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Development, Manufacture and Preclinical evaluation and efficacy studies of a melanin IgM antibody labeled with Rhenium-188-Labeled against experimental human metastatic melanoma in nude mice

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Reference Articles

- E. Dadachova, E Revskaya, M. Sesay, H. Damania, R.Boucher et al., Pre-clinical evaluation and efficacy studies of a melanin-binding IgM antibody with 188-Re against experimental human metastatic melanoma in nude mice. *Cancer Biology and Therapy*, 2008; 7:7, 1116-1127
- E. Dadachova, E. Revskaya, M.Sesay, R. Howell, G. Thornton, et al., Pre-clinical development of a 188-Re labeled melanin-binding antibody for phase I clinical trial in patients with metastatic melanoma, 2008; *J. Nucl. Med.* 2008; 49 (Supplement):327P
- E. Dadachova, A Casadevall, M. Sesay, H. Damania, and P. Smariga, Radiolabeled monoclonal antibodies using TCEP, and uses thereof, US Patent Application number 61/000,795; 2008. (Patent Pending)





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Presentation Overview

Introduction – Radio immunotherapy, melanin pigment and melanoma cancer

Overview of melanin binding IgM antibody cell culture and purification development and manufacture

Melanin binding IgM antibody conjugation (activation) and ^{188}Re labeling development

Pre-Clinical development of melanin binding IgM antibody labeled with ^{188}Re for Phase I clinical trials in patients with metastatic melanoma

Conclusion





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What is Radio immunotherapy (RIT)?

RIT involves the delivery of cytotoxic radiation to the cells by linking radionuclides to target-specific antibodies.

RIT of cancer has been in development for 30 years.

Two RIT agents for treatment of patient with refractory and recurrent non-Hodgkin lymphoma (Zevalin (90-Yttrium) and Bexxar (131-Iodine)) have been approved by the FDA.





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Melanin as a novel intracellular target for RIT of melanoma cancer

Melanin owes its name to the presence of the pigment melanin.

This pigment presents a potential target for the development of radionuclide therapy of metastatic melanoma.

Historically, melanin is not considered a target for RIT due to intracellular pigment outside the reach of specific antibody.

Because melanomas are rapidly growing, cell turnover releases melanin pigment into the extracellular space that can be targeted for delivery of cytotoxic radiation by radiolabeled melanin-binding antibodies.

Recent studies have established the feasibility of targeting melanin released from dead melanoma cells in tumors with radiolabeled antibodies and peptides (Dadachova et al. *PNAS USA* 2004 101:14865-14870; Dadachova et al. *Cancer Bioter. Radiopharm.* 2006 21:117-129)





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Therapeutic Product Candidate

Designation:	188Re-6D2-IgM
Therapeutic class:	Radiopharmaceutic
Indication:	Metastatic melanoma
Source:	Murine hybridoma cell line
mAb Species:	Murine
mAb Class:	IgM
Antigen Target:	Melanin
Radionuclide Isotope:	¹⁸⁸ Re
Emission Characteristic(s):	2.1 MeV β^- , 155 Kev, 15% γ -ray
Range in tissue:	10 mm
Half-life:	16.9 hrs
Source:	Tungsten/Rhenium generator
Manufacturer:	Oak Ridge National Labs

Other long-range beta emitters are 90-Yttrium (used in Zevalin); 131-Iodine (used in Bexxar); 177-Lutetium (in clinical trials).



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Development and Manufacturing considerations ("Naked" Antibody and Radiolabelled Antibody)

Most IgM's do not bind to Protein A affinity column (conventional) as do IgG's, sIgAs and fragmented Mabs

Build quality into the process for therapeutic usage

Product safety

Identity

Potency

Purity

Purification Process Economics (use of commercially available non-affinity resins)

Novel conjugation (activation) process that is fast, simple, efficient and minimizes extensive reduction of full length IgM

Novel radiolabeling Kit for the generation of ^{188}Re -IgM drug product that is also fast, efficient and simple





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Overview of Cell Culture Manufacturing Process for 6D2 IgM



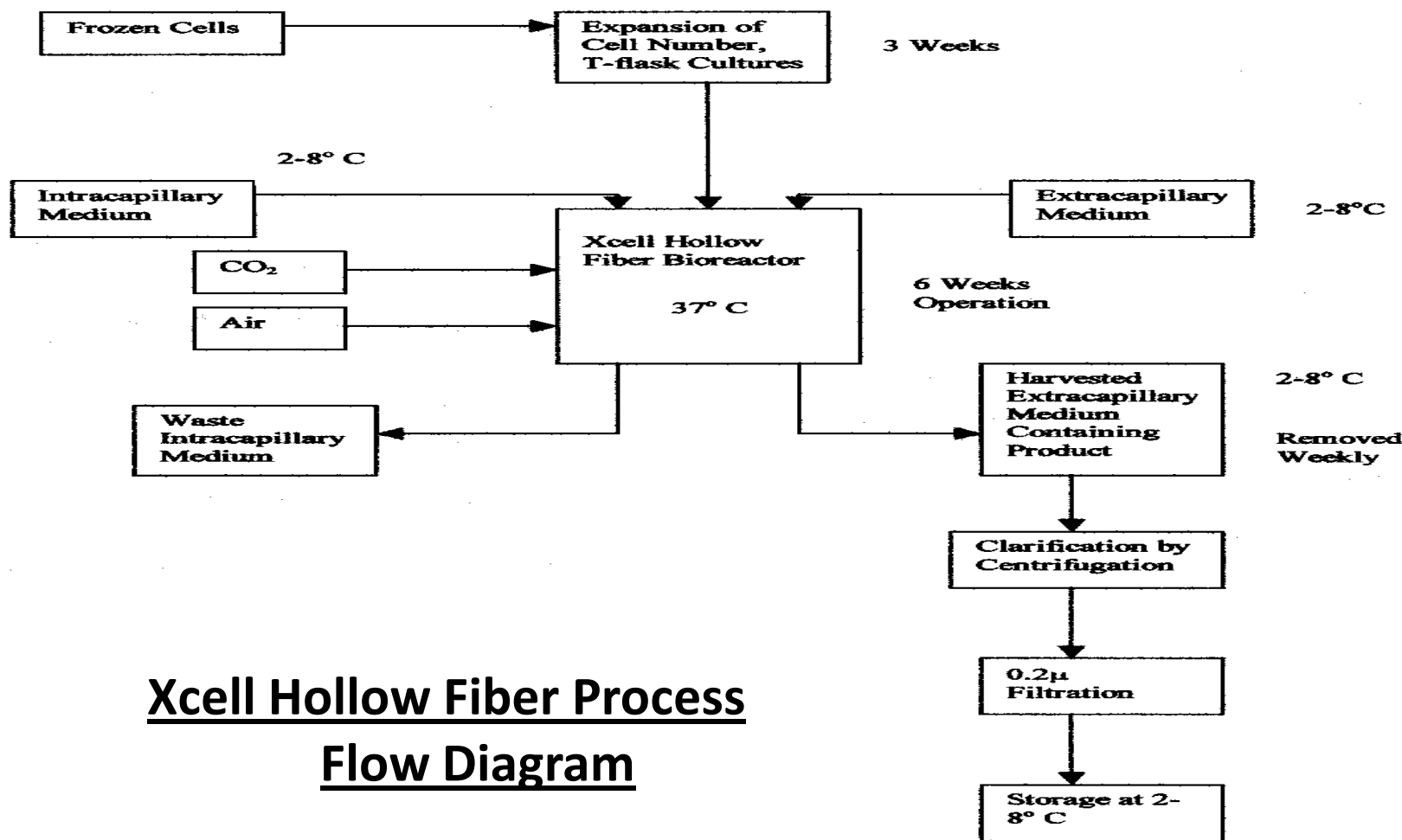


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Xcell Hollow Fiber Process
Flow Diagram



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6D2-IgM Bioreactor (Xcell) Production

Maximum media flow: 3500 mL/Hr.

Productivity (several harvests) ranged from ~0.2 mg/ml to 0.44 mg/ml.

Purify pooled harvest within 1-2 weeks of manufacture.

IgM may lose up to 50% of potency after 1 month in the pooled harvest at 2-8 °C.





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Purification Process flow

Crude Cell Culture harvest



Initial Capture Step



Low pH Viral Inactivation



Purification Step



Polishing



Viral (nano) Filtration



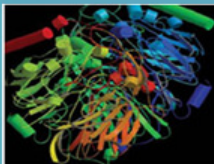
Final polishing



TFF



Drug Substance



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Purified 6D2-IgM Characterization/Manufacturing Summary

Overall process recovery >30%

Concentration of the 6D2 IgM drug substance (antigen specific ELISA) = 5.0 mg/ml

Purification process includes two orthogonal viral reduction steps and without the use of expensive conventional Protein A column resin

pI = Acidic IgM

SDS-PAGE (R) : Kappa Light chain ~30kDa; major mu heavy chain ~65kDa and minor mu heavy chain at ~55kDa

HPLC-SEC >95% purity

HCPs , nucleic acids and other media contaminants in the low part per million or below (ng HCP /mg of 6D2IgM) range

Endotoxin (LAL) <0.012 EU/mL

Achieved a therapeutic grade purified 6D2-IgM for preclinical and Phase I studies





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Conjugation Development and Manufacture of Re-188 labeling of 6D2-IgM

Development Strategy:

1) Developed novel conjugation and radiolabeling processes

Fast (^{188}Re half life ~17 hours)

Utilize TCEP.HCl (Tris(2-Carboxyethyl) Phosphine Hydrochloride) as non-sulfo containing reducing agent for generating –SH (sulfhydryl) groups on the 6D2-IgM via reduction of disulfides

Perform reduction of the 6D2-IgM, ^{188}Re labeling of 6D2-IgM and purification of the drug product in one step

2) Drug product manufacturing kit is simple and easy to use

3) Efficient process – Good recovery of radiolabeled drug product and ^{188}Re incorporation in the drug product

4) Cost effective processes





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Generation of sulfhydryl (-SH) of groups on the 6D2-IgM via TCEP reduction

The influence of TCEP molar excess over 6D2-IgM on Mab structural integrity and on radiolabeling yields was evaluated .

The kinetics of generating –SH groups on 6D2-IgM via TCEP reduction at constant TCEP to 6D2 molar ratio was established.





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The influence of TCEP molar excess over 6D2-IgM on Mab structural integrity and on radiolabeling yields was initially evaluated as follows:

Incubate 6D2-IgM (in PBS) for 1 hr at room temp with 0, 2, 10, 50 and 100 molar excess of TCEP (in PBS) over 6D2-IgM.

After incubation, each sample is split into 2 equal aliquots.

The first aliquot is treated with N-Ethylmaleimide (in PBS) to protect –SH groups from recombining.

The second aliquot was radiolabeled with ^{188}Re as described later (50 molar excess).



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Results and Discussions

Table 1: Influence of TCEP amount (molar excess over 6D2 Mab) on the subsequent radiolabeling yields with ^{188}Re . Incubation was carried out at room temperature for 1 hr.

TCEP molar excess over 6D2	Radiolabeling yield, %
0	10
2	25
10	25
50	72
100	70



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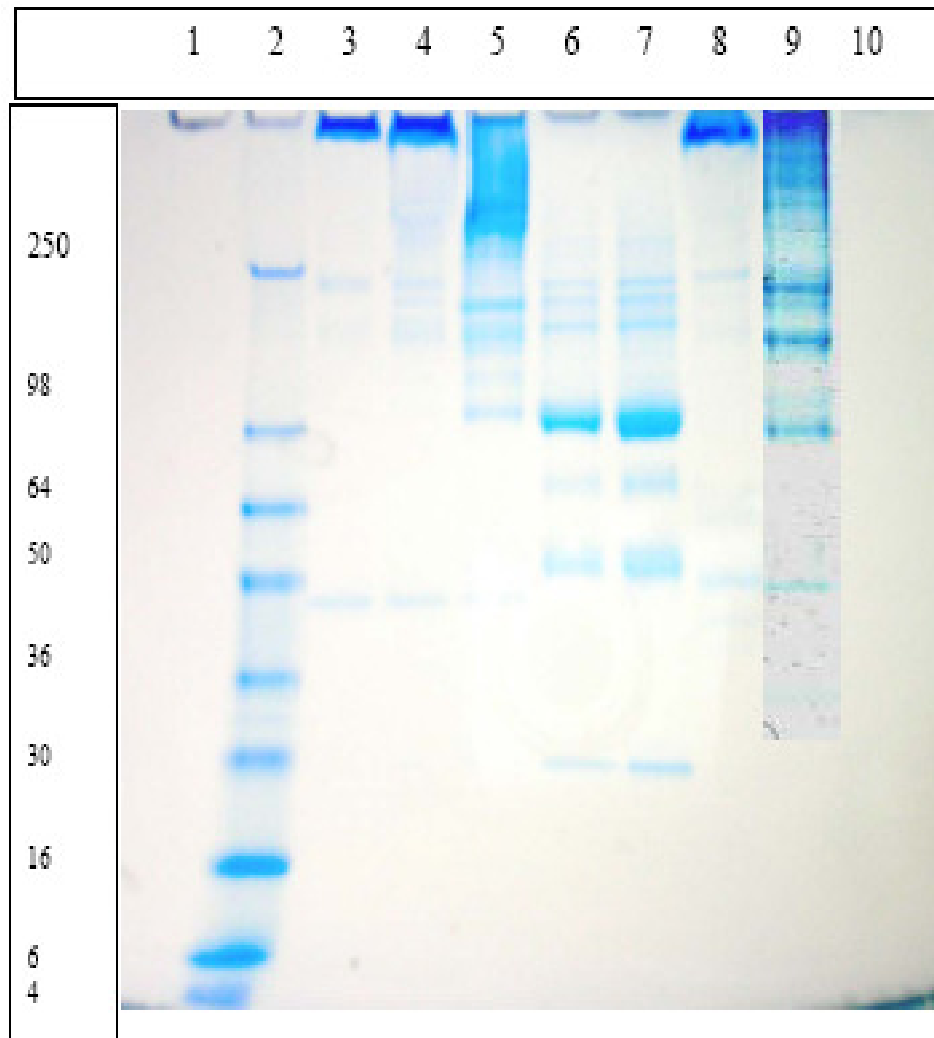
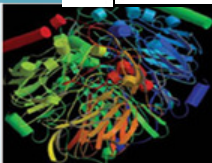


Figure 1: Structural integrity of 6D2 mAb after treatment with TCEP. For comparative purposes samples of 6D2 treated with DTT as in (5) are also shown. Non-reducing SDS-PAGE (4-20% Tris-Glycine gel) was used. Lane # 1: empty; Lane # 2: Pre-stained MW markers (see designations in the left column); Lane # 3: affinity purified 6D2 standard; Lane # 4: 6D2:TCEP, 1:10 molar ratio; Lane # 5: 6D2:TCEP, 1:100 molar ratio; Lane # 6: 6D2 treated with DTT; Lane # 7: the same; Lane # 8: mouse myeloma IgM standard; Lane # 9: 6D2:TCEP, 1:50 molar ratio; Lane # 10: empty.



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The kinetics of generating –SH groups on 6D2-IgM via TCEP reduction at constant TCEP to 6D2 molar ratio

Incubate the second aliquot from above concentration with 50 molar excess of TCEP over 6D2-IgM for 5 mins to 240 mins at room temperature.

Each sample was split into two aliquots.

The first aliquot was labeled with “cold” Sodium Perrhenate described later and analyzed by non-reducing SDS-PAGE and HPLC-SEC.

The second aliquot was radiolabeled with ^{188}Re (as described later) and dependence of radiolabeling yields on the reduction time with TCEP was determined.



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Results and Discussions

Table 2: Influence of incubation time of 6D2 Mab with 50 molar excess of TCEP on the subsequent radiolabeling yields with ¹⁸⁸Re. Incubation was carried out at room temperature.

Time of reduction with TCEP, min	Radiolabeling yields, %
5	42
15	41
30	72
60	71
120	66
240	33



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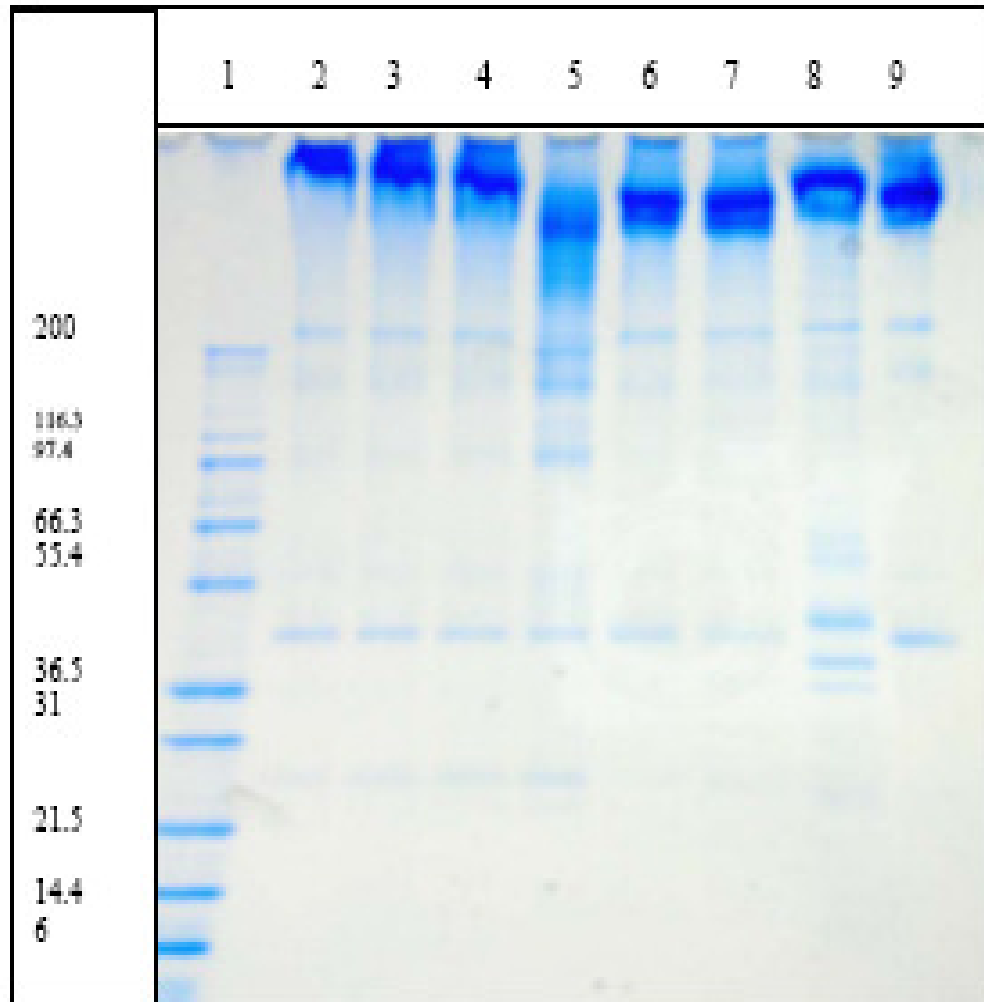


Figure 2: Structural integrity of 6D2 mAb after treatment with TCEP at a TCEP:mAb ratio of 50:1 for various periods of time. The samples were subsequently labeled with "cold" rhenium. Non-reducing SDS-PAGE (4-20% Tris-Glycine gel) was used. A) SDS-PAGE: Lane # 1: Pre-stained MW markers (see designations in the left column); Lane # 2: 6D2, 5 min treatment; Lane # 3: 6D2, 15 min treatment; Lane # 4: 6D2, 30 min treatment; Lane # 5: 6D2, 120 min treatment; Lane # 6: 6D2, 30 min treatment; Lane # 7: 6D2, 60 min treatment; Lane # 8: Sigma Std. IgM; Lane # 9: 6D2 reference standard.

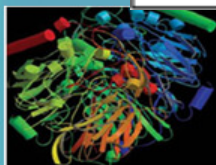
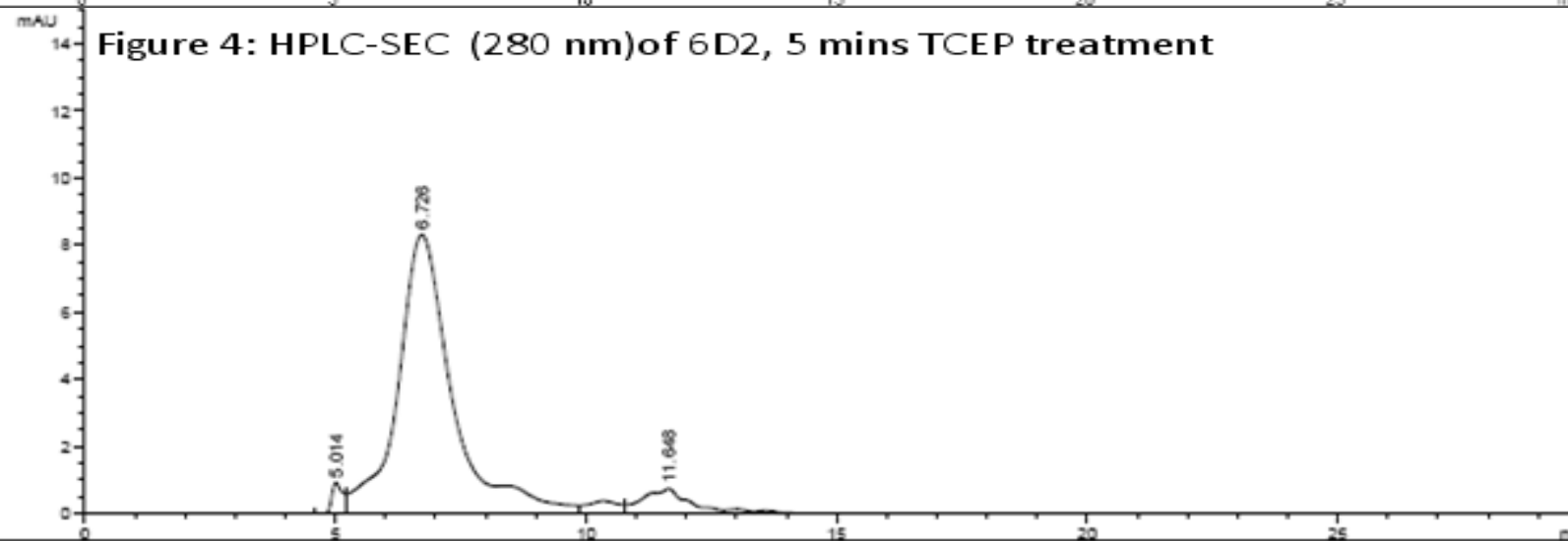
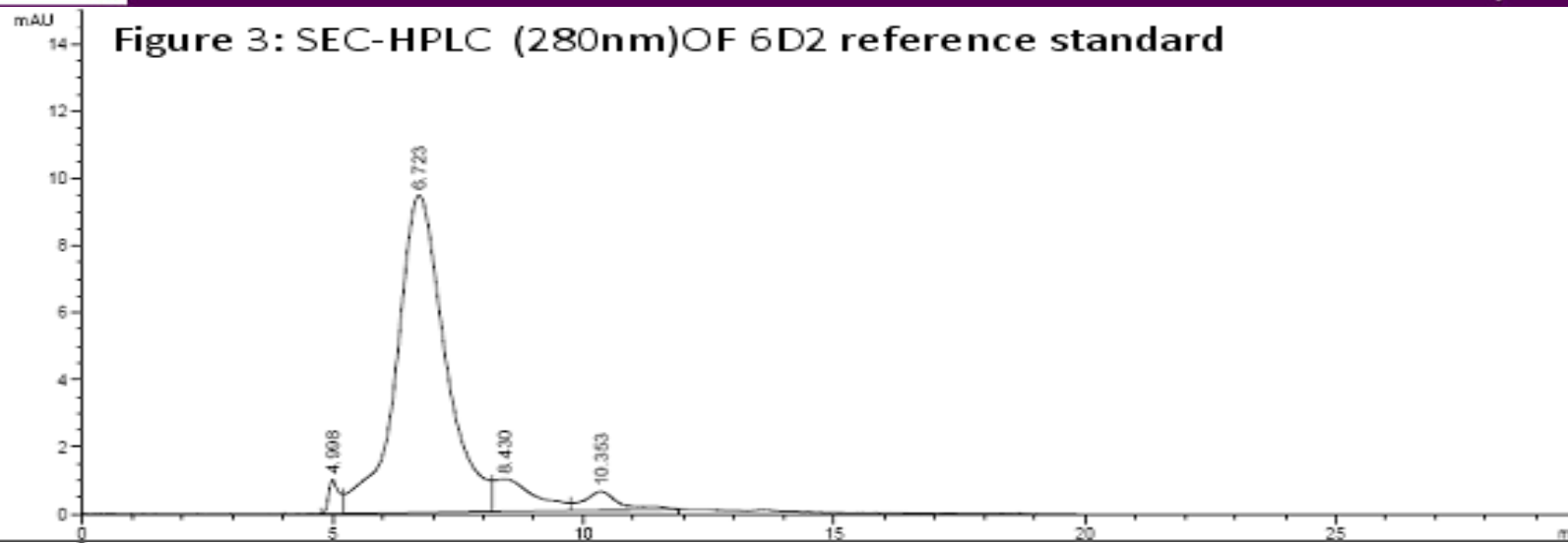


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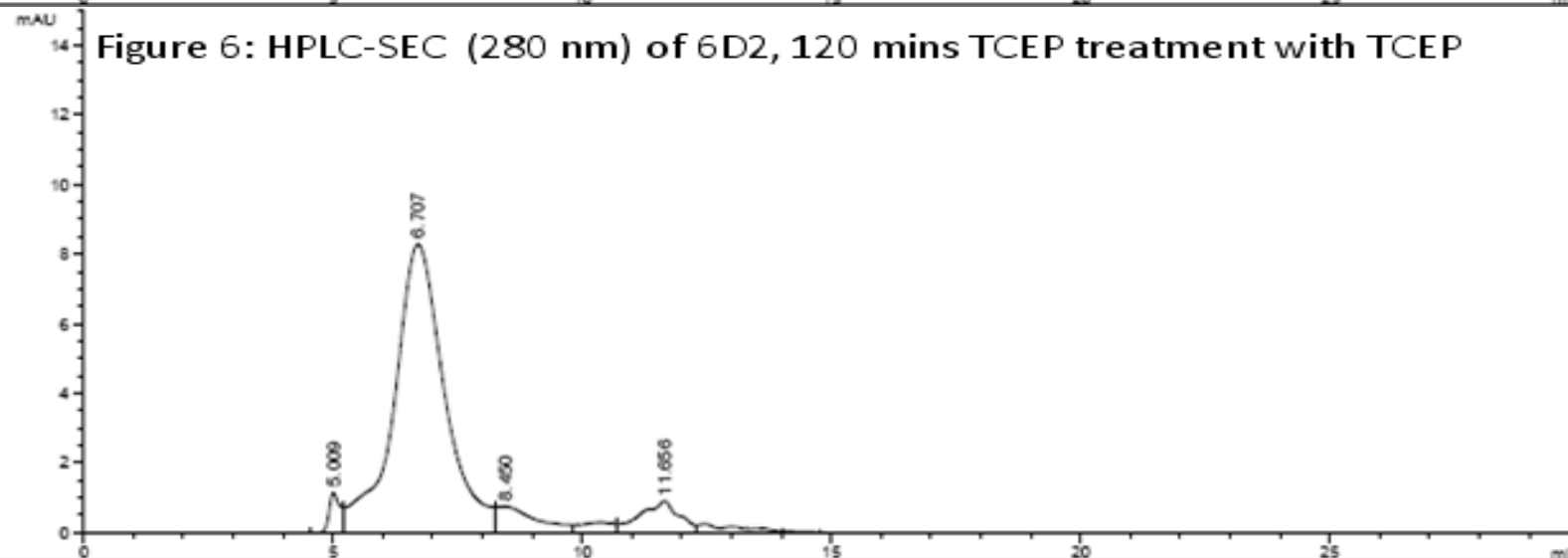
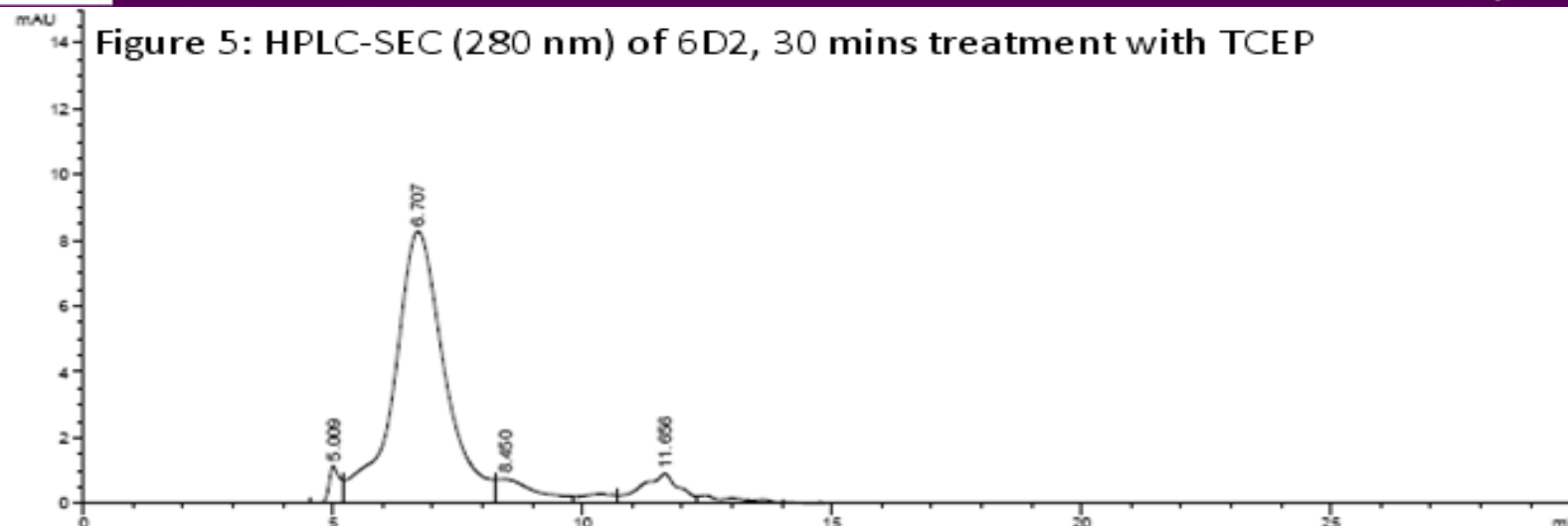


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Summary of Sulfhydryl (-SH) group generation via TCEP reduction

Based upon radiolabeling yield, SDS-PAGE analysis, HPLC-SEC analysis, and incubation time of 30 minutes at room temperature, 50 mM TCEP was utilized for activation and subsequent 188-Re radiolabeling onto 6D2-IgM.



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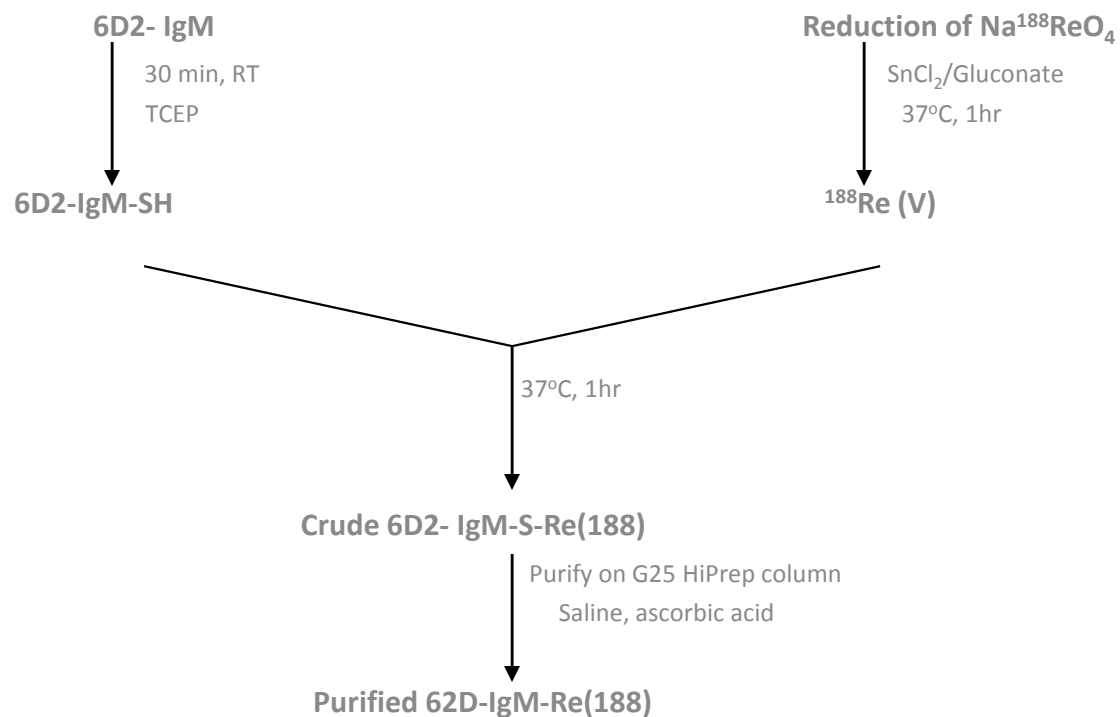
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Radiolabeling of Reduced 6D2-IgM with ^{188}Re , purification and Stabilization of the Drug Product

Labeling Procedure:



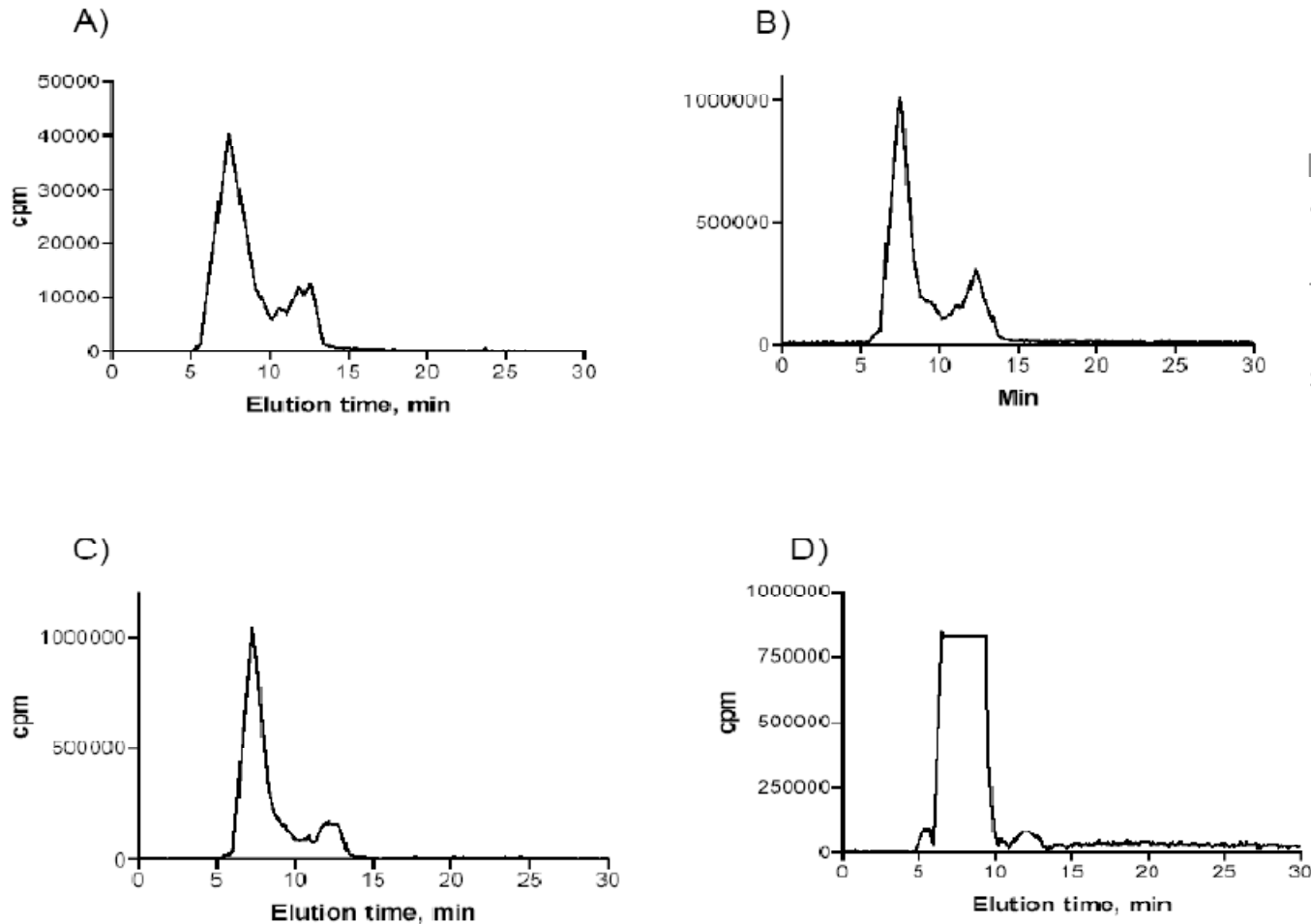


Figure 7: Radiochromatography profiles of ^{188}Re -IgM preparations: a) eluted from HiPrep column and stabilized with L-ascorbic acid; b) the same preparation as in (a) but frozen for 24 hr in a rein vial; (c) stabilized with L-ascorbic acid and "cold" IgM, stored at 4 °C for 6 hr and passed through infusion set; d) eluted from HiPrep column with L-ascorbic acid in saline.

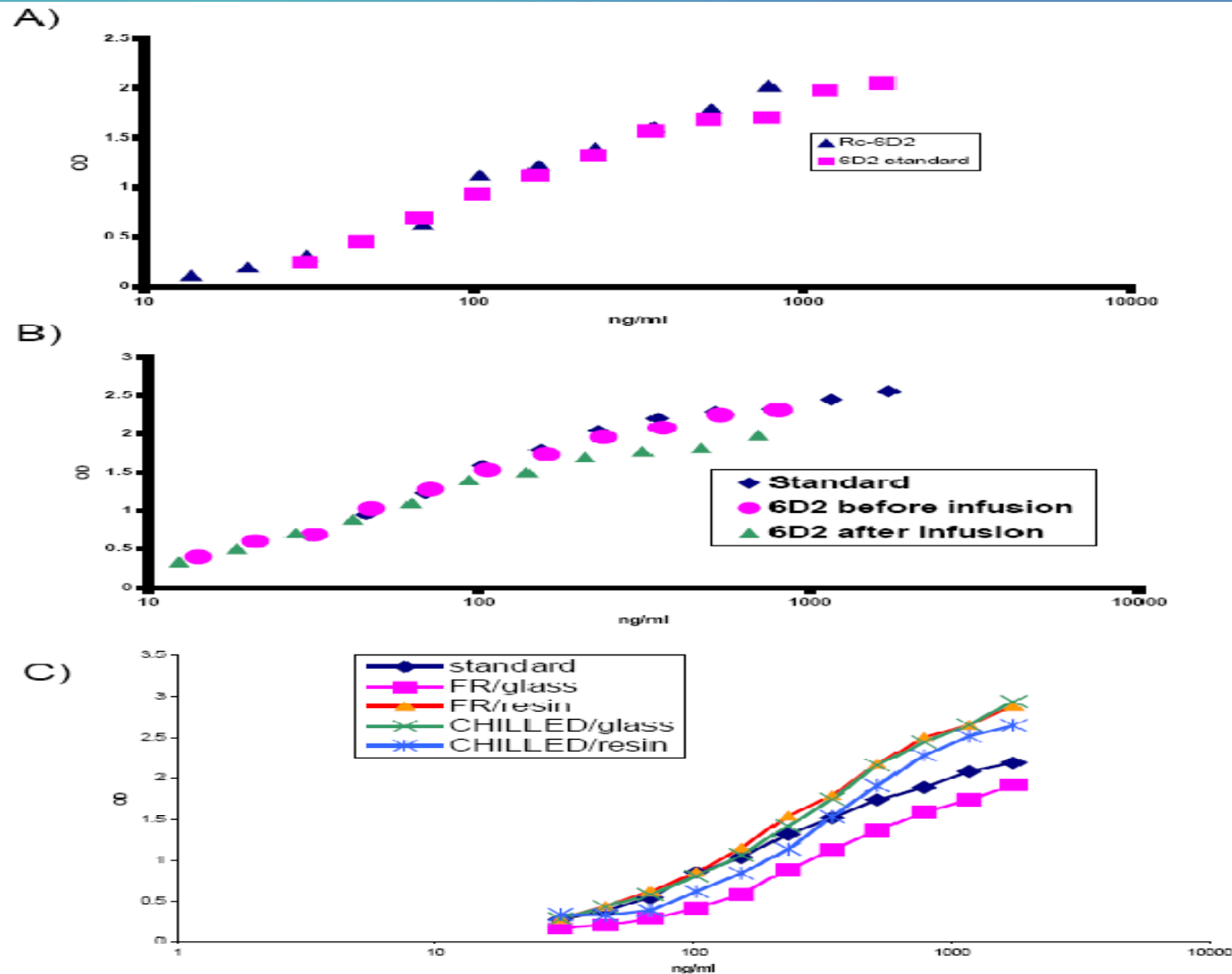


Fig 8: Immunoreactivity of radiolabeled 6D2-IgM to ensure fidelity of radiolabeled D6-IgM to bind to its respective antigen. A) ^{188}Re -6D2 bound to melanin to the same degree as native 6D2. B) Storage of ^{188}Re -6D2 for 6 hr at 4°C (proposed shelf-life of ^{188}Re -6D2 in clinical trials) followed by passing it through infusion set did not cause any significant decrease in immunoreactivity. C) Overnight freezing of ^{188}Re -6D2 at -80°C in a resin vial did not affect its ability to bind melanin.



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Radiolabeling with ^{188}Re , purification and Stabilization of the Drug Product

Summary and Discussion

Simple, fast and efficient procedure was developed to generate the reduced 6D2-IgM, followed by generation of the reduced ^{188}Re and coupling to the reduced 6D2-IgM to the ^{188}Re and subsequent purification in <3 hrs.

Recovery of the 6D2 antibody in the ^{188}Re -6D2 drug product >70%.

Radiochemical purity of ^{188}Re -6D2 was 97% (as per ITLC and radiochromatography) when L-ascorbic acid is added in the elution buffer during purification on the HiPrep column.

Immunoreactivity of the purified ^{188}Re -6D2 drug product bound to melanin to the same degree as native 6D2.

Storage of ^{188}Re -6D2 drug product for 6 hr at 4°C (proposed shelf life of ^{188}Re -6D2 drug product in clinical trials) followed by passing it through infusion did not cause any significant decrease in its immunoreactivity.





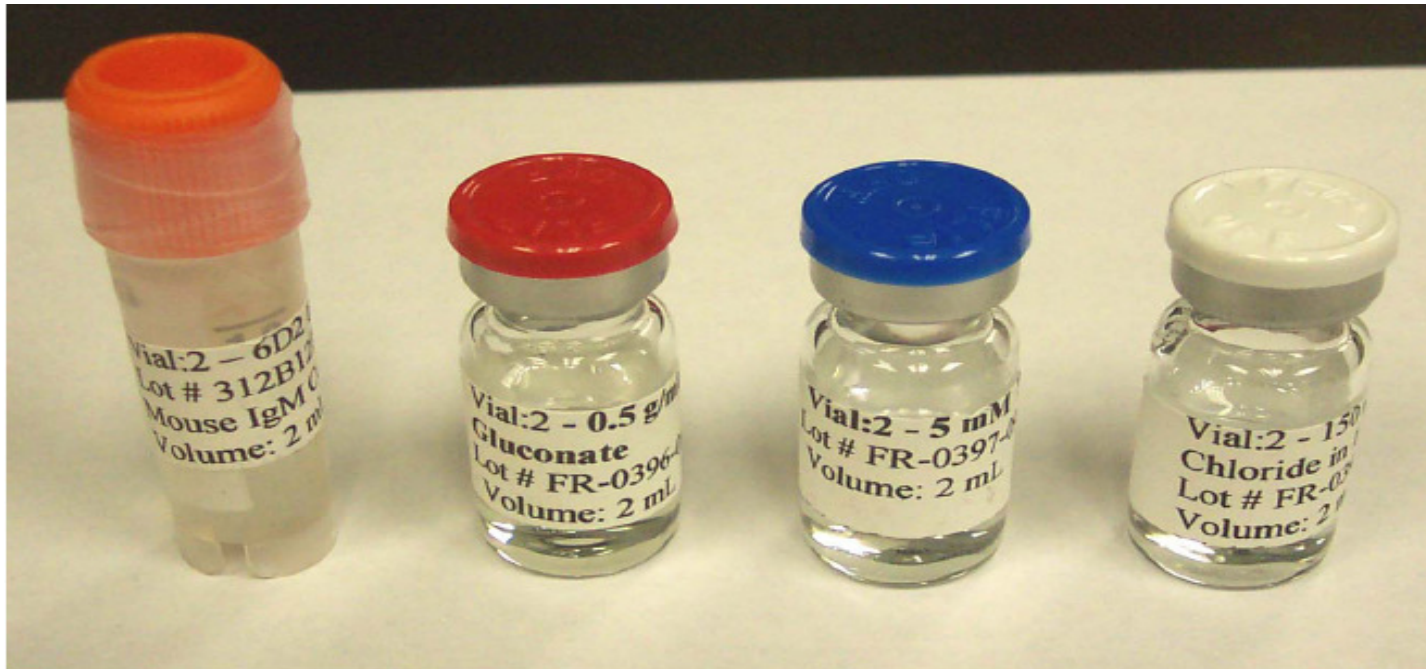
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PreClinical Development of melanin binding 6D2-IgM labeled with ^{188}Re



^{188}Re -Labeled melanin-binding 6D2 reagents

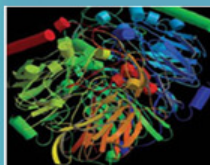


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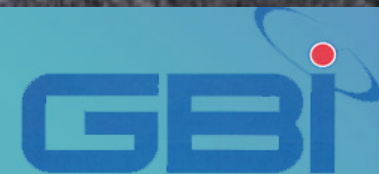
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Biodistribution (Pharmacokinetics) and WBAR of 188-Re-6D2

The biodistribution and whole body autoradiography of 188-Re-6D2 were evaluated in nude mice bearing tumors derived from the A2058 human metastatic melanoma cell line





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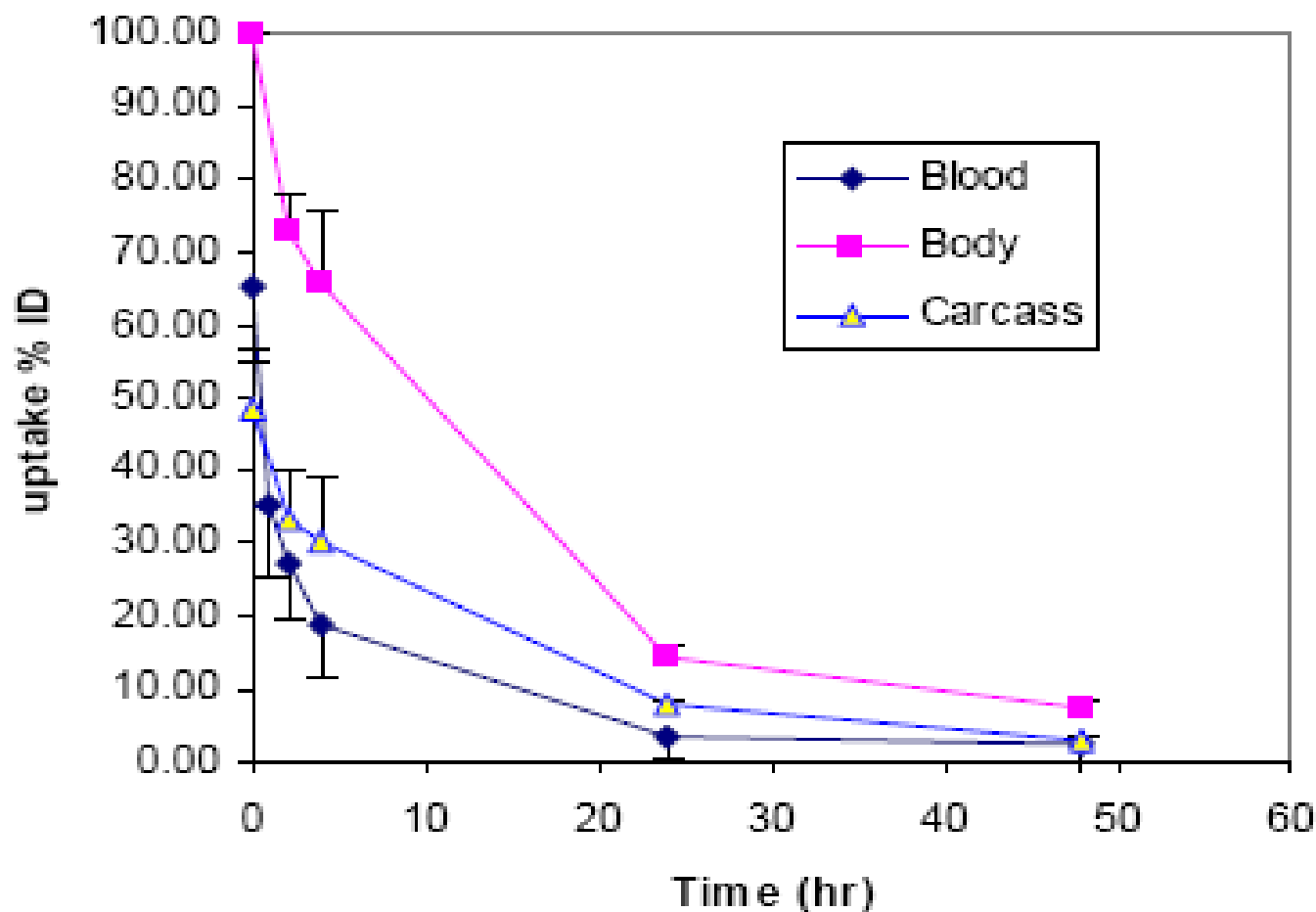


Figure 9A : Biodistribution of ^{188}Re -6D2 in nude mice bearing A-2058-derived melanoma tumors after IV administration - blood, whole body and carcass



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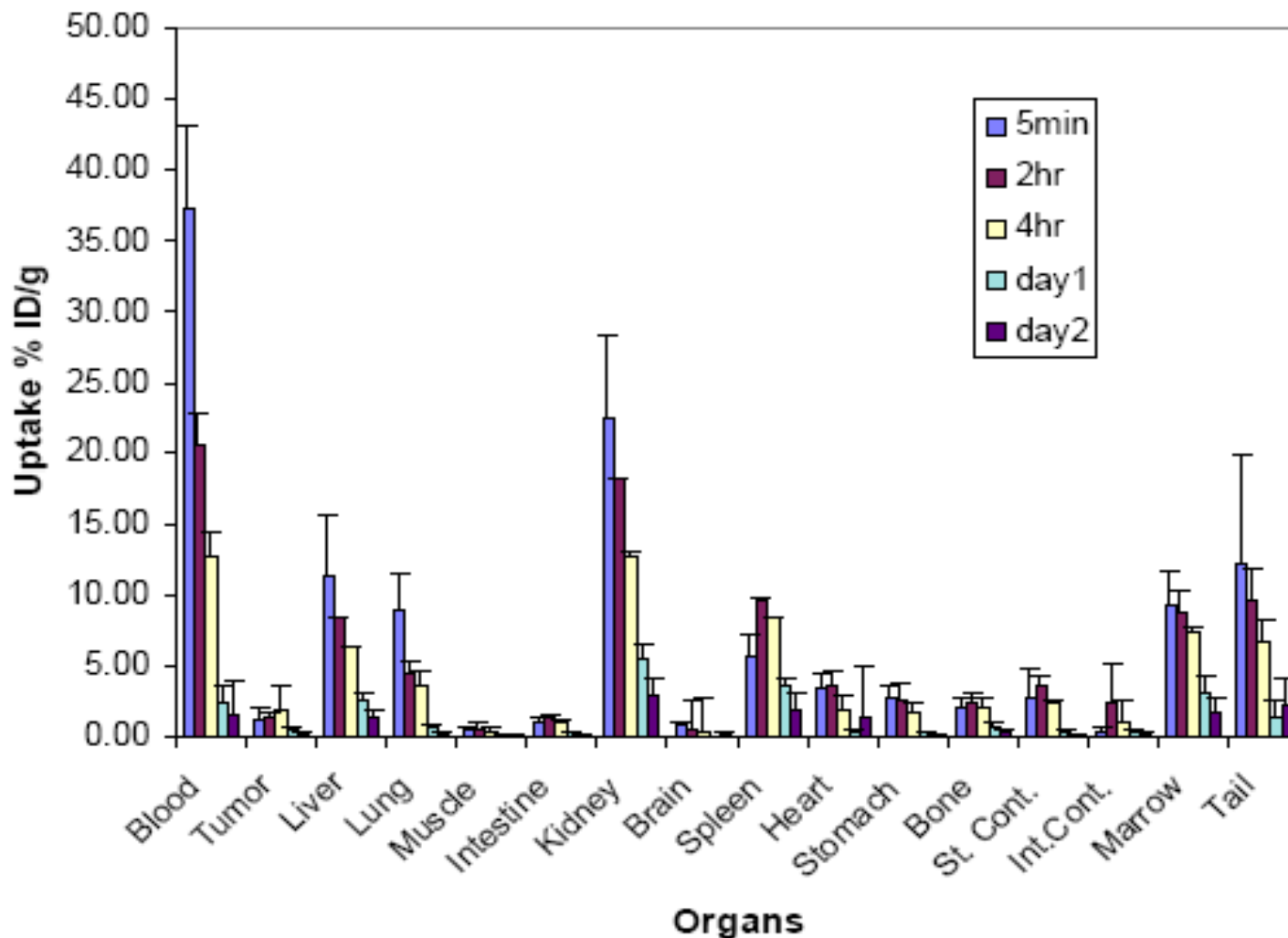
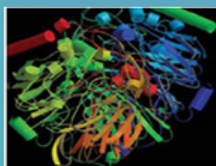


Figure 9B :
Biodistribution of ^{188}Re -6D2 in nude mice bearing A-2058-derived melanoma tumors after IV administration – distribution in major organs and tumors



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A)

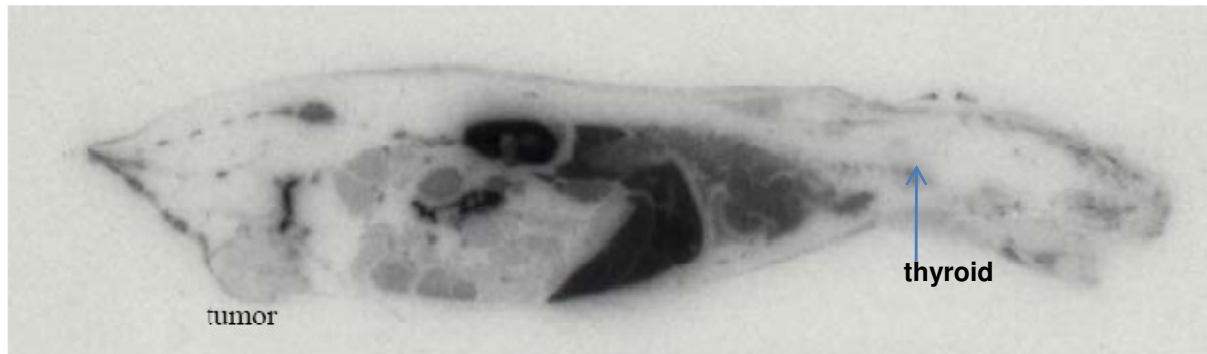
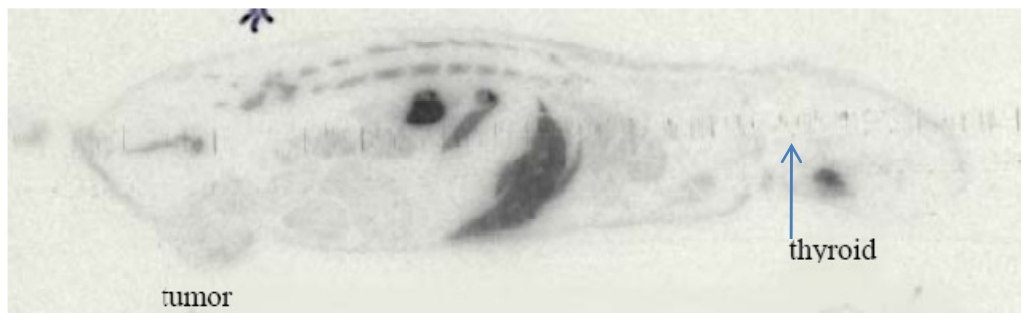


Figure 10: Whole body autoradiography of ^{188}Re -6D2 in A2058 human melanoma-bearing nude mice after IV administration: A) 4 hr; B) 24 hr

B)





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Summary of Biodistribution and WBAR of 188-Re-6D2

188-Re-6D2 was quickly cleared with a half life of approximately 5 hours from the blood and 10 hours from the body

The overall clearance of 188-Re-6D2 from all major organs was rapid and mirrored the clearance from the blood

Due to 8% of free 188-Re-perrhenate in the preparation, the WBAR at 4 hours showed some uptake in the thyroid that disappeared at 24 hours due to the inability of the 188-Re-perrhenate to accumulate in the thyroid tissue

Tumor uptake was modest with a maximum uptake of 1.94% ID/g reached at 4 hours and decreasing to 0.51 and 0.21 %, at 24 and 48 hours, respectively





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Therapy for A2058 human metastatic melanoma tumors in nude mice, tumor histology and evaluation of acute hematologic toxicity of ^{188}Re -6D2





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A)

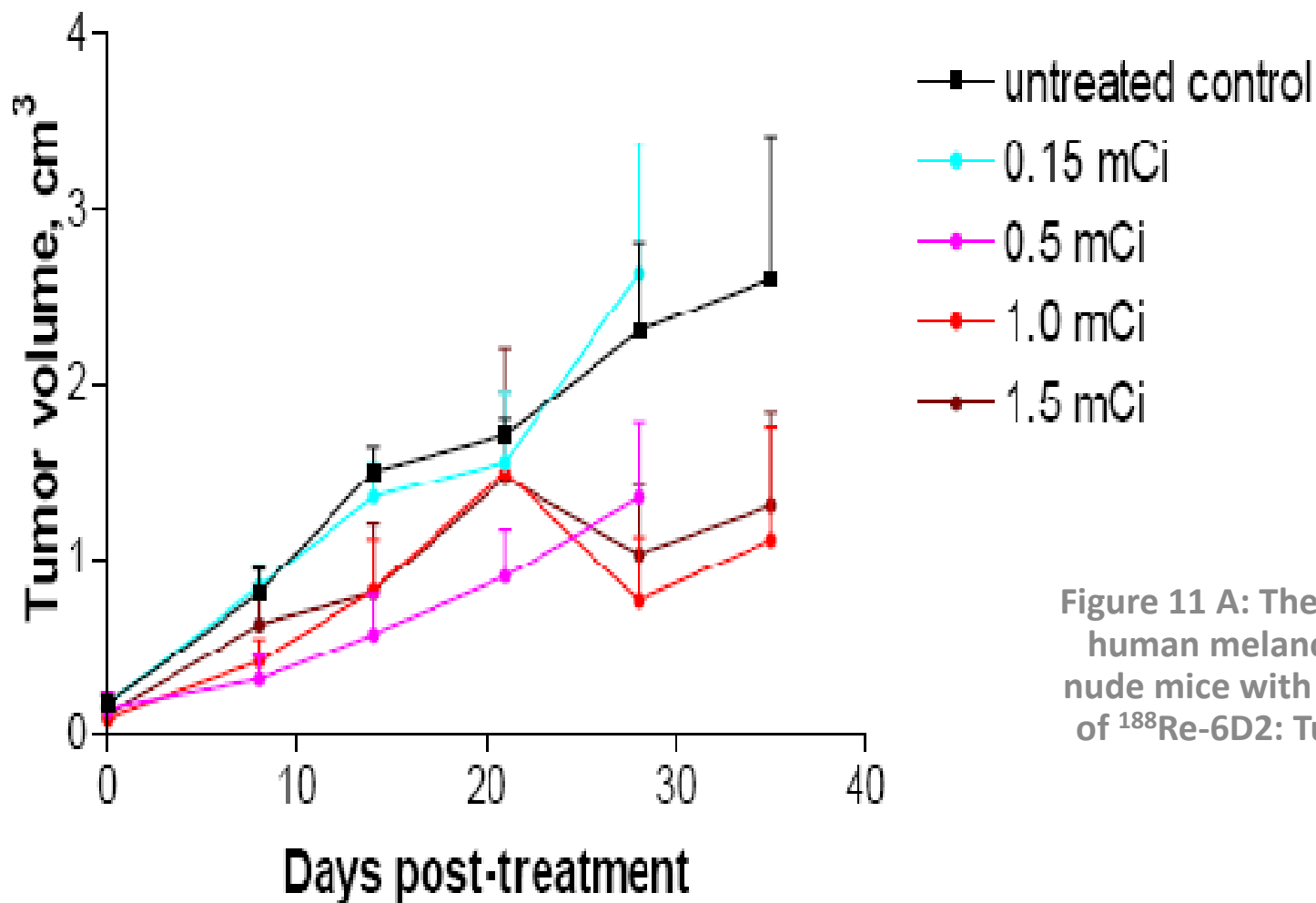


Figure 11 A: Therapy of A2058 human melanoma-bearing nude mice with various doses of ¹⁸⁸Re-6D2: Tumor volume



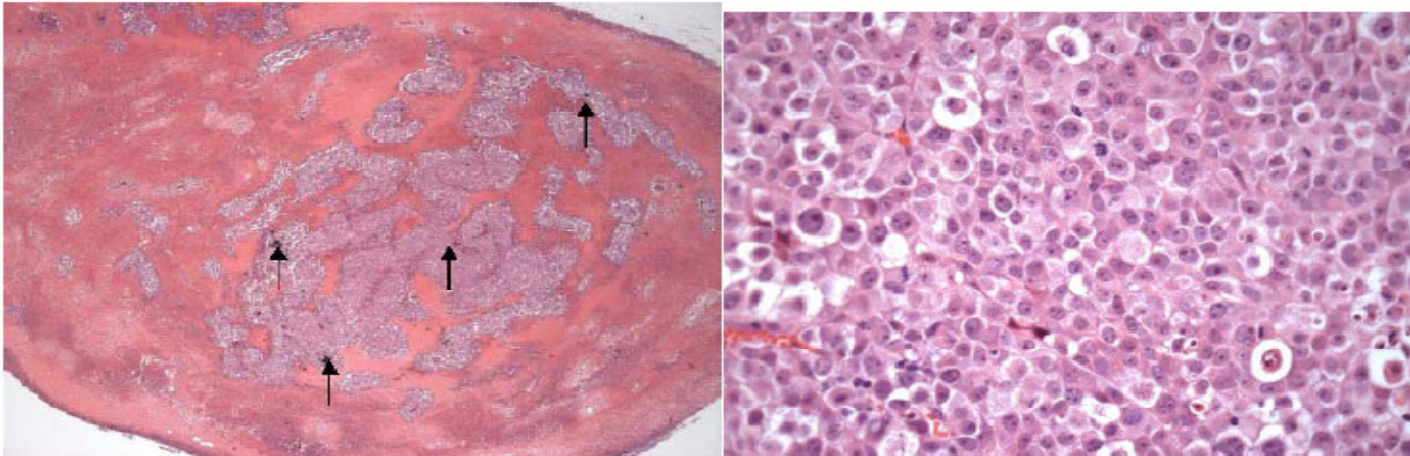
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B)



C)

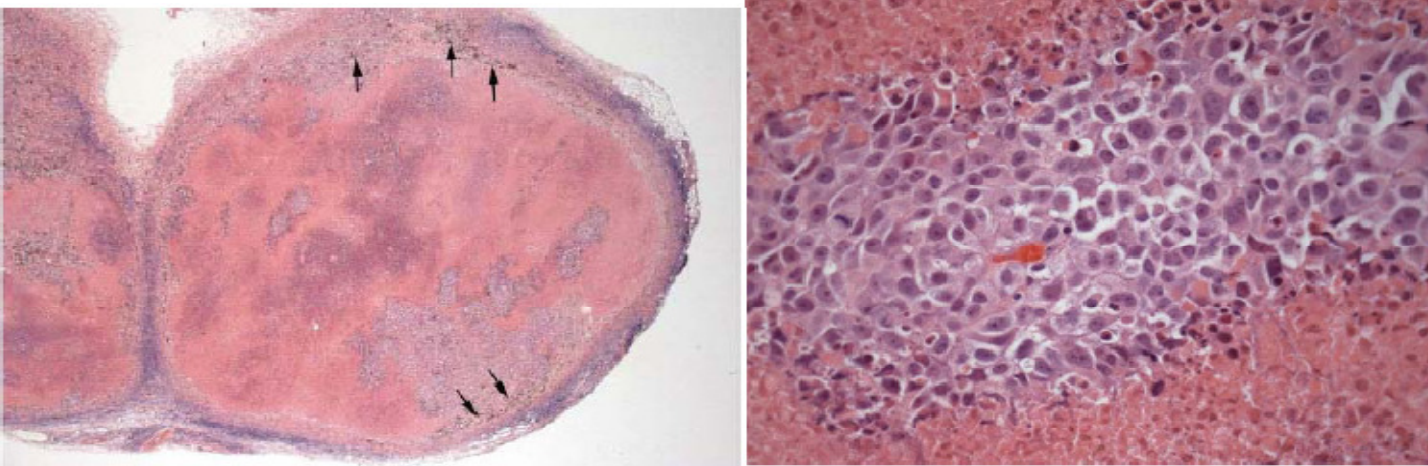


Figure 11B and C:
Therapy of A2058
human melanoma-
bearing nude mice with
various doses of ^{188}Re -
6D2 – Histology of the
tumors: B) tumor from
untreated mouse; C)
tumor from a mouse
treated with 1.5 mCi.
Tissues were stained
with hematoxylin and
eosin. Melanin granules
are marked with black
arrows. Left panel in B)
and C) – 25X
magnification; right
panel – 400X
magnification



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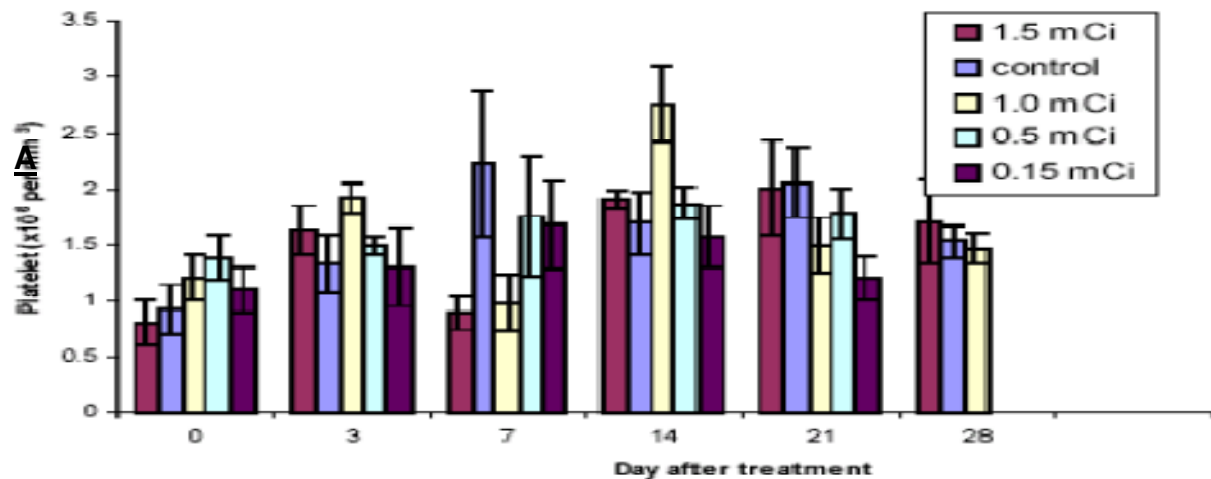
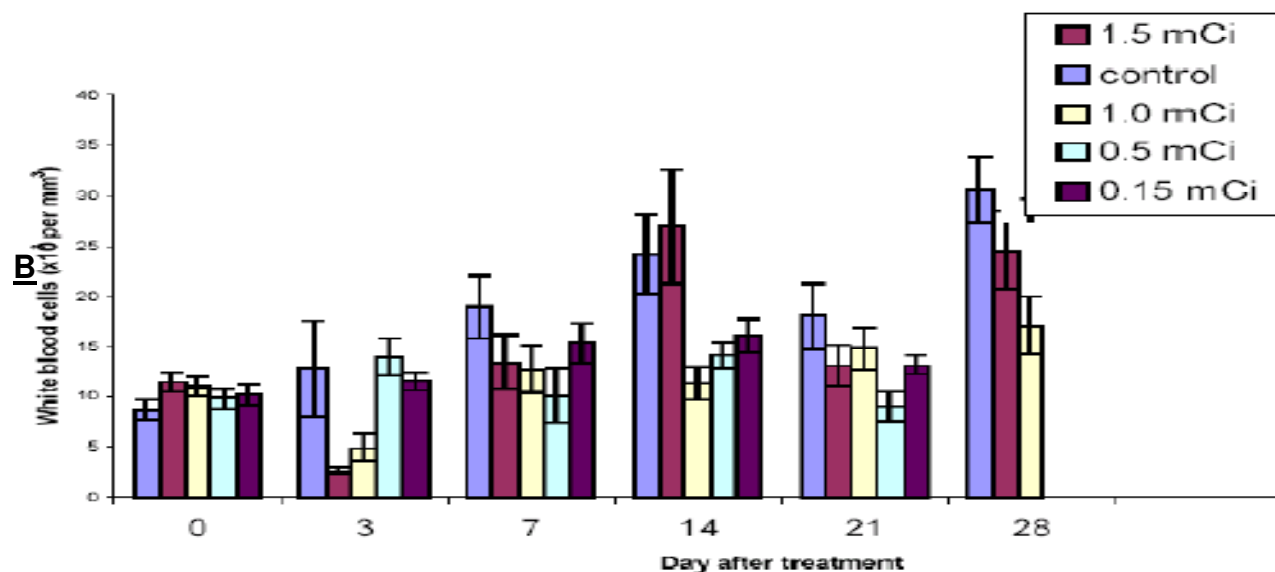


Figure 12 : Platelet and white blood counts in A2058 human melanoma-bearing nude mice treated with various doses of ¹⁸⁸Re-6D2: A) platelet; B) white blood count





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Summary of Therapy for A2058 human metastatic melanoma tumors in nude mice, tumor histology and evaluation of acute hematologic toxicity of 188-Re-6D2

While the lowest dose of 188-Re-6D2 had no effect on tumor progression relative to the untreated mice, higher doses significantly ($p < 0.05$) slowed tumor growth

On day 35 post-treatment, histological analysis of the untreated mice and mice treated with 1.0 and 1.5 mCi indicate that the tumors from the mice treated with 188-Re-6D2 had more extensive central necrosis and fewer viable tumor cells than the tumors from the untreated control mice



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Project Conclusions

A generic purification process has been developed to purify therapeutic grade IgMs from mammalian cell culture harvest without the use of conventional Protein A column (affinity) resin

A fast, simple and efficient reduction (activation) of 6D2-IgM via TCEP has been developed

Radiolabeling and quality control procedures for ^{188}Re -labeled melanin binding for use in Phase I clinical trial in patients with metastatic melanoma has been developed

Phase I clinical trials on the use of ^{188}Re -6D2-IgM in patients with metastatic melanoma has been initiated and is ongoing.





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Thanks you for your attention:

Questions?

Contact M. Sesay, PhD

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