Astatine-211and Astatinated Radiopharmaceuticals

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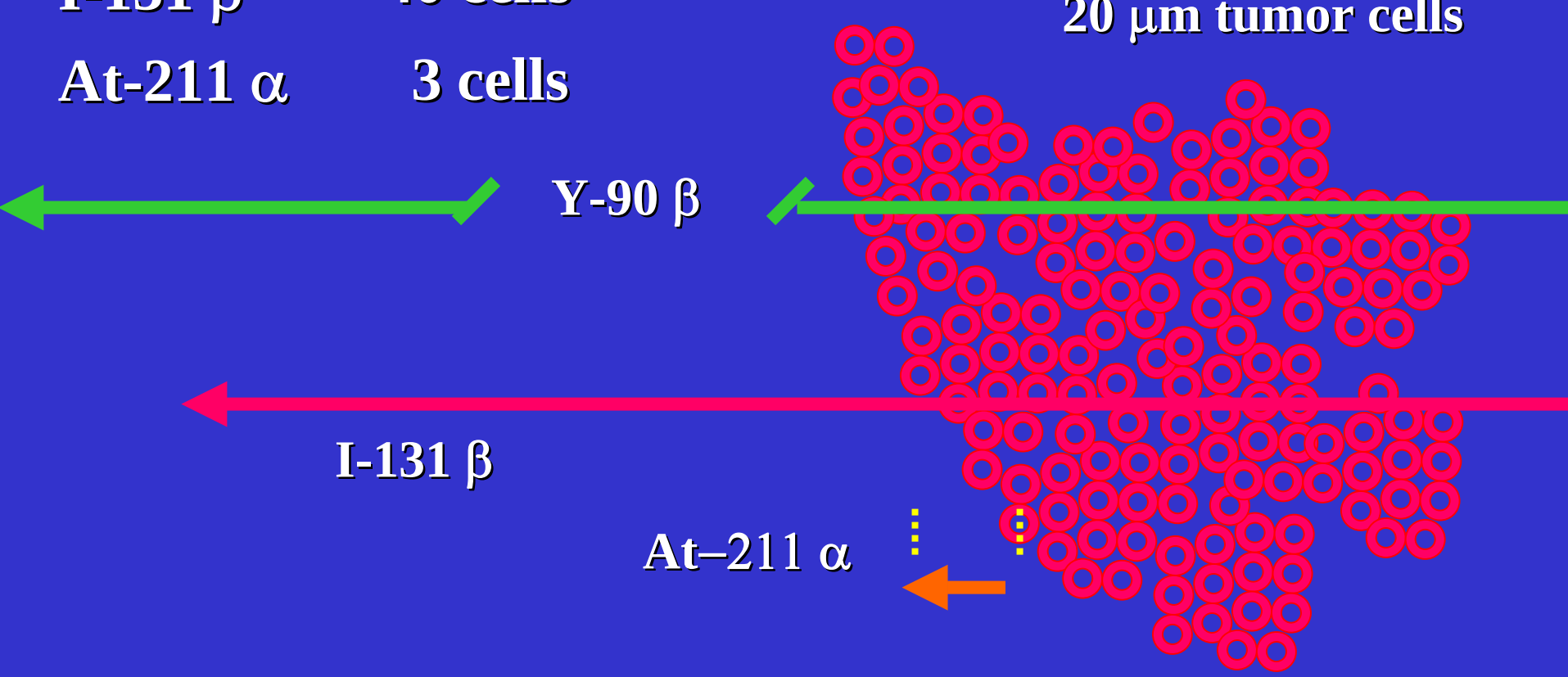
Range of α- and β-Particles

	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**

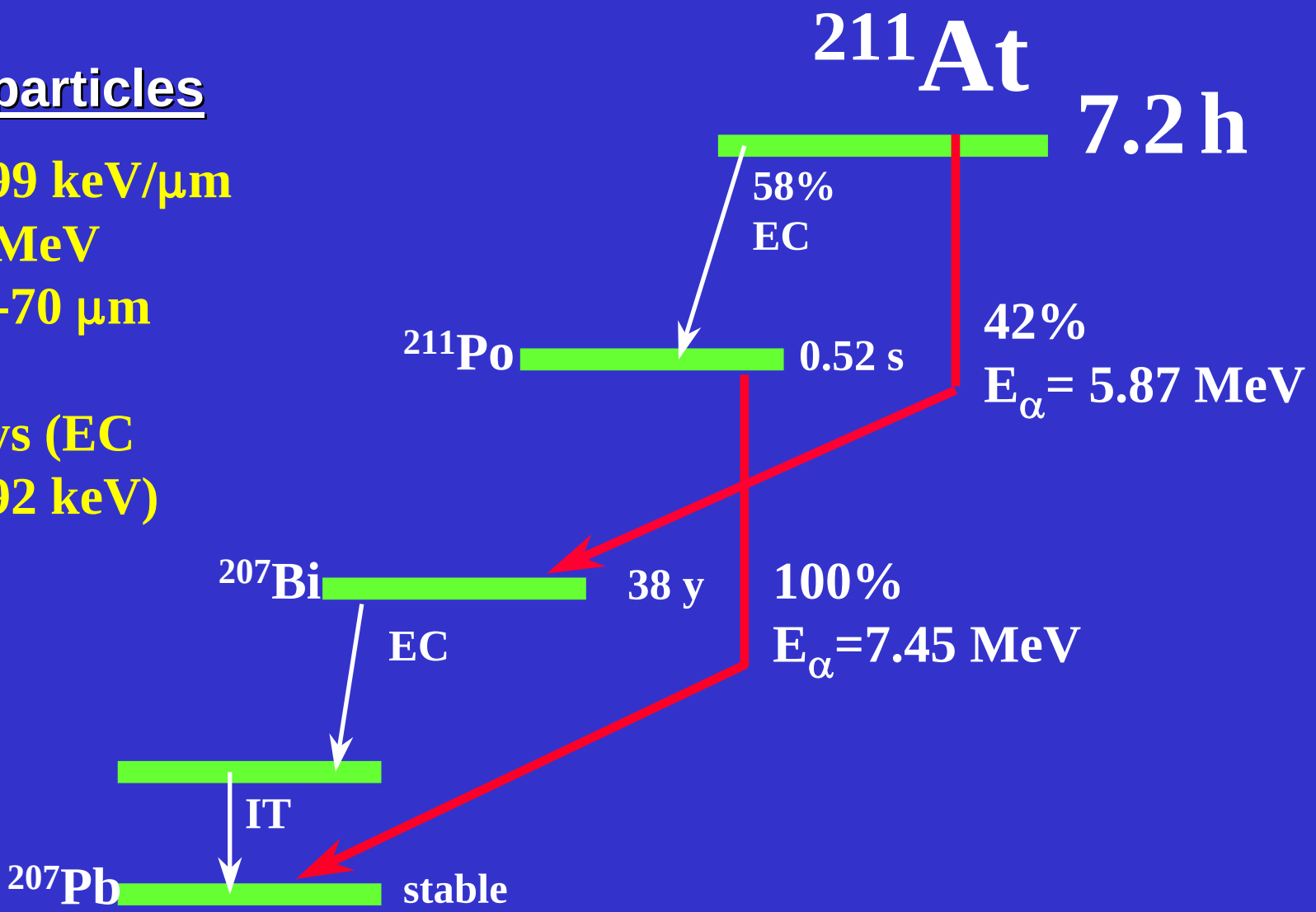
Radiobiological Advantages of Alpha Particles

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

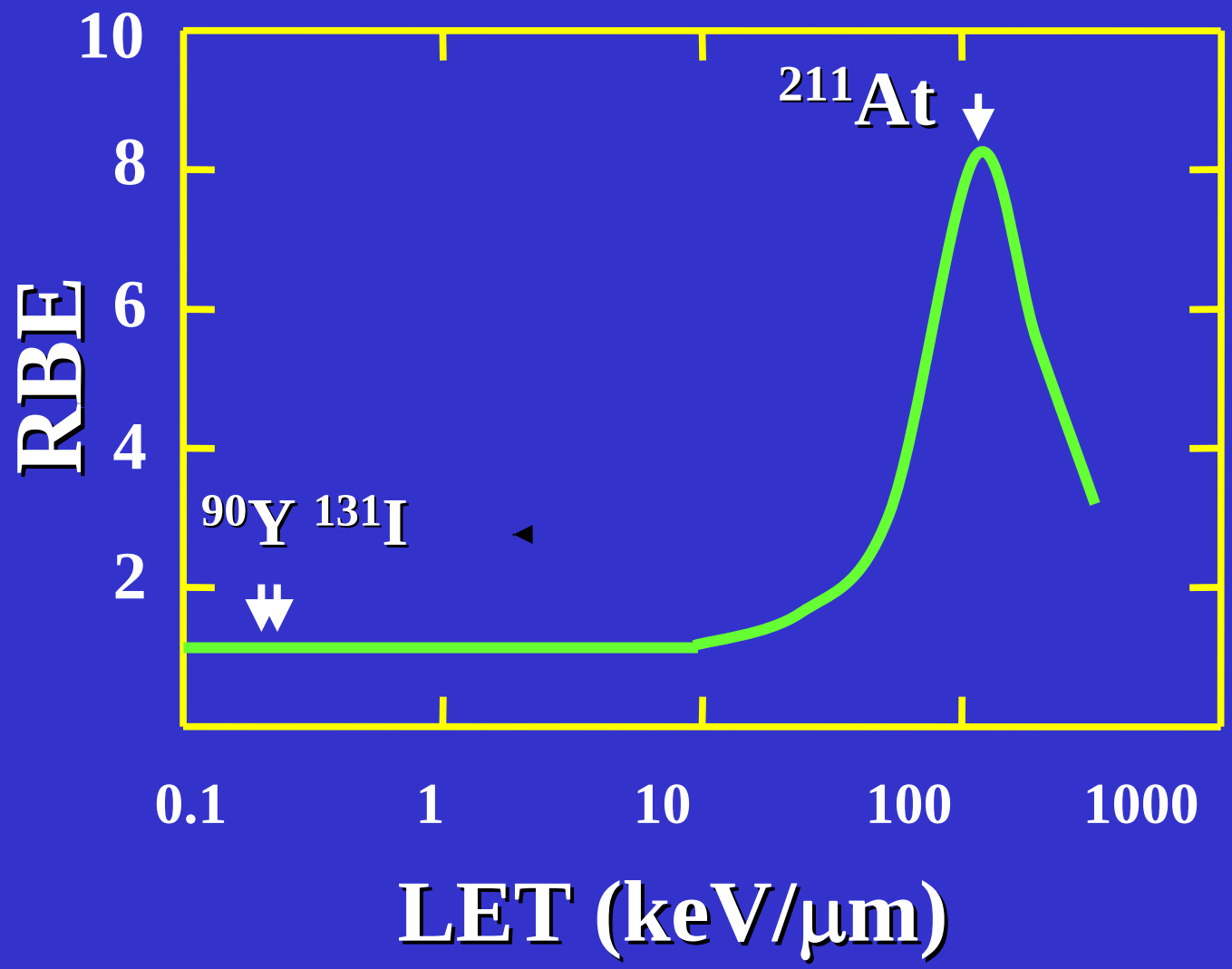
Astatine-211 Decay Scheme

At-211 α particles

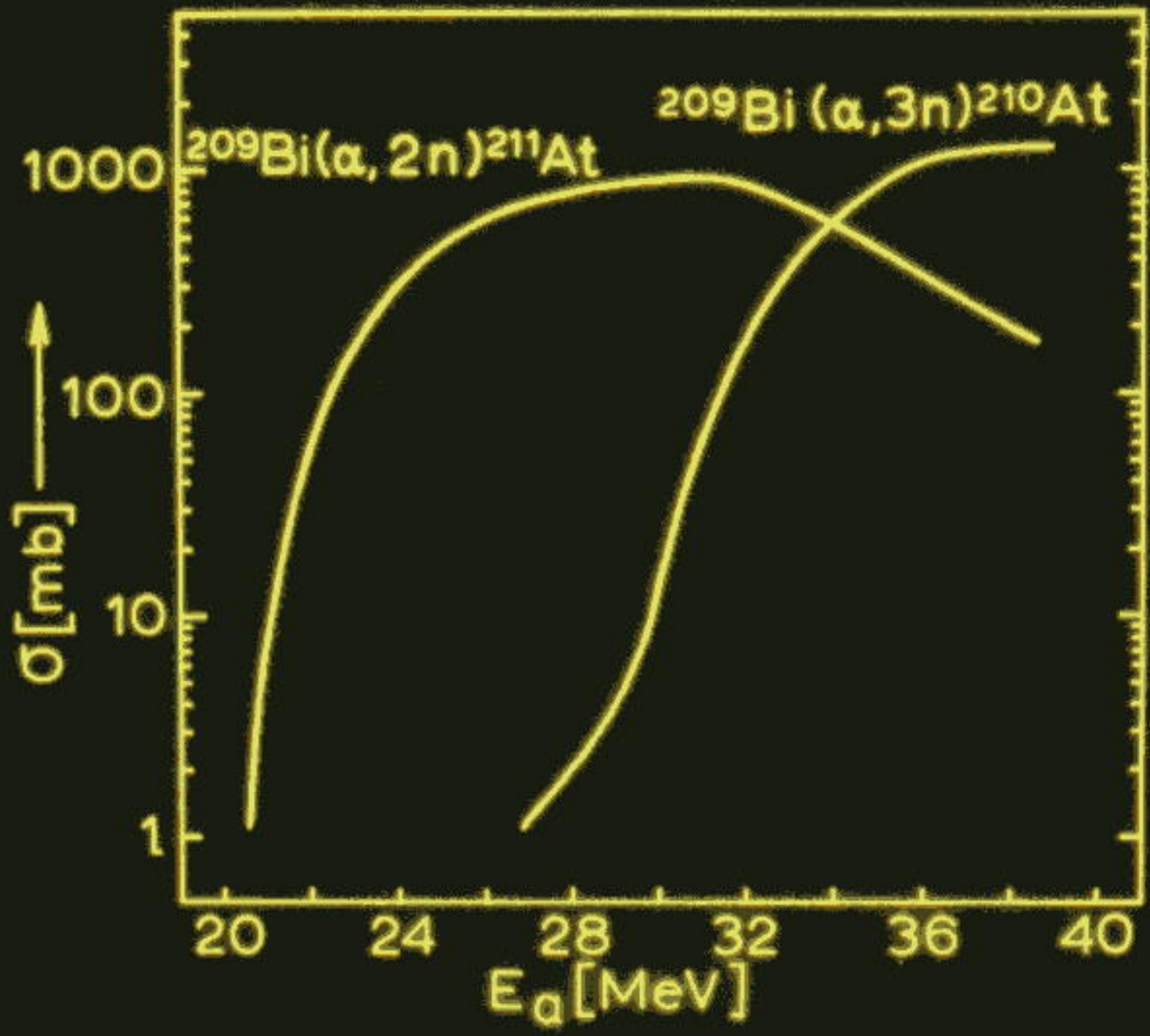
LET_{mean} = 99 keV/μm
E_{ave} = 6.79 MeV
range = 55-70 μm
Imaging:
^{211}Po X-rays (EC decay; 77-92 keV)



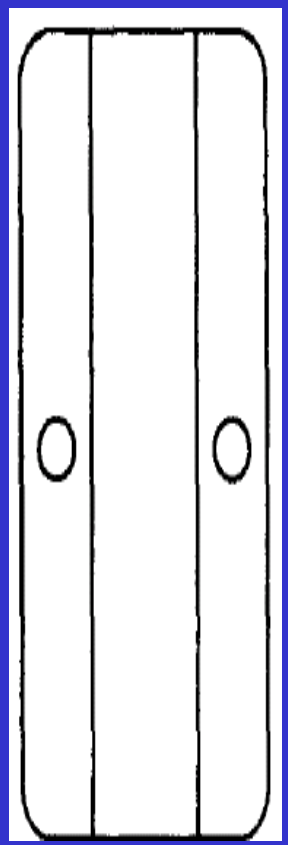
Relative Biological Effectiveness vs. LET



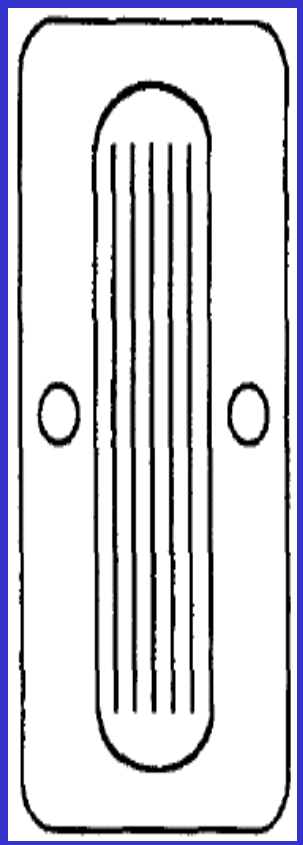
- **Astatine-211 Production**
- **Astatinated Monoclonal Antibodies**
- ***Meta*-[^{211}At]Astatobenzylguanidine**
- **5-[^{211}At]Astato-2’-deoxyuridine**
- **Astatinated octreotide Analogues**


209Bi(α, 2n)211At
209Bi (α,3n)210At
1000
100
10
1
σ[mb]
20
24
28
32
36
40
Eα[MeV]

^{211}At-Internal Target



Top/Front



Bottom/Back

- Grazing angle at 1.5^{o} 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

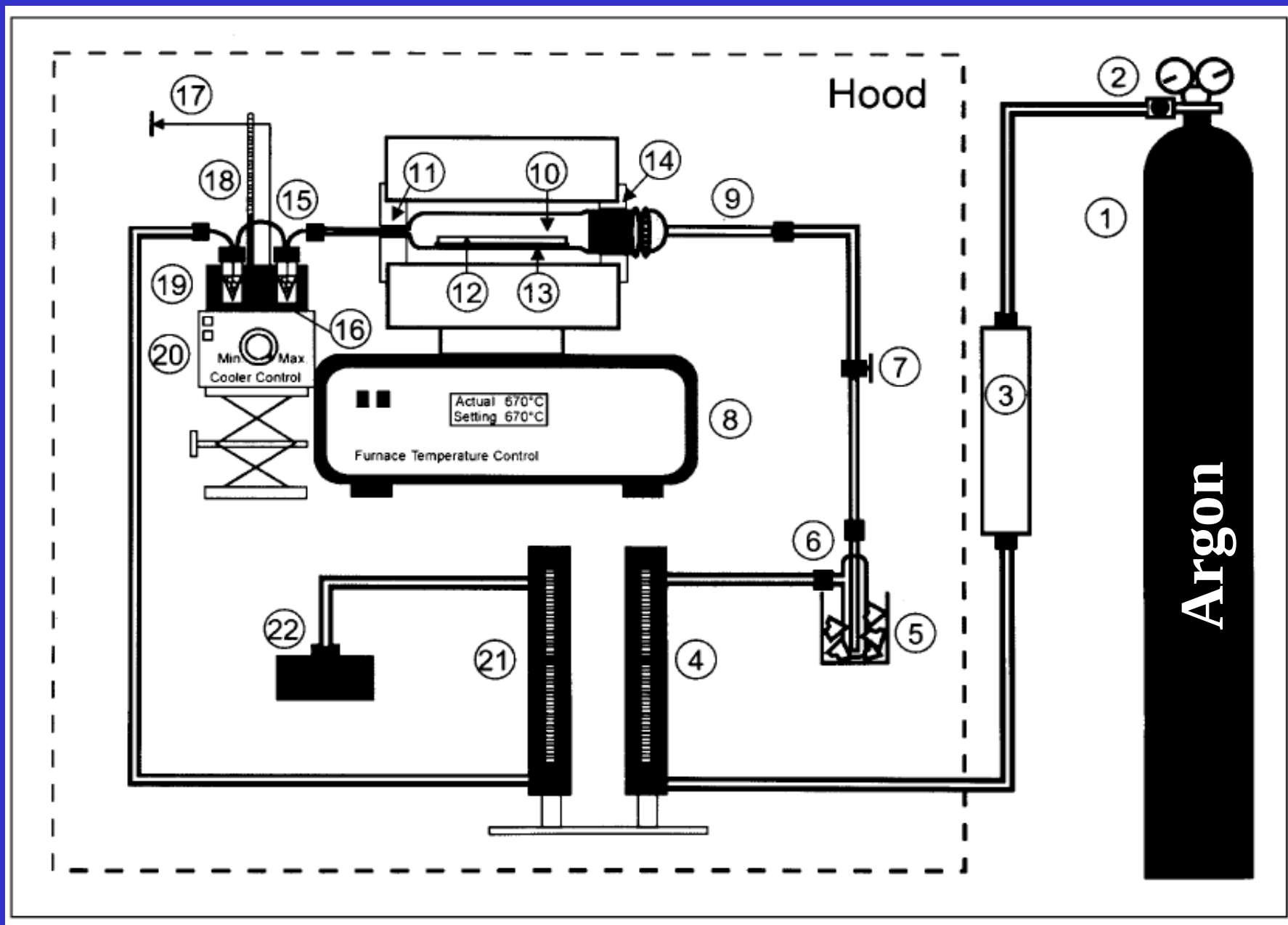
  -background activity

  -distillation efficiency

- **Target Configuration**

  -yield

  -still size, yield


Hood
Argon
Min
Max
Cooler Control
Actual 670°C
Setting 670°C
Furnace Temperature Control
1
2
3
4
5
6
7
8
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22

^{211}At Internal Target

^{209}Bi(α,2n)^{211}At

- Beam currents of 50-60 μA, 28 MeV α-particles, and 1.5-4.5 hr runs
- 0.8 ± 0.1 mCi/μA·h
- Maximum to date:

  55 μA × 4.0 h = 178 mCi
- 67 ± 16% distillation yield

- **Astatine-211 Production**
- **Astatinated Monoclonal Antibodies**
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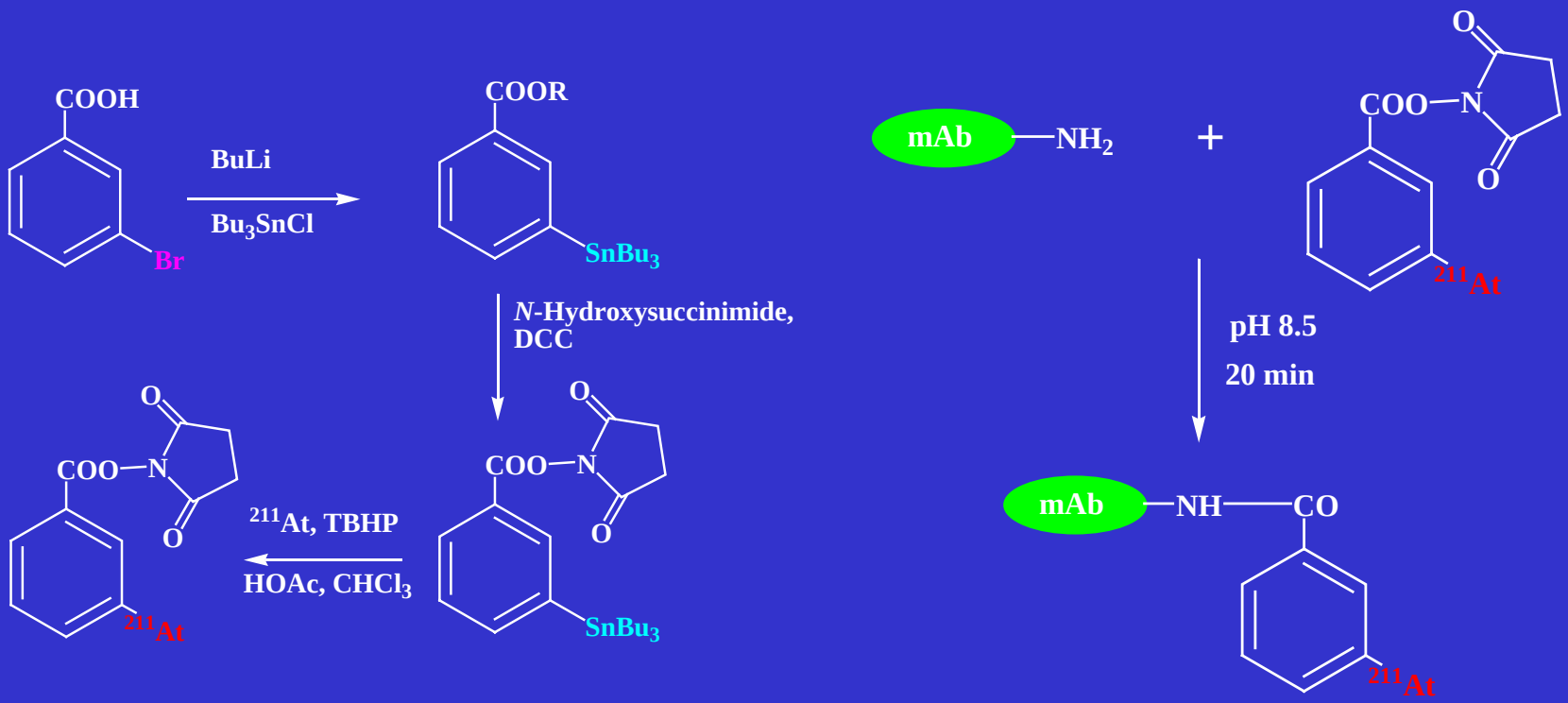
- **5-[^{211}At]Astato-2’-deoxyuridine**
- **Astatinated octreotide Analogues**

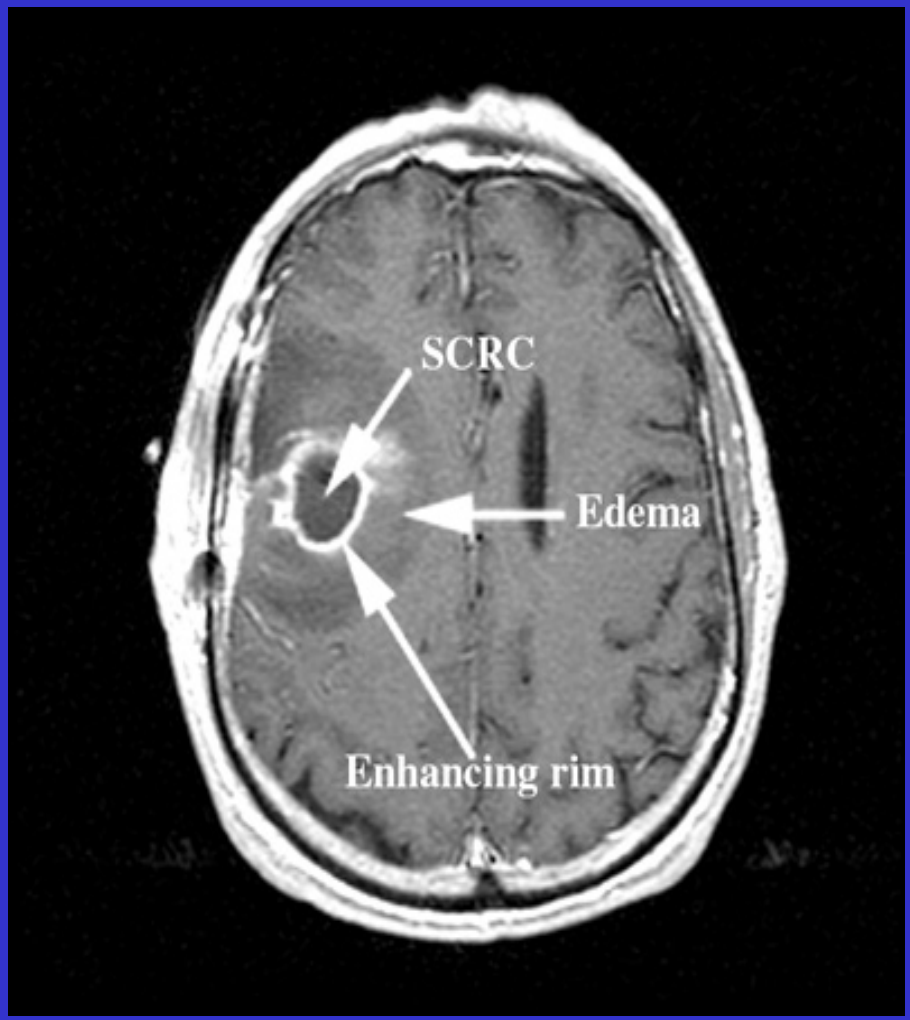
N-Succinimidyl 3-[^{211}At]Astatobenzoate ([^{211}At]SAB) for mAb Labeling

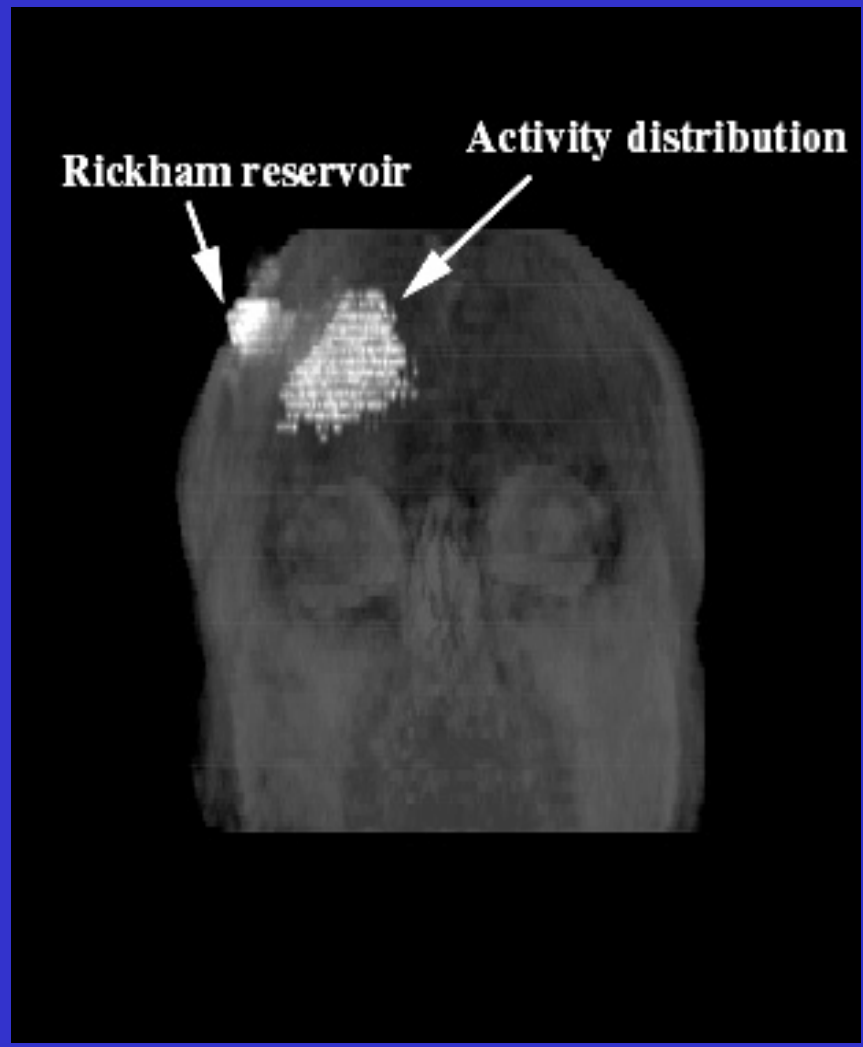


Clinical Batches of Chimeric 81C6 mAb labeled with ^{211}At Using [^{211}At]SAB

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

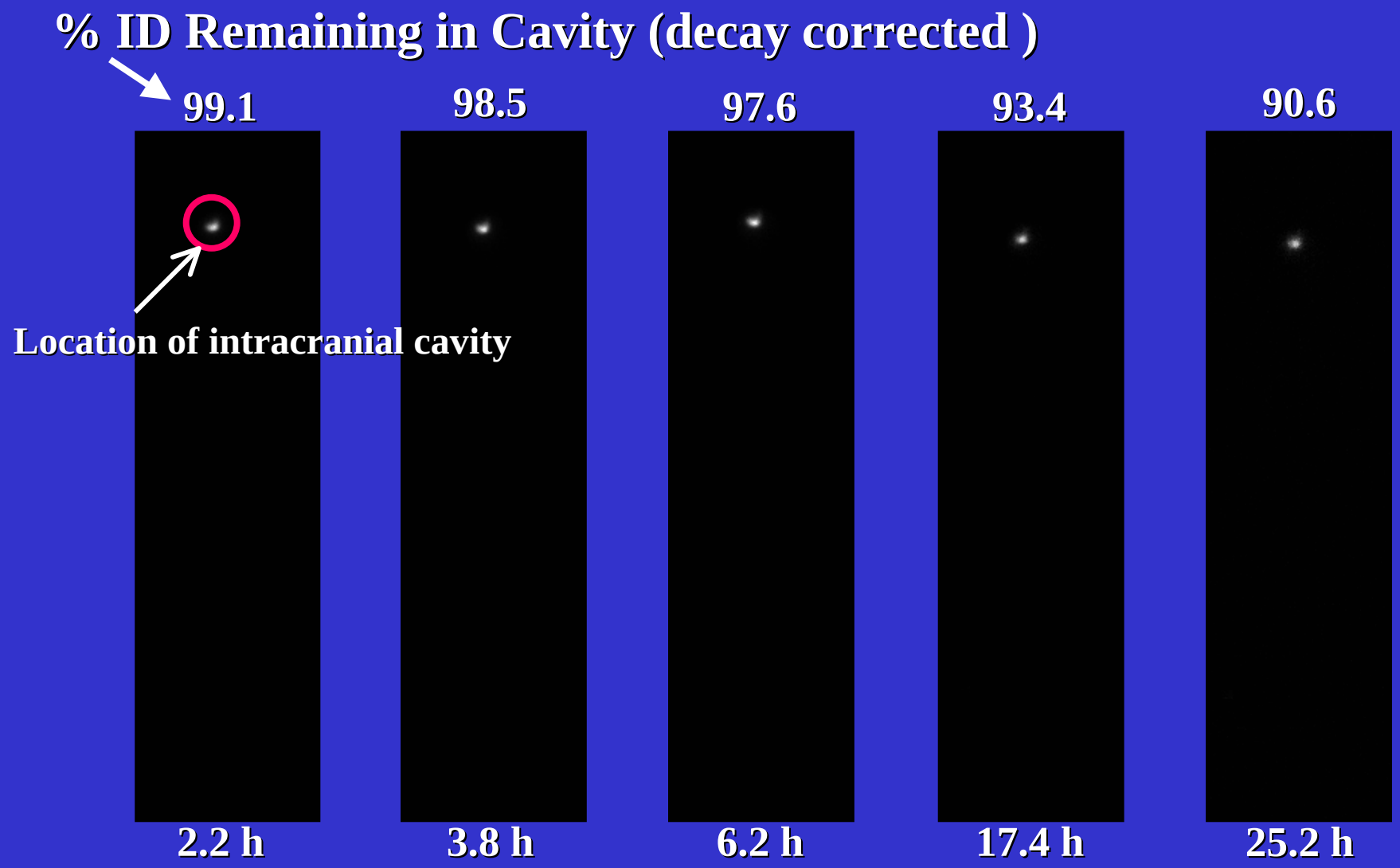
  -Single dose of 10 mg of ch81C6

  -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



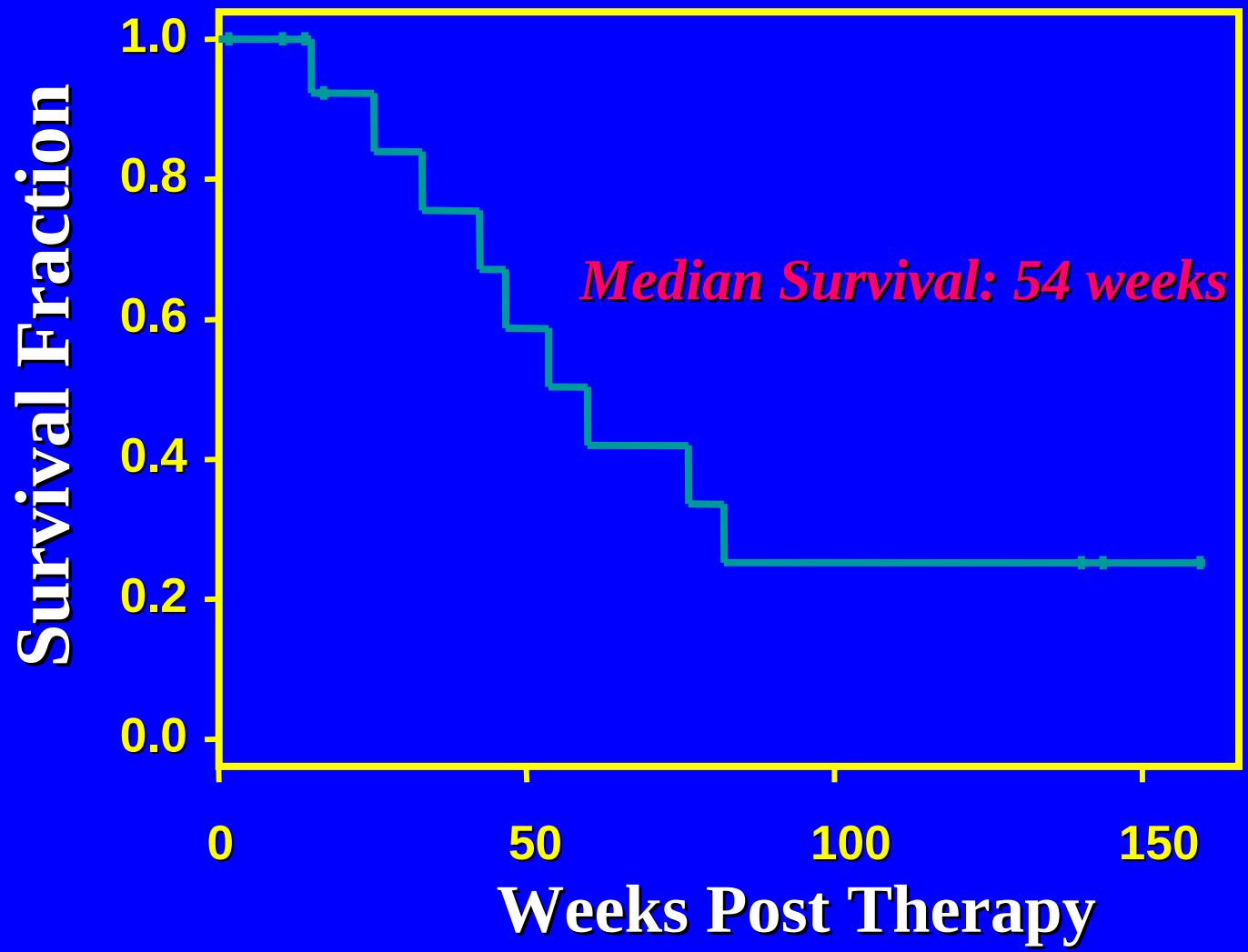
At-211 Activity Distribution in Patients

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

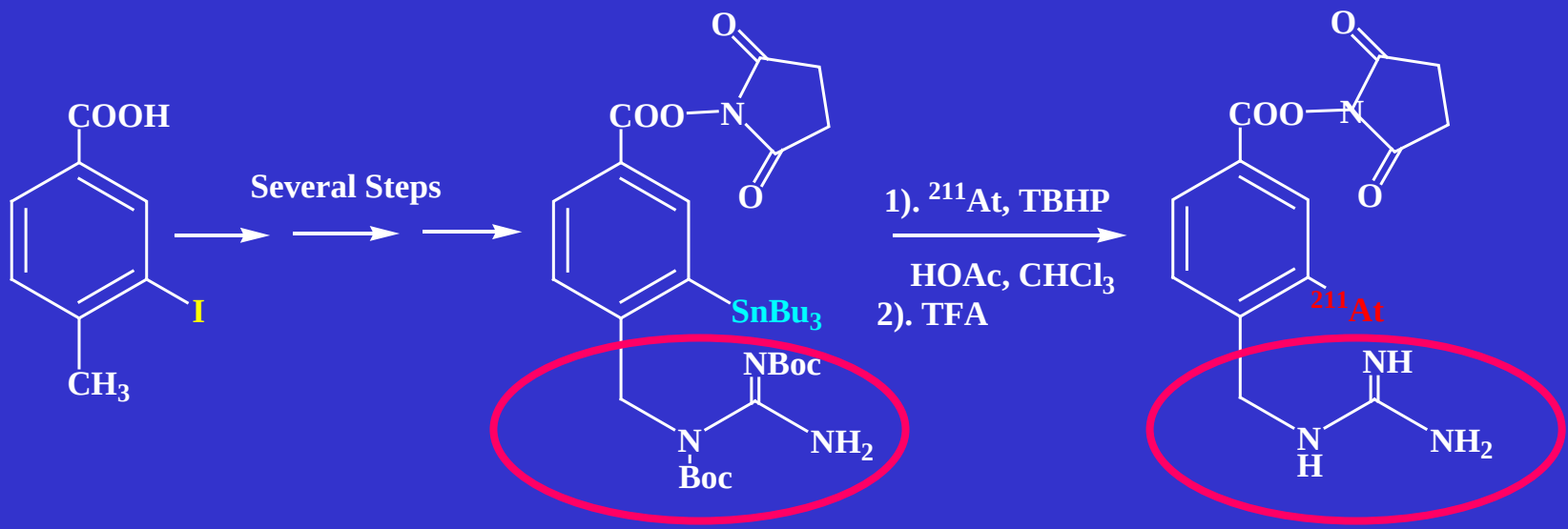
At-211 Chimeric 81C6: Radiation Dosimetry

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

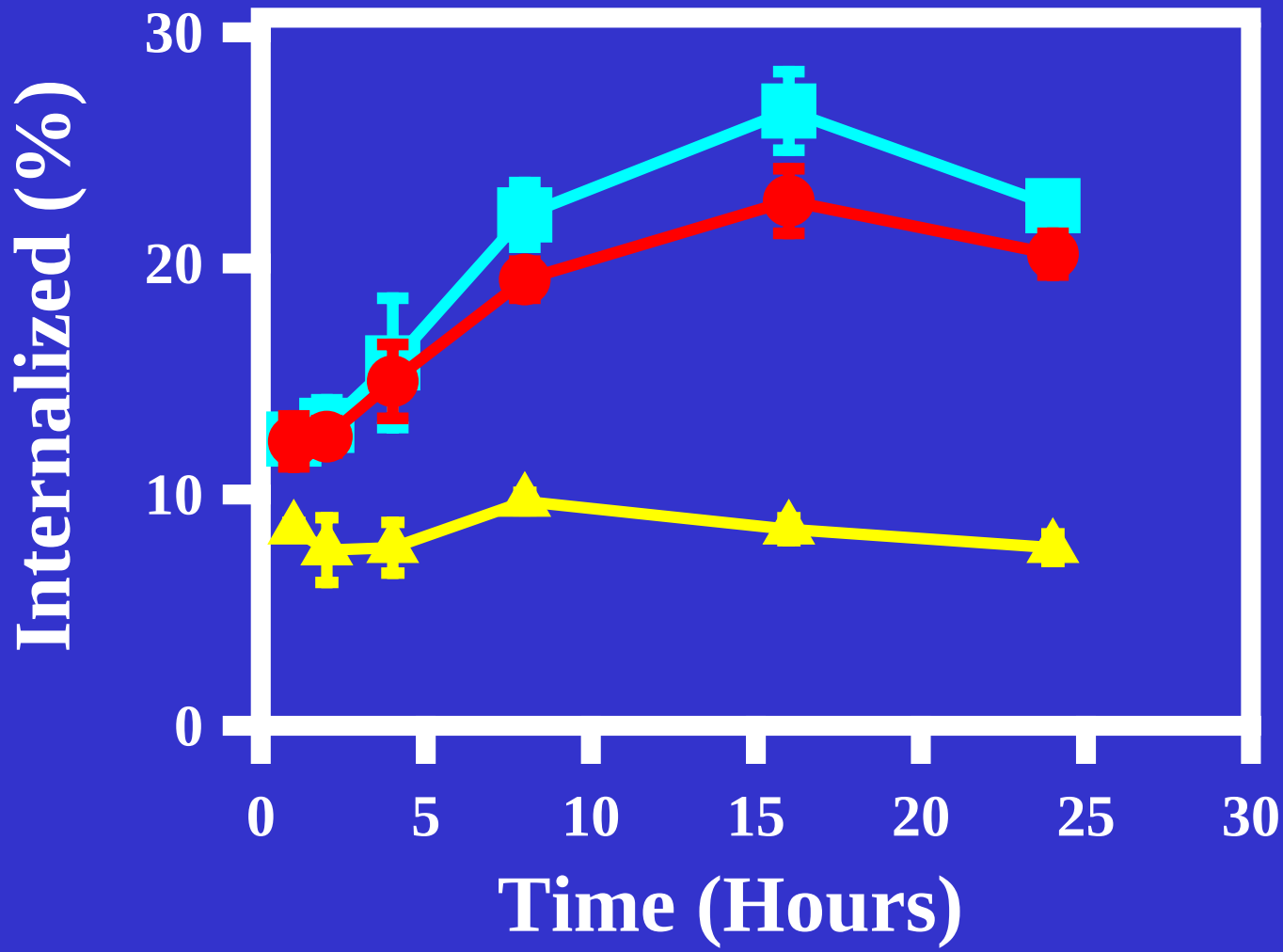


N-Succinimidyl 3-[^{211}At]Astato-4-Guanidinomethylbenzoate ([^{211}At]SAGMB)

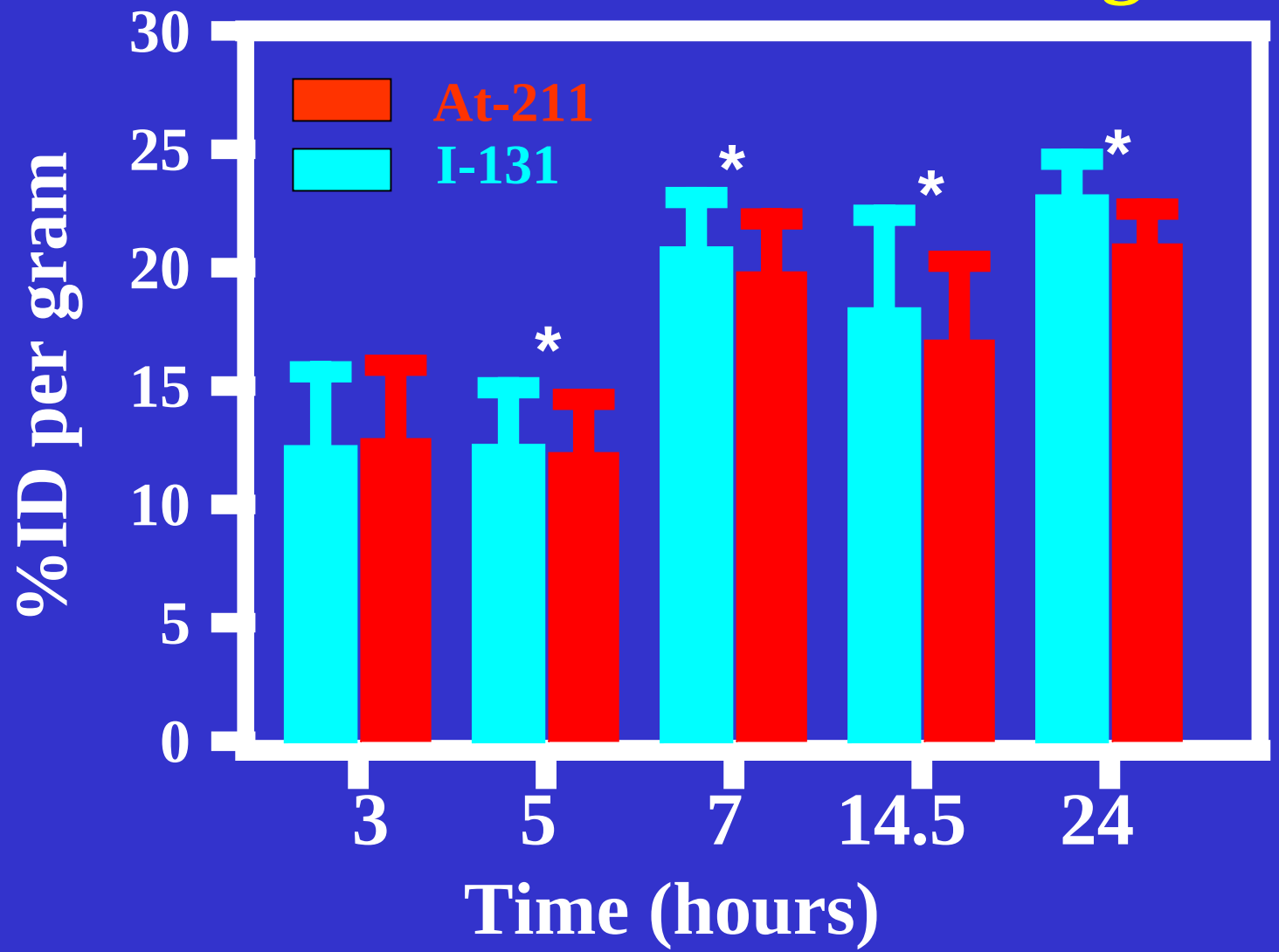


- **Radiochemical yield: 61.7 ± 13.1%**
- **L8A4 (anti-EGFR vIII mAb) conjugation yield: 36.1 ± 1.9%**
- **Immunoreactive Fraction: 65.2 ± 1.5%**

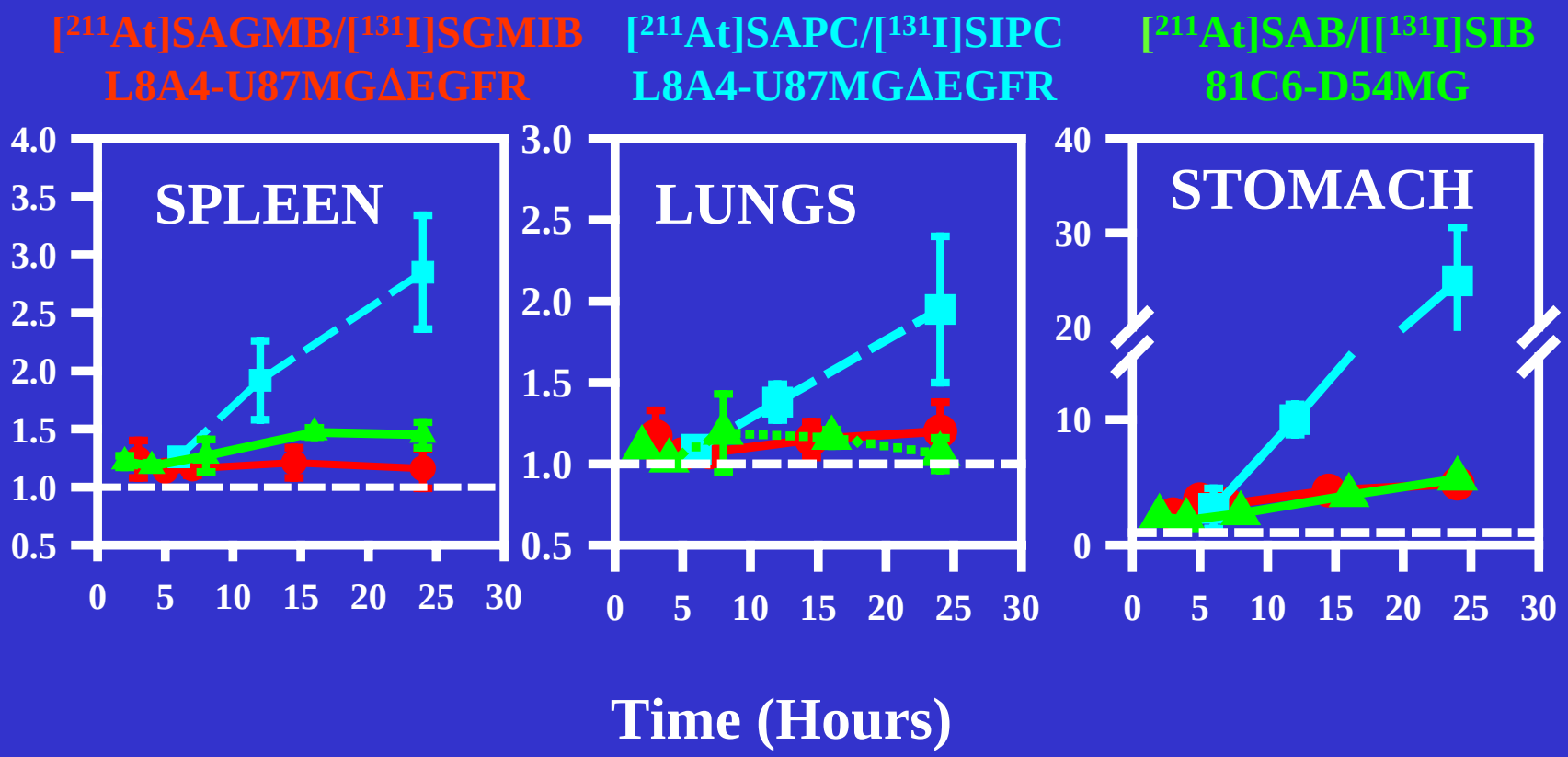
In vitro internalization by U87MGEGFR Glioblastoma Cells: L8A4-[^{211}At]SAGMB vs. [^{131}I]L8A4 (Iodogen and [^{131}I]SGMIB)



Paired-label Tumor Uptake of [^{131}I]SGMIB-L8A4 and [^{211}At]SAGMB-L8A4 in U87MGΔEGFR Xenografts



^{211}At/^{131}I Uptake Ratios



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

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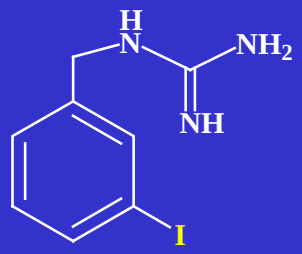
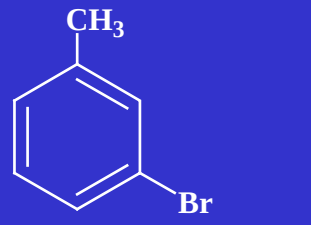
Meta-[^{211}At]Astatobenzylguanidine ([^{211}At]MABG)



Meta-Iodobenzylguanidine (MIBG)

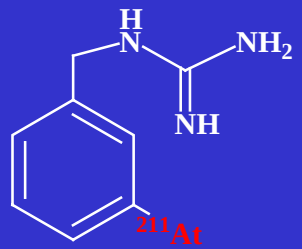


Several steps

^{211}At, NCS

TFA, 70°C

RCY = 85%



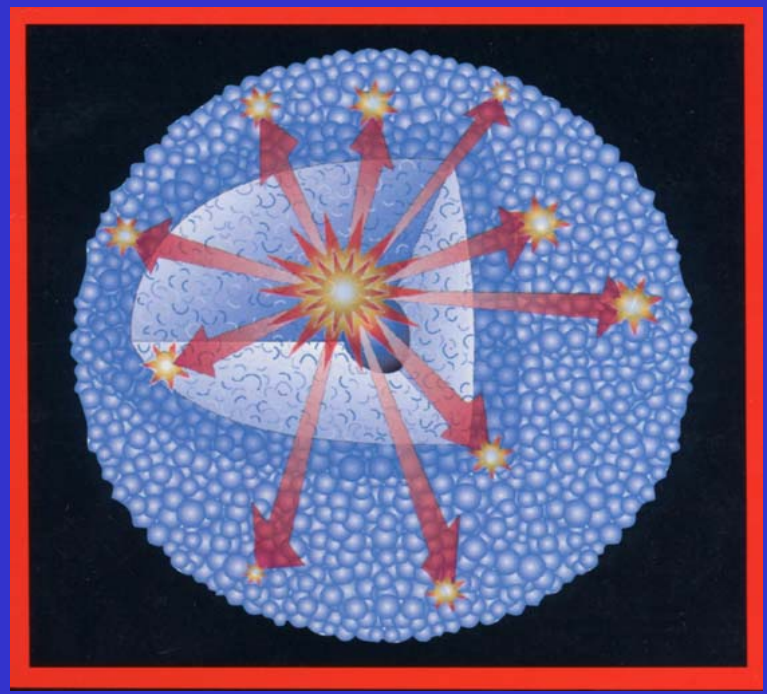
Meta-[^{211}At]Astatobenzylguanidine ([^{211}At]MABG

[^{211}At]MABG

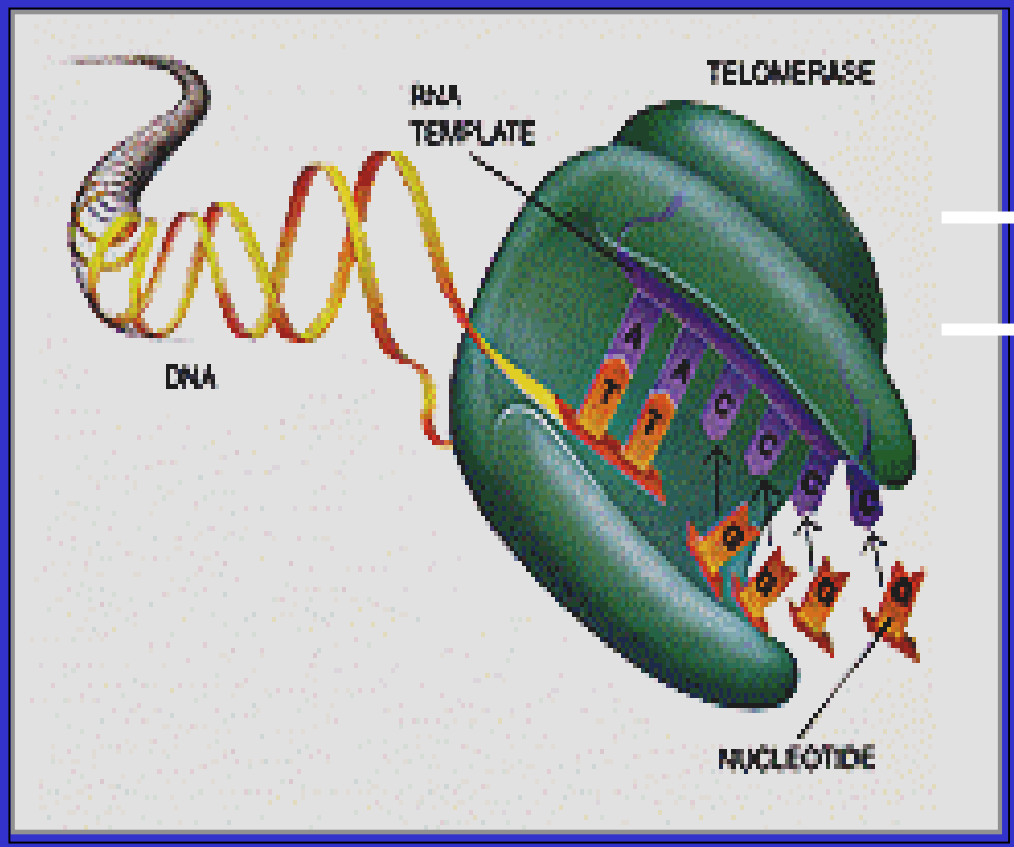
- **Uptake in SK-N-SH neuroblastoma cells *in vitro* and tissue distribution in mice *in vivo* very similar to MIBG**
- **Minimal *in vivo* dehalogenation**
- **Exquisite cytotoxicity (more than 1000-fold compared to n.c.a. [^{131}I]MIBG and equivalent to only a few ^{211}At decays per cell in monolayers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

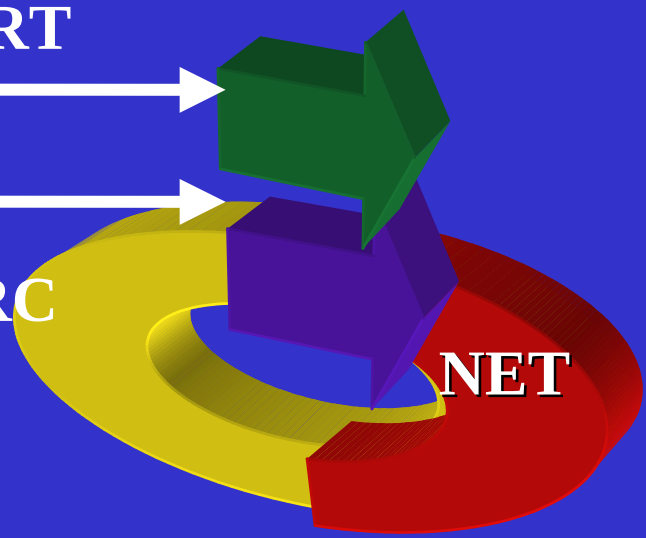


MABG and Gene Therapy

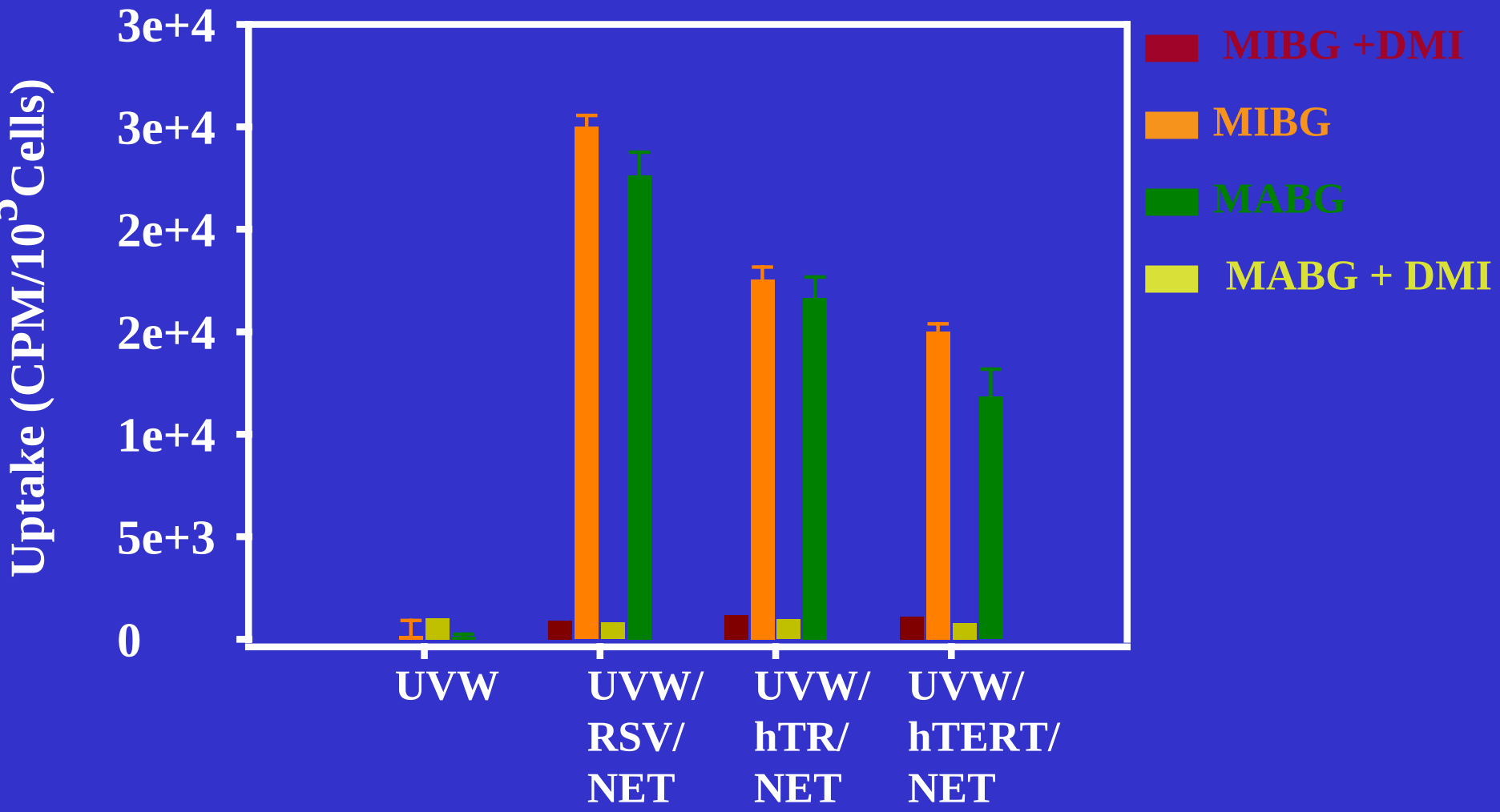


hTERT

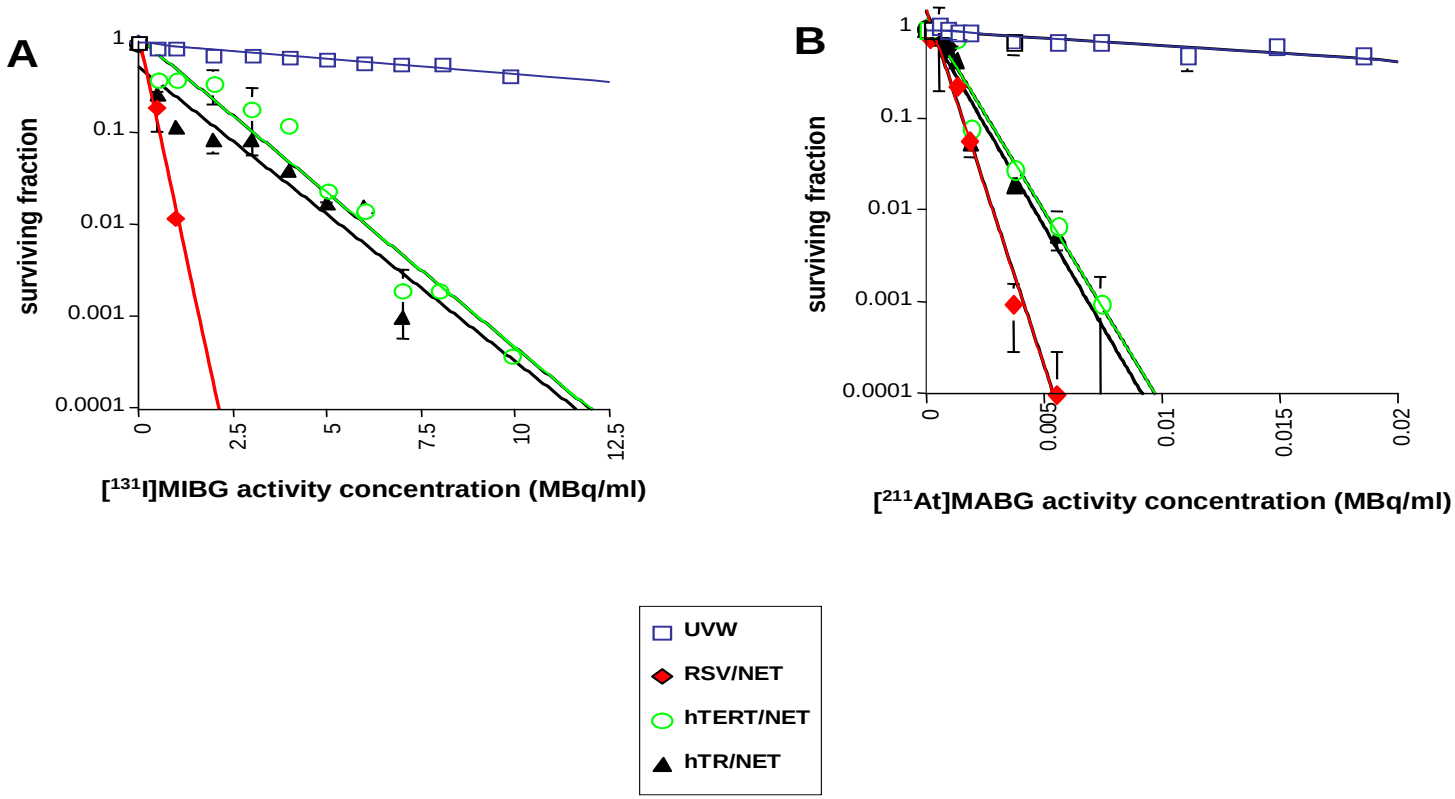
hTERC



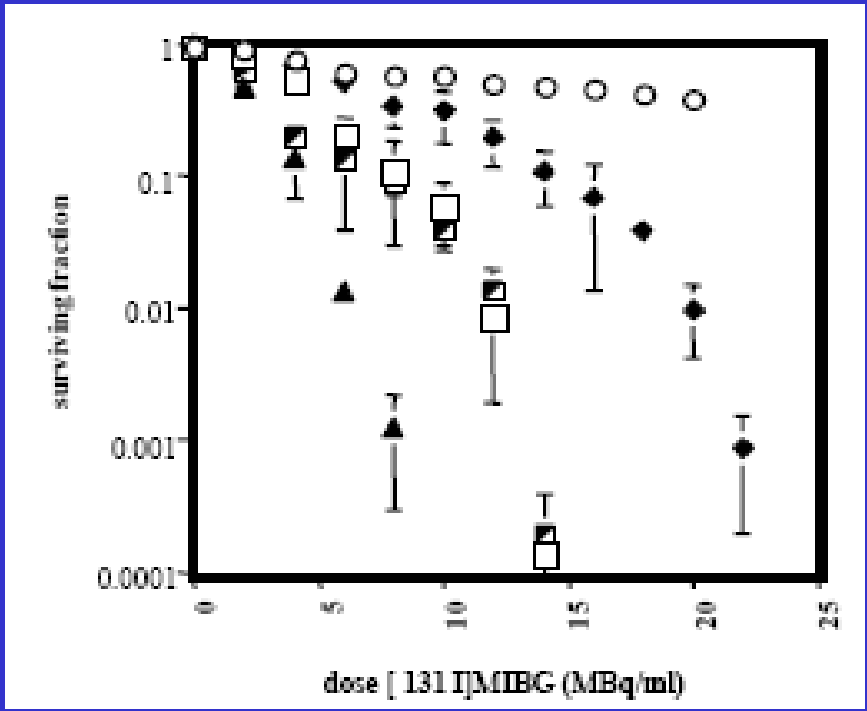
[^{211}At]MABG and Gene Therapy: Uptake in Glioma cells transfected with NET Gene Under the Control of Various Promoters

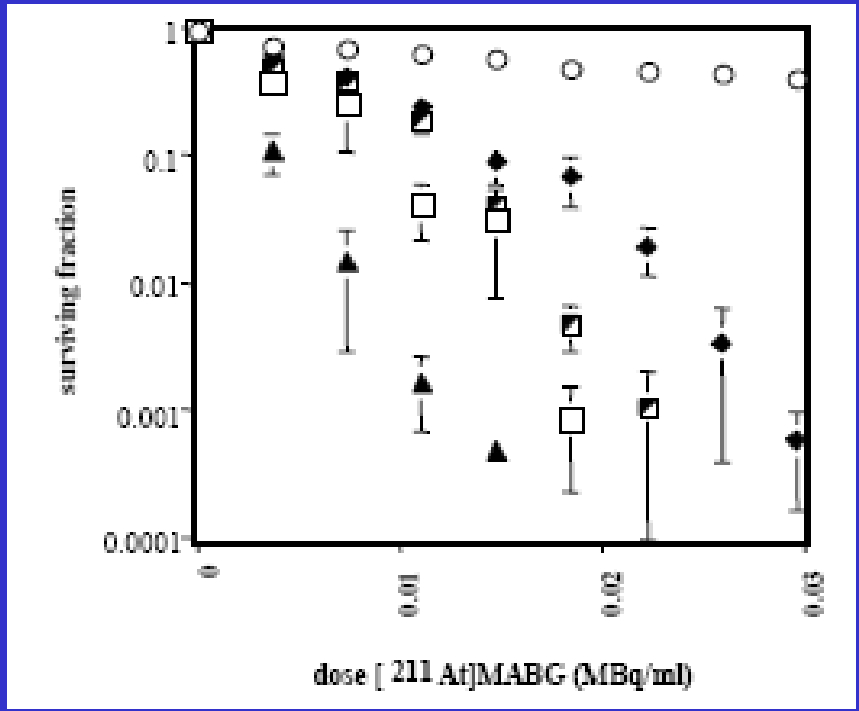


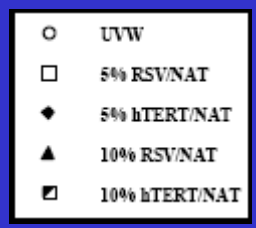
[^{211}At]MABG and Gene Therapy: Clonogenic Survival of UVW Spheroids: 100% Cells Transfected with NET



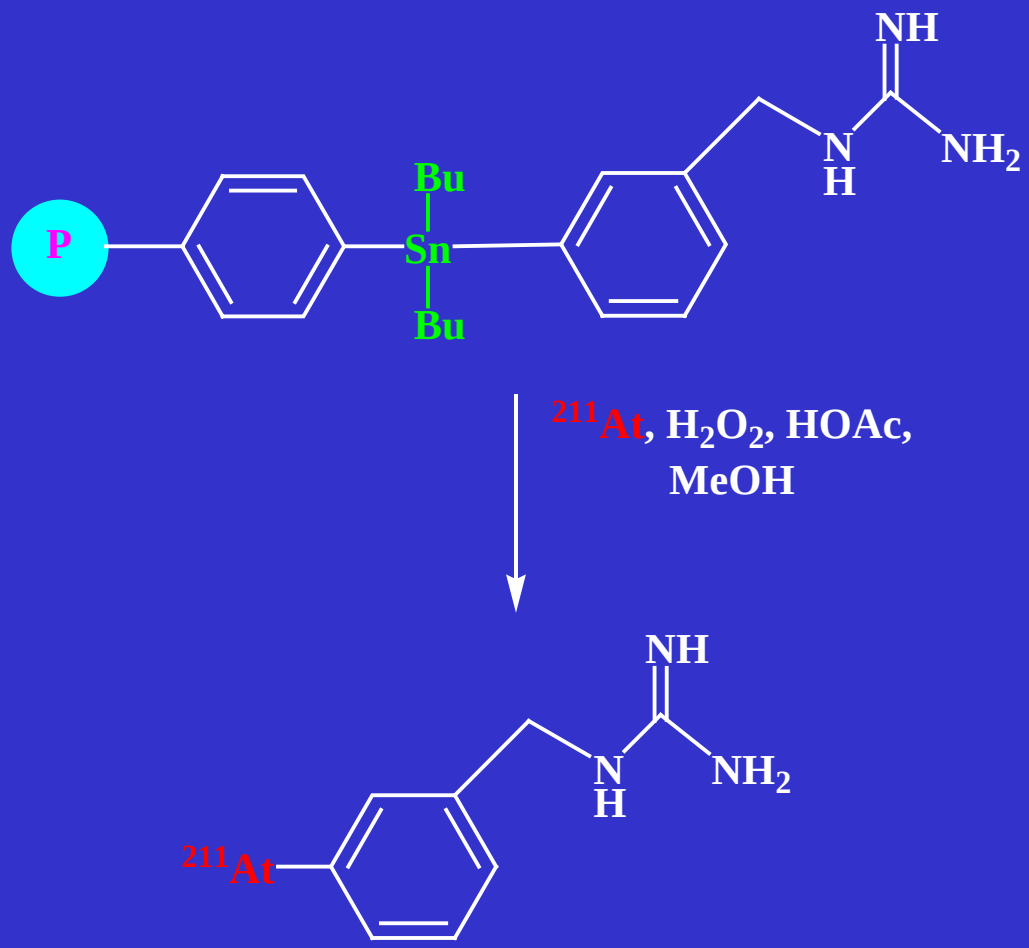
[^{211}At]MABG and Gene Therapy: Clonogenic Survival of Mosaic Spheroids: Various Percent of Cells Transfected with NET



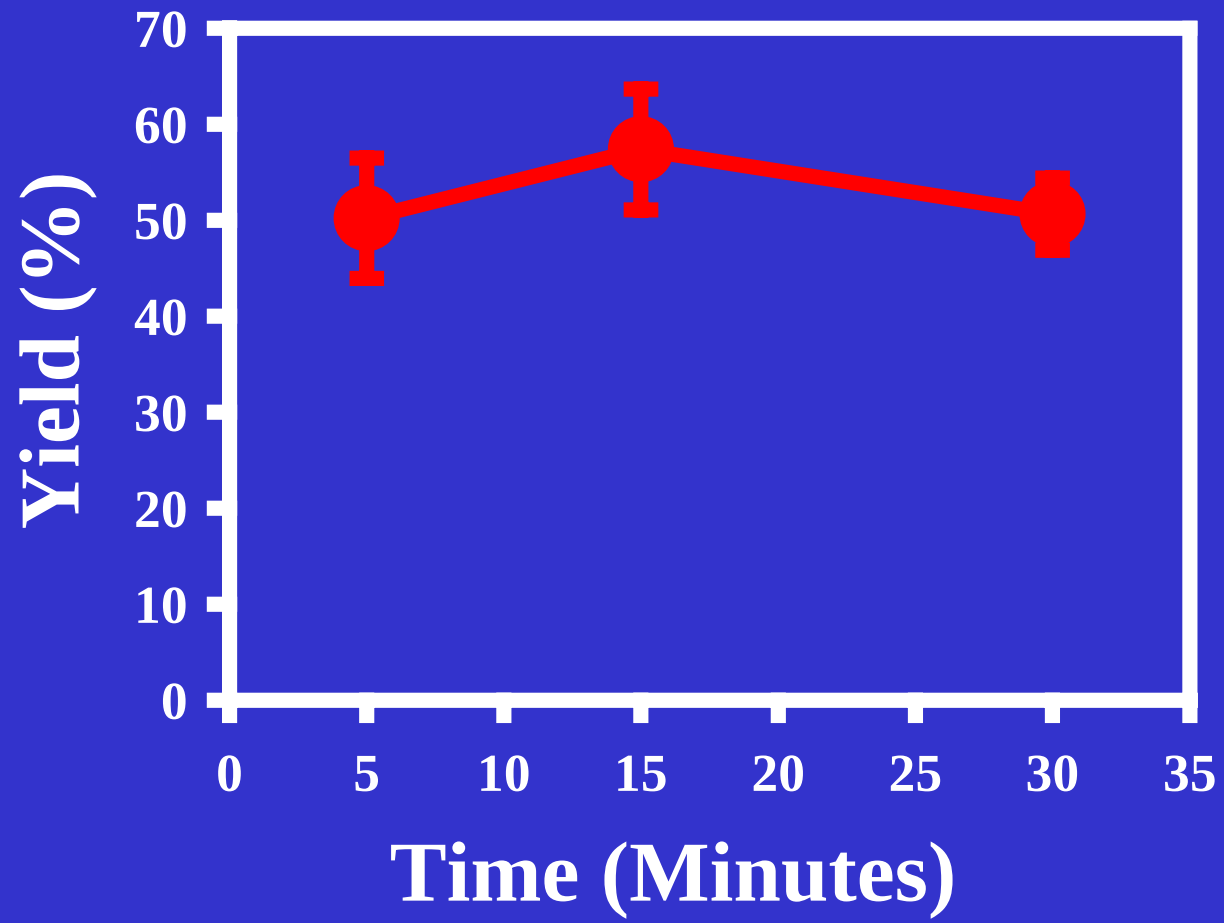




Solid-phase synthesis of [^{211}At]MABG



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



Resin: 5 mg

^{211}At: 200-300µCi in 50 µl MeOH

H_2O_2 (30%):HOAc 17:10 (v/v): 10 µl

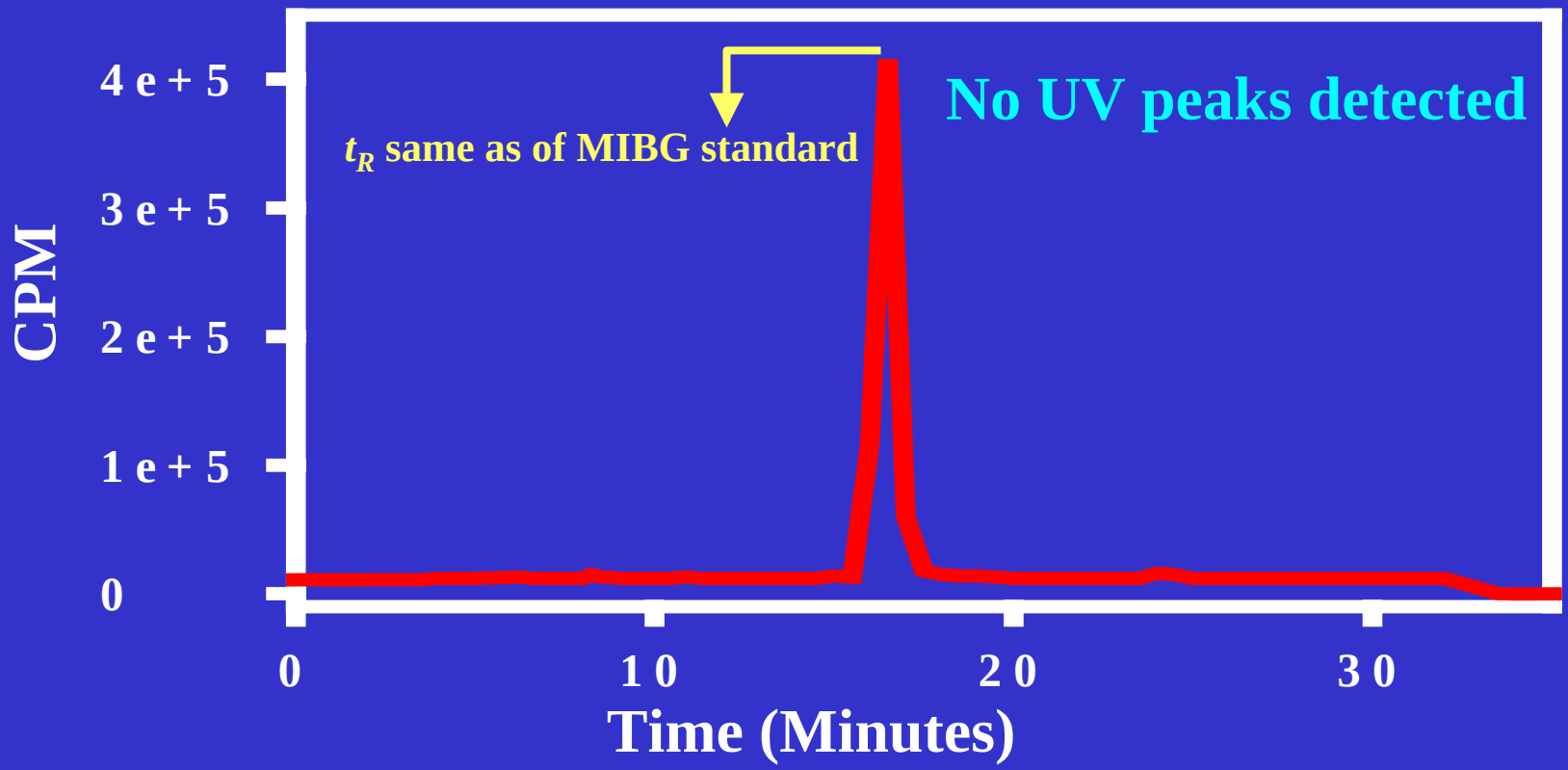
Room temp.

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

- 10 mg resin
- 1.0 – 8.0 mCi or ^{211}At in ~100 µl MeOH
- 20 µl of H_2O_2/HOAc mixture
- 10 min @ RT
- Purification by C18 solid-phase extraction

- Radiochemical yield: 56.4 ± 9.8% (n = 5)
- Maximum [^{211}At]MABG produced: 5 mCi

HPLC of [^{211}At]MABG from Kit Method



Waters X-Terra RP18 (4.6 × 250 mm; 5µ) column
0.1%TFA in 20/80 CH_3CN/Water; 1 ml/min

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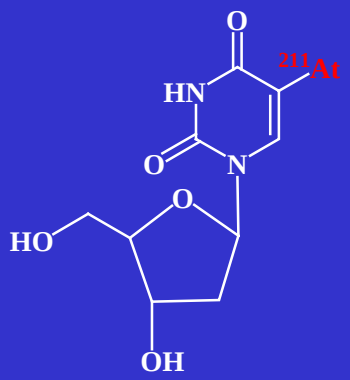
AUdR

5-Iodo-2'-deoxyuridine (IUdR)



5-[^{211}At]Astato-2'-deoxyuridine

$(Me_3Sn)_2$, $(Ph_3P)_2PdCl_2$

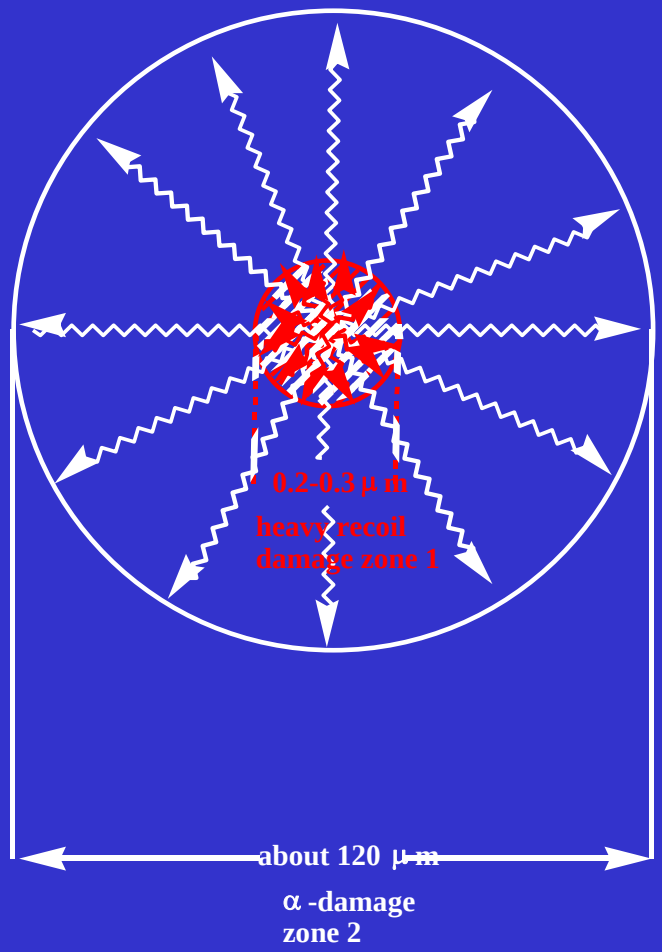
Dioxane

^{211}At, H_2O_2, HOAc,

$CHCl_3$, Sonication 20 sec

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

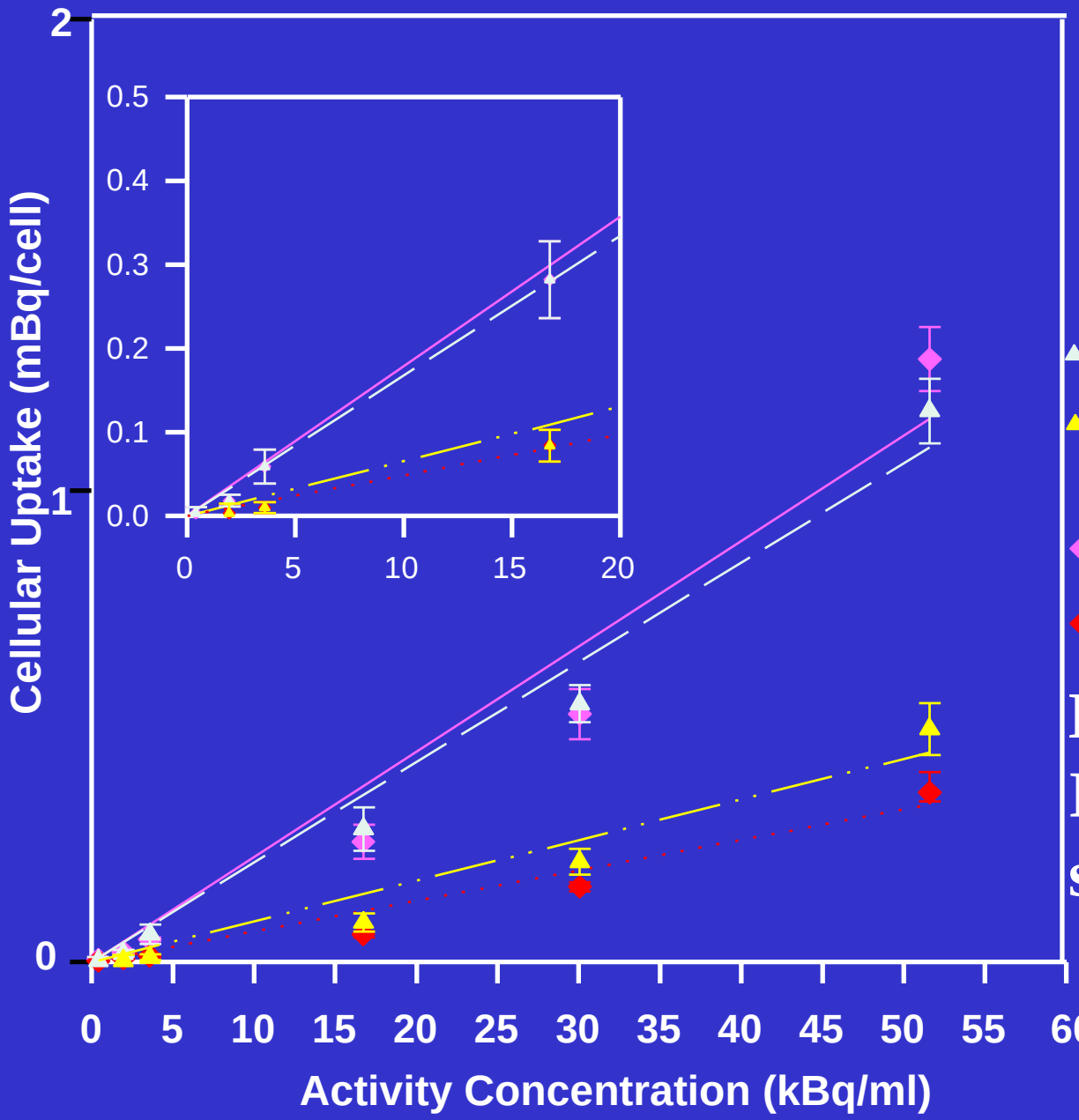
[^{211}At]AUdR: Rationale



Two potential zones of high-LET kill:

1) a-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 [^{211}At]AUdR in Rat Neoplastic Meningitis Models

- **Experiment 1:**

D341 Med human medulloblastoma

43 mCi AUdR

45 mCi free [^{211}At]astatide or saline

- **Experiment 2:**

A431p human epidermoid carcinoma

28 mCi AUdR
61 mCi AUdR
or saline

- **Experiment 3:**

D341 Med human medulloblastoma

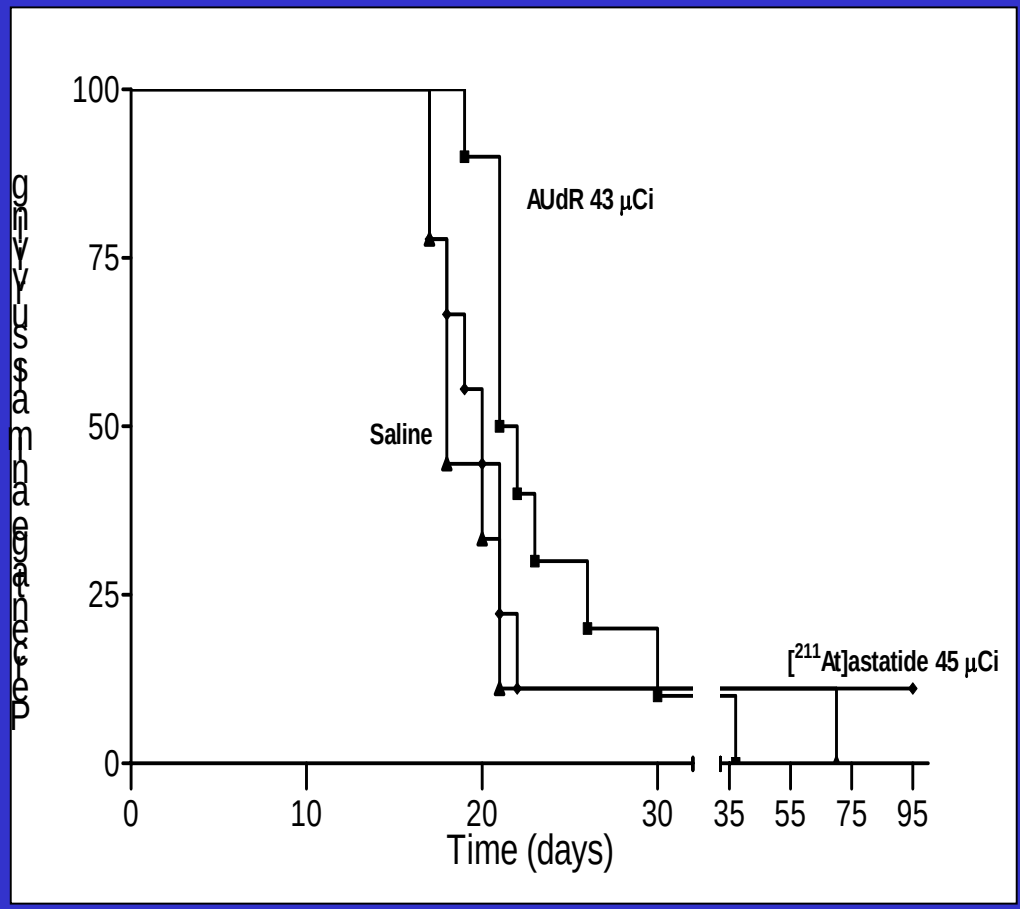
3 × 30 mCi AUdR
3 × 57 mCi AUdR
or 3 × saline

1 injection daily for 3 days

Results: Experiment 1

Median Survival

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



Results: Experiment 2

Median Survival

saline 10 d

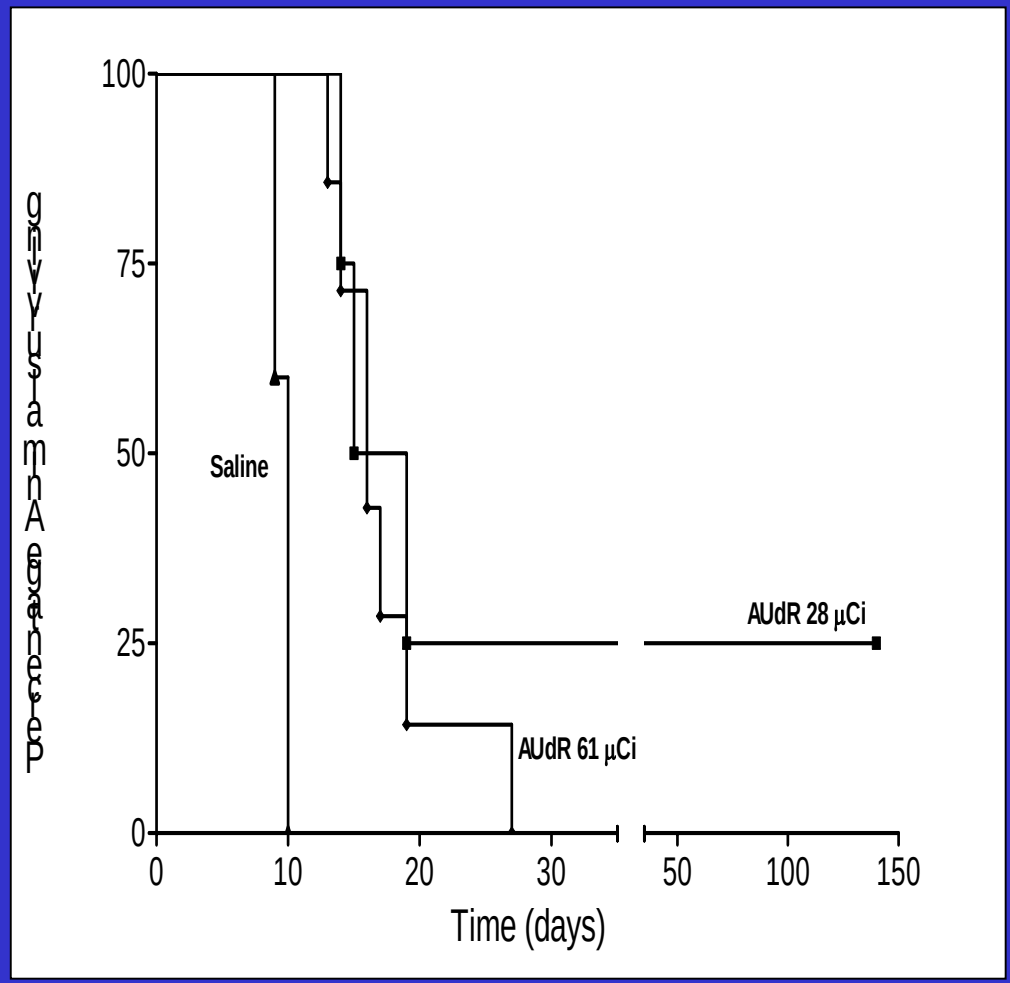
AUdR (28 mCi) 17 d

(p=0.01)

AUdR (61 mCi) 16 d

(p=0.004)

(p=0.7)



Results: Experiment 3

Median Survival

saline (3×) 22 d

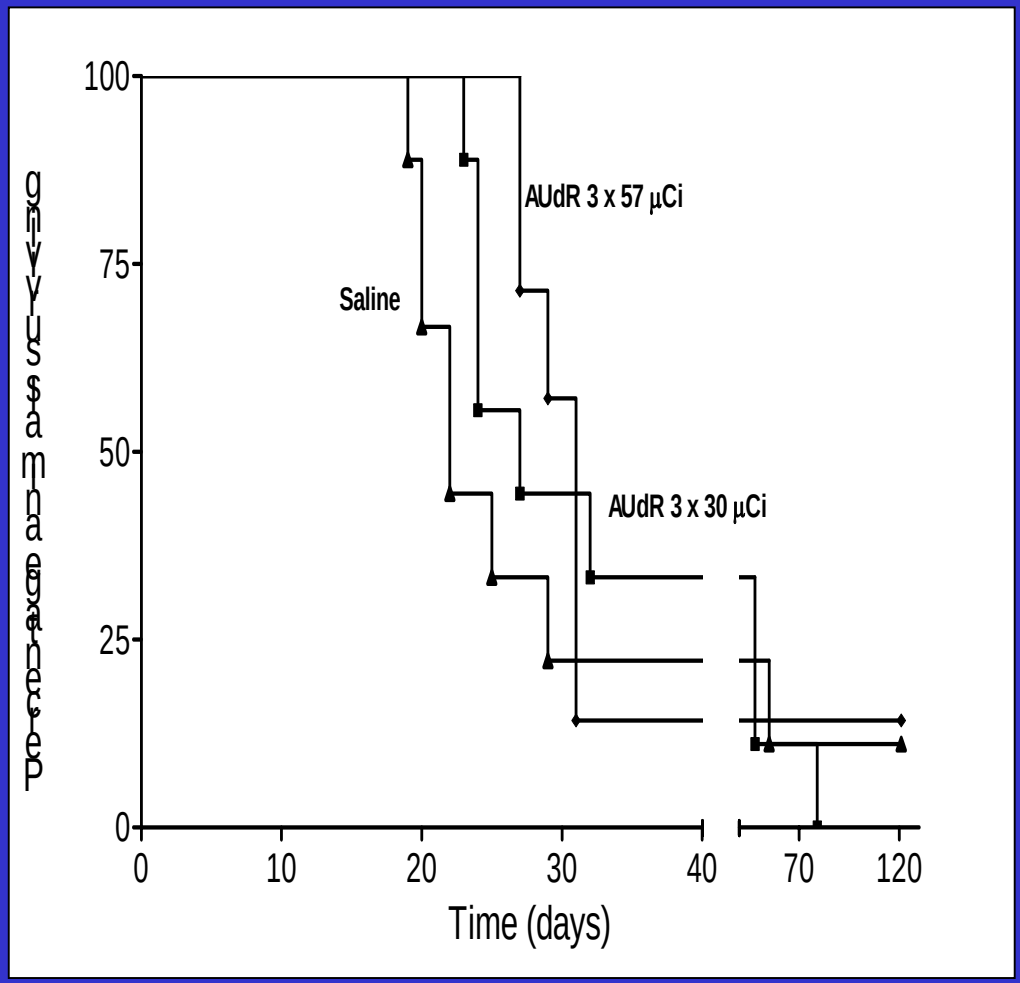
AUdR (3× 30 mCi) 27 d

(p=0.02)

AUdR (3× 57 mCi) 31 d

(p=0.08)

(p=0.49)



AUdR-Summary

- **Intrathecal administration of [^{211}At]AUdR significantly improved the median survival of athymic rats with medulloblastoma neoplastic meningitis**
- **The therapeutic efficacy of [^{211}At]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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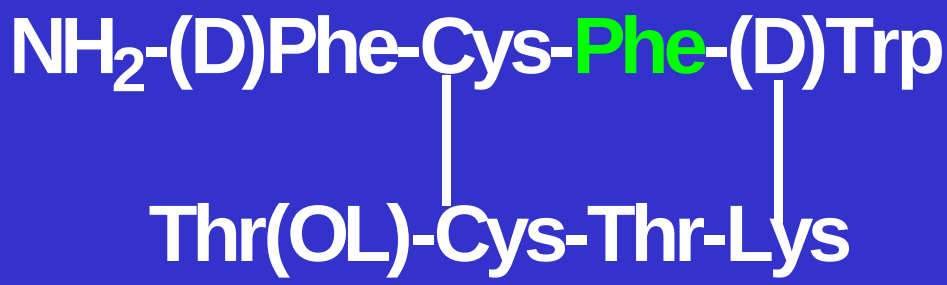
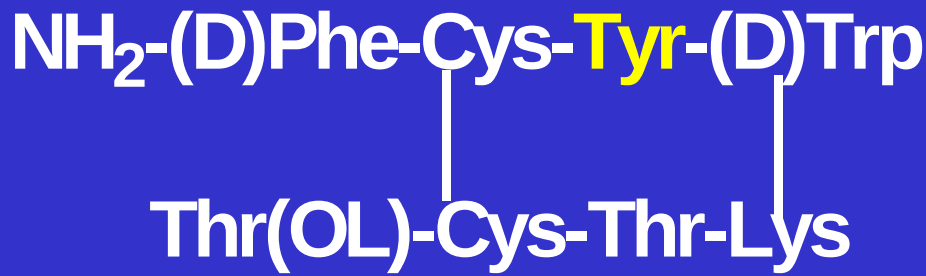
- **5-[^{211}At]Astato-2’-deoxyuridine**
- **Astatinated octreotide Analogues**



OCTREOTIDE

Cannot be radioiodinated directly



Tyr^3-OCTREOTIDE

Tyrosine residue can be radioiodinated directly; however, unstable *in vivo*

STRUCTURES OF IBO, INO AND ABO

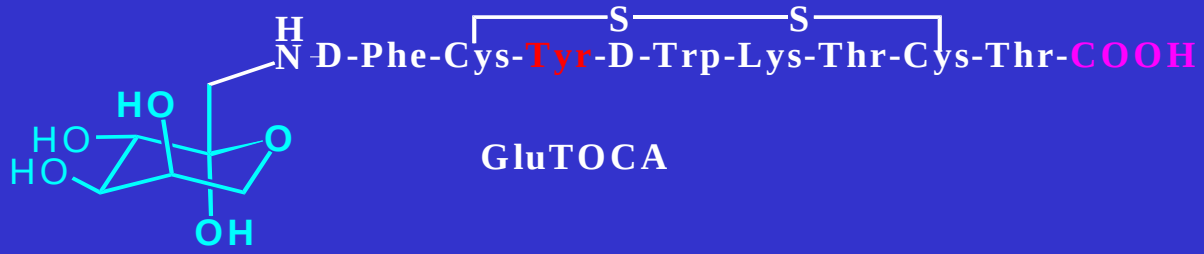
IODOBENZOYLOCTREOTIDE (IBO): X = I, Y = CH

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

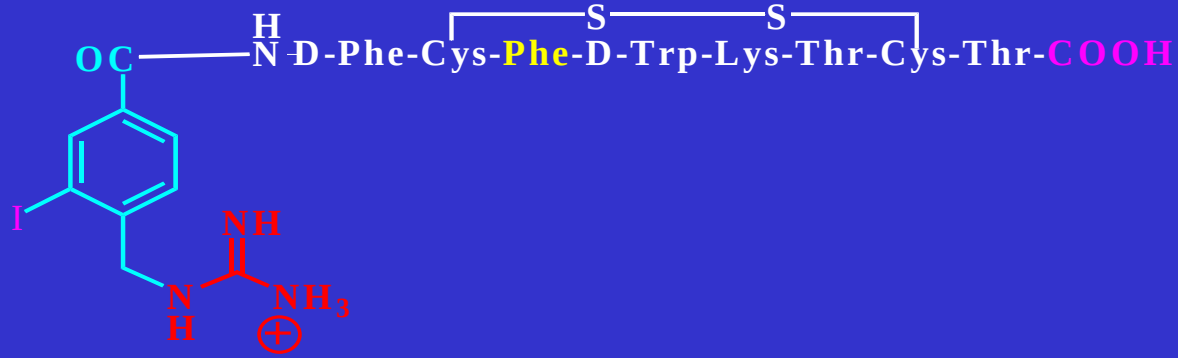
ASTATOBENZOYLOCTREOTIDE (ABO): X = ^{211}At, Y = CH

Structures of Octreotide, Glu-TOCA and GMIBO

Octreotide



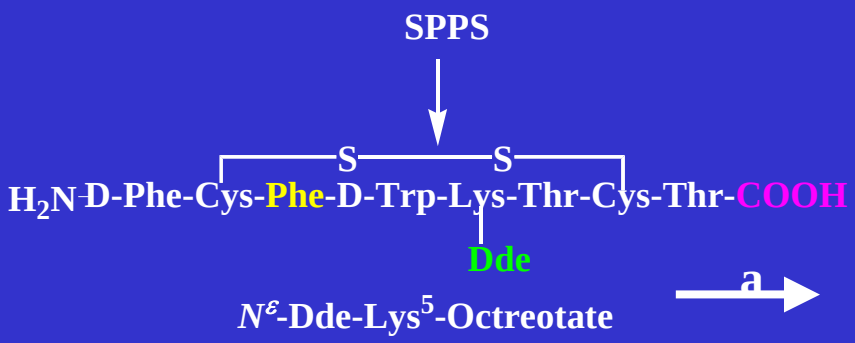
GluTOCA



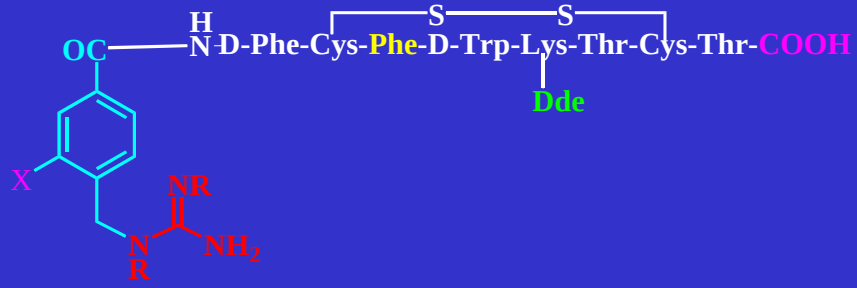
N-(4-Guanidinomethyl-3-iodobenzoyl)Phe^{1}-Octreotate (GMIBO)

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

SPPS



a



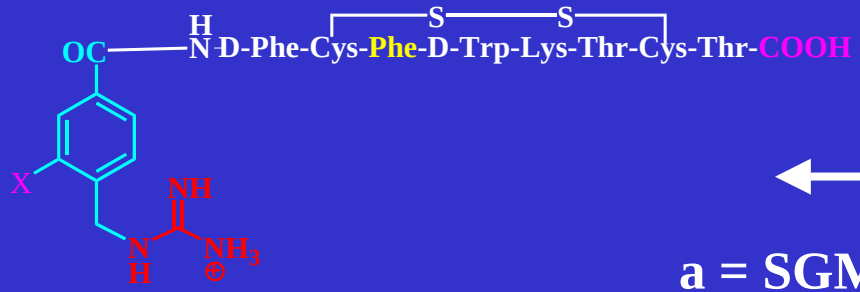
b

GMIBO

Boc-Dde-GMIBO; X = I, R = Boc

Boc-Dde-GMTMSBO: X = $SnMe_3$, R = Boc

c



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

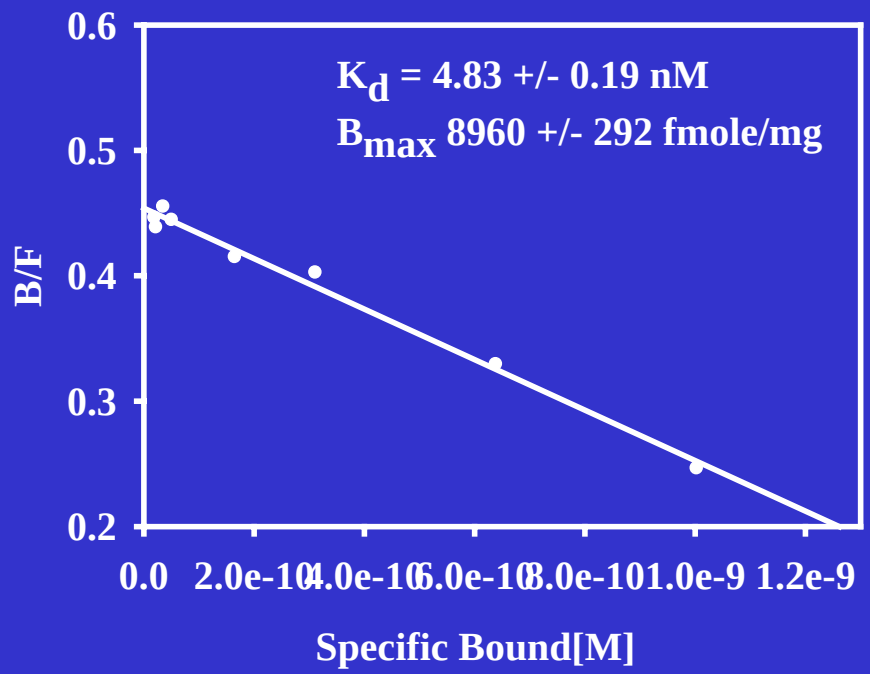
[^{211}At]AGMBO; X = ^{211}At (15-20%)

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

c = i) ethanolamine ii) radioiodine or ^{211}At, 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 [^{131}I]Glu-TOCA and [^{125}I]GMIBO by D341 Med Cells

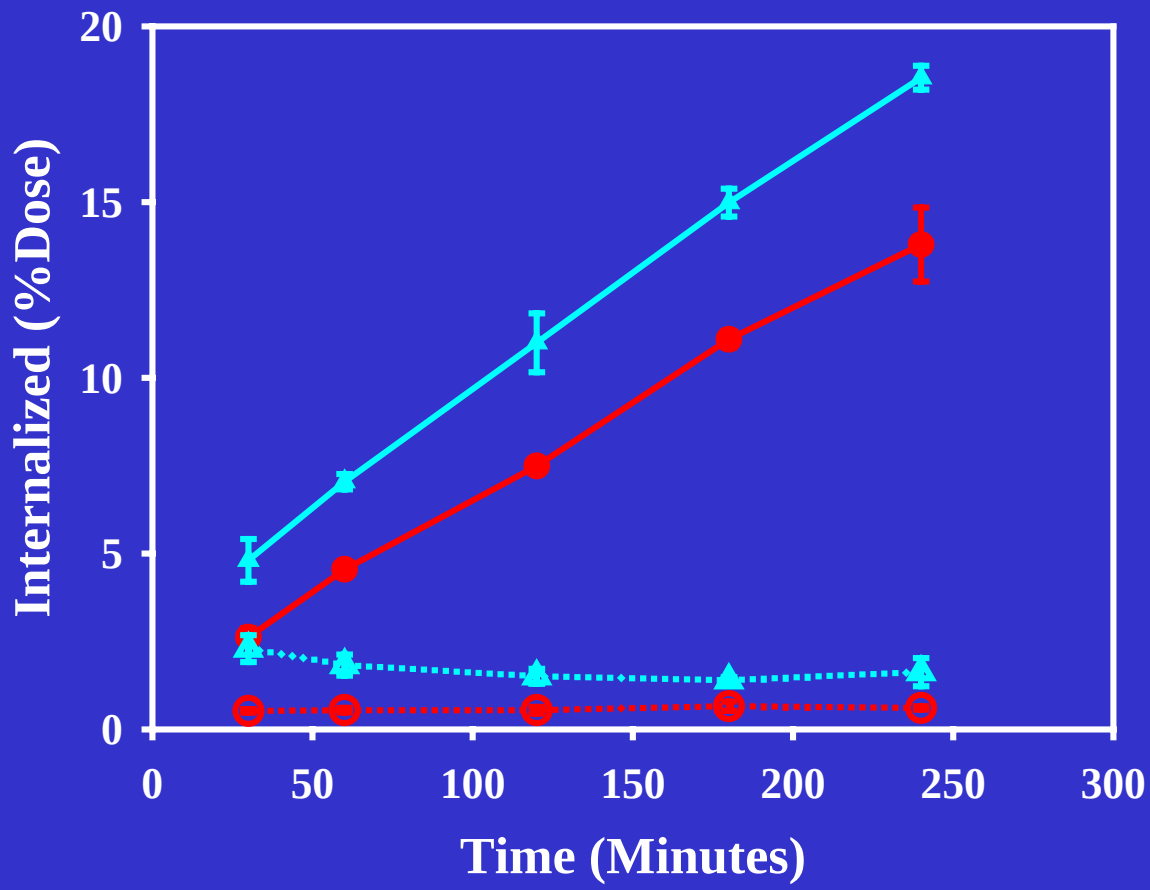
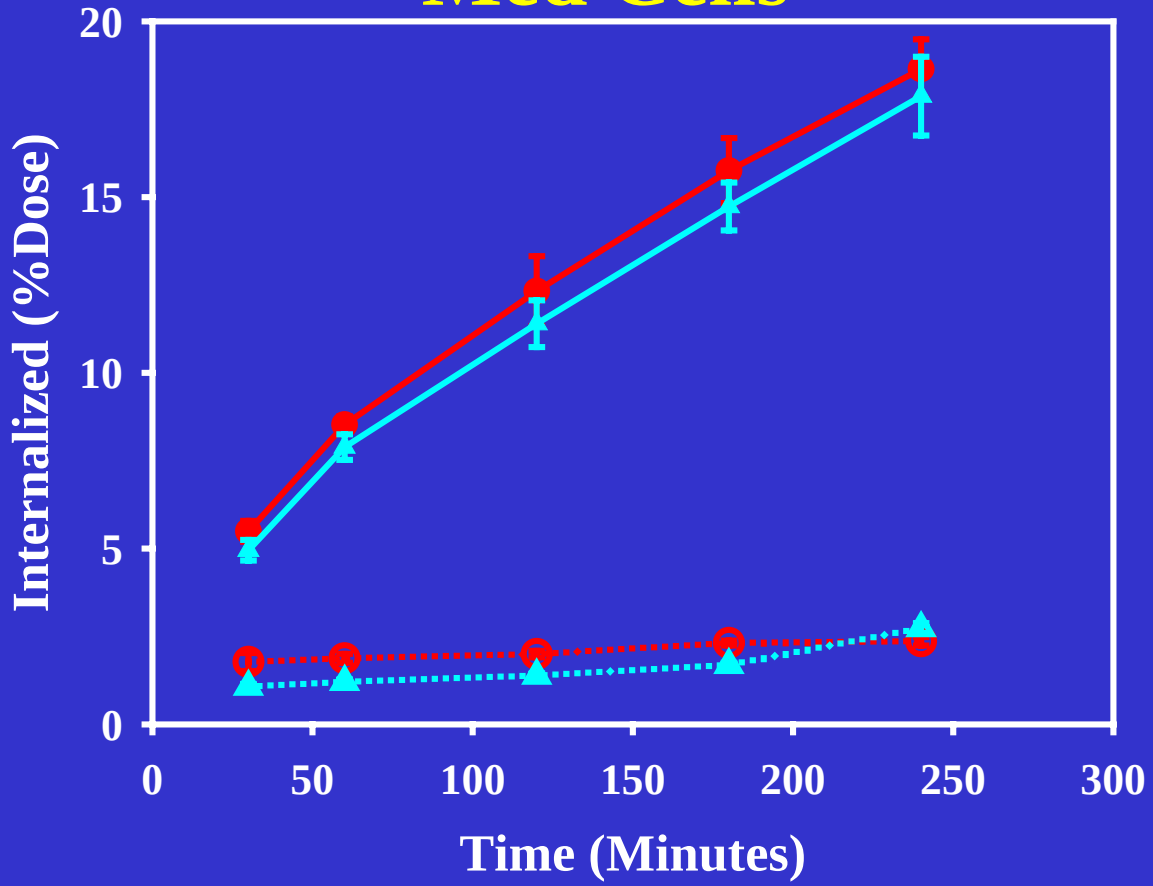


Figure 5. Paired-label Internalization of [^{131}I]GMIBO and [^{211}At]AGMBO by D341 Med Cells



[^{211}At]AGMBO

[^{211}At]AGMBO + Octreotide

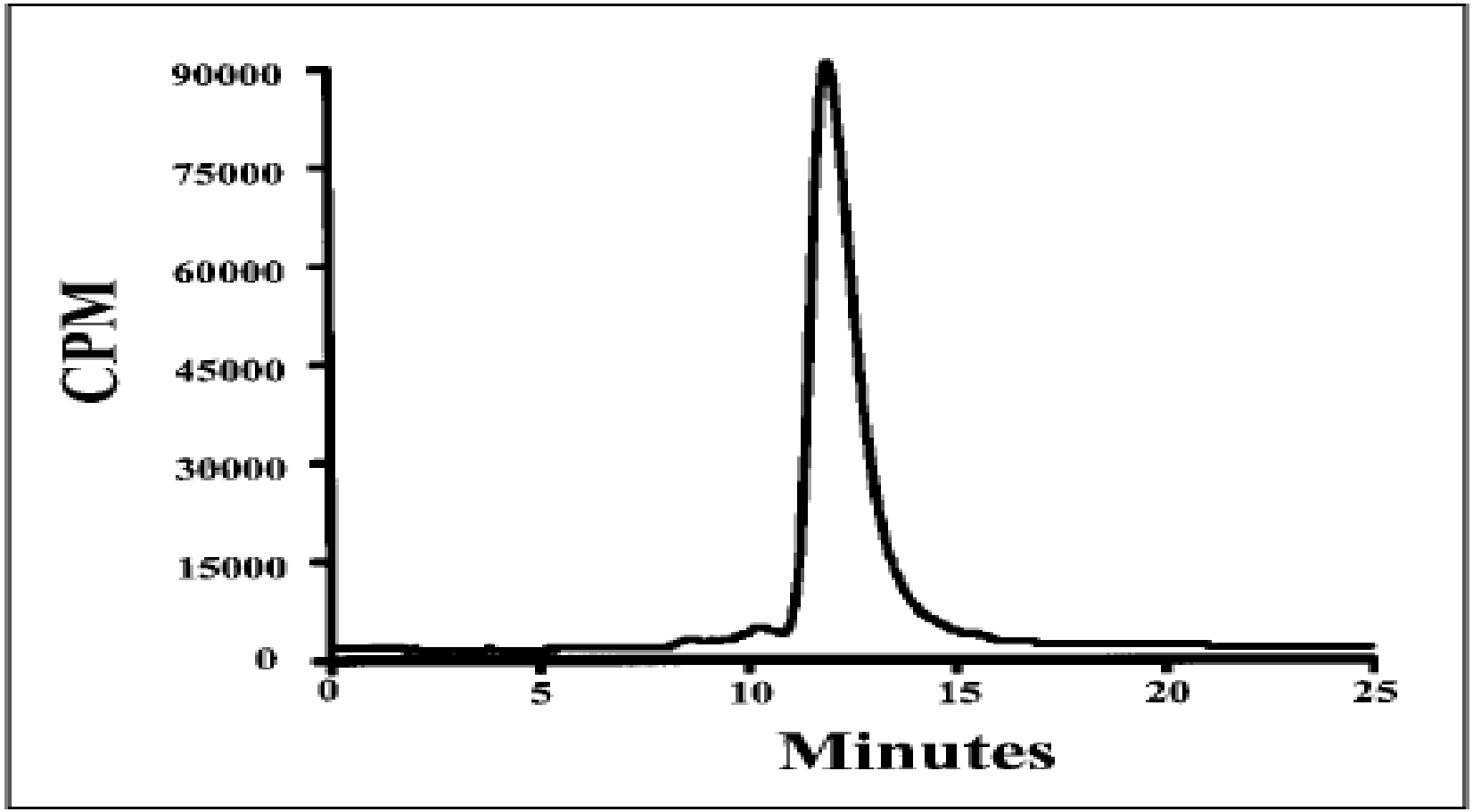
[^{131}I]GMIBO

[^{131}I]GMIBO + Octreotide

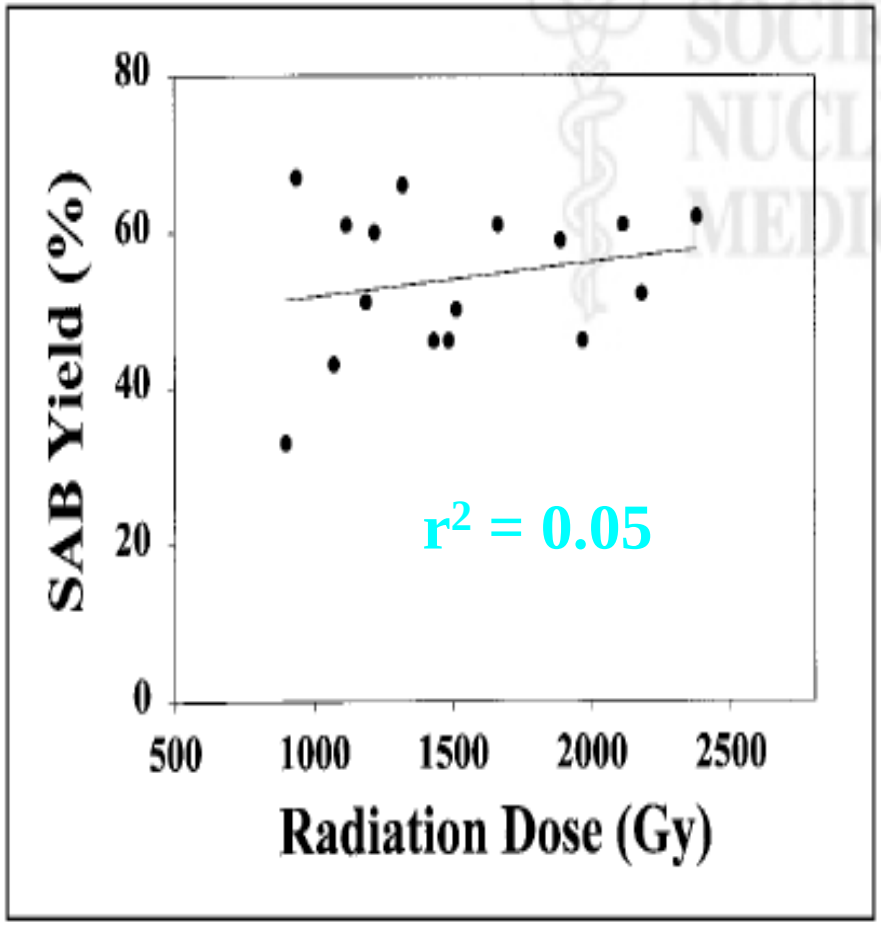
Summary

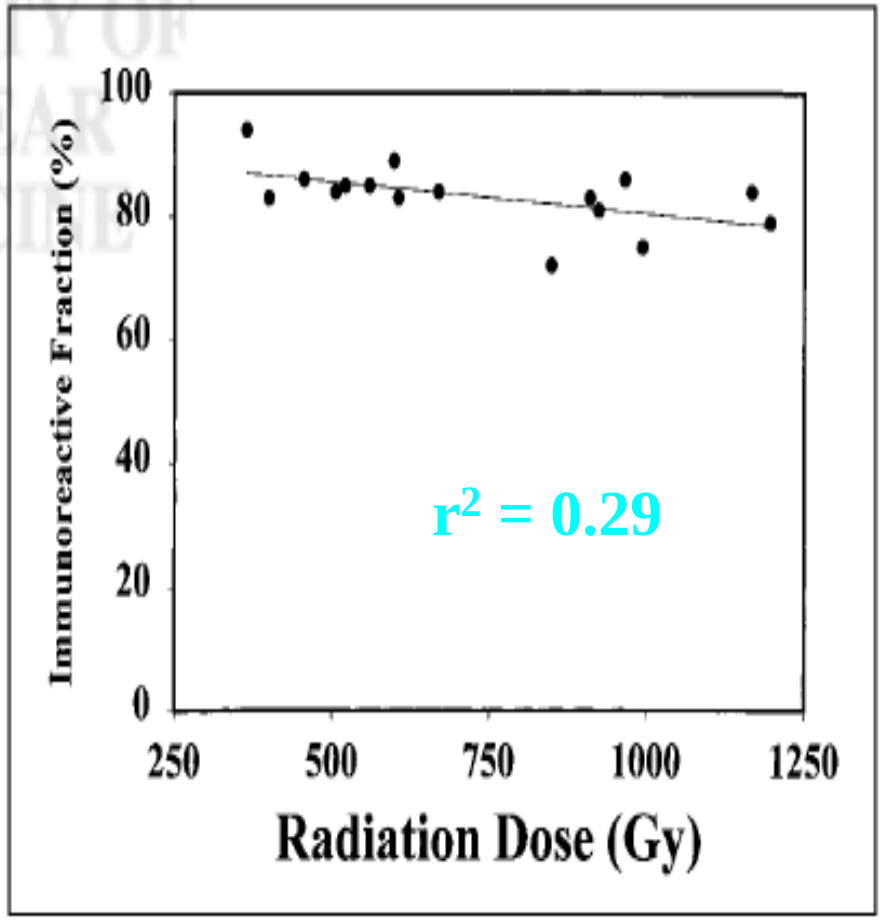
- Astatine-211 in quantities sufficient for the synthesis of clinical batches of astatinated radiopharmaceuticals can be produced
- Synthesis of a number of astatinated radiopharmaceuticals, some in quantities sufficient for clinical trails have been achieved
- Radiolysis may be the biggest issue; split 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 × 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
  - **100 mCi**
  - **1 cyclotron × 1 run**
- **Phase I/II clinical:**
  -25 centers
  -1 patient/wk
  -10 mCi/dose
  - **250 mCi**
  - **1 cyclotron × 2 runs**
- **Clinical:**
  -100 centers
  -3 patients/wk
  -10 mCi/dose
  - **3,000 mCi**
  - **6 cyclotrons × 3 runs**

Astatine-211 Rationale

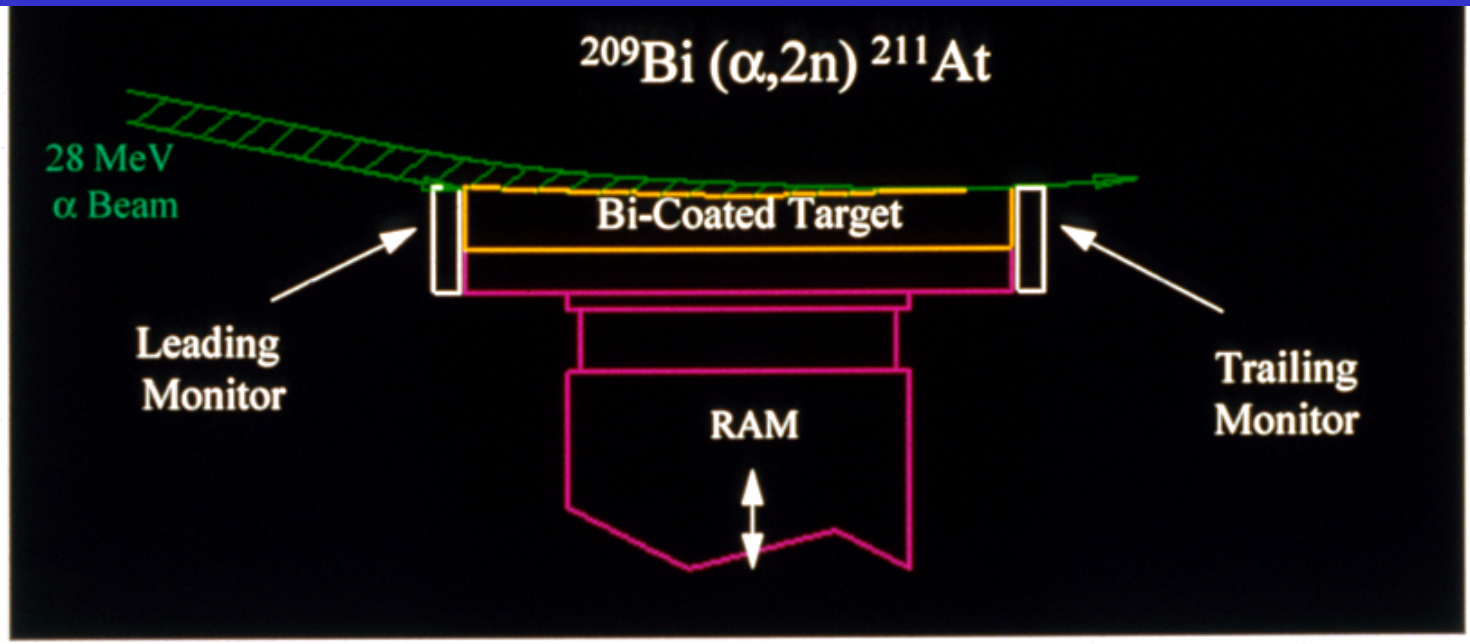
- 7.2 h Half life
- α-emission with each decay
- Po K x-rays for imaging

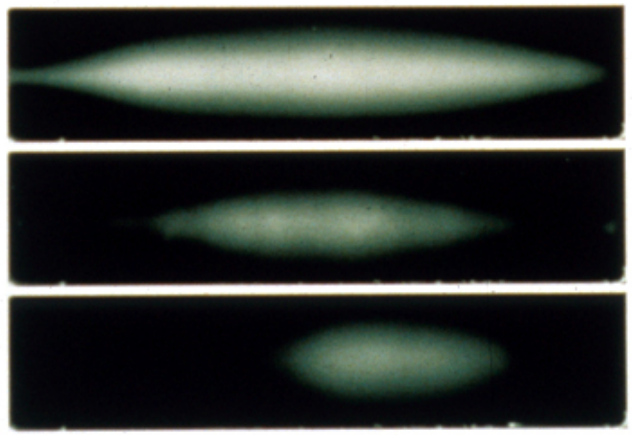
- Half life compatible with variety of carriers
- Chemistry amenable to biomolecules
- Minimal problem with transformation during decay

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