

Astatine-211 and Astatinated Radiopharmaceuticals

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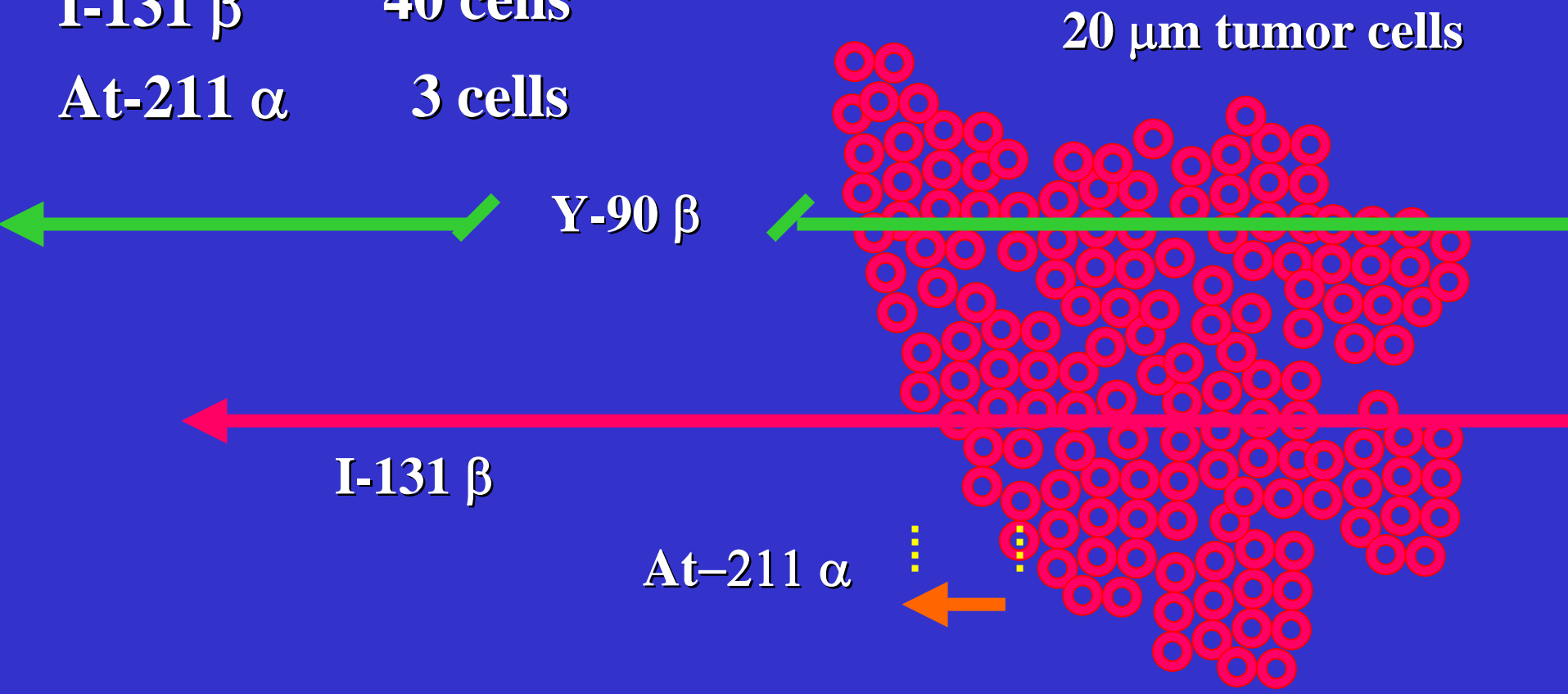
Range of α - and β -Particles

Mean range

Y-90 β 215 cells

I-131 β 40 cells

At-211 α 3 cells



Potential Applications of α -Particle Therapy

- **Micrometastases**
- **Tumors of Circulation**
 - Lymphoma
 - Leukemia
- **Compartmental Tumors**
 - Cystic
 - Ovarian
 - Neoplastic Meningitis

Radiobiological Advantages of Alpha Particles

- **High relative biological effectiveness (RBE)**
- **Low oxygen enhancement ratio**
- **Absence of dose rate effects**
- **Not dependent on cell cycle**

Astatine-211 Decay Scheme

At-211 α particles

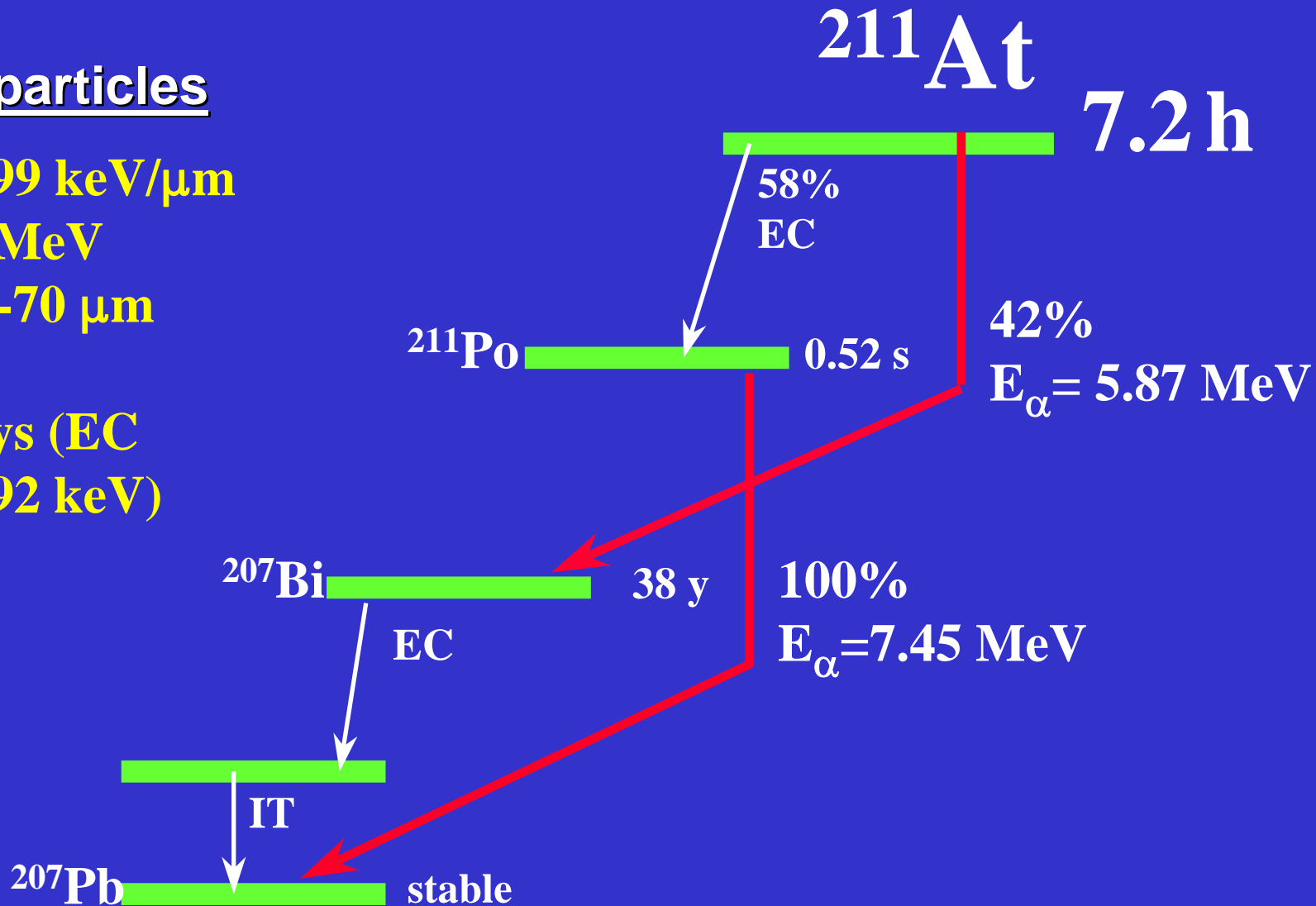
$LET_{\text{mean}} = 99 \text{ keV}/\mu\text{m}$

$E_{\text{ave}} = 6.79 \text{ MeV}$

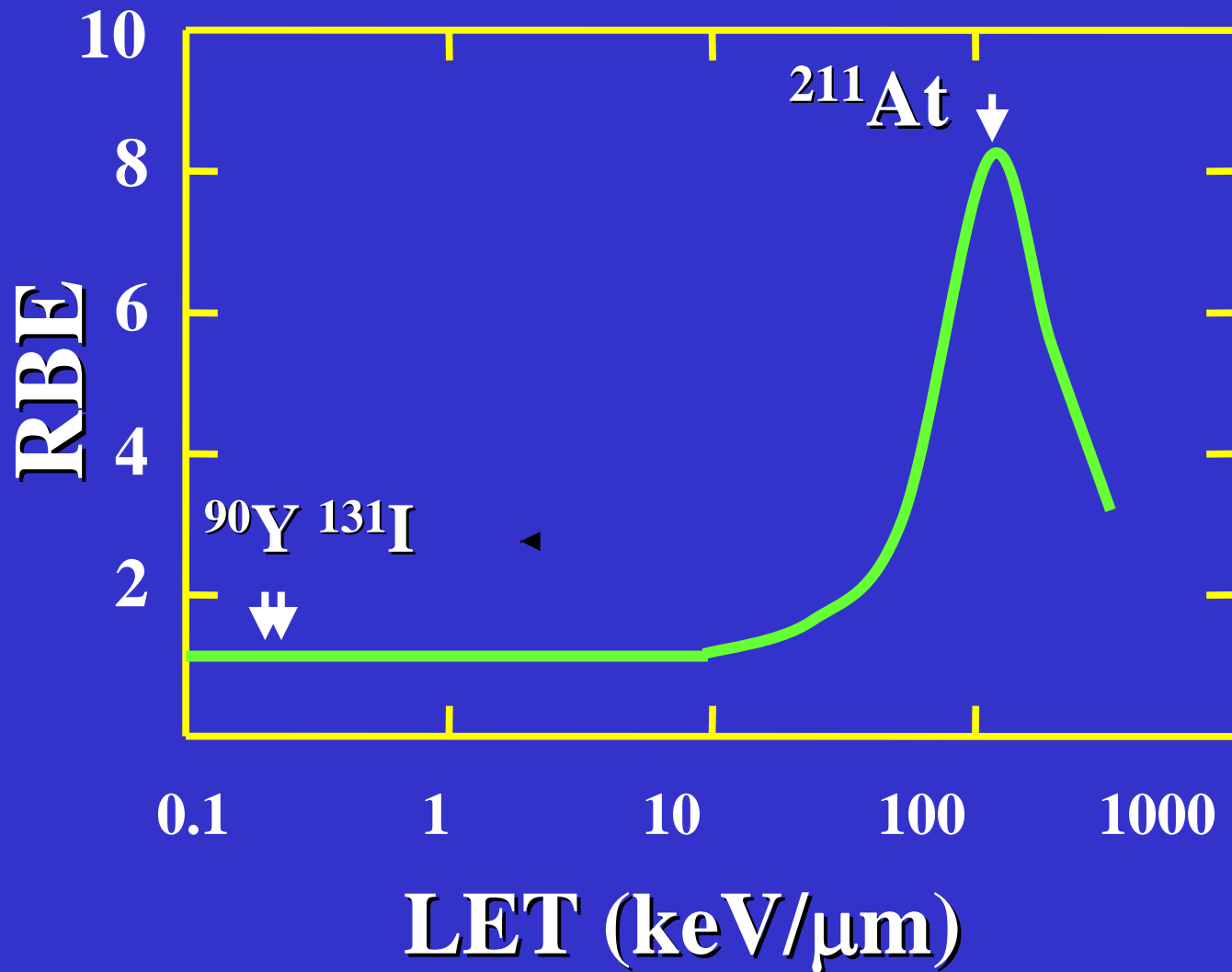
range = 55-70 μm

Imaging:

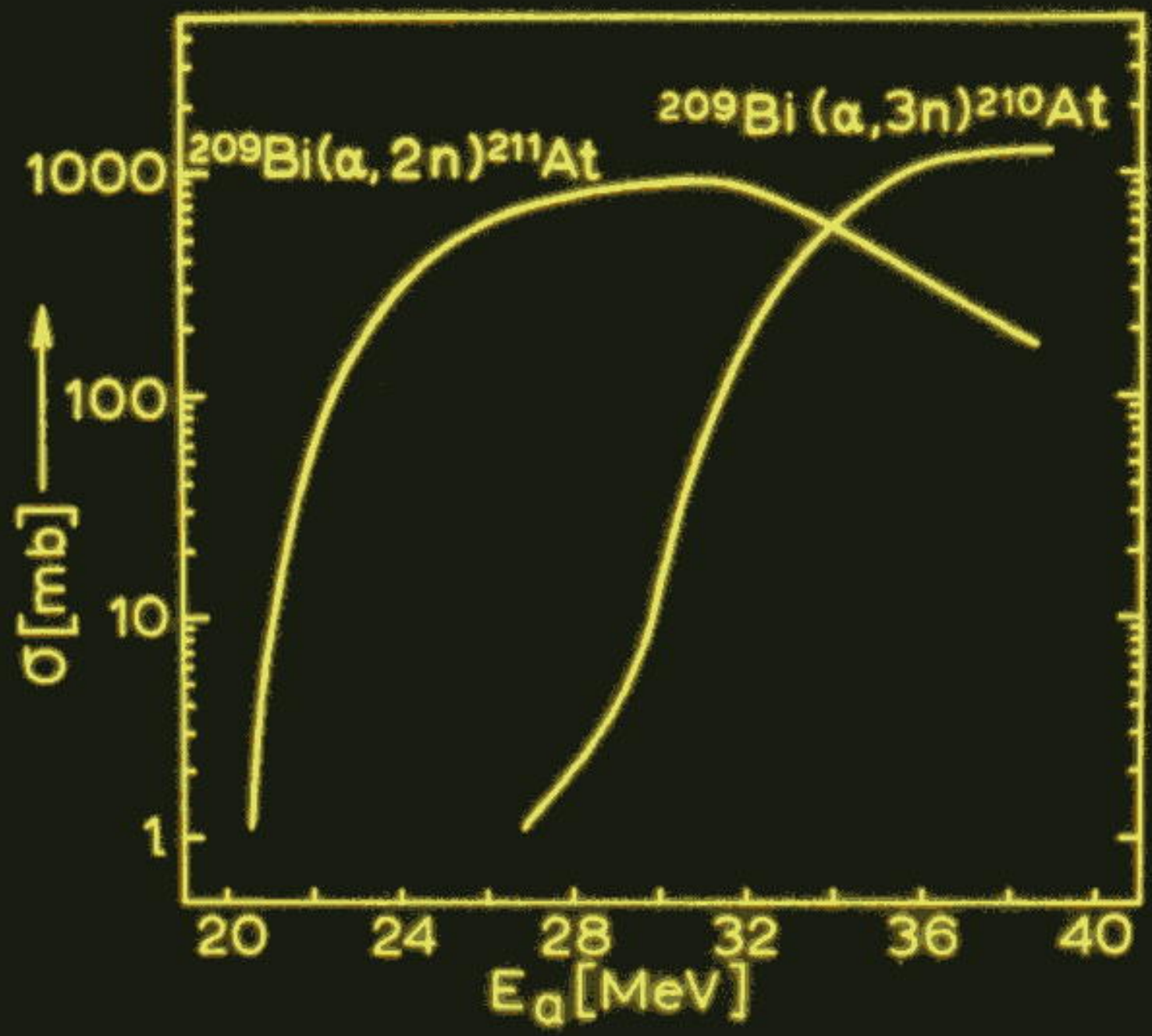
^{211}Po X-rays (EC decay; 77-92 keV)



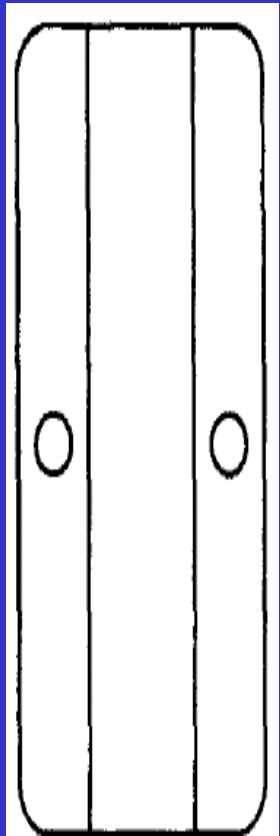
Relative Biological Effectiveness vs. LET



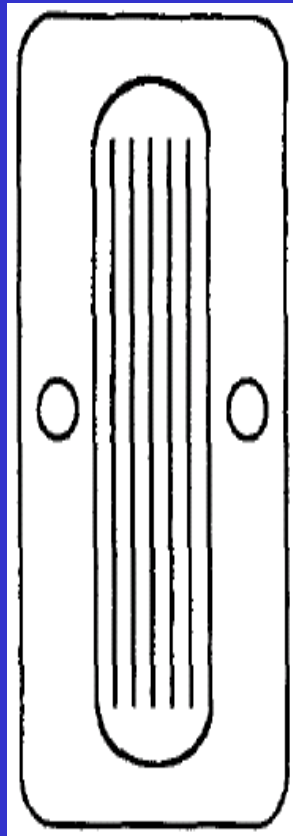
- **Astatine-211 Production**
- **Astatinated Monoclonal Antibodies**
- *Meta*-[²¹¹At]Astatobenzylguanidine
- 5-[²¹¹At]Astatato-2'-deoxyuridine
- **Astatinated octreotide Analogues**



²¹¹At-Internal Target



Top/Front

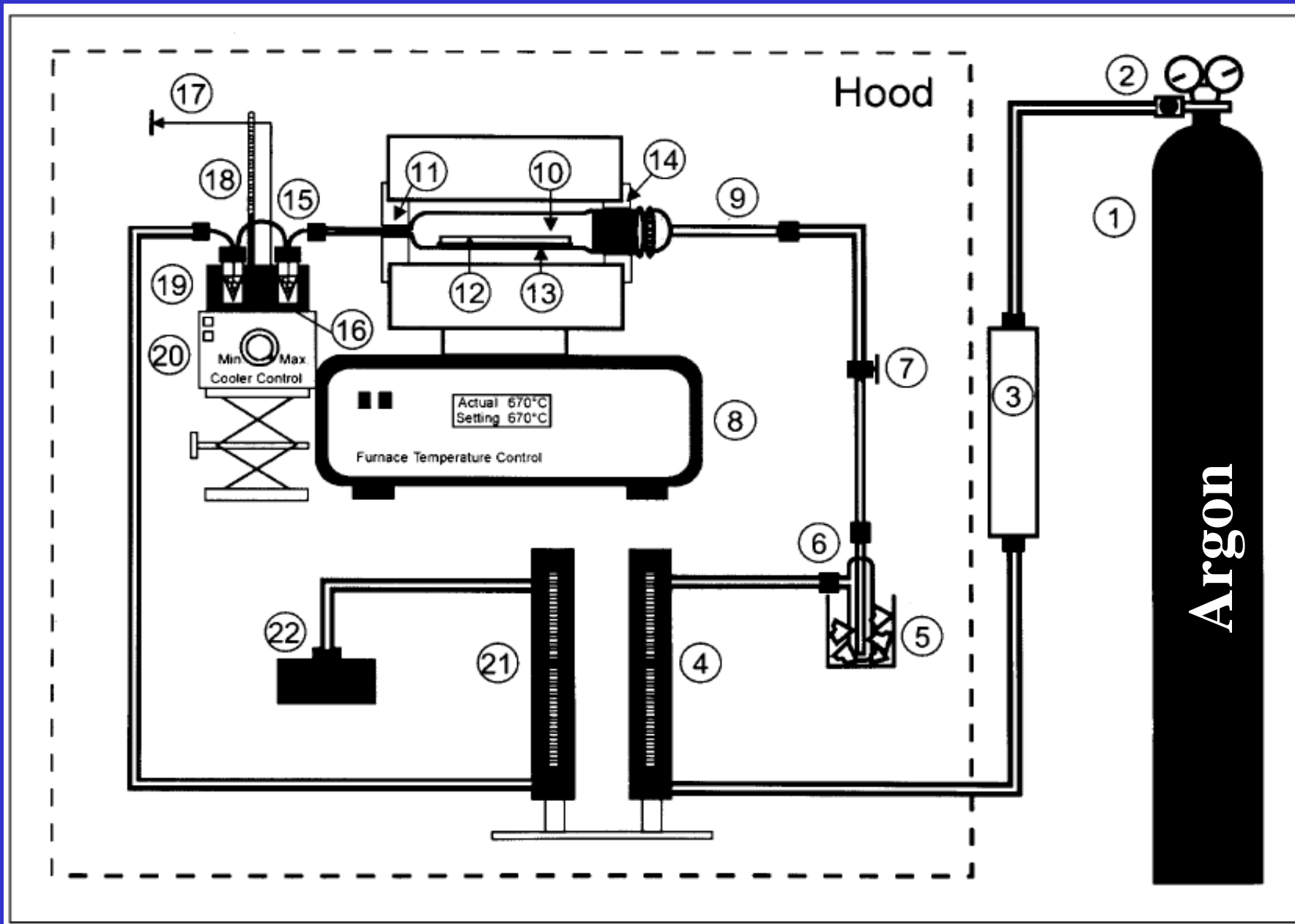


Bottom/Back

- Grazing angle at 1.5° using curved target face
 - increases beam strike length from 2.5 to 10 cm
 - improves heat transfer

At-211 Internal Target

- **Al vs. Cu backing material**
 - yield
 - background activity
 - distillation efficiency
- **Target Configuration**
 - yield
 - still size, yield



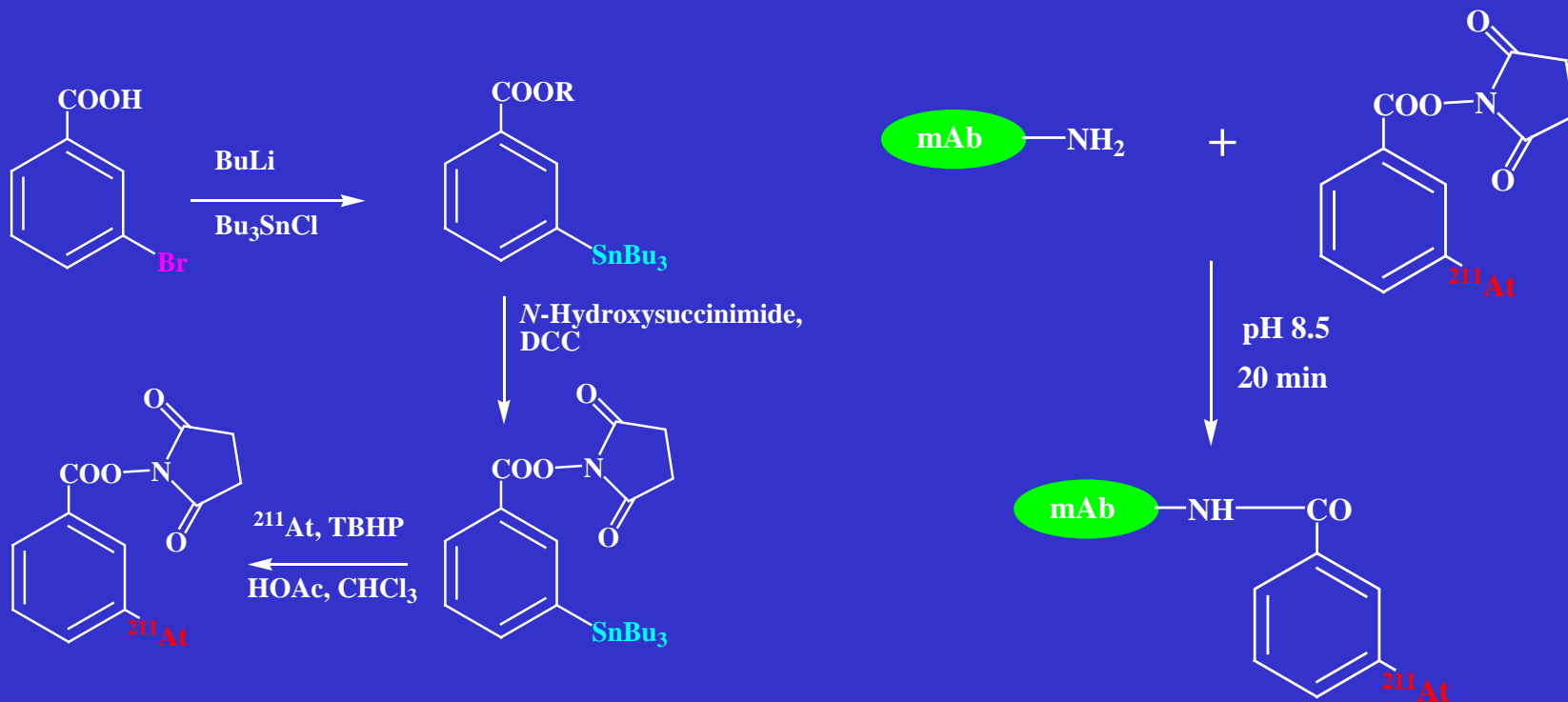
^{211}At Internal Target



- Beam currents of 50-60 μA , 28 MeV α -particles, and 1.5-4.5 hr runs
- 0.8 ± 0.1 mCi/ $\mu\text{A}\cdot\text{h}$
- Maximum to date:
 $55 \mu\text{A} \times 4.0 \text{ h} = 178 \text{ mCi}$
- $67 \pm 16\%$ distillation yield

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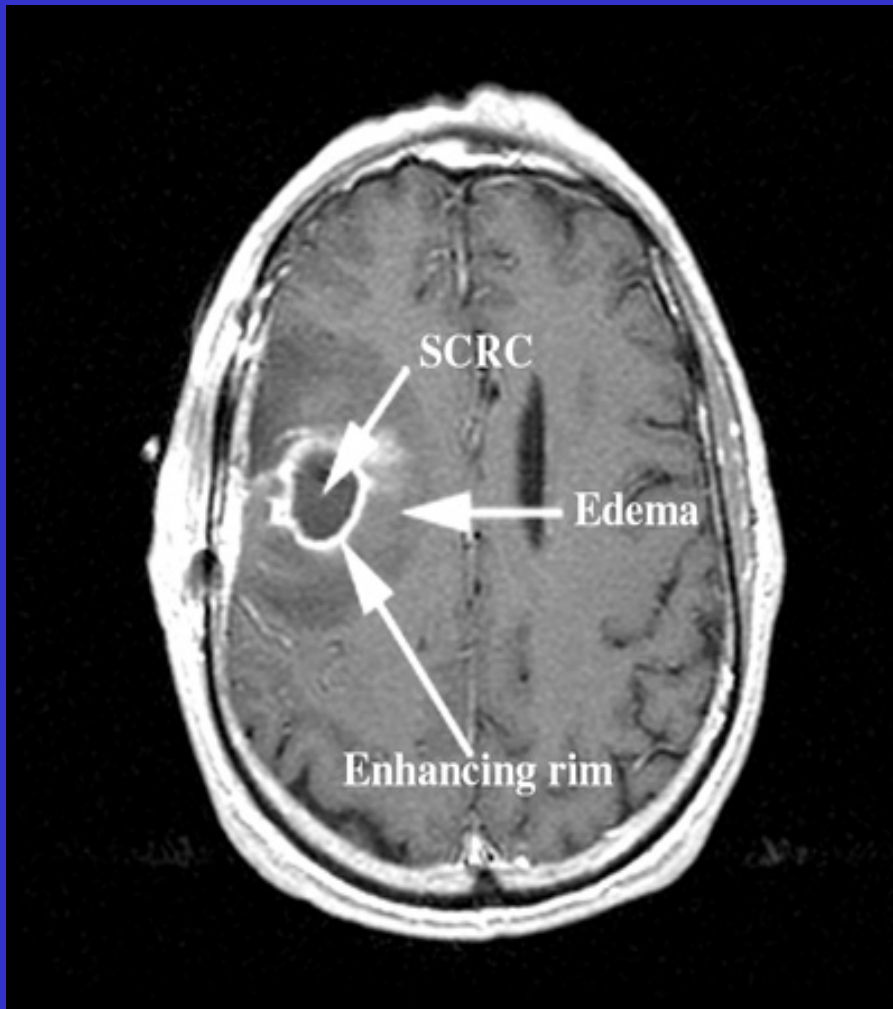
N-Succinimidyl 3-[²¹¹At]Astatobenzoate ([²¹¹At]SAB) for mAb Labeling



Clinical Batches of Chimeric 81C6 mAb labeled with ^{211}At Using $[^{211}\text{At}]\text{SAB}$

- **17 batches (2.8 – 14.0 mCi)**
- **$[^{211}\text{At}]\text{SAB}$ yield: $54 \pm 10\%$ (30% EtOAc fraction from Sep-pak)**
- **Conjugation yield: $76 \pm 10\%$**
- **Radiochemical purity: $96.0 \pm 2.5\%$
(Size exclusion HPLC)**
- **Immunoreactive fraction: $83.3 \pm 5.3\%$
(Lindmo method)**

Compartmental Administration: Surgically Created Resection Cavity (SCRC)



At-211 Labeled Chimeric 81C6: Clinical Protocol

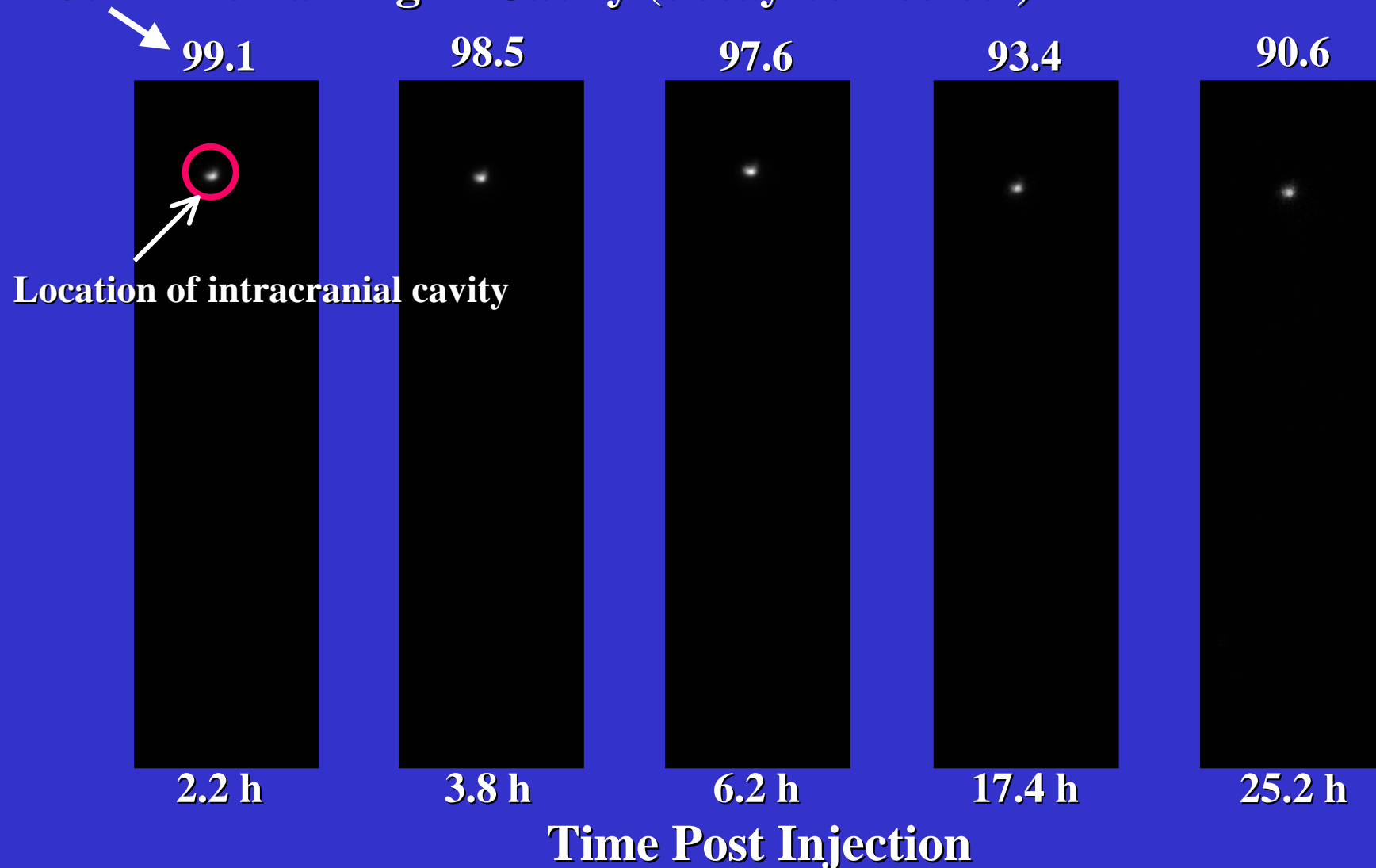
- **Thyroid blocking with SSKI and Cytomel beginning 48 hr prior to therapy**
- **Dose administration via indwelling catheter**
 - Single dose of 10 mg of ch81C6
 - Escalating doses of ^{211}At {2 (n=5), 4 (n=6), 6.7 (n=5), and 10 mCi (n=1)}
- **Blood sampling at 1, 2, 4, 8, 12, 18 and 24 hr**
- **SPECT of head and whole body imaging at 2, 4, 8, 18 and 24 hr**

Patient Characteristics

Characteristic	Number (%)
Median Age (years)	50
Range	28-76
Gender	
Male	6 (35%)
Female	11 (65%)
Karnofsky Performance Score	
100	11 (65%)
90	2 (12%)
80	3 (18%)
70	1 (6%)
Histology	
GBM	14 (82%)
AO	3 (18%)

Serial whole-body gamma camera images after injection of ^{211}At -labeled 81C6 in surgical resection cavity

% ID Remaining in Cavity (decay corrected)



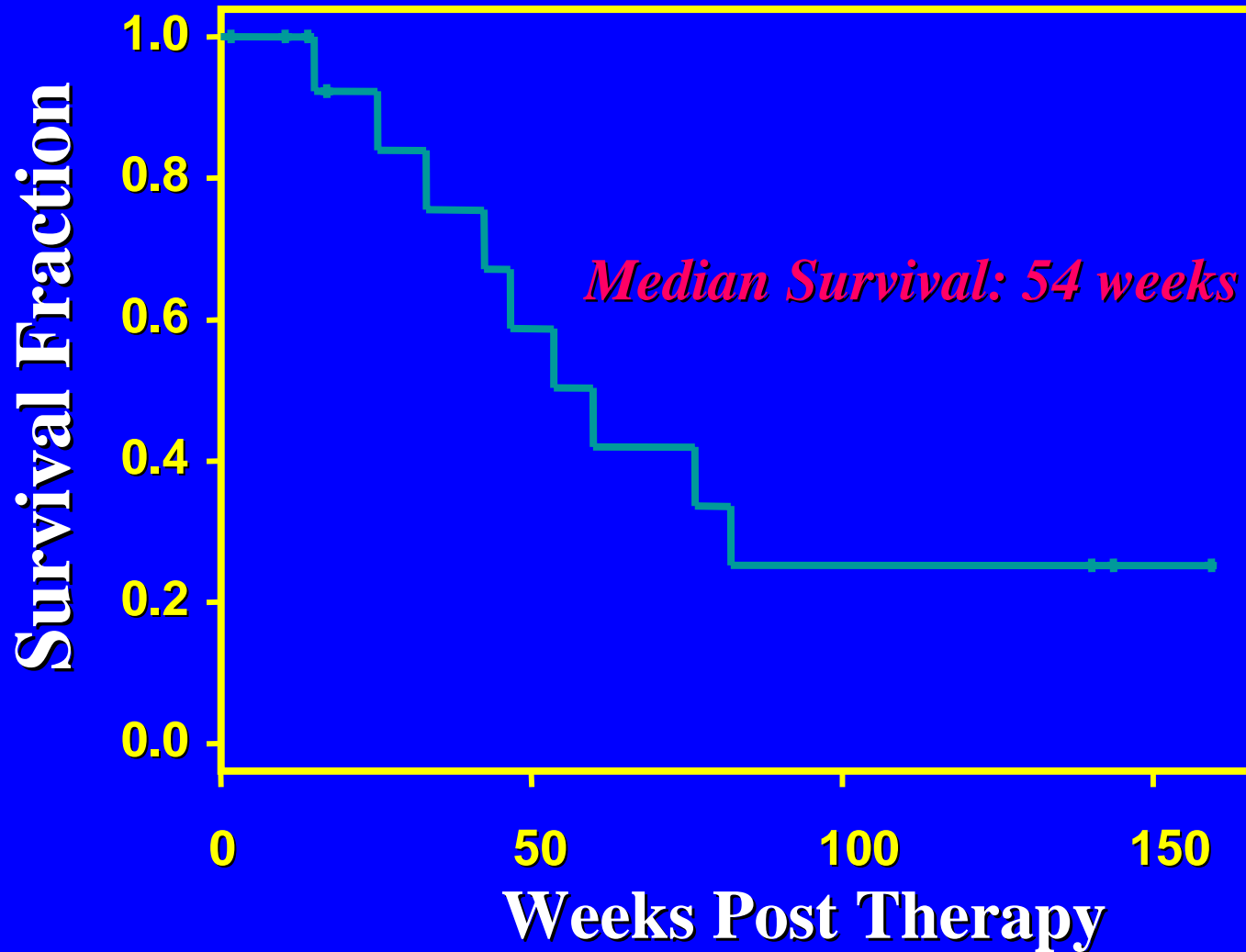
At-211 Activity Distribution in Patients

- Median cavity biological clearance half time: **218 hr**
- Percent decays in cavity: **$99.1 \pm 0.9\%$**
- Blood pool activity (decay corrected):
 - **$0.032 \pm 0.025\%$ ID at 2 hr**
 - **$0.26 \pm 0.43\%$ ID at 24 hr**

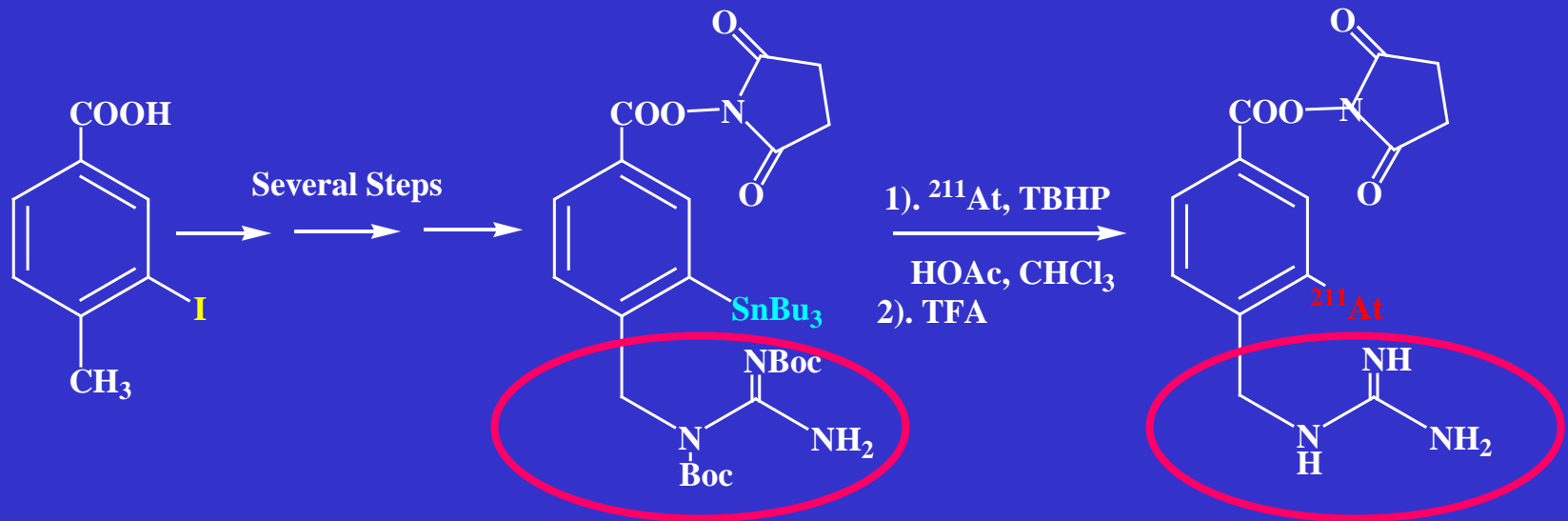
At-211 Chimeric 81C6: Radiation Dosimetry

Tissue	Dose (Gy)
SRC Margin	1041
Bone Marrow	0.040
Brain	0.020
Liver	0.017
Spleen	0.016

Phase I ^{211}At -labeled Chimeric 81C6: Outcome

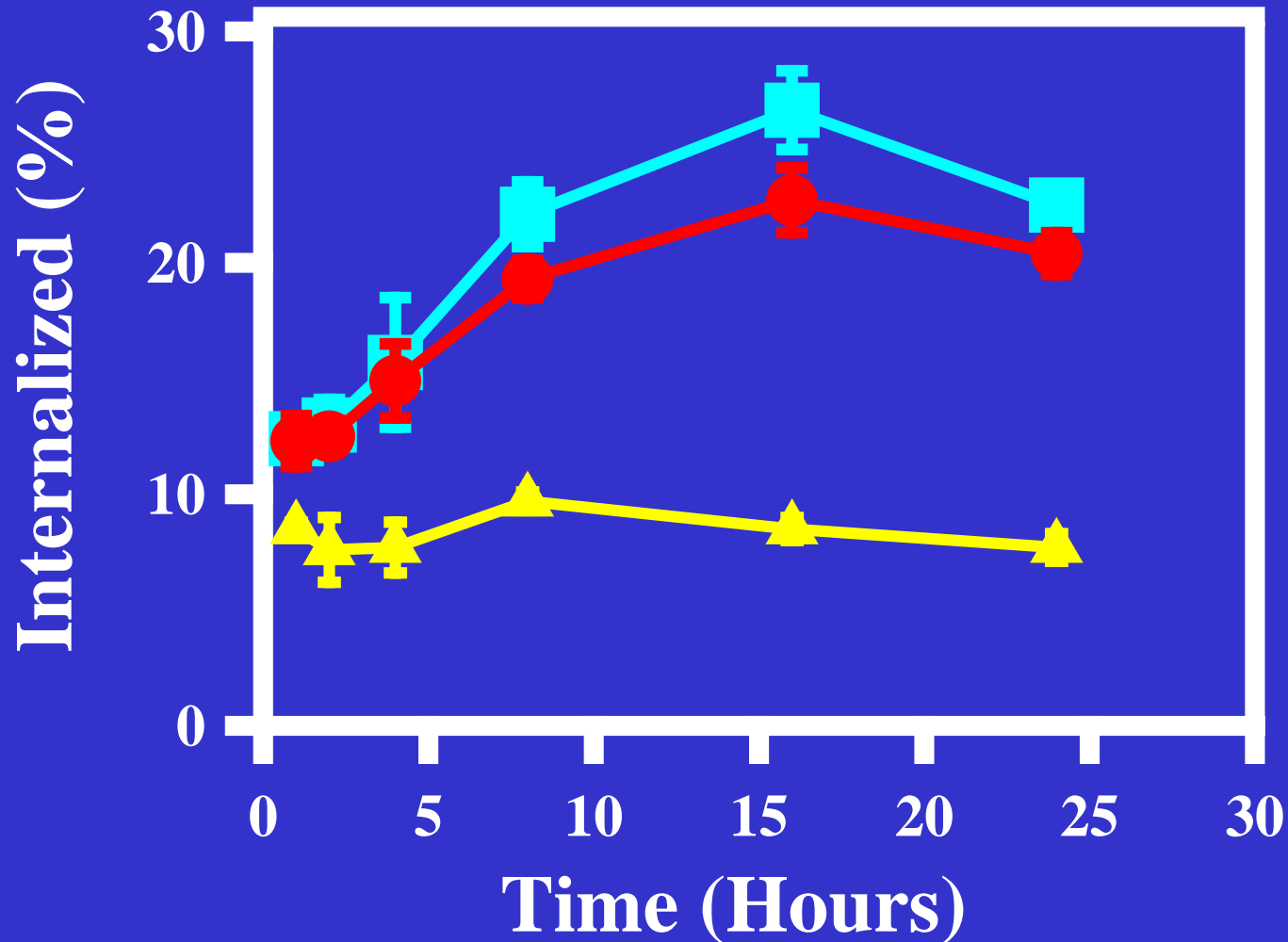


N-Succinimidyl 3-^[211At]Astato-4-Guanidinomethylbenzoate (^[211At]SAGMB)

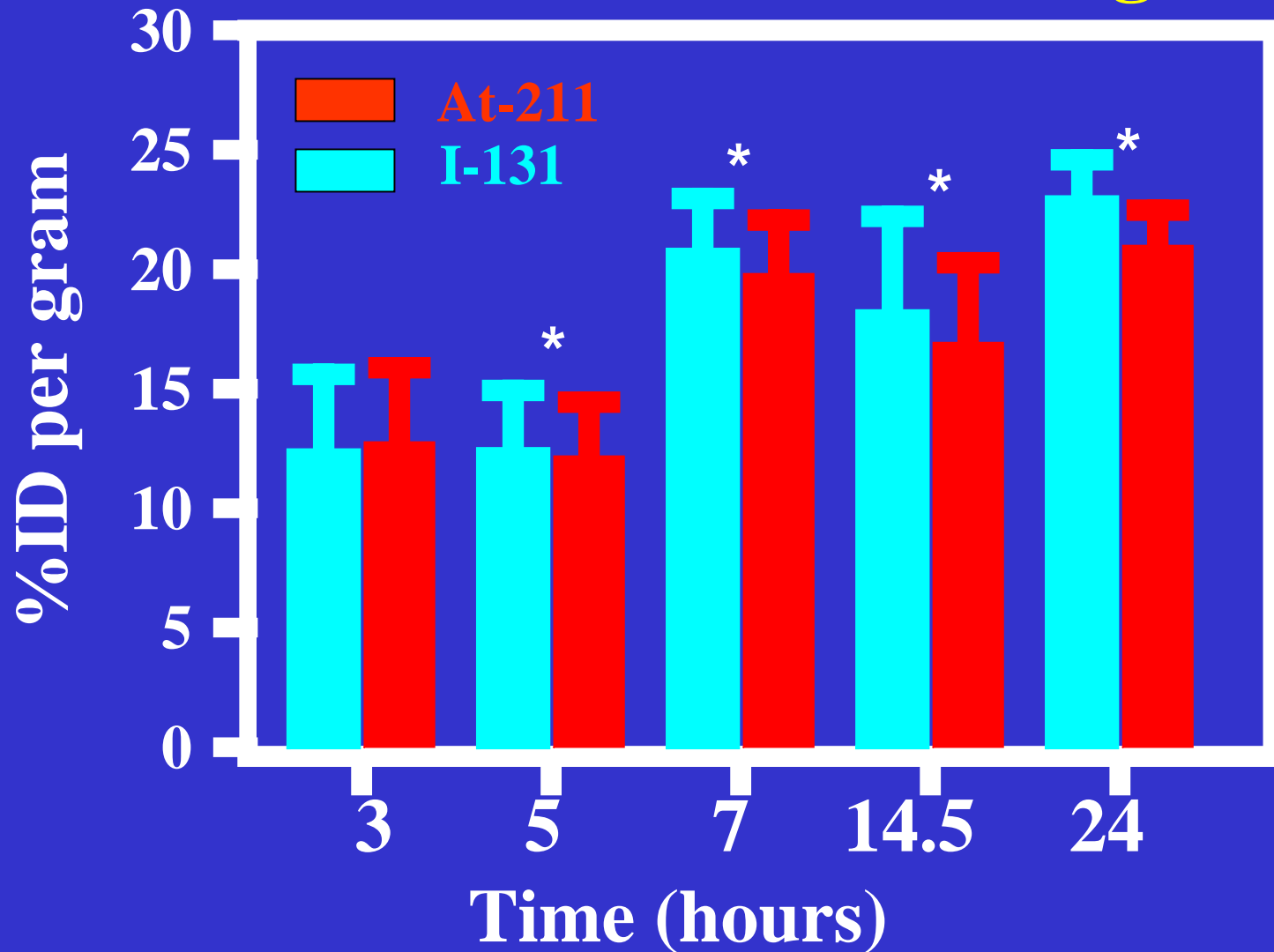


- Radiochemical yield: $61.7 \pm 13.1\%$
- L8A4 (anti-EGFR vIII mAb) conjugation yield: $36.1 \pm 1.9\%$
- Immunoreactive Fraction: $65.2 \pm 1.5\%$

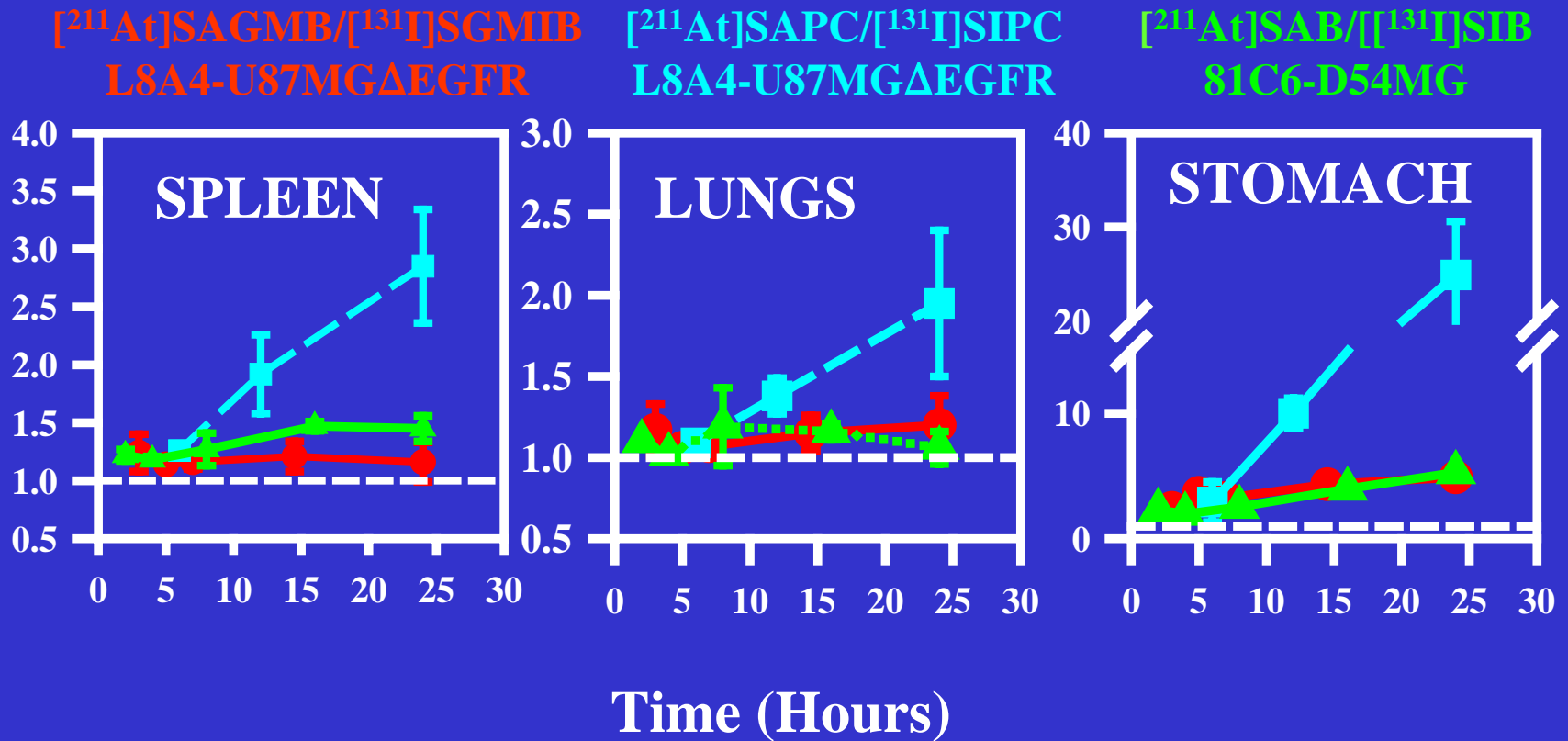
In vitro internalization by U87MGEGFR
Glioblastoma Cells: L8A4- $[^{211}\text{At}]$ SAGMB vs.
 $[^{131}\text{I}]$ L8A4 (Iodogen and $[^{131}\text{I}]$ SGMIB)



Paired-label Tumor Uptake of $[^{131}\text{I}]\text{SGMIB-L8A4}$ and $[^{211}\text{At}]\text{SAGMB-L8A4}$ in U87MG ΔEGFR Xenografts



$^{211}\text{At}/^{131}\text{I}$ Uptake Ratios



Relative $^{211}\text{At}/^{131}\text{I}$ selectivity over 24 h: Lung 5-9; Spleen 5-17; Stomach 1-3

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- **Astatinated octreotide Analogues**

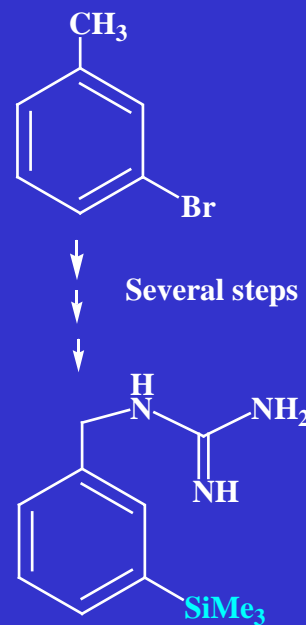
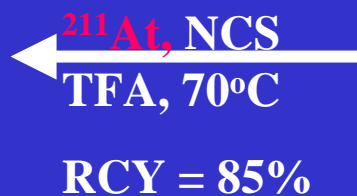
Meta-[²¹¹At]Astatobenzylguanidine ([²¹¹At]MABG)



Meta-Iodobenzylguanidine (MIBG)



Meta-[²¹¹At]Astatobenzylguanidine ([²¹¹At]MABG)

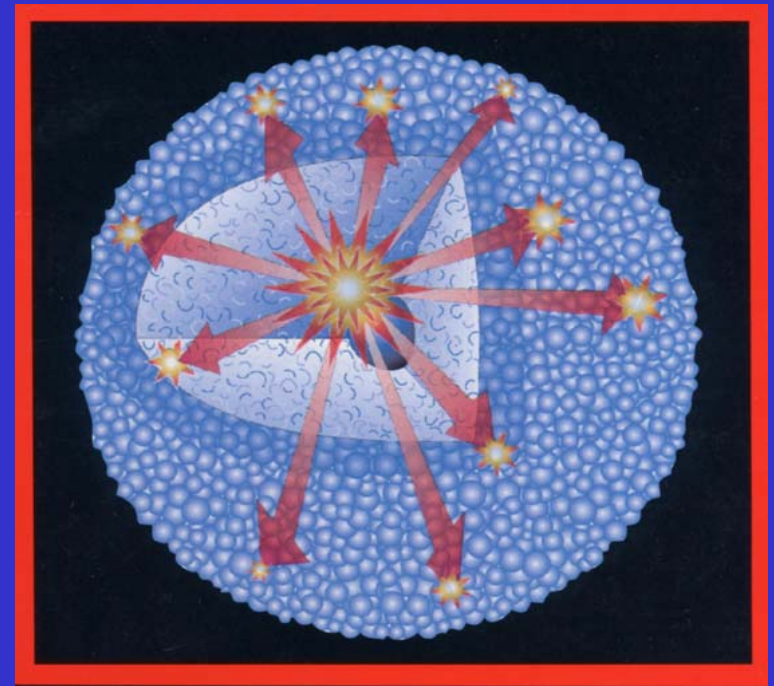


[²¹¹At]MABG

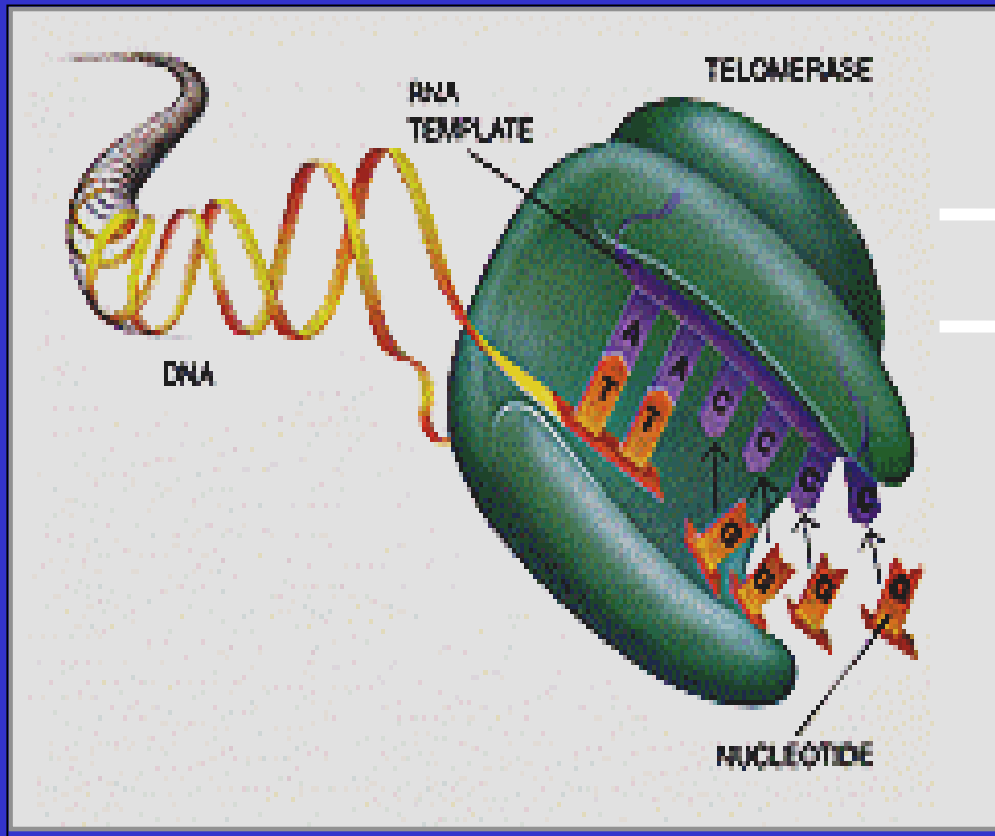
- **Uptake in SK-N-SH neuroblastoma cells *in vitro* and tissue distribution in mice *in vivo* very similar to MIBG**
- **Minimal *in vivo* dehalogenation**
- **Exquisite cytotoxicity (more than 1000-fold compared to n.c.a. [¹³¹I]MIBG and equivalent to only a few ²¹¹At decays per cell in monolayers and multi-cellular spheroids)**

Gene Therapy plus Targeted α -Particle Radiotherapy

- *Hypothesis* – Alpha particle emitting compounds can be targeted to tumors via gene expressed markers, and effectively compensate for the heterogeneous nature of gene therapy via **Bystander Effect**

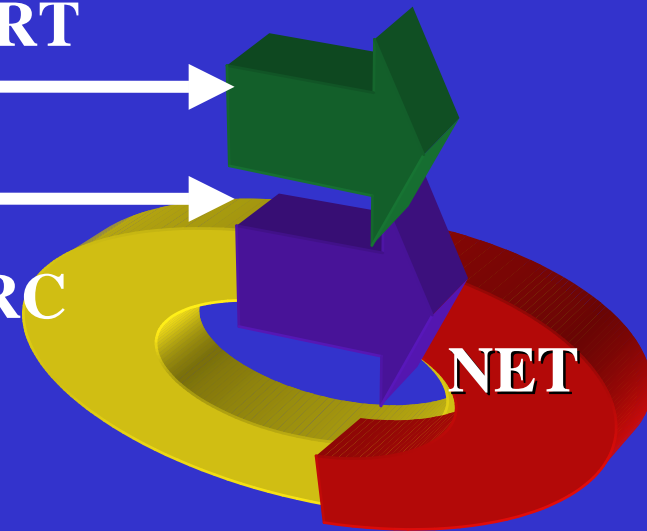


MABG and Gene Therapy

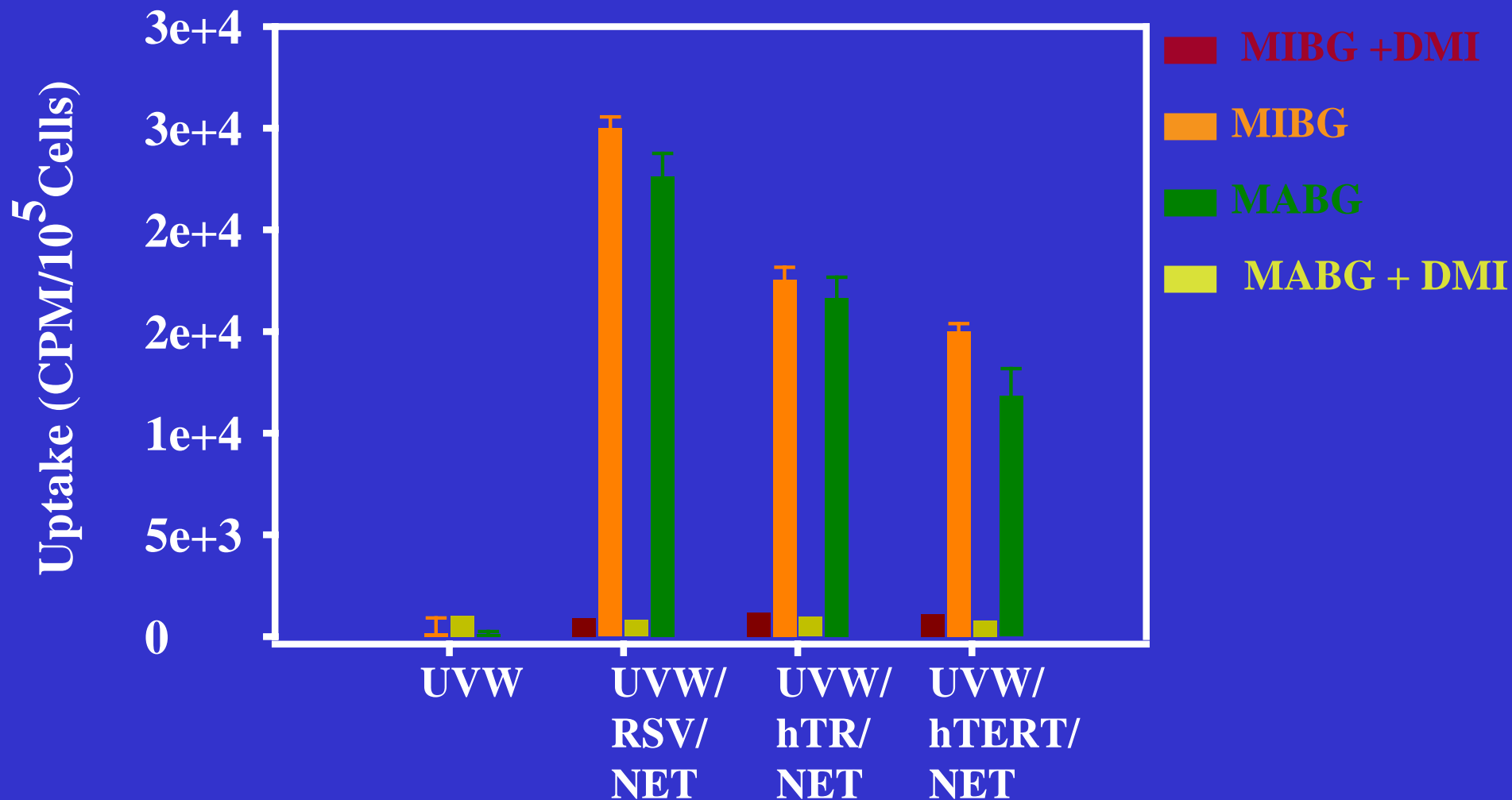


hTERT

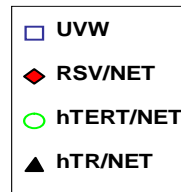
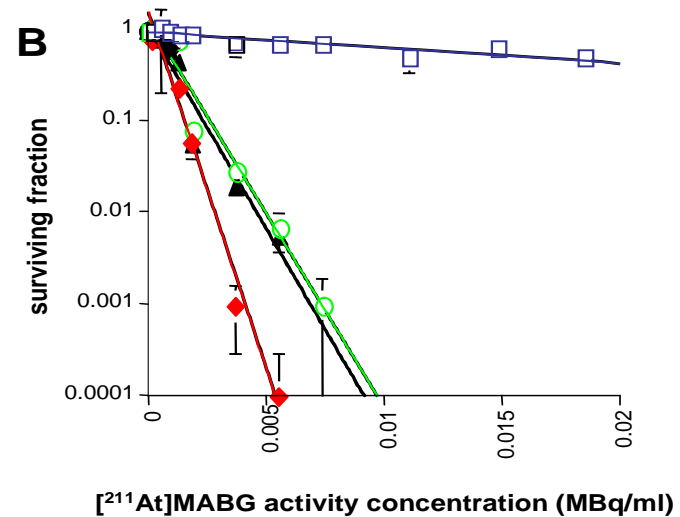
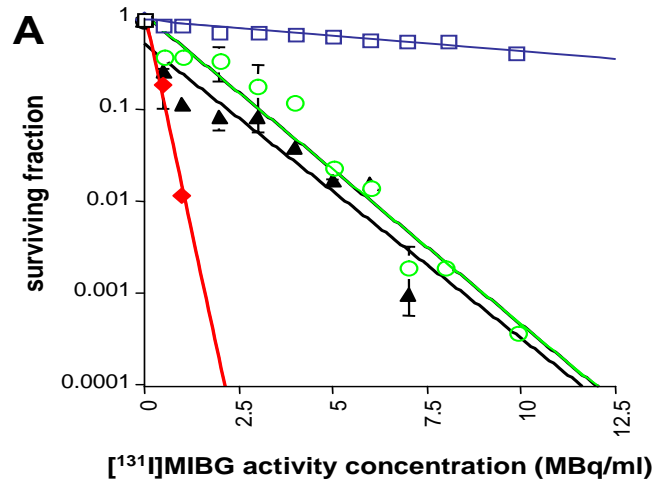
hTERC



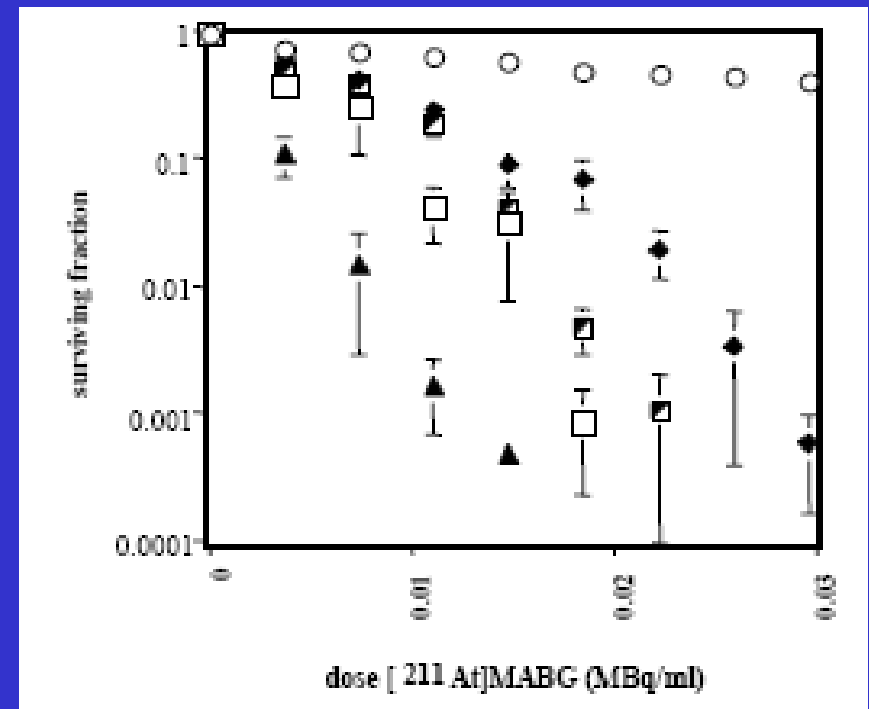
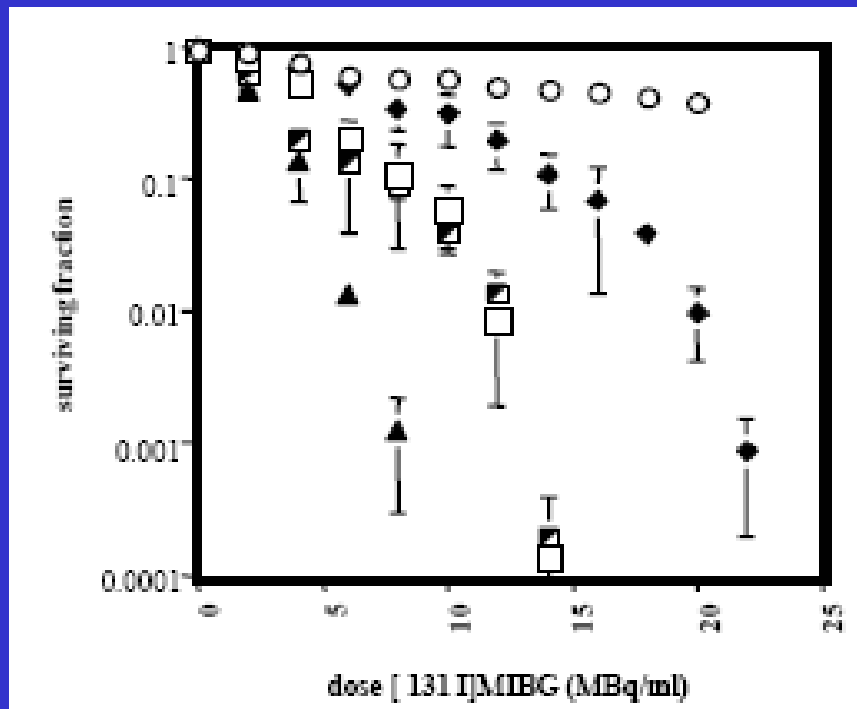
[²¹¹At]MABG and Gene Therapy: Uptake in Glioma cells transfected with NET Gene Under the Control of Various Promoters



[²¹¹At]MABG and Gene Therapy: Clonogenic Survival of UVW Spheroids: 100% Cells Transfected with NET

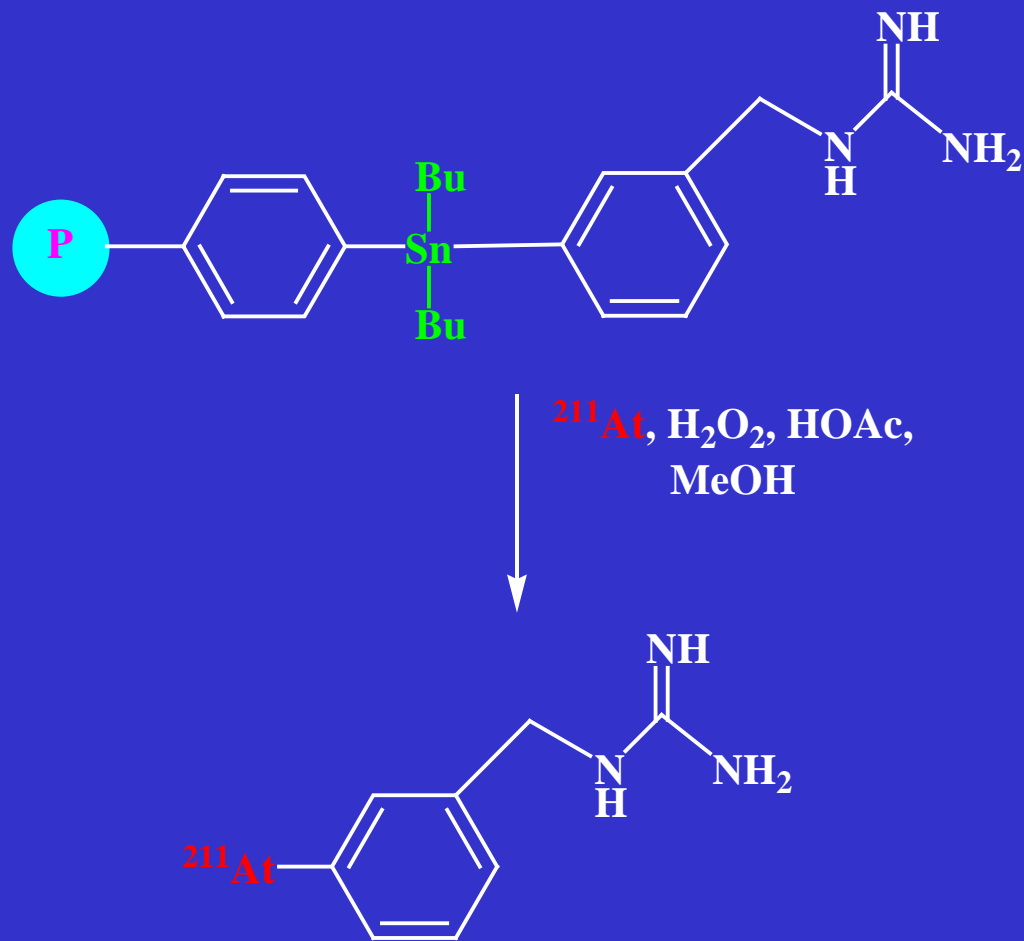


$[^{211}\text{At}]$ MABG and Gene Therapy: Clonogenic Survival of Mosaic Spheroids: Various Percent of Cells Transfected with NET

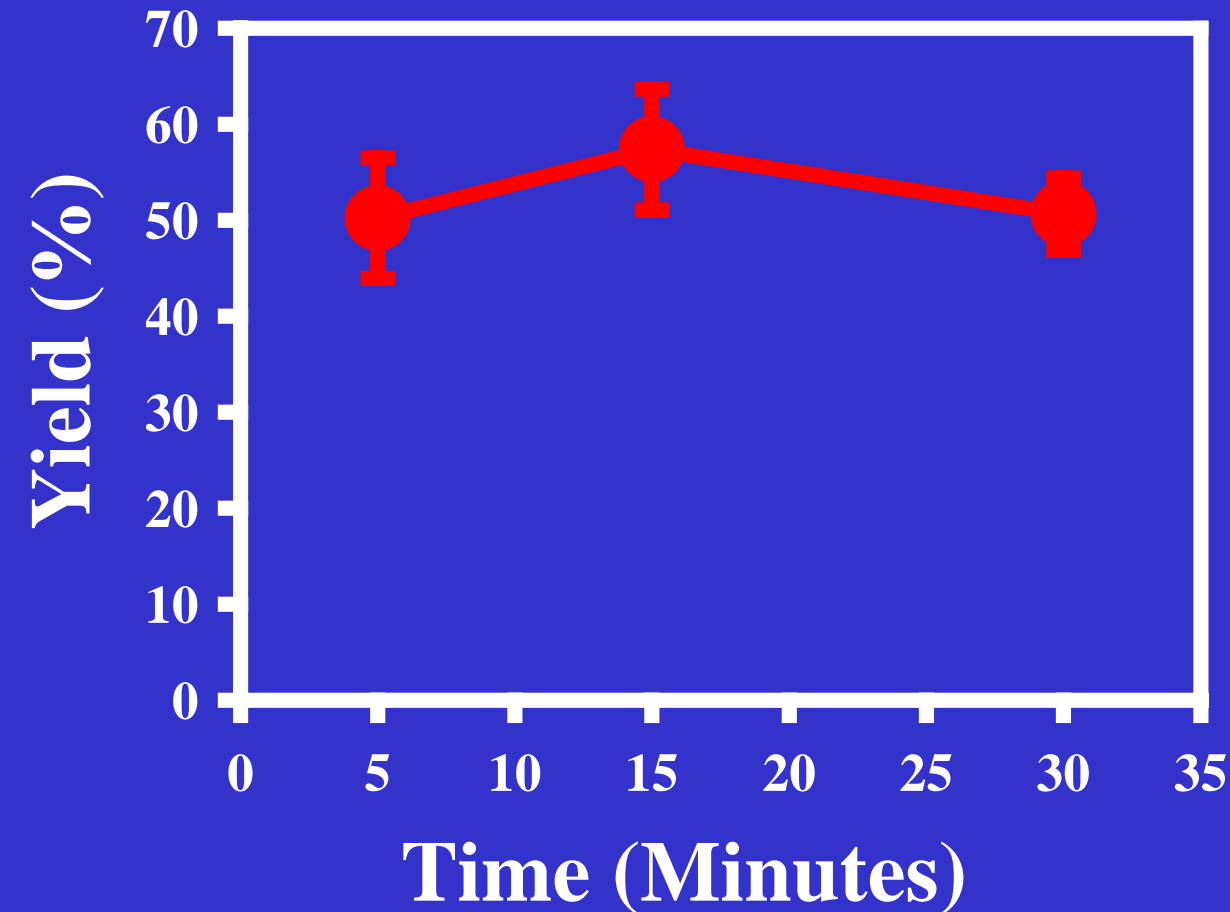


- UVW
- 5% RSV/NAT
- ◆ 5% hTERT/NAT
- ▲ 10% RSV/NAT
- 10% hTERT/NAT

Solid-phase synthesis of [²¹¹At]MABG



[²¹¹At]MABG from Polymer-supported Precursor: Radiochemical yield vs time



Resin: 5 mg

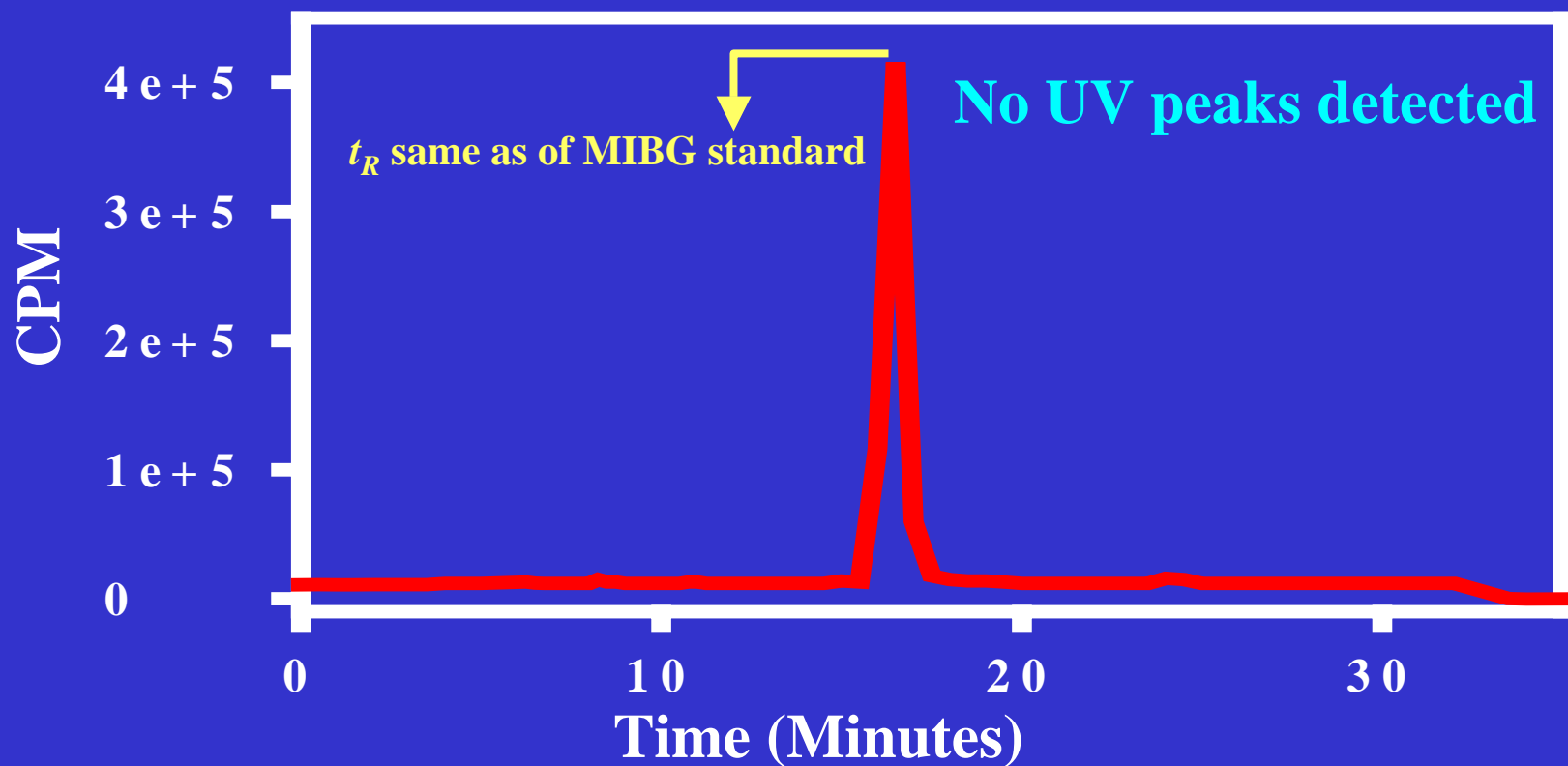
**²¹¹At: 200-300 μCi
in 50 μl MeOH**

**H₂O₂ (30%):HOAc
17:10 (v/v): 10 μl
Room temp.**

[²¹¹At]MABG: Production at Higher Levels by A Kit Method

- **10 mg resin**
- **1.0 – 8.0 mCi or ²¹¹At in ~100 µl MeOH**
- **20 µl of H₂O₂/HOAc mixture**
- **10 min @ RT**
- **Purification by C18 solid-phase extraction**
- **Radiochemical yield: 56.4 ± 9.8% (n = 5)**
- **Maximum [²¹¹At]MABG produced: 5 mCi**

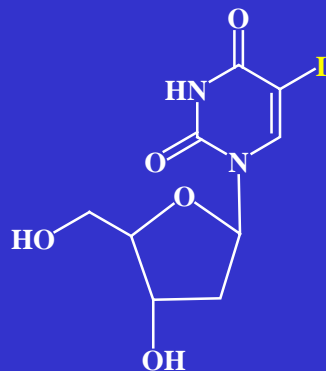
HPLC of [²¹¹At]MABG from Kit Method



**Waters X-Terra RP18 (4.6 × 250 mm; 5 μ) column
0.1% TFA in 20/80 CH₃CN/Water; 1 ml/min**

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- **Astatinated octreotide Analogues**

AUdR



5-Iodo-2'-deoxyuridine (IUdR)



5-[²¹¹At]Atrato-2'-deoxyuridine



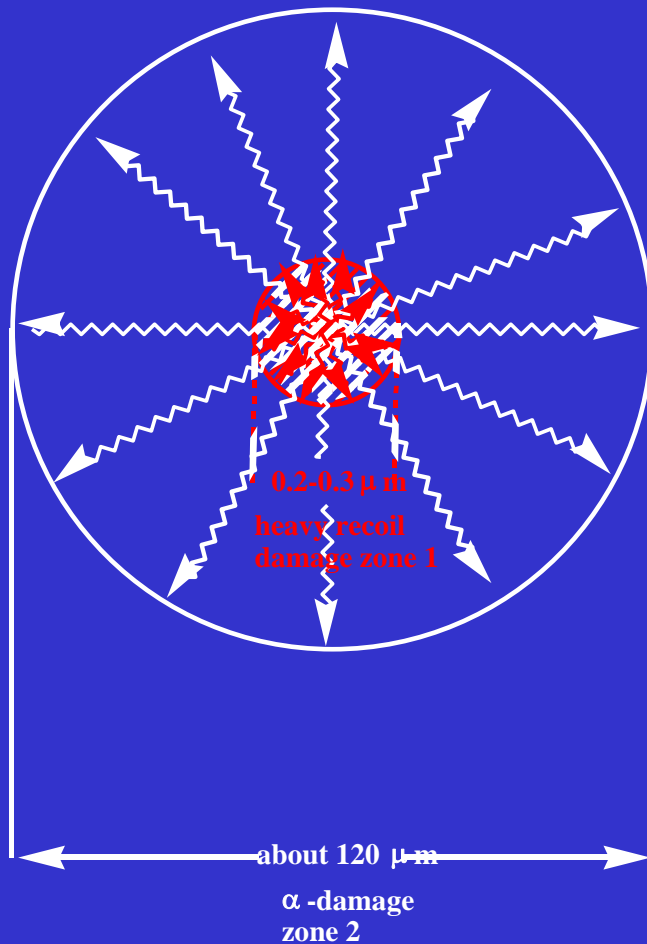
Dioxane



CHCl_3 , Sonication 20 sec

RCY = 85-90%; Max produced: 2.7 mCi

[²¹¹At]AUdR: Rationale

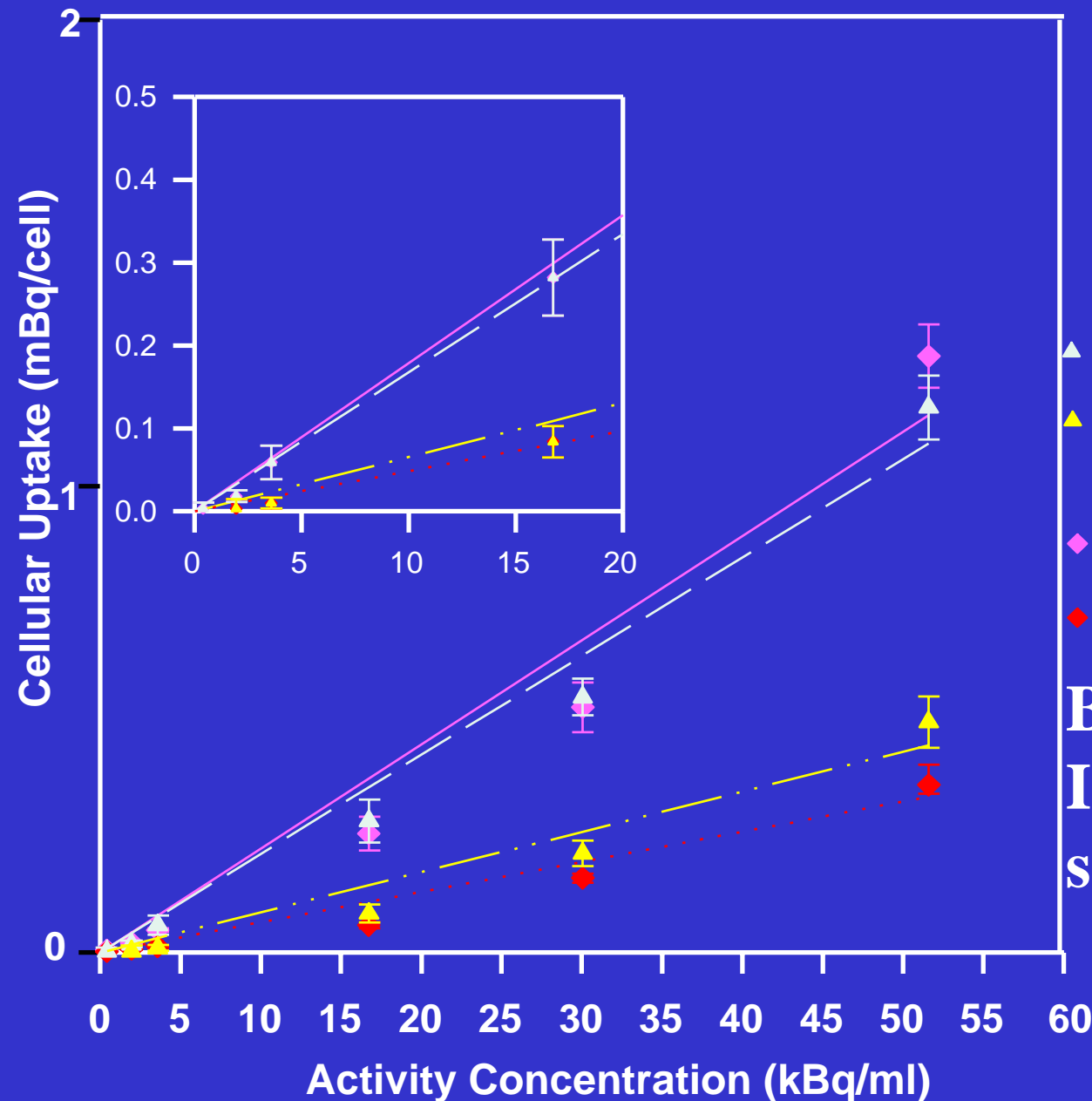


Two potential zones of high-LET kill:

- 1) α-recoil nuclei (range <0.1 μm) for cells in S-phase taking up AUdR
- 2) α-particles (range 55-70 μm) for killing of non S-phase cells by cross fire

Uptake of [^{211}At]AUdR and [^{131}I]IUdR in D-247 MG

human glioma cells



Blocking with excess IUdR demonstrates saturability of uptake

Radiotherapy with [²¹¹At]AUdR in Rat Neoplastic Meningitis Models

- Experiment 1:

D341 Med human medulloblastoma

43 mCi AUdR

45 mCi free [²¹¹At]astatide
or saline

- Experiment 2:

A431p human epidermoid carcinoma

28 mCi AUdR

61 mCi AUdR
or saline

- Experiment 3:

D341 Med human medulloblastoma

3 × 30 mCi AUdR

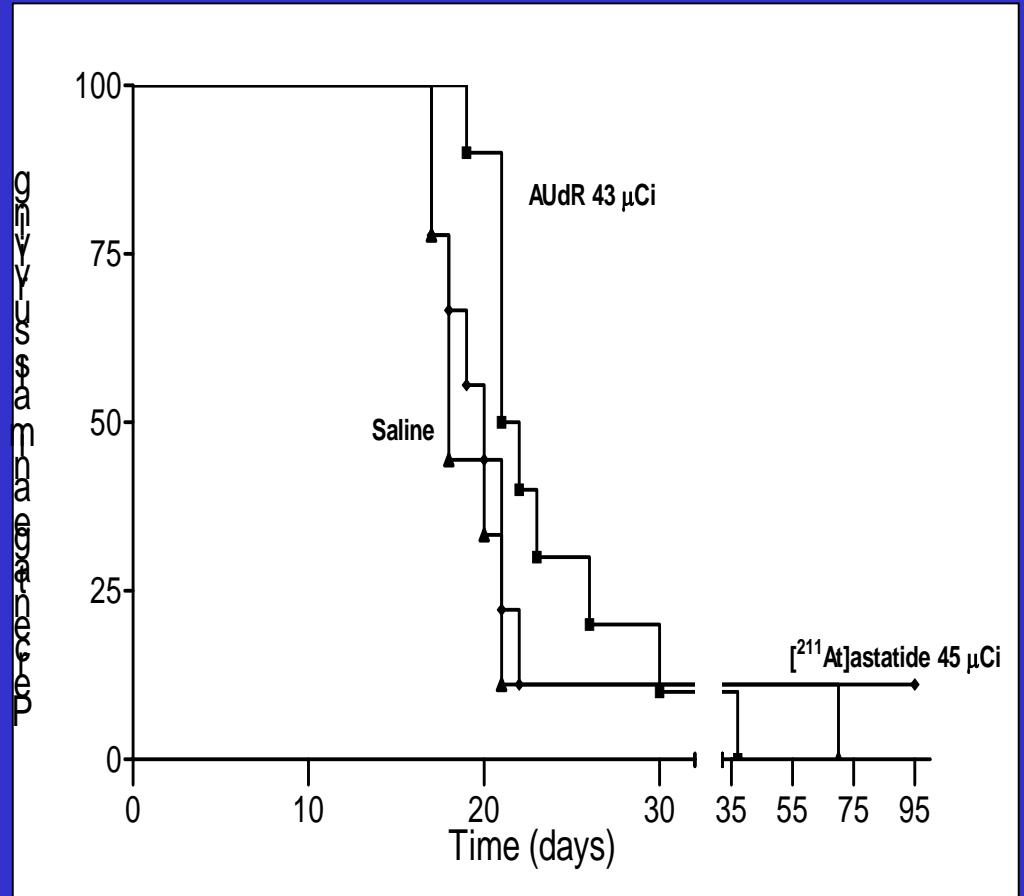
3 × 57 mCi AUdR
or 3 × saline

1 injection daily for 3 days

Results: Experiment 1

Median Survival

saline **18 d**
[²¹¹At]astatide **20 d**
(p=0.56)
AUdR **21.5 d**
(p=0.02)
(p=0.07)



Results: Experiment 2

Median Survival

saline 10 d

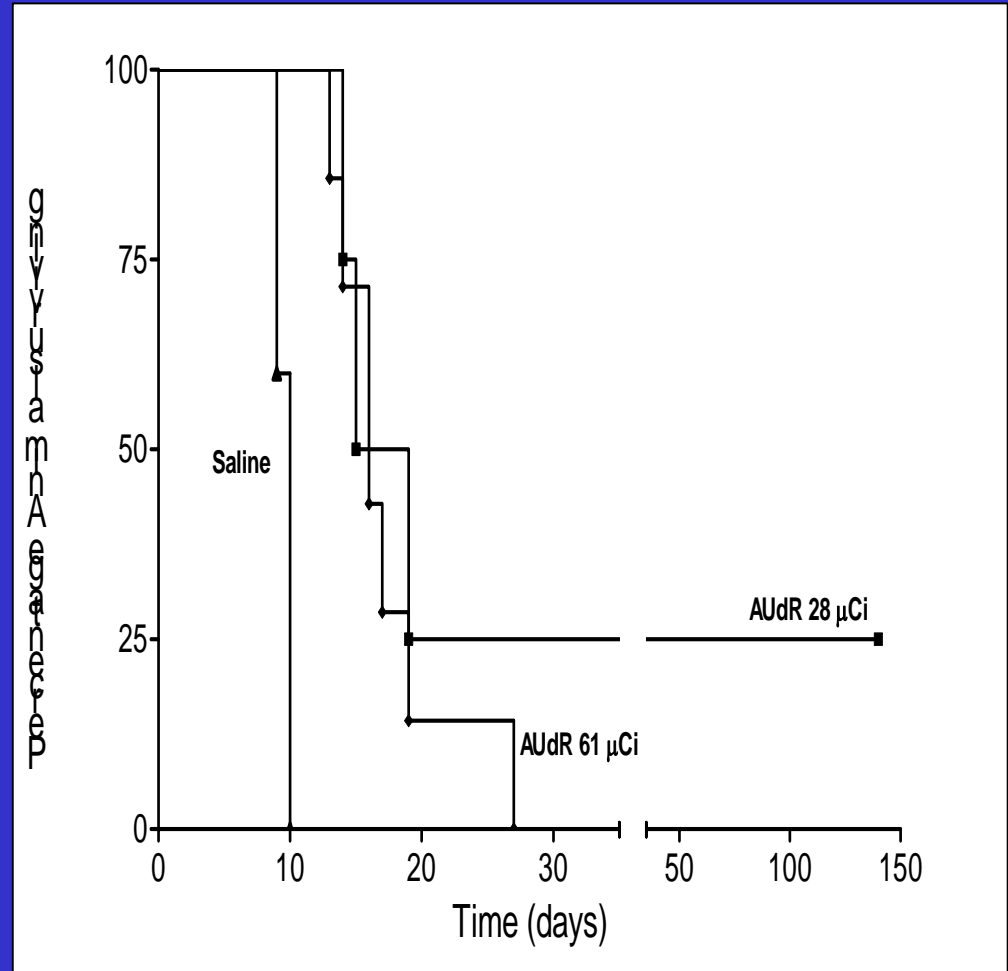
AUdR (28 mCi) 17 d

($p=0.01$)

AUdR (61 mCi) 16 d

($p=0.004$)

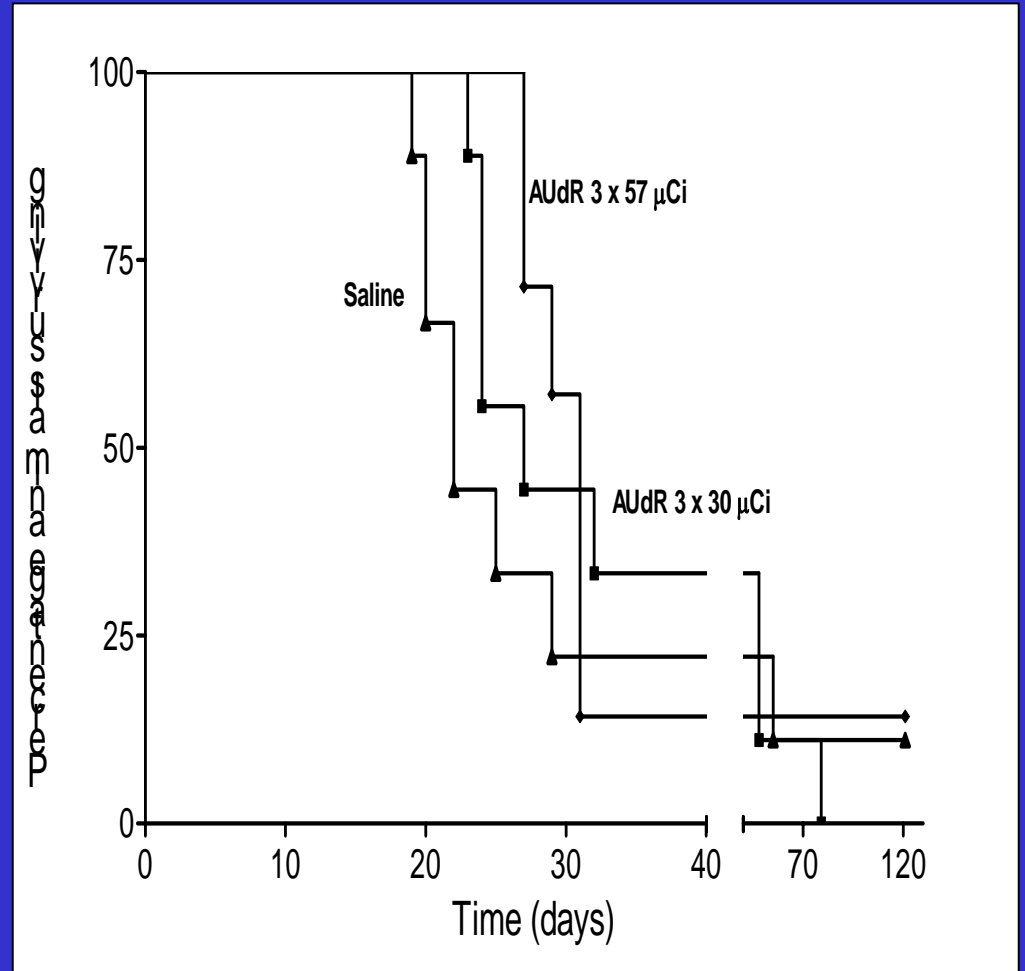
($p=0.7$)



Results: Experiment 3

Median Survival

saline (3×) 22 d
AUdR (3× 30 mCi) 27 d
(p=0.02)
AUdR (3× 57 mCi) 31 d
(p=0.08)
(p=0.49)

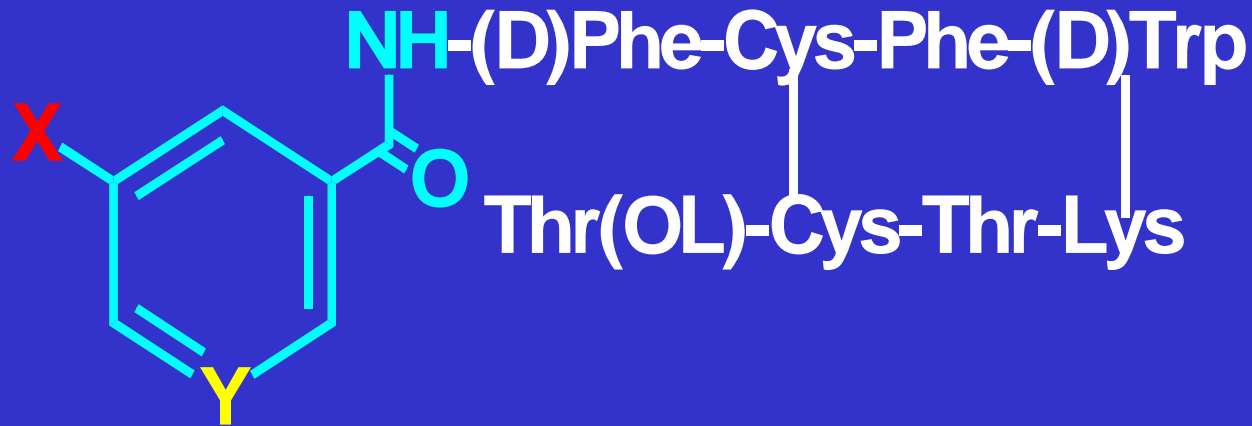


AUdR-Summary

- **Intrathecal administration of [²¹¹At]AUdR significantly improved the median survival of athymic rats with medulloblastoma neoplastic meningitis**
- **The therapeutic efficacy of [²¹¹At]AUdR was specific and could be increased by multiple dose protocols**
- **There was no evidence of toxicity either in intact animals or on histopathological analysis of the neuroaxis**

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STRUCTURES OF IBO, INO AND ABO



IODOBENZOYLOCTREOTIDE (**IBO**): X = I, Y = CH

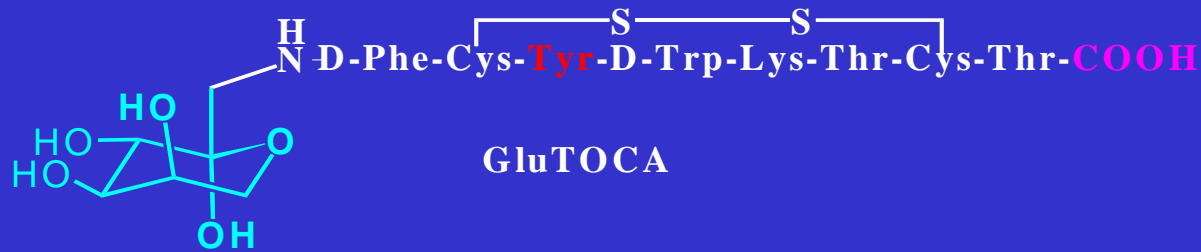
IODONICOTINYLOCTREOTIDE (**INO**): X = I, Y = N

ASTATOBENZOYLOCTREOTIDE (**ABO**): X = ^{211}At , Y = CH

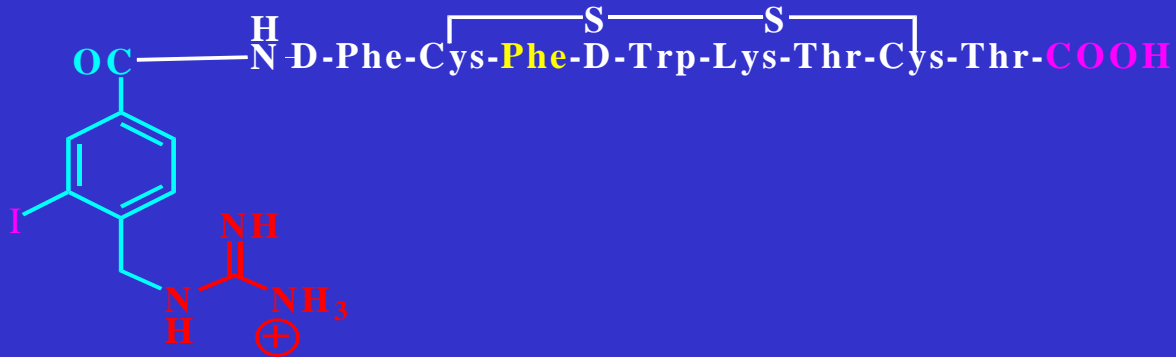
Structures of Octreotide, Glu-TOCA and GMIBO



Octreotide

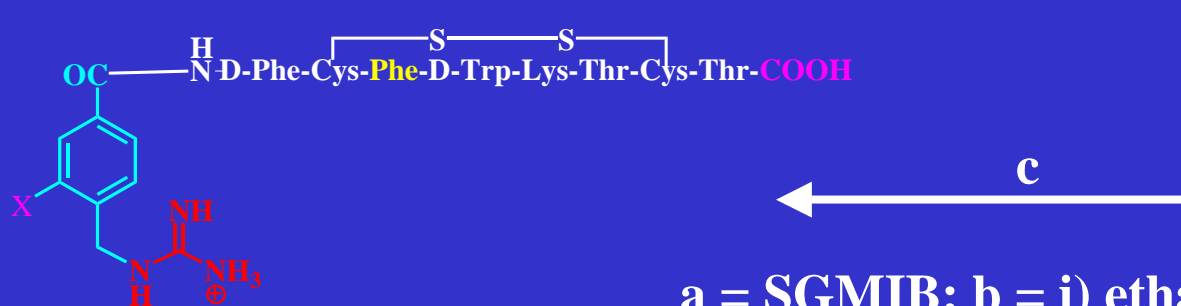
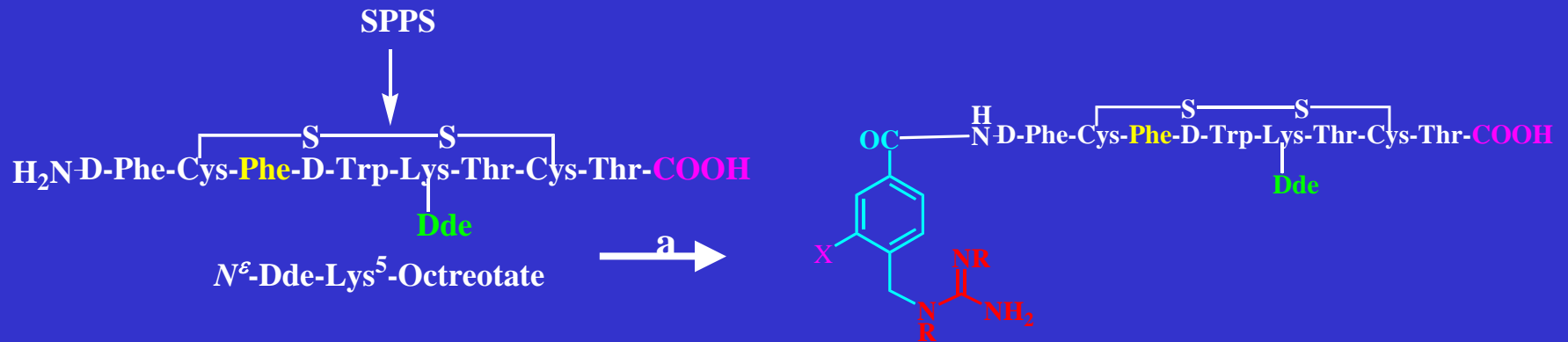


GluTOCA



N-(4-Guanidinomethyl-3-iodobenzoyl)Phe¹-Octreotate
(GMIBO)

Synthesis of the Tin Precursor (Boc-GMTMSBO), [^{*}I]GMIBO and [²¹¹At]AGMBO



[^{*}I]GMIBO; X = ^{*}I (30-35%)

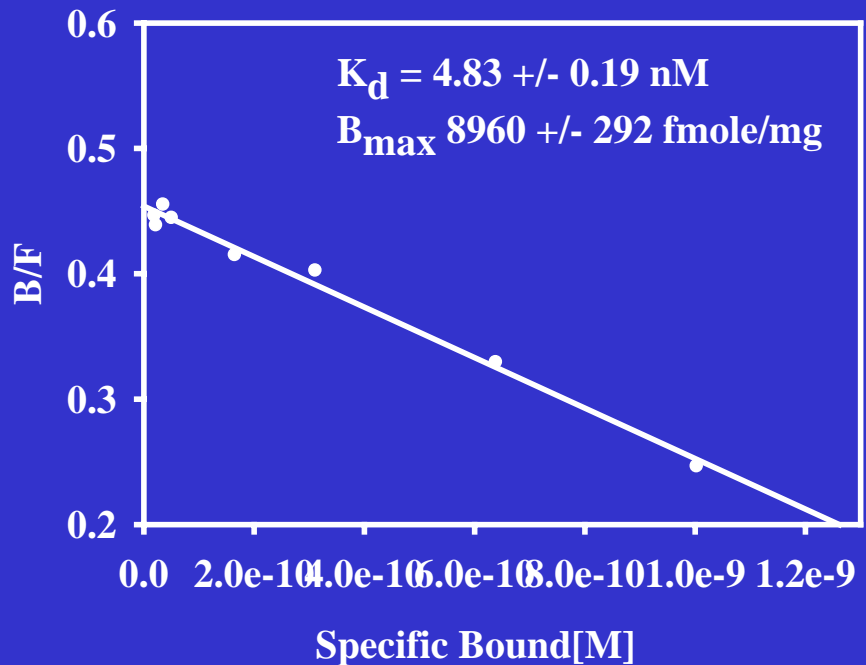
[²¹¹At]AGMBO; X = ²¹¹At (15-20%)

a = SGMIB; b = i) ethanolamine ii) TFA;

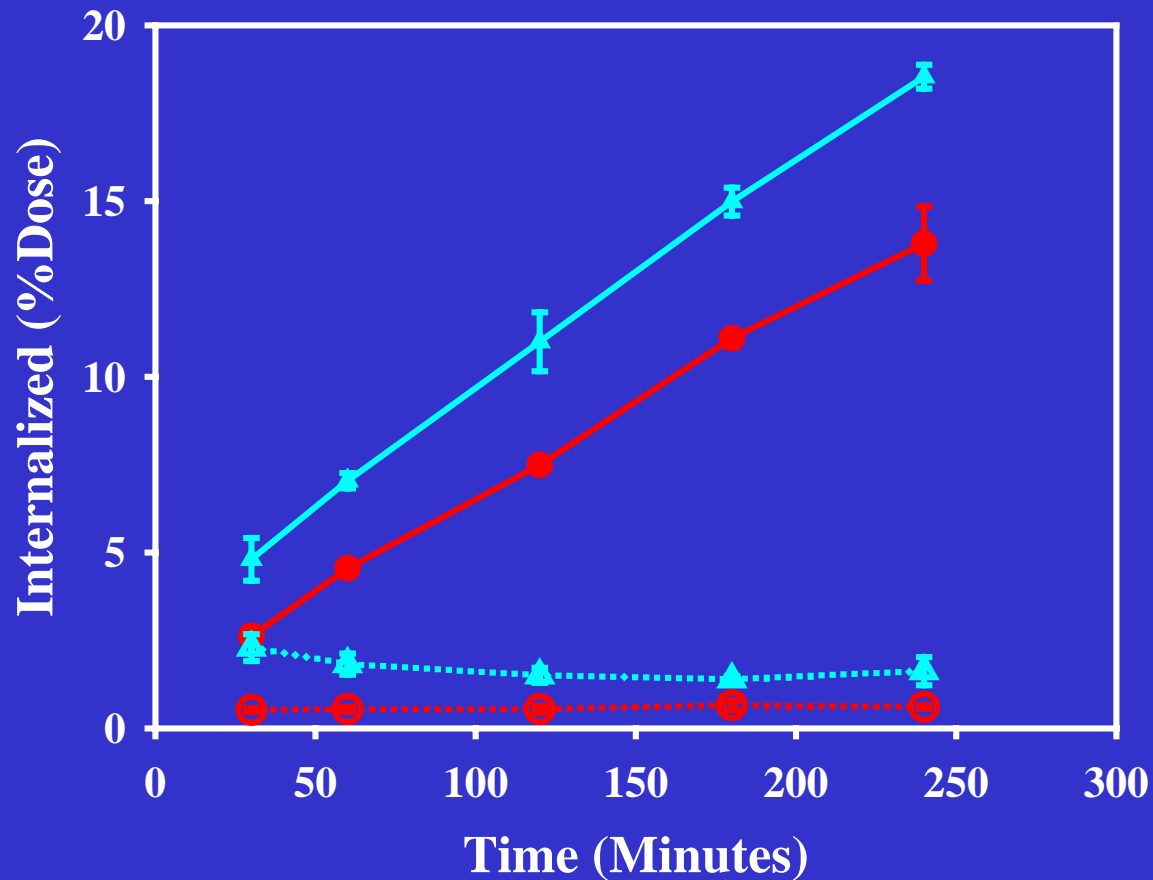
c = i) ethanolamine ii) radioiodine or ²¹¹At, TBHP, HOAc, 70°C

Receptor-binding Assay

The affinity of GMIBO was determined using the SSTR-expressing AR42 J rat pancreatic carcinoma cell membranes via a cold-saturation assay. The Scatchard analysis of the data yielded a K_d value of 4.8 nM

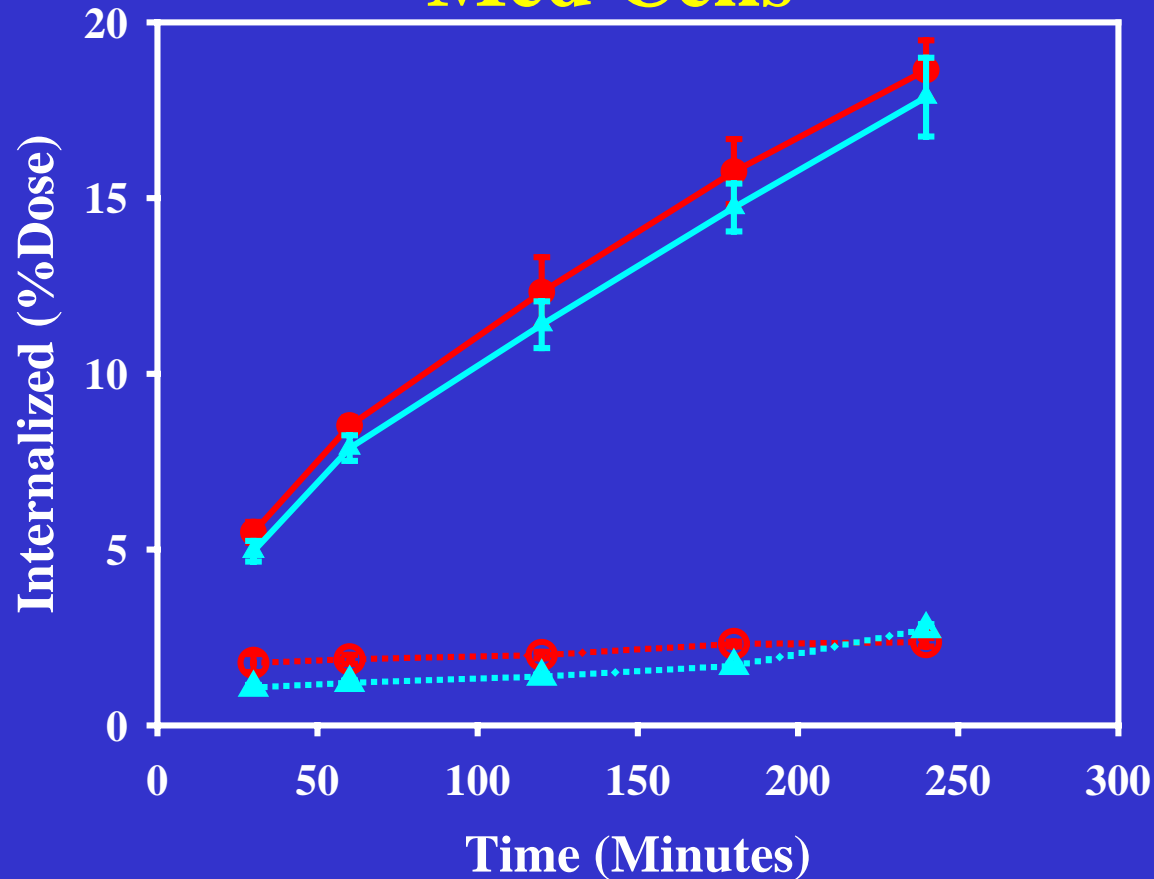


Paired-label Internalization of [¹³¹I]Glu-TOCA and [¹²⁵I]GMIBO by D341 Med Cells



▲ — [¹²⁵I]GMIBO △ ... [¹²⁵I]GMIBO + Octreotide
● — [¹³¹I]Glu-TOCA ○ ... [¹³¹I]Glu-TOCA + Octreotide

Figure 5. Paired-label Internalization of $[^{131}\text{I}]\text{GMIBO}$ and $[^{211}\text{At}]\text{AGMBO}$ by D341 Med Cells

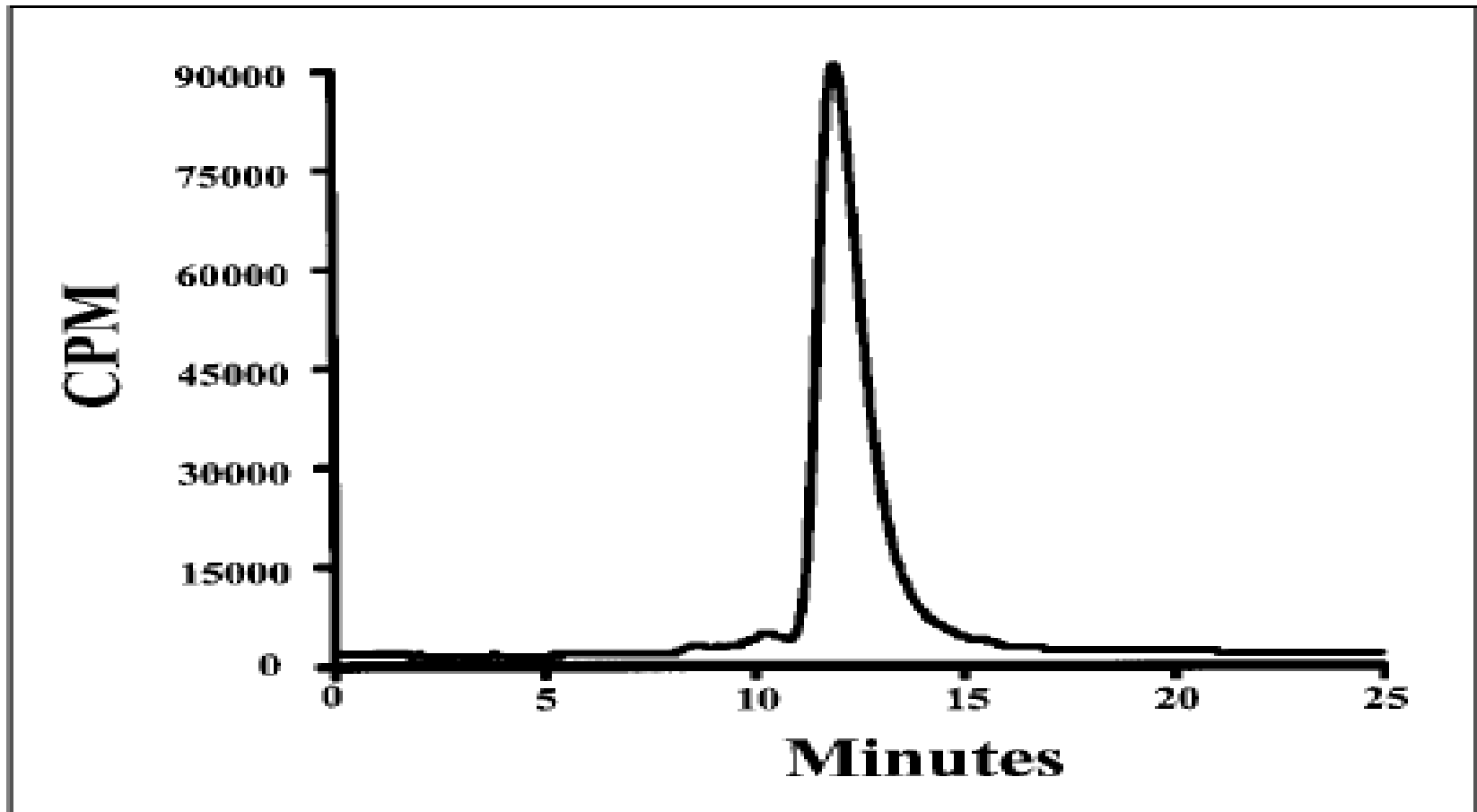


▲ — $[^{211}\text{At}]\text{AGMBO}$ △ ... $[^{211}\text{At}]\text{AGMBO} + \text{Octreotide}$
● — $[^{131}\text{I}]\text{GMIBO}$ ○ ... $[^{131}\text{I}]\text{GMIBO} + \text{Octreotide}$

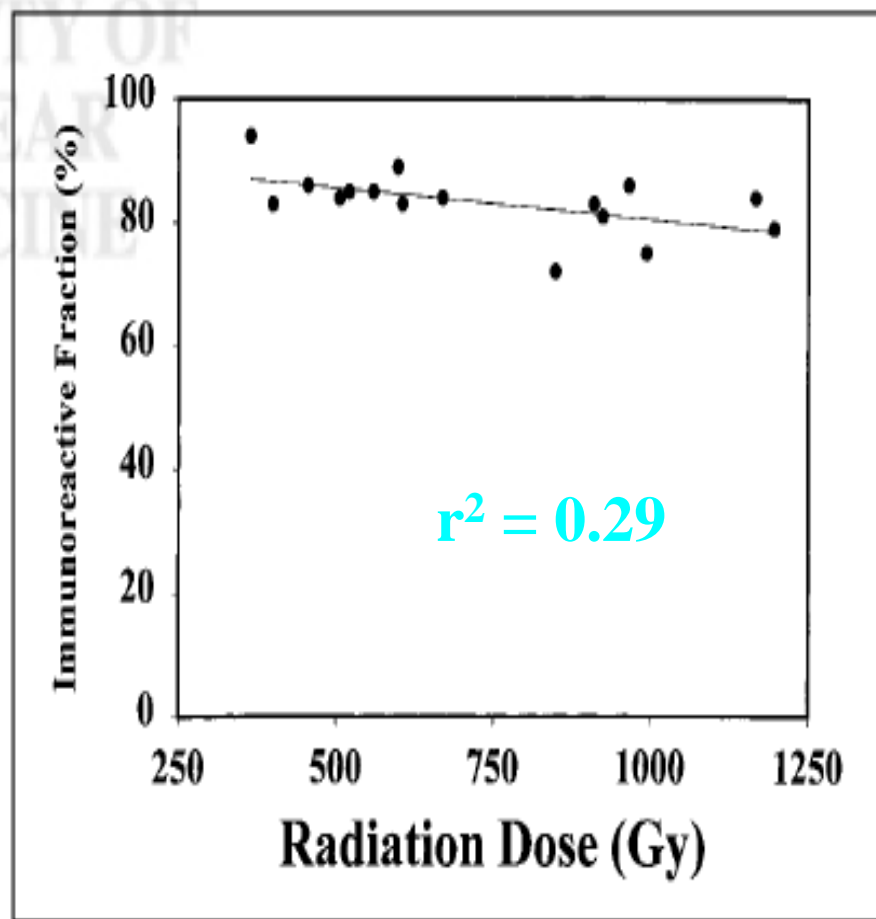
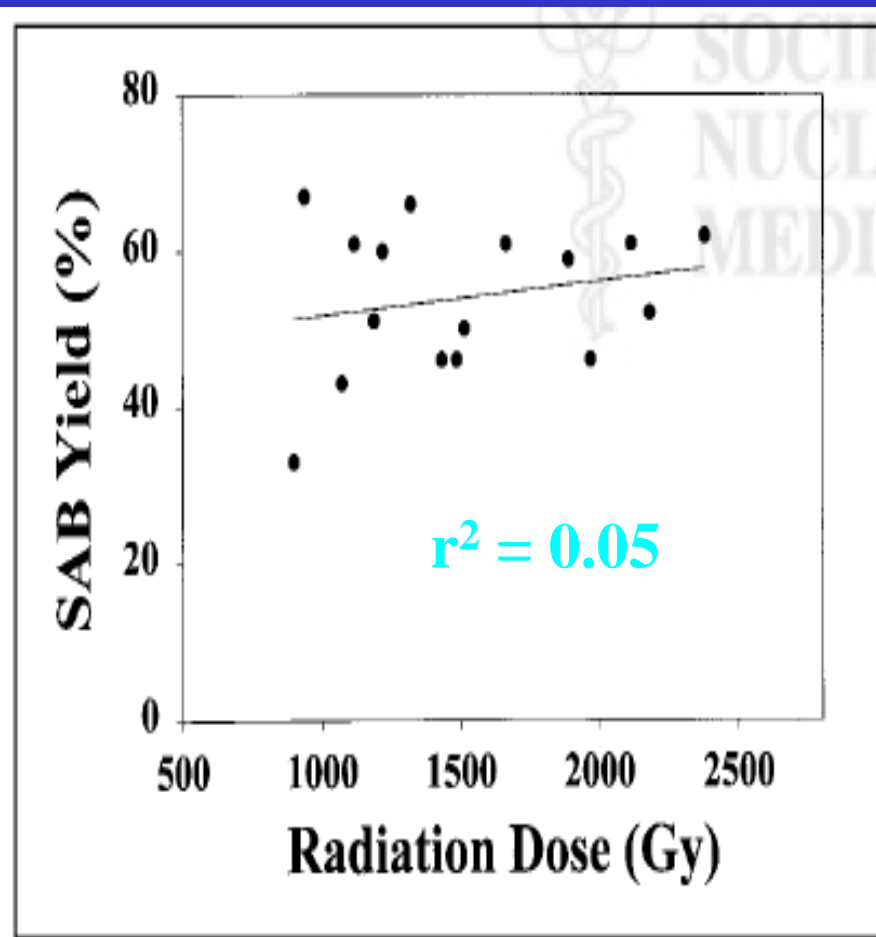
Summary

- Astatine-211 in quantities sufficient for the synthesis of clinical batches of astatinated radiopharmaceuticals can be produced
- Synthesis of a number of astatinated radiopharmaceuticals, some in quantities sufficient for clinical trials have been achieved
- Radiolysis may be the biggest issue; split and pool approach may be necessary

Typical Size-exclusion HPLC of ^{211}At -ch81C6



SAB Yield And Immunoreactive Fraction Of Labeled ch81C6 As A Function Of Radiation Dose



At-211 Production If Clinical Rationale

- **200 μA internal current**
- **90% distillation efficiency**
- **4 h decay during transportation**

720 mCi vs. 160 mCi

At-211 Therapy: Future?

- Assume: 720 mCi/run

5 runs/wk \times 50 wk/yr

50% radiochemical yield

5-20 mCi/patient dose

One Cyclotron yields 4,500 to 18,000 patient
doses per year

Ovarian 22,000 cases/yr

Brain 18,000 cases/yr

Astatine-211 Needs

- **Research**
 - 10 centers
 - 10 mCi/center/wk
 - **Phase I/II clinical:**
 - 25 centers
 - 1 patient/wk
 - 10 mCi/dose
 - **Clinical:**
 - 100 centers
 - 3 patients/wk
 - 10 mCi/dose
- **100 mCi**
 - **1 cyclotron × 1 run**
 - **250 mCi**
 - **1 cyclotron × 2 runs**
 - **3,000 mCi**
 - **6 cyclotrons × 3 runs**

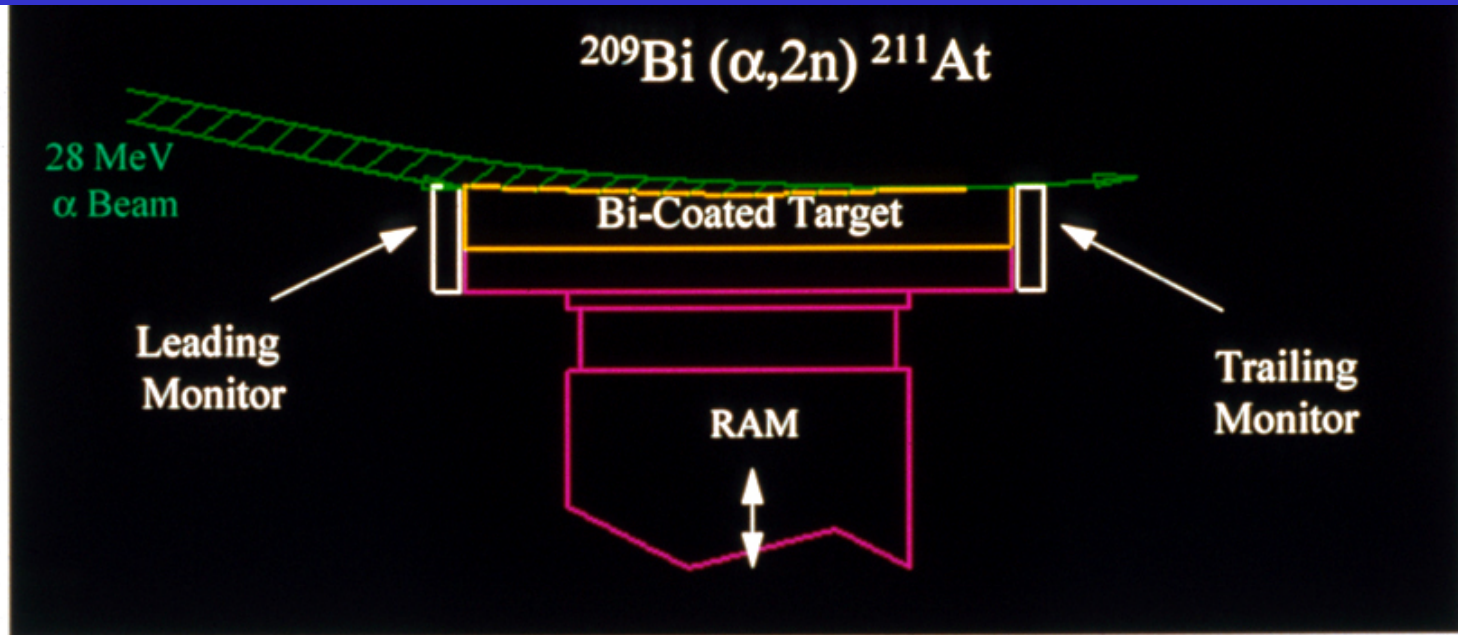
Astatine-211 Rationale

- 7.2 h Half life
- α -emission with each decay
- Po K x-rays for imaging
- Half life compatible with variety of carriers
- Chemistry amenable to biomolecules
- Minimal problem with transformation during decay

Increasing ^{211}At Production

- Design of internal target system
- Development of bismuth electroplating method
- Design of induction heating system
- Design of specialized cyclotron for ^{211}At production

At-211 Internal Target

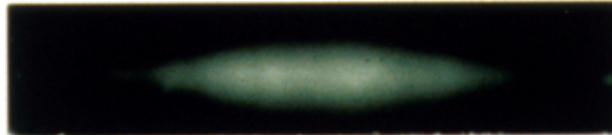


306 mg Bi - JVD
32 in radius



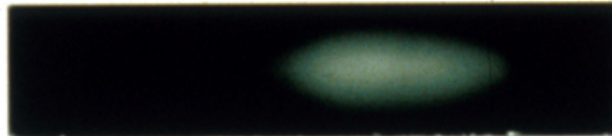
2.5 / 36 / 0.1 μA
45 μA , 90 min

380 mg Bi - Hand
32 in radius



3.0 / 40 / 0.1 μA
50 μA , 90 min

551 mg Bi - Hand
Flat



? / 42 / ? μA
50 μA , 60 min