.1 ± 1.6	2.3 ± 1.5	96.8 ± 1.6	0.9 ± 0.1
	Day 7	62.7 ± 3.3	96.7 ± 0.6	2.9 ± 1.3	95.2 ± 1.4	1.9 ± 0.4
In Vivo Pooled Human Serum	Day 0	70.6 ± 1.1	96.5 ± 1.3	1.4 ± 0.3	98.3 ± 1.0	0.3 ± 0.3
	Day 1	-	97.9 ± 0.7	0.7 ± 0.7	98.2 ± 0.5	1.1 ± 0.2
	Day 2	-	99.3 ± 0.8	1.5 ± 1.1	98.1 ± 1.3	0.4 ± 0.3
	Day 7	65.4 ± 0.4	98.2 ± 1.5	2.5 ± 0.5	96.2 ± 0.6	1.4 ± 0.3

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Table 3. Nofetumomab Radiolabeled with I-125 • 1mCi/5mg						
	Time	Immunoreactivity Net Bound	ITLC	HPLC		
				Aggregates	Bound	Free I-125
In Vitro	Day 0	-	98.5 ± 1.1	0.8 ± 0.1	98.7 ± 0.7	0.5 ± 0.6
	Day 1	72.3 ± 2.2	-	-	-	-
	Day 8	72.8 ± 3.2	97.7 ± 0.8	0.7 ± 0.03	98.8 ± 0.2	0.4 ± 0.2
	Day 15	73.6 ± 2.9	98.1 ± 0.2	0.8 ± 0.1	97.9 ± 0.1	1.3 ± 0.1
	Day 22	69.9 ± 0.5	98.7 ± 0.5	1.1 ± 0.5	97.8 ± 1.3	1.1 ± 1.0
	Day 29	72.2 ± 0.9	97.4 ± 0.4	0.8 ± 0.1	97.7 ± 0.2	1.5 ± 0.2
	Day 36	73.9 ± 1.8	97.7 ± 0.04	0.7 ± 0.2	97.8 ± 0.2	1.6 ± 0.1
In Vivo PBS Control	Day 0	74.6 ± 1.2	98.7 ± 1.2	0.5 ± 0.3	99.4 ± 0.3	0.1 ± 0.1
	Day 1	-	99.8 ± 0.1	0.3 ± 0.3	98.7 ± 1.7	1.0 ± 1.5
	Day 2	-	98.7 ± 0.7	0.6 ± 0.1	98.1 ± 0.1	1.4 ± 0.1
	Day 7	68.6 ± 2.4	96.8 ± 1.3	1.9 ± 1.1	97.1 ± 1.0	1.0 ± 0.1
In Vivo Pooled Human Serum	Day 0	75.7 ± 2.4	98.6 ± 0.8	0.1 ± 0.1	99.9 ± 0.1	0.0 ± 0.0
	Day 1	-	97.8 ± 1.7	1.3 ± 0.4	95.8 ± 0.4	2.9 ± 0.7
	Day 2	-	98.5 ± 0.7	0.4 ± 0.1	98.5 ± 0.3	1.1 ± 0.4
	Day 7	73.7 ± 2.6	97.2 ± 0.2	3.1 ± 0.9	95.9 ± 0.8	0.9 ± 0.1

Table 4. Nofetumomab Radiolabeled with I-125 • 1mCi/1mg						
	Time	Immunoreactivity Net Bound	ITLC	HPLC		
				Aggregates	Bound	Free I-125
In Vitro	Day 0	-	99.7 ± 0.3	1.0 ± 0.3	98.9 ± 0.3	0.1 ± 0.1
	Day 1	75.5 ± 1.5	-	-	-	-
	Day 8	71.0 ± 0.9	98.7 ± 0.3	0.8 ± 0.2	98.8 ± 0.2	0.3 ± 0.02
	Day 15	72.2 ± 0.7	98.6 ± 0.3	0.7 ± 0.02	98.7 ± 0.07	0.6 ± 0.05
	Day 22	72.2 ± 1.2	98.1 ± 0.7	1.1 ± 0.2	97.6 ± 0.4	1.3 ± 0.3
	Day 29	69.4 ± 0.6	98.1 ± 0.6	0.7 ± 0.2	98.1 ± 0.3	1.2 ± 0.2
	Day 36	75.6 ± 0.9	97.6 ± 0.7	0.8 ± 0.2	97.9 ± 0.2	1.4 ± 0.1
In Vivo PBS Control	Day 0	73.9 ± 1.9	98.3 ± 1.3	0.6 ± 0.2	99.5 ± 0.2	0.0 ± 0.0
	Day 1	-	98.0 ± 1.4	0.9 ± 0.4	98.4 ± 1.1	0.8 ± 0.7
	Day 2	-	97.8 ± 1.2	0.5 ± 0.3	99.4 ± 0.4	0.2 ± 0.3
	Day 7	61.9 ± 0.3	98.1 ± 0.8	0.6 ± 0.3	99.4 ± 0.3	0.0 ± 0.0
		-	-	-	-	-
In Vivo Pooled Human Serum	Day 0	76.6 ± 1.4	98.3 ± 1.2	1.0 ± 0.7	98.3 ± 1.9	0.8 ± 1.2
	Day 1	-	98.2 ± 1.3	1.3 ± 0.6	98.7 ± 0.6	0.0 ± 0.0
	Day 2	-	98.5 ± 0.9	2.0 ± 2.9	97.8 ± 2.9	0.1 ± 0.2
	Day 7	67.6 ± 2.1	98.2 ± 0.3	0.9 ± 0.2	97.9 ± 1.4	1.1 ± 1.5

The test of *in vivo* stability using pooled human serum as the incubation medium was completed to determine stability in patients (31). At Day 0, radiochemical purity ranged from 96-98% for ITLC and >98% for HPLC. Immunoreactivity on Day 0 ranged

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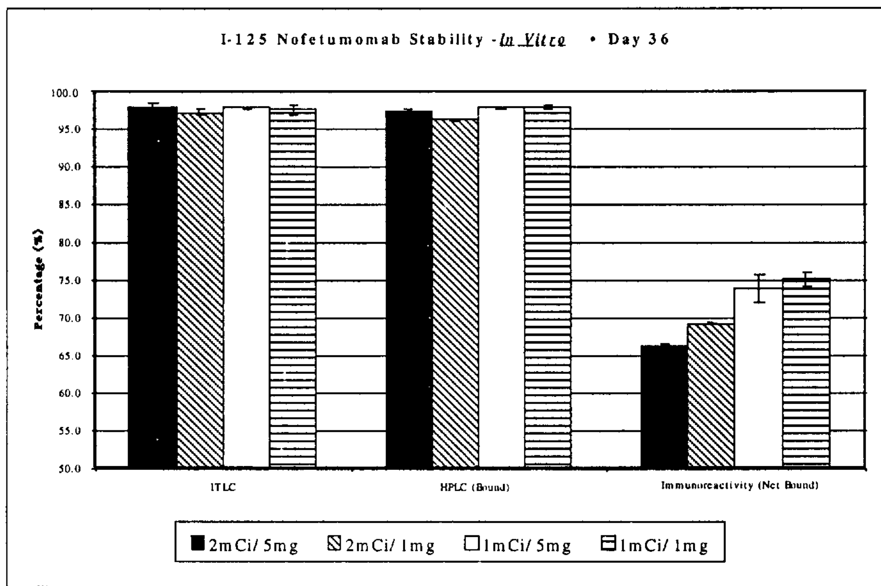
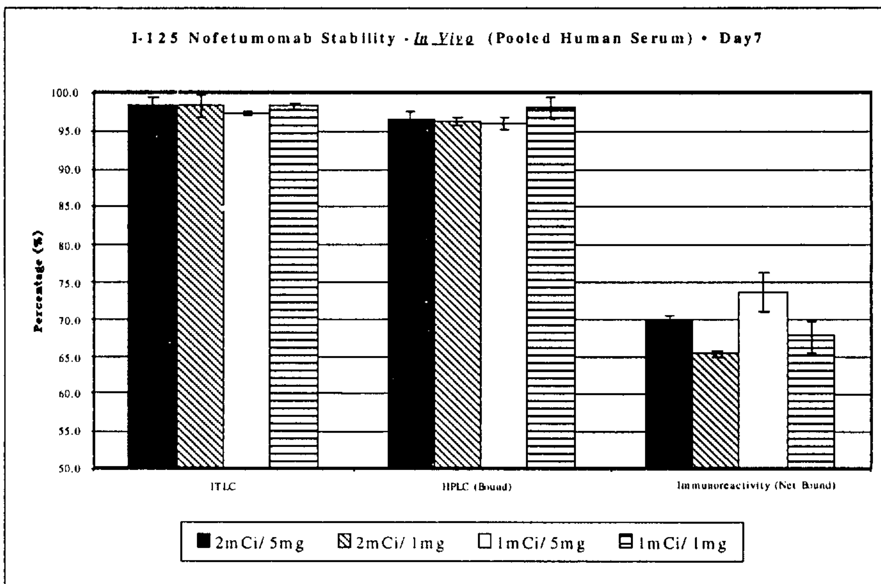
from 70-76% for all samples. After incubation for one week in human serum, ITLC radiochemical purity ranged from 97-98%, HPLC fell to 95-97% while immunoreactivity dropped to 65-73%. All values exceeded criteria for acceptance. Incubation of the final product in phosphate buffered saline in a manner identical to the pooled human serum samples as a negative control showed similar results as seen in Figures 1-2.





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DISCUSSION

In order to reduce production costs associated with commercially available radiopharmaceuticals containing relatively long half-life radionuclides such as iodine-125, the product should have a long shelf-life or expiration time. In addition to decreasing the number of production runs for the manufacturer, a long shelf-life would accomodate patient infusions by making the drug available on short notice if stocked by the nuclear pharmacy or nuclear medicine department.

Unlike traditional drugs, radiopharmaceuticals, especially those composed of radiolabeled proteins, have an inherent mechanism for self-destruction through the action of radiolysis or the breaking of chemical bonds through the action of radiation energy. Radioiodinated compounds have shown greater in vitro stability when a stabilizing compound such as albumin is added. This study indicates little radiolysis or separation of the iodine-125 from the antibody protein over the time period studied. If deiodination of the antibody occurs, the specific activity of the labeled compound will be reduced. Consequently, a greater number of unlabeled antibody molecules will be available to compete with radioiodinated antibody for binding to the tumor associated antigen. The ultimate effect is a reduction of the radioactivity on the tumor and a lower tumor to normal tissue ratio. The free, unbound iodine-125 will localize in thyroid, stomach, salivary glands and other sites which increases the background "noise" encountered during the intraoperative detection procedure.

An earlier stability study provided evidence of consistency with the live cell immunoassay procedure. Although the growth, harvesting and utilization of the live LS-180 cells for immunoreactivity determination proved to be amenable to modifications which improved the reproducibility of the method, a solid-phase binding assay should be developed for use as the product is developed for commercial use. This type of assay has fewer potential errors from techniques associated with different laboratory users.

The method of radioiodination, using the water-insoluble powder 1,3,4,6-tetrachloro-3α,6α-diphenylglycouril (IODO-GEN®, Pierce Chemicals) was chosen based on the simplicity, elimination of the required reducing agents associated with the use of strong oxidizing agents such as chloramine-T and other advantages over earlier

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methods which are time-consuming, inefficient and require strongly oxidizing environments which may lead to protein degradation.

CONCLUSION

The results of this study indicate the I-125 nofetumomab can be prepared in a manner that results in a very stable product. The physical decay of I-125 may be the limiting factor in the establishment of the expiry date of this product. This will allow for a long shelf-life for this material which will prove beneficial during commercialization of the product.

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REFERENCES

1. Aitken DR, Hinkle GH, Thurston MO, Tuttle SE, Martin DT, Olsen JO, Haagensen DE Jr, Houchens D, Martin EW Jr.-*Dis Col Rect* 27:279-282(1984).

2. Martin DT, Hinkle GH, Tuttle S, Olsen JO, Nabi H, Houchens D, Thurston M, Martin EW Jr.-*Am J Surg* 150:672-675(1985).

3. O'Dwyer PJ, Mojzisik CM, Hinkle GH, Rousseau M, Olsen J, Tuttle SE, Barth RF, Thurston MO, McCabe DP, Farrar WB, Martin EW Jr.-*Arch Surg* 121:1391-1394(1986).

4. Martin EW Jr., Mojzisik CM, Hinkle GH Jr., Sampsel J, Siddiqi MA, Tuttle SE, Sickle-Santanello B, Colcher D., Thurston MO, Bell JG, Farrar WB, Schlom J.-*Am J Surg* 156:386-392(1988).

5. Nieroda CA, Mojzisik C, Sardi A, Farrar WB, Hinkle G, Siddiqi MA, Ferrara PJ, James A, Schlom J, Thurston MO, Martin EW Jr.-*Surg Gynecol Obstet* 169:35-40(1989).

6. Bell J, Mojzisik C, Hinkle G, Derman H, Baumert J, Schlom J, Martin EW Jr. *Obstet Gynecol* 76(4):607-611(1990).

©1998 John Wiley & Sons, Ltd.

7. Martin EW Jr., Hinkle G, Mojzisik C, Thurston MO.-In Goldenberg DM, ed. Cancer Imaging with Radiolabeled Antibodies. Boston, MA: Kluwer Academic Publishers, 1990:387-411. Also appeared in *Cancer Treat Res* 51:387-411(1990).

8. Nieroda CA, Martin E Jr.-In Ceriani, RL, ed. Breast Epithelial Antigens. New York: Plenum Press, 1991:195-209.

9. Badellino F, Percivale P, Bartolomeo S, Meszaros P, Bertoglio S.-*Oncoscope* 5:3(1992).

10. Badalament RA, Burgers JK, Petty LR, Berens A, Mojzisik CM, Marsh W, Hinkle GH, Martin EW Jr.-*Cancer* 71(7):2268-2275(1993).

11. Woltering EA, Barrie R, O'Dorisio TM, O'Dorisio S, Nance R, Cook DM.-*Surgery* 116:1139-1147(1994).

12. Martinez DA, King DR, Romshe C, Lozano RA, Morris JD, O'Dorisio MS, Martin EW Jr. *J Ped Surg* 9:1-5(1995).

13. Albertini J, Cruse CW, Rapaport D, Wells K, DeConti R, Berman C, Jared K, Messina J, Lyman G, Glass F, Fenske N, Reintgen D.-*Ann Surg* 223;2:217-224(1996).

14. Sickle-Santanello BJ, O'Dwyer PJ, Mojzisik CM, Tuttle SE, Hinkle GH, Rousseau M, Schlom J, Colcher D, Thurston MO, Neiroda C, Sardi A, Farrar WB, Minton JP, Martin EW Jr.-*Dis Col Rect* 30:761-764(1987).

15. Tuttle SE, Jewell SD, Mojzisik CM, Hinkle GH, Colcher D, Schlom J, Martin EW.-*Int J Cancer* 42:352-358(1988).

16. Benevento A, Carcano G, Dominioni L, Dionigi R.-*J Esp Clin Cancer Res* 8(3):126-127(1989).

17. Schlom J, Colcher D, Roselli M, Carasquillo JA, Reynolds JC, Larson SM, Sugarbaker P, Tuttle SE, Martin EW Jr.-*Nucl Med Biol* 16:137-142(1989).

18. Benevento A, Dominioni L, Carcano G, Lovisolo J, Riva C, Giuliani G, Roncari G, Dionigi R.-*Surg Res Comm* 9(4):249-258(1990).

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19. Bertoglio S, Percivale P, Bartolomeo S, Schenone F, Meszaros P, Conzi GF, Spina B, Nicolo G, Spagnolo W, Badellino F.-In Klapdor R, ed. Recent Results in Tumor Diagnosis and Therapy. Munchen:W. Zuckschwerdt Verlag, 437-443(1990).

20. Cohen AM, Martin,Jr. EW, Lavery I, Daly J, Sardi A, Aitken D, Bland K, Mojzisik C, Hinkle G.-*Arch Surg* 126:349-351(1991).

21. Arnold MW, Schneebaum S, Berens A, Petty L, Mojzisik C, Hinkle G, Martin EW Jr. *Ann Surg* 216:627-632(1992).

22. Burak WE Jr, Schneebaum S, Kim JA, Arnold MW, Hinkle G, Berens A, Mojzisik C, Martin EW Jr.-*Surgery* 118(1):103-108(1995).

23. Cote RJ, Houchens DP, Hitchcock CL, Saad AD, Nines RG, Greenson JK, Schneebaum S, Arnold MW,Martin EW Jr.-*Cancer* 77(4):613-620(1996).

24. Okabe T, Kaizu T, Fujisawa M.-*Cancer Res* 44:5273-5278(1984).

25. Varki NM, Reisfield RA, Walker LE.-*Cancer Res* 44:681-687(1984).

26. Breitz HB, Sullivan K, Nelp WB.-*Sem Nucl Med* XXIII, No.2(April):127-132(1993).

27. Vansant JP, Johnson DH, O'Donnell DM, Stewart JR, Sonin AH, McCook BM, Powers TA, Salk DJ, Frist WH, Sandler MP.-*Clin Nucl Med* 17:431-438(1992).

28. Balaban EP, Walker BS, Cox JV, Bordlee RP, Salk DJ, Abrams PG, Sheehan RG, Frenkel EP.-*Clin Nucl Med* 17:439-445(1992).

29. Package insert for Verluma™-Du Pont Pharma.

30. Beaumier PL, Neuzil D, Yang HM, Noll EA, Kishore R, Eary JF, Krohn KA, Nelp WB, Hellström KE, Hellström I.-*J Nucl Med* 27:824-828(1986).

31. Cole WC, DeNardo SJ, Meares CF, McCall MJ, DeNardo GL, Epstein AL, O'Brien HA, Moi MK.-*J Nucl Med* 28:83-90(1987).

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