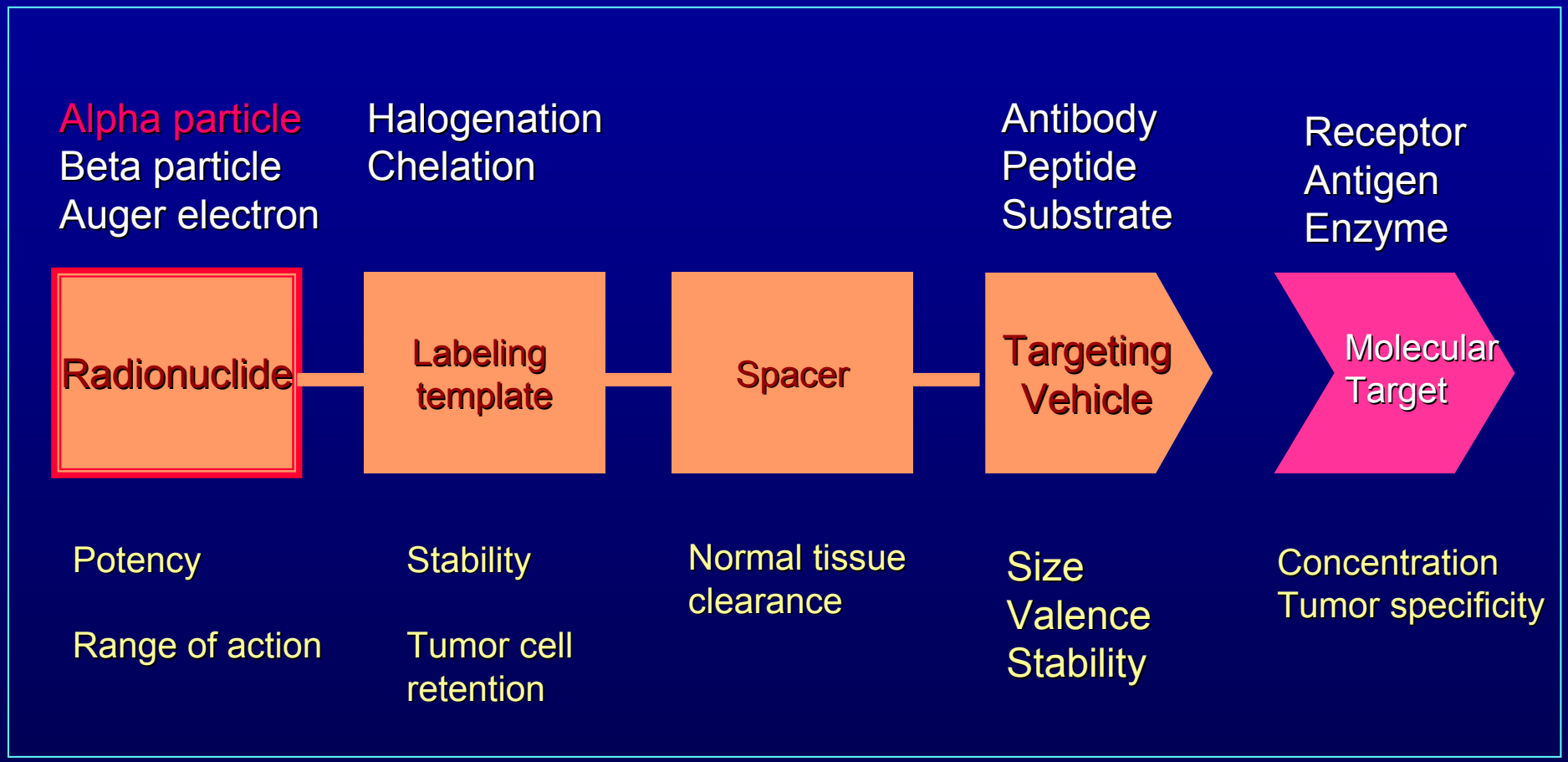
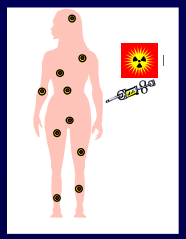
Targeted Radiotherapeutics



The characteristics of the radioactive drug can be varied to optimize the treatment of a particular type of cancer and ultimately, the needs of an individual patient

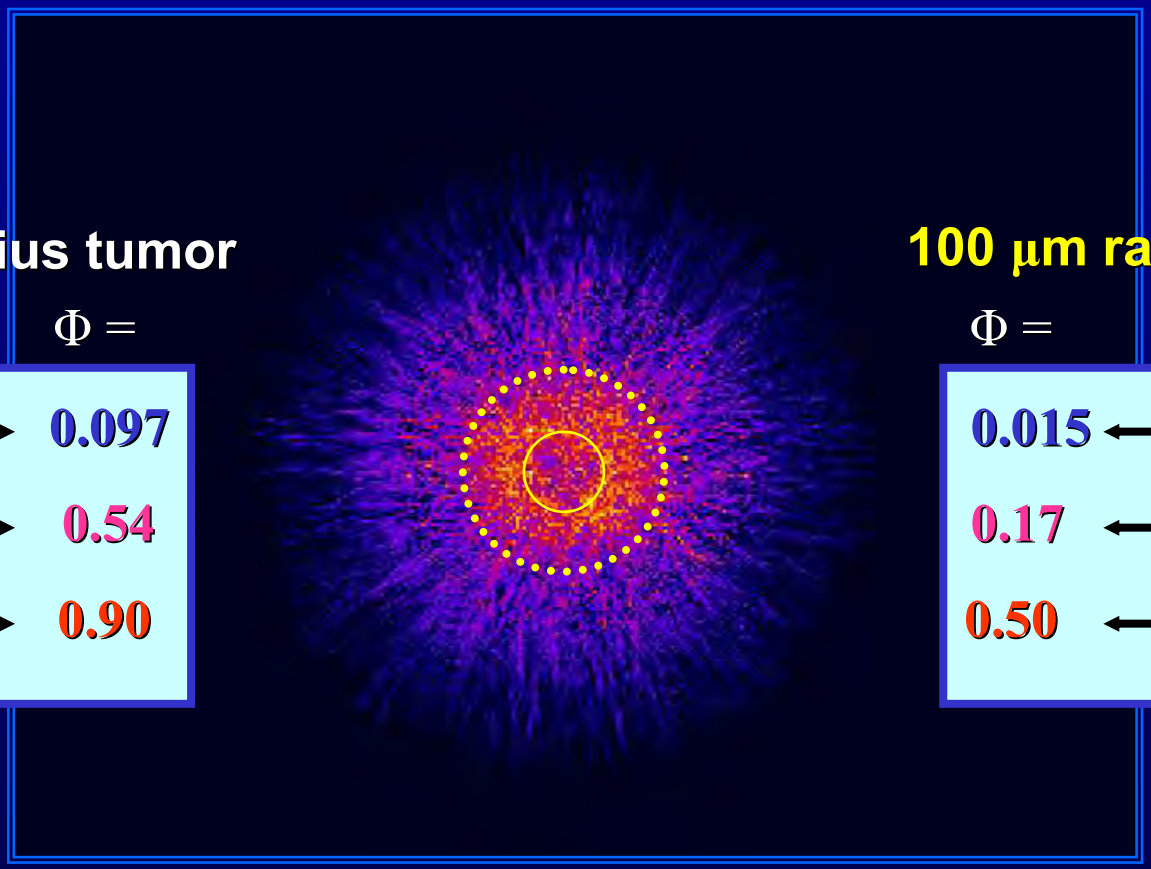
Radionuclides for Targeted Radiotherapy

Decay Mode	Range	LET	Example
Beta	Multi-cellular 0.5-15 mm	Low 0.2 kev/μm	^{131}I
Alpha	Cellular 30-80 μm	High 100 kev/μm	^{211}At
Auger electron	Subcellular <0.1 μm	High/Low	^{125}I



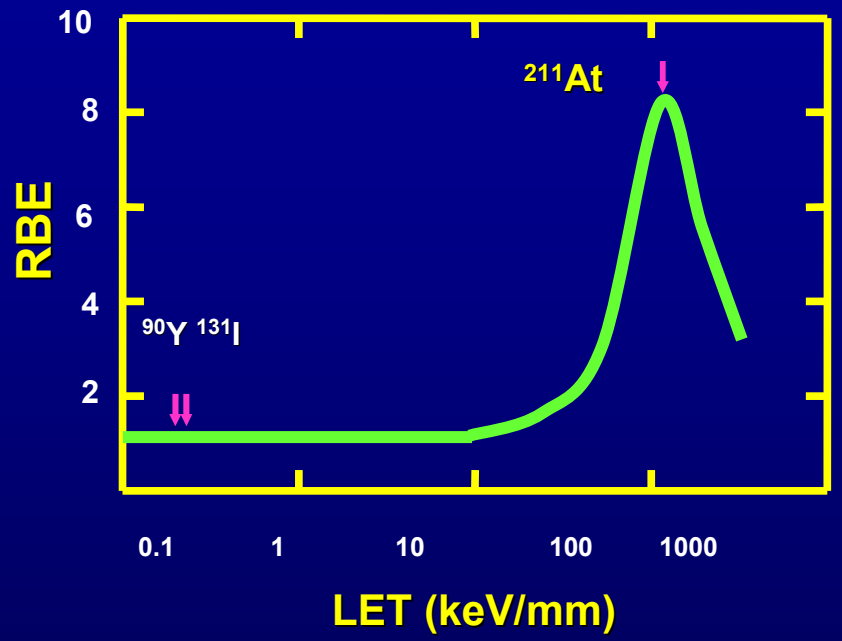
Rationale for α Emitters: Particle Range

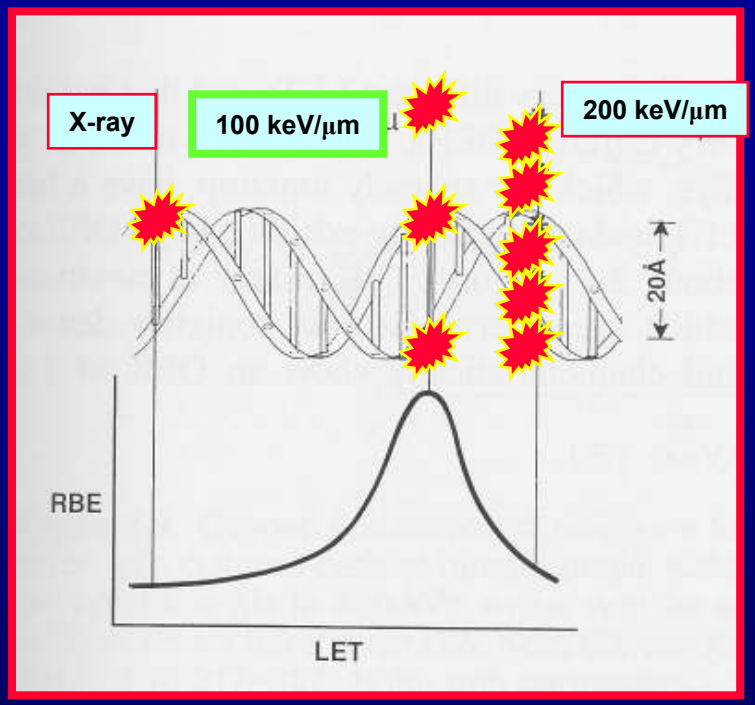
Minimum residual disease settings are where targeted radiotherapy has the best opportunity of having a meaningful clinical impact



Particularly for smaller tumors, use of short range alpha particles is optimal

Rationale for α Emitters: LET



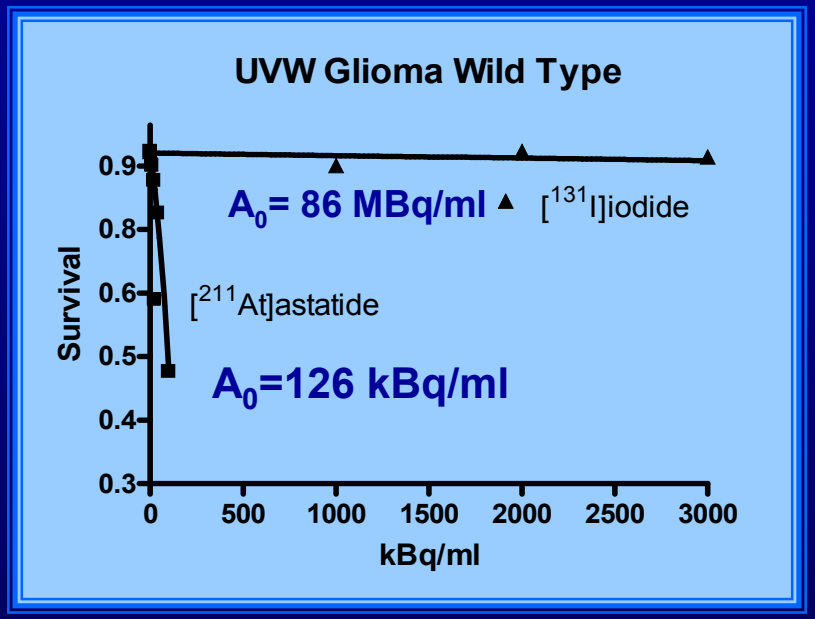


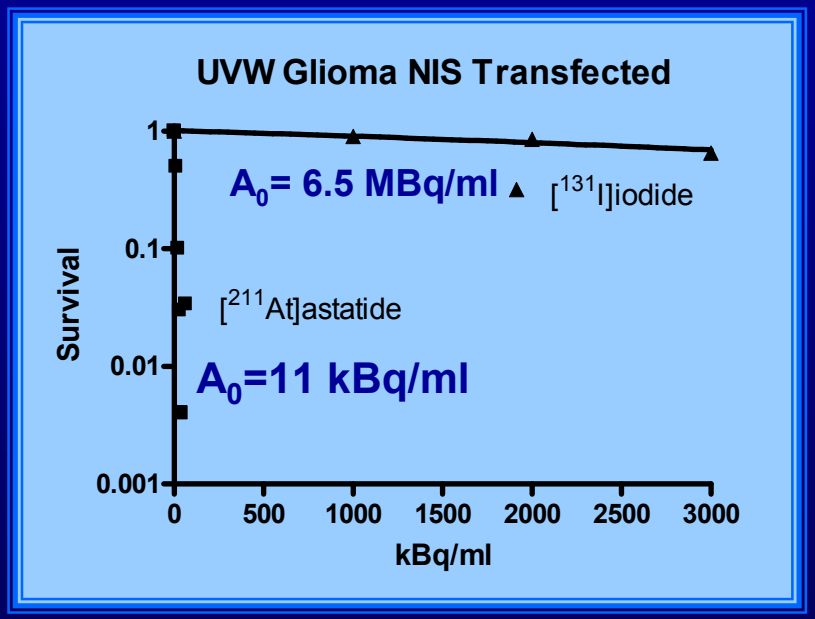
- RBE is highest at about 100 keV/mm
  - the average distance between ionizing events is about the same as diameter of DNA double helix
- Highest probability of DNA double strand breaks

Alpha Particles Are More Effective in Killing Glioma Cells than Beta Particles

Increased cytotoxicity mediated By NIS specific uptake

Based on Radioactivity Concentration





Based on Radiation Dose

	^{131}I (Gy)	^{211}At (Gy)	Toxicity Ratio
UVW	5.27	0.27	19.5
NIS6	4.75	0.28	17.0

Carlin et al. JNM, 2003

Selected α-Particle Emitting Radionuclides

Radionuclide	Daughters	Half-life	α-particle Energy (MeV)	Yield per 100 decays
^{149}Tb		4.15 h	3.97	17
^{211}At		7.21 h	5.87	42
	^{211}Po	516 msec	7.44	58
^{212}Bi		61 min	6.05	36
	^{212}Po	298 nsec	8.78	64
^{213}Bi		45.6 min	5.84	36
	^{213}Po	4.2 sec	8.38	64
^{225}Ac		10 days	5.75	100
	^{221}Fr	4.9 min	6.36	100
	^{217} At	32 msec	7.07	100
	^{213}Bi	45.6 min	5.84	2
	^{213}Po	4.2 sec	8.38	98

Clinical Settings for α-Particle Radiotherapy

- Minimal Residual Disease
  - Location of tumor difficult to detect (XRT difficult)
  - Smaller size minimizes heterogeneity problems
- Compartmentally-Spread Cancers
  - Neoplastic meningitis
  - Ovarian cancer
  - Non-resected tumor margins

Bismuth-213

^{225}Ac

10 d

^{221}Fr

4.8 m

^{217}At

0.032 s

^{213}Bi

46 m

^{213}Po

0.004 ms

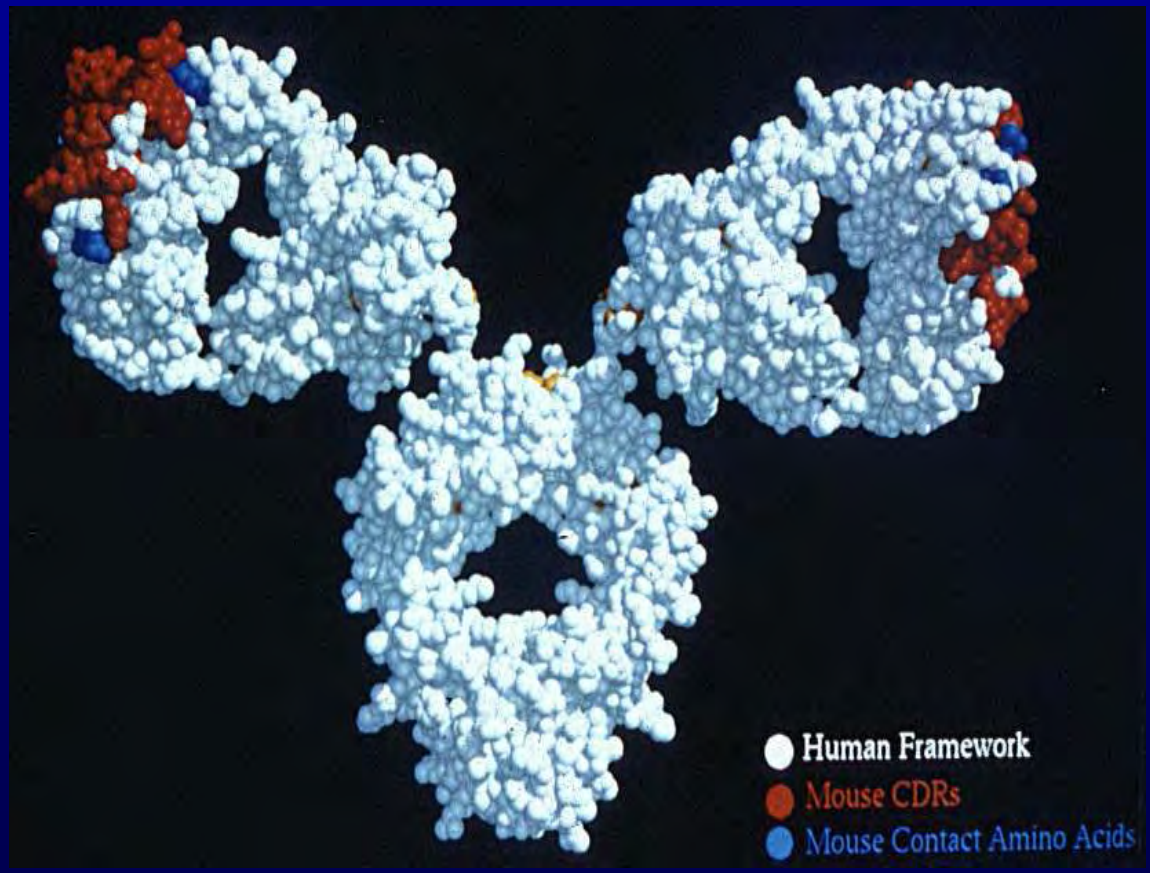
^{209}Pb

3.3 h

^{209}Bi

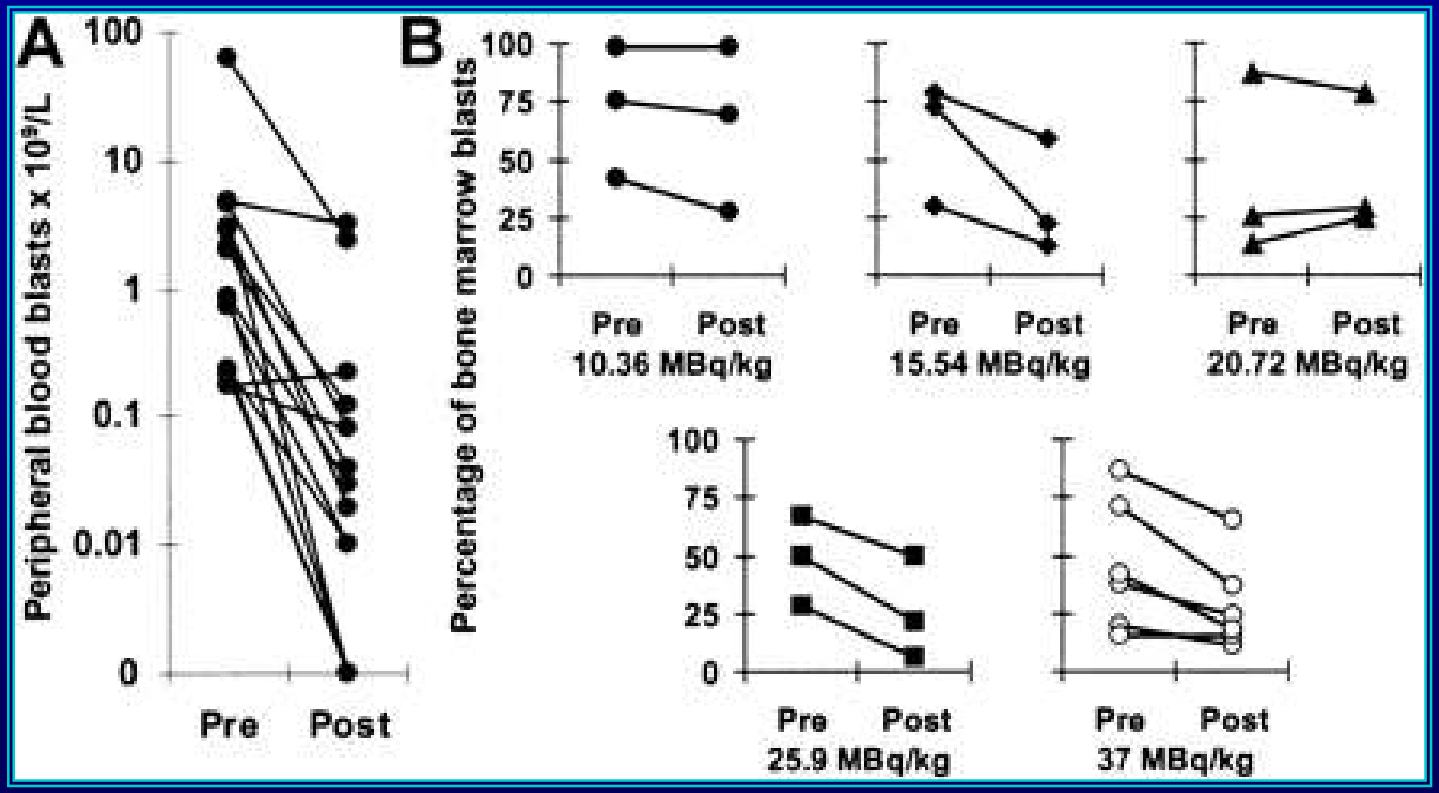
Bi-213-Labeled Hu195

- Reacts with CD33 antigen over expressed on acute myelogenous leukemia
- CHX-A-DTPA chelate



Scheinberg, MSKCC

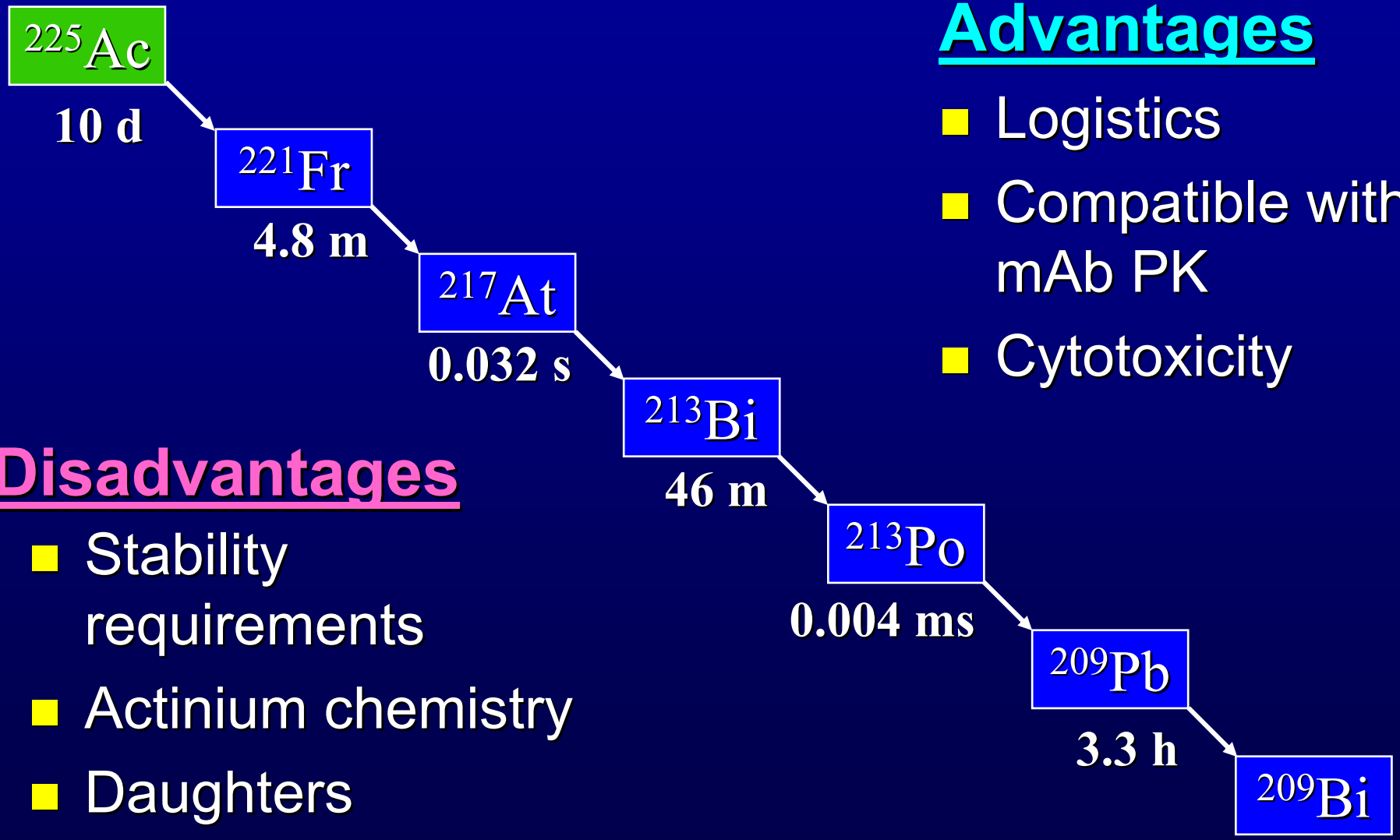
Anti-Leukemic Effects of ^{213}Bi-Hu195



A) 14 of 15 evaluable patients had reduction in the number of peripheral blood blasts

B) 14 of 18 patients had reductions in the percentages of bone marrow leukemia cells after 7 to 10 days

Actinium-225



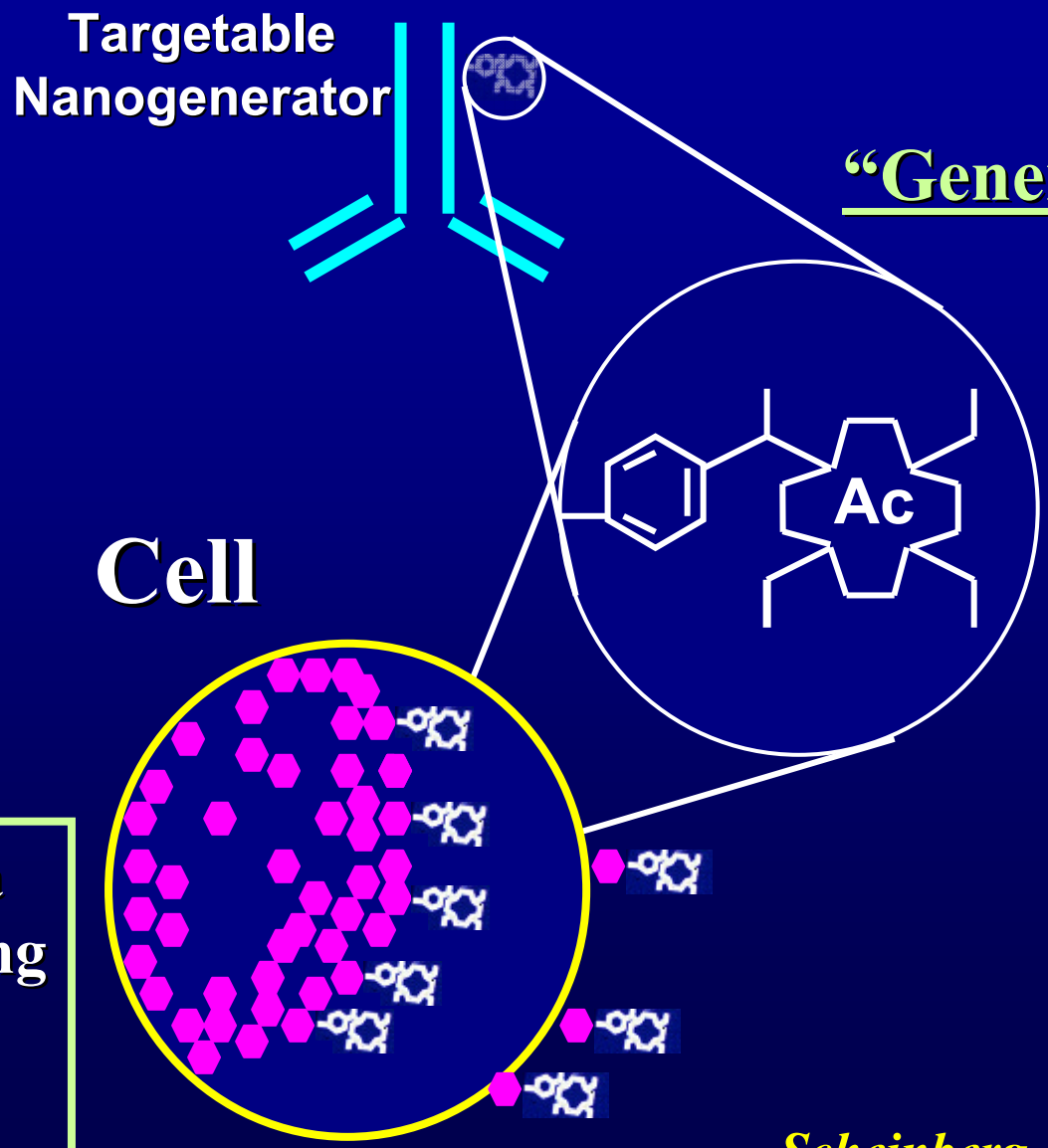
Advantages

- Logistics
- Compatible with mAb PK
- Cytotoxicity

Disadvantages

- Stability requirements
- Actinium chemistry
- Daughters

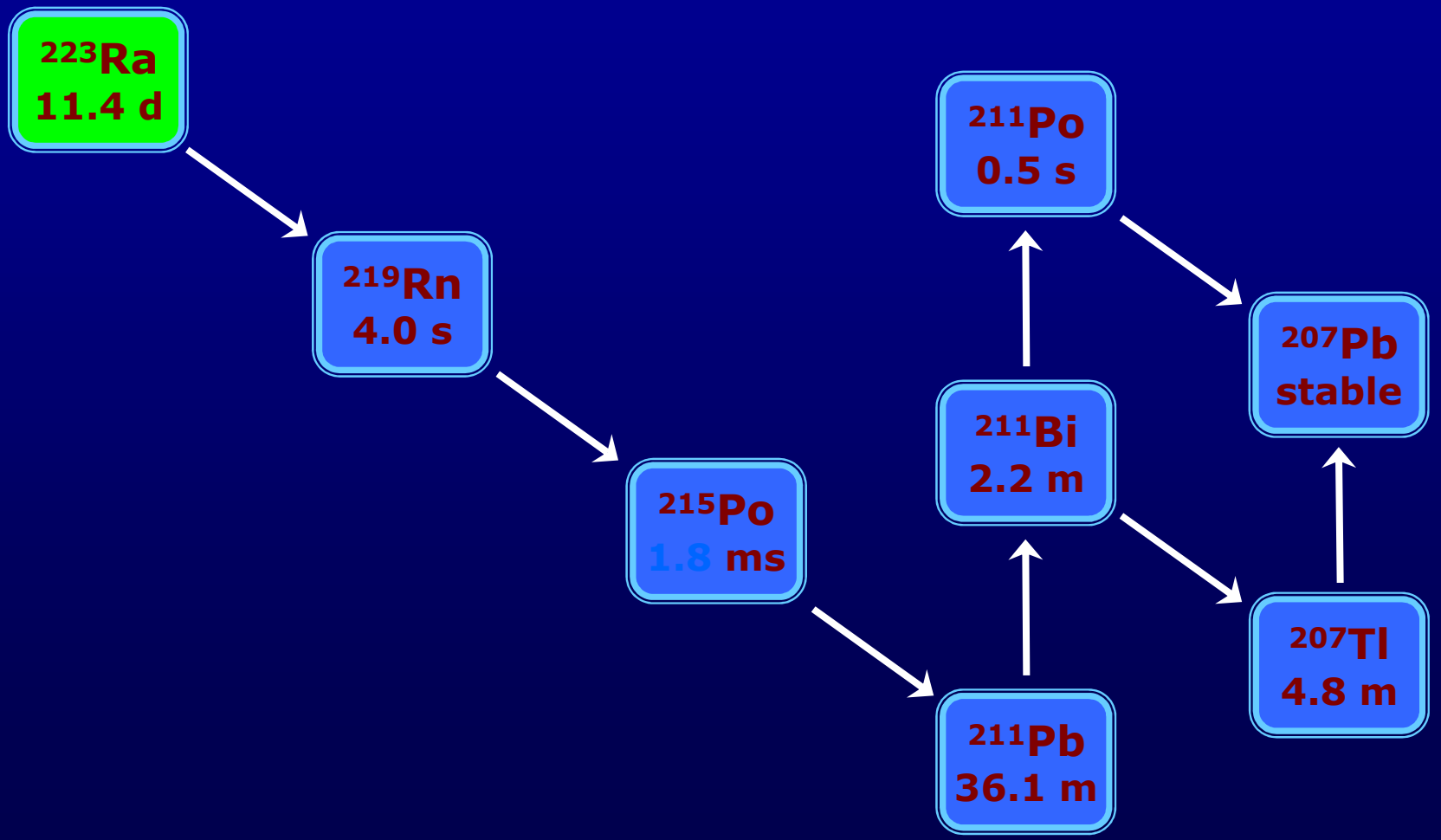
Targetable Atomic Nanogenerators



Internalization as a strategy for trapping daughter radionuclides

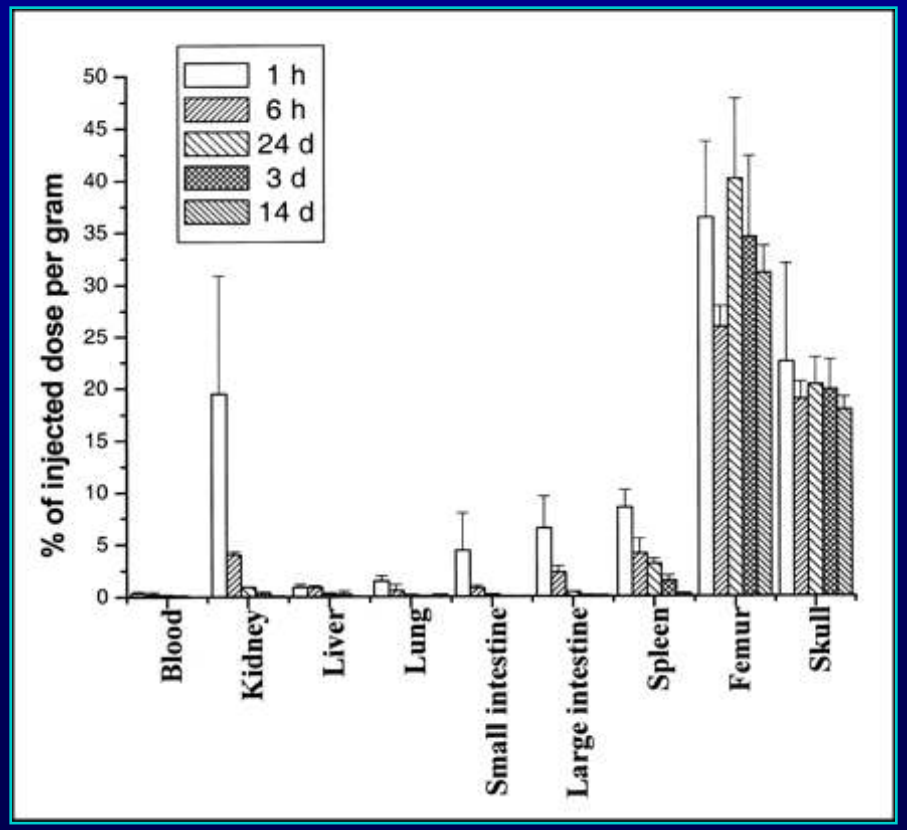
Scheinberg, MSKCC

Radium-223 Decay Cascade

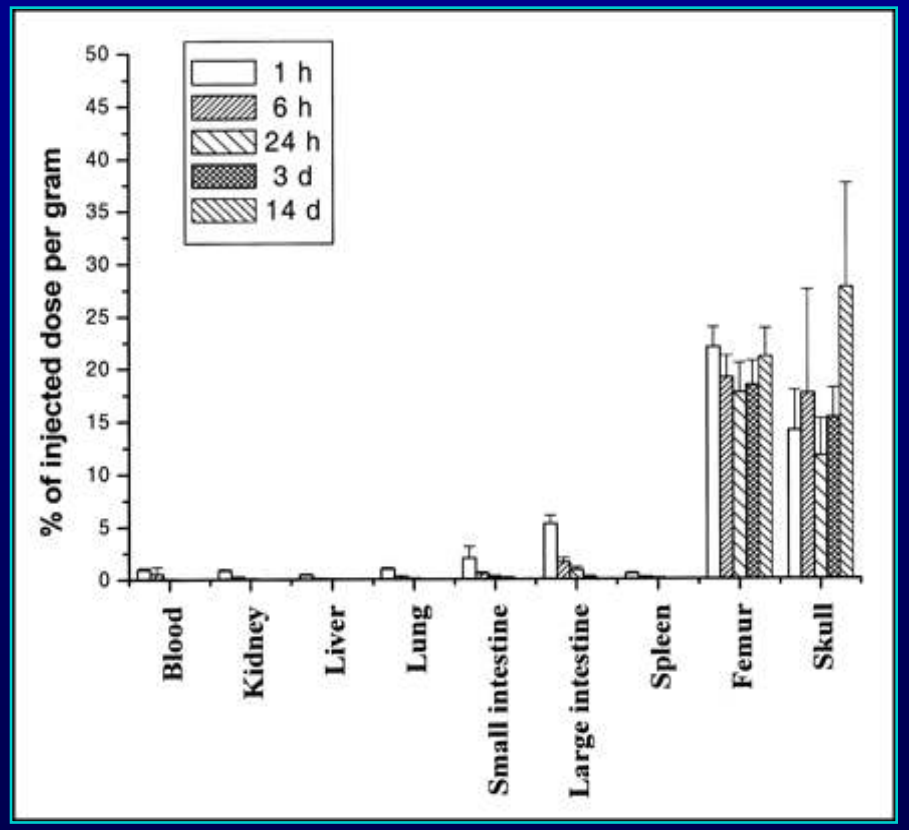


Tissue Distribution of $^{223}RaCl_2$ and $^{89}SrCl_2$ in Mice

^{223}Ra



^{89}Sr



Henriksen et al, JNM 2003

Completed phase I clinical study with Alpharadin ($^{223}RaCl_2$)

- *Experimental Design:* 15 prostate and 10 breast cancer patients enrolled in a phase I trial received a single iv. injection of ^{223}Ra.
- Five patients were included at each of the dosages; 46, 93, 163, 213 or 250 kBq/kg and followed for 8 weeks.
- Palliative response was evaluated according to the pain scale of the EORTC-QLQ C30 questionnaire at baseline and at 1, 4 and 8 weeks after injection.

Nilsson et al., Clin Cancer Res 2005

Results from ^{223}Ra phase I clinical trial

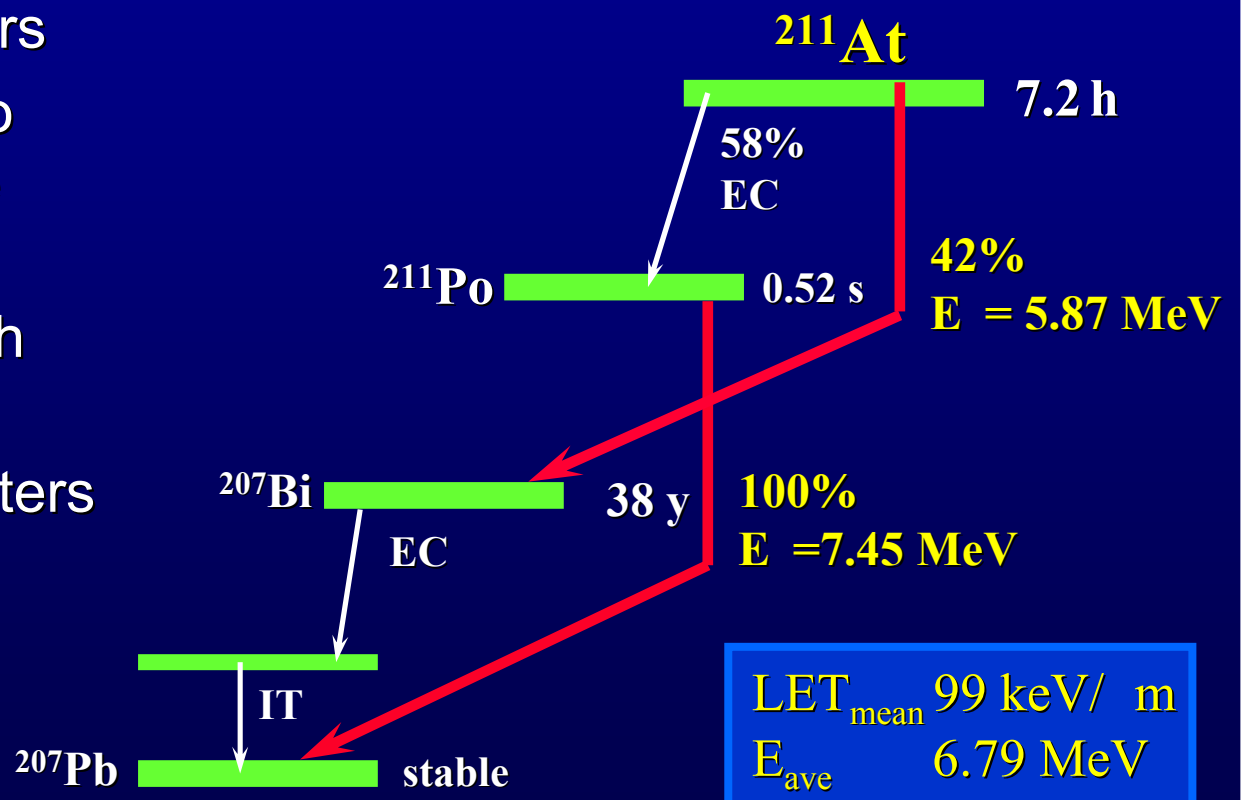
- Rapid blood clearance observed
- No dose limiting toxicity
- Strong reduction in ALP levels, particularly in patients with elevated levels due to osteoblastic metastases (ALP-alkaline phospatase, a biomarker often elevated due to skeletal metastases)
- Responses in the form of pain reduction and/or reduction in ALP levels observed in the majority of the patients
- No significant reduction in quality of life due to the treatment

Nilsson et al., Clin Cancer Res 2005

Astatine-211

Rationale

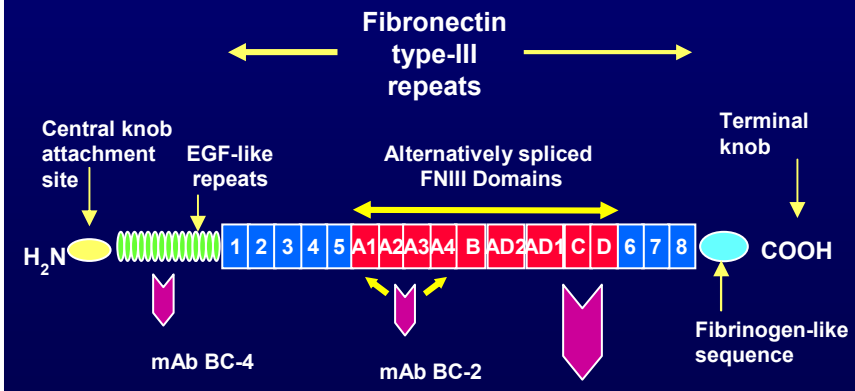
- 7.2 hr half-life compatible with variety of carriers
- Chemically similar to iodine but with more metallic character
- α-emission with each decay
- No long-lived daughters
- Po K x-rays permit imaging



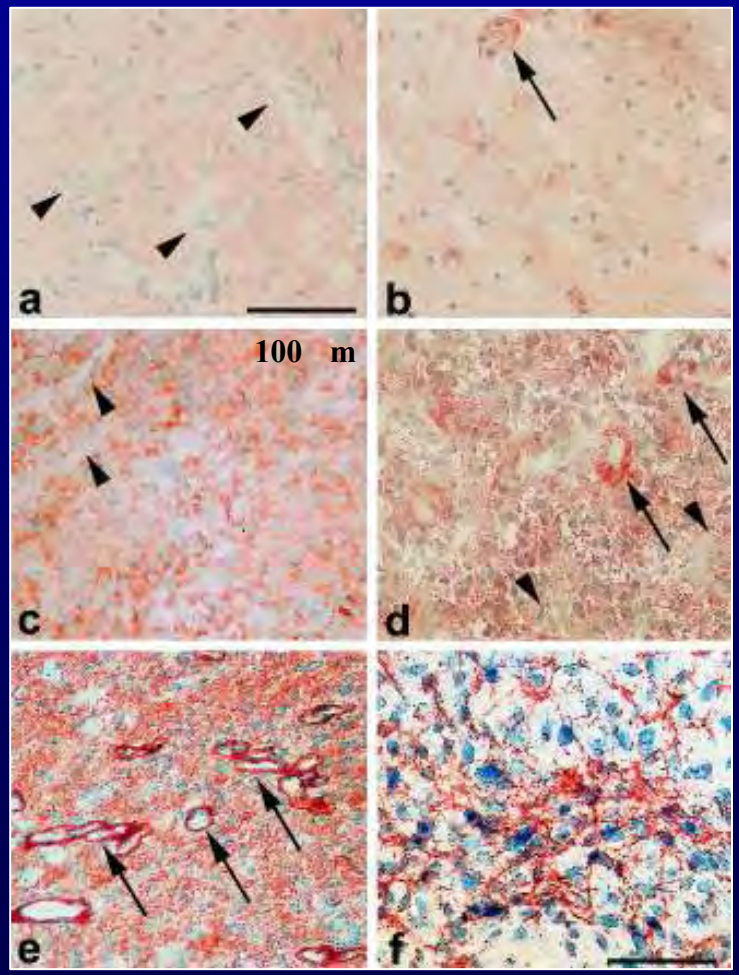
LET_{mean}	99 keV/ m
E_{ave}	6.79 MeV
range	55-70 m

Tenascin Expression in Brain Tumors

- Extracellular matrix glycoprotein
- Expressed on >95% of GBM
- Hexamer with 200-300 kDa arms



mAb 81C6



WHO Grade II

Perivascular 11/25

WHO Grade III

Perivascular 9/13

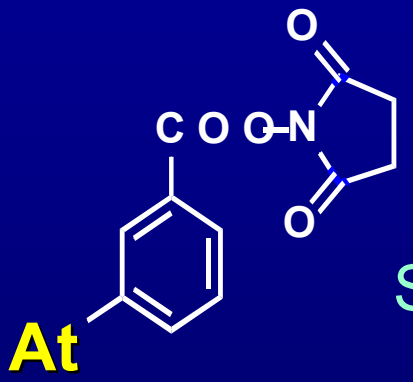
WHO Grade IV

Perivascular 48/48

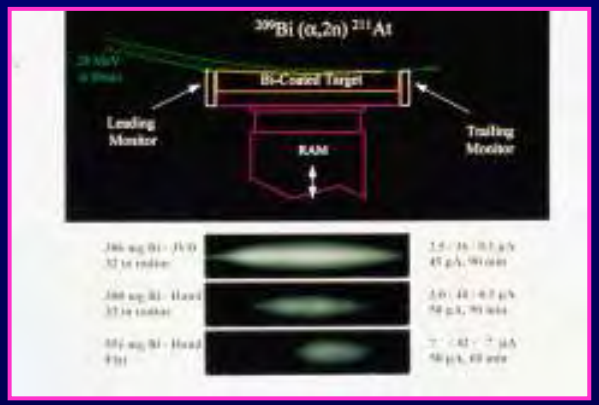
Herold-Mende, 2002

Hurdles to Initiating Clinical Trials with ^{211}At-labeled mAbs

- Labeling method yielding good *in vivo* stability
- Develop capability for high level ^{211}At production and chemistry
- Determination of chronic and acute radiotoxicity

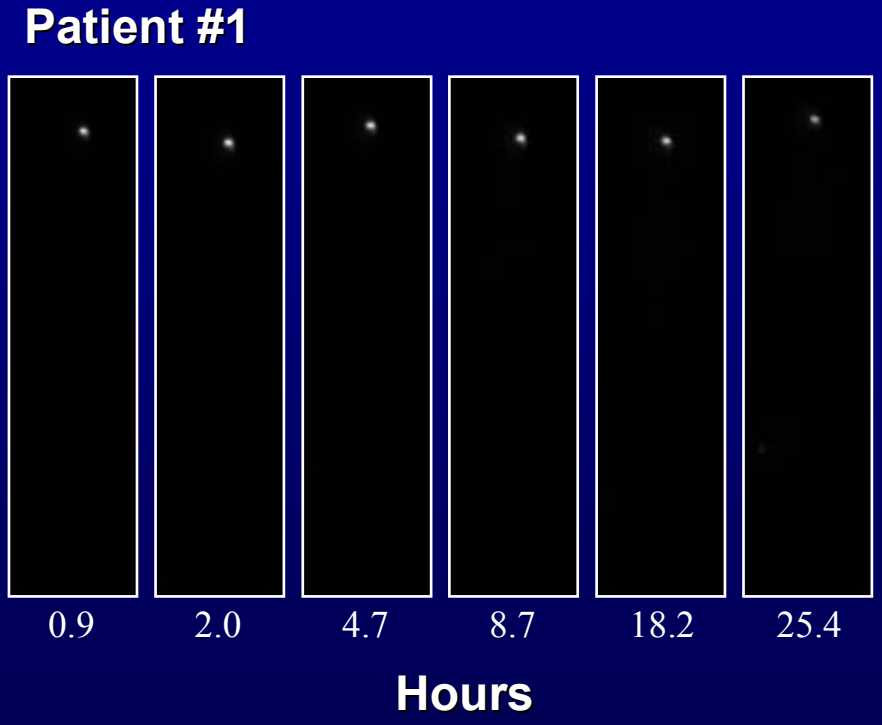


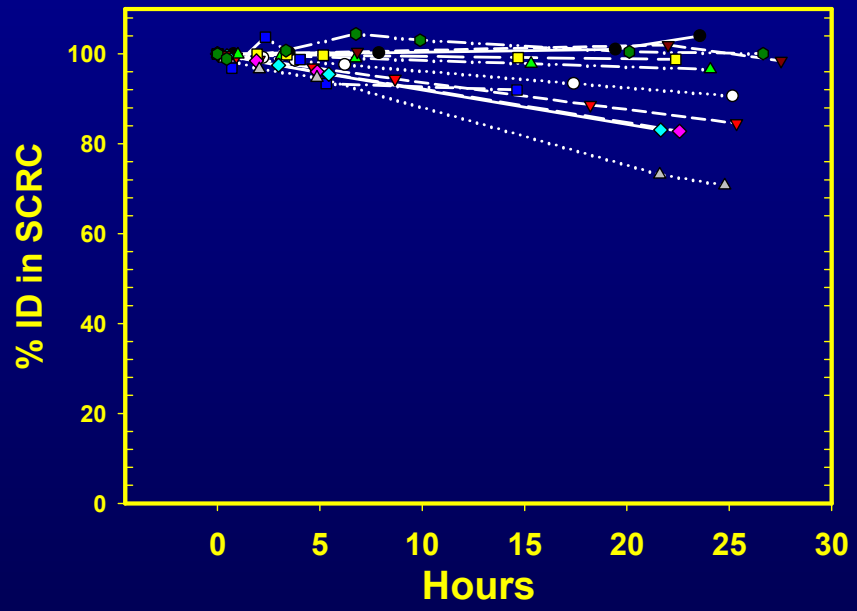
SAB



Internal Target for ^{211}At

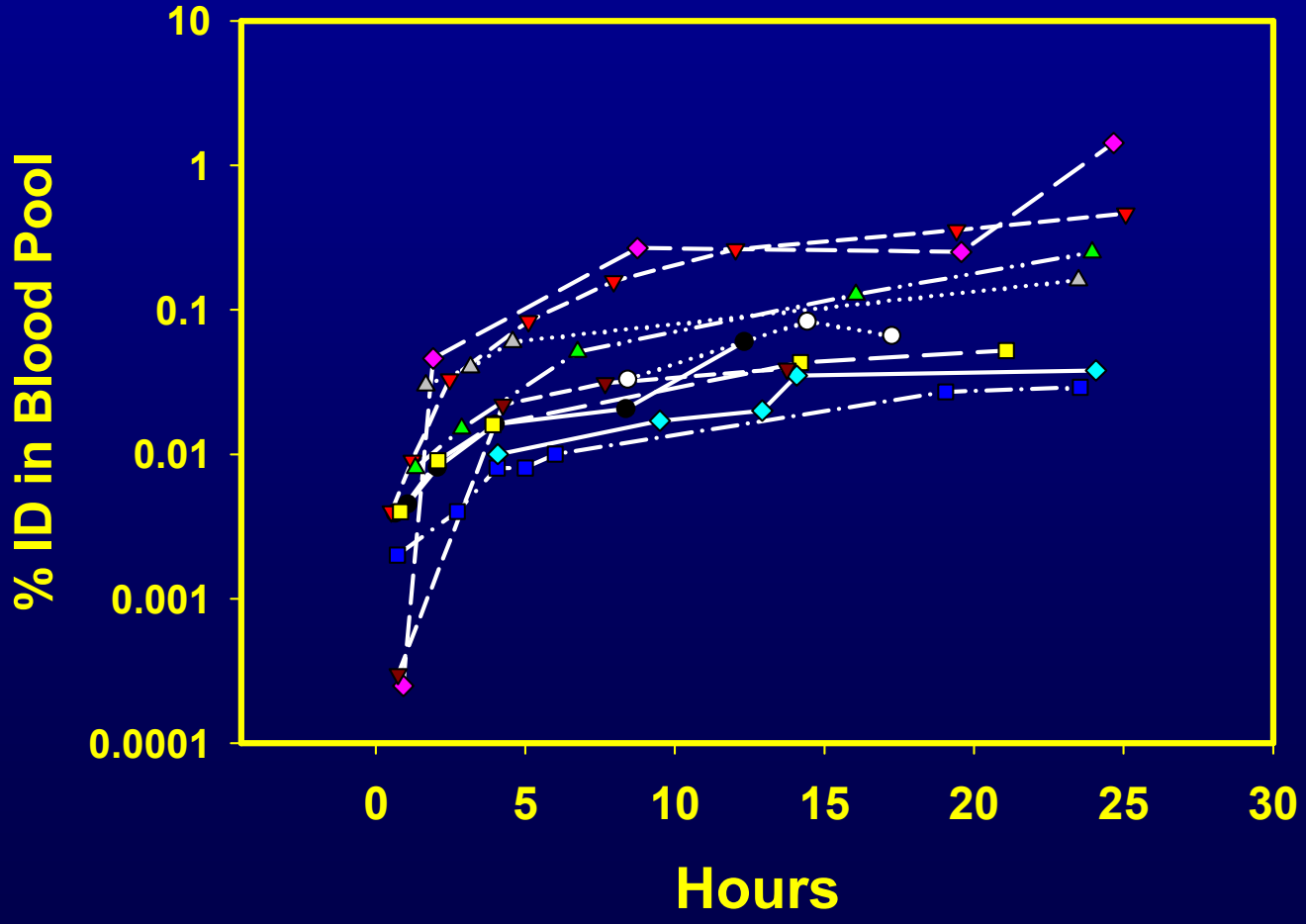
Clearance of ^{211}At from the SCRC





96.7 ± 3.6% ^{211}At decays occur in the SCRC

Blood Pool Activity following Injection of ^{211}At-Labeled Chimeric 81C6 mAb via the SCRC

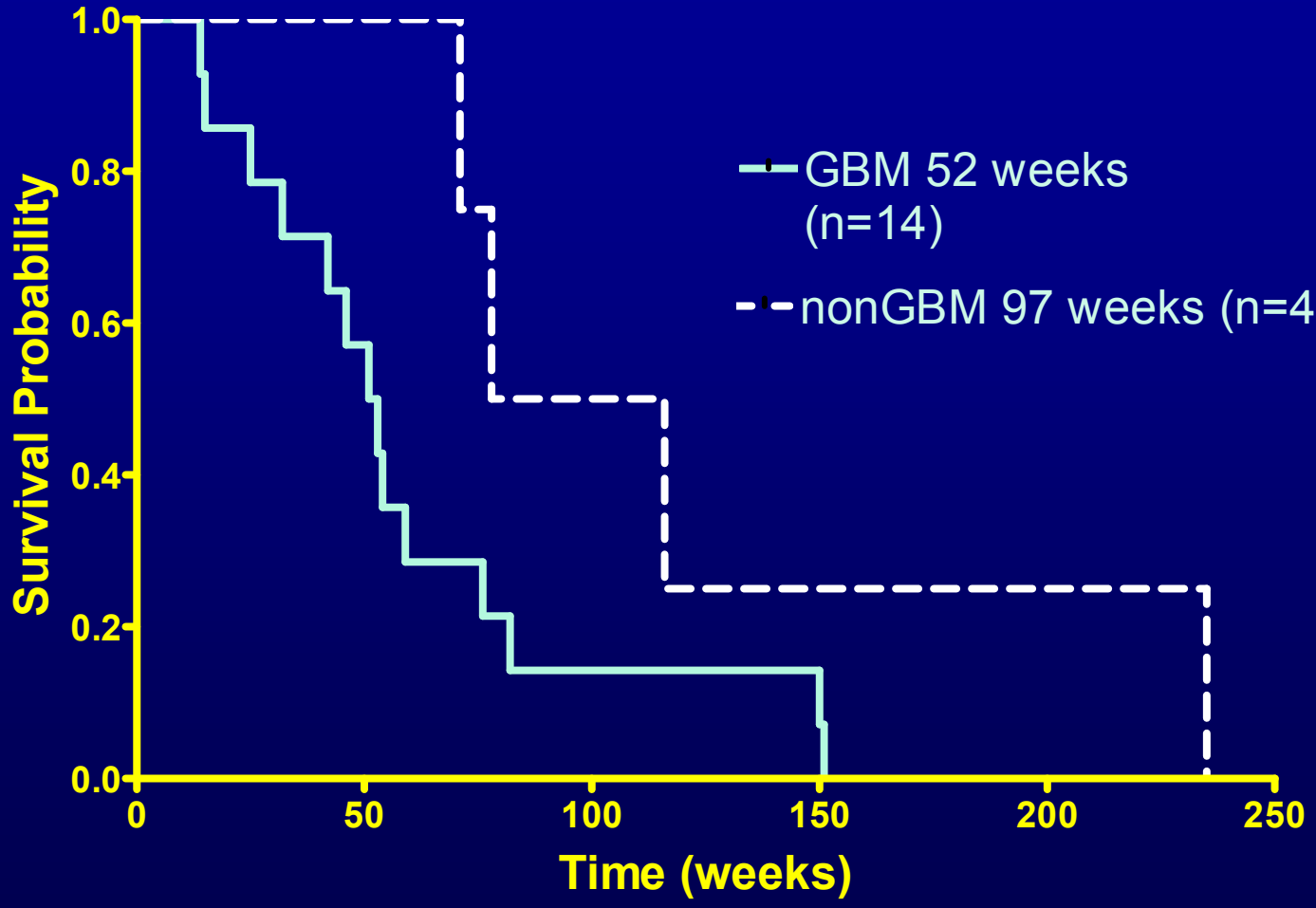


% ID in Blood Pool

Time	mean ± SD
6 h	0.044 ± 0.043
12 h	0.067 ± 0.069
max	0.258 ± 0.432

N = 10

Phase 1 ^{211}At-Labeled Chimeric 81C6 in Recurrent Brain Tumor Patients: Outcome



Historical Control: GBM 31 weeks
Brem et al. 1995

Impediments to the Development of ^{211}At-Labeled Targeted Radiotherapeutics: Radiochemistry

- Decline in labeling yield at higher activity levels observed in clinical trial
- Decline in labeling yields with time of day
- At-211 shipped from one site to another exhibits poor reactivity

Implications:

- ➔ *Preparation of clinical doses unreliable at >350 MBq*
- ➔ *Commercialization not feasible*

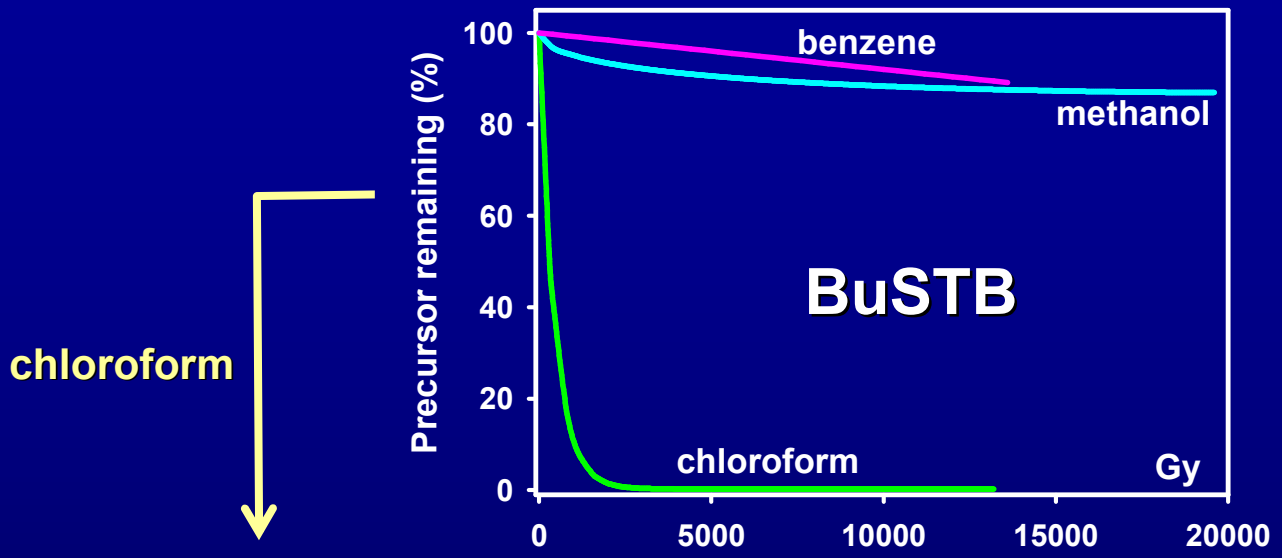
Radiolysis

- Radiation induced decomposition of molecules, generating ions, free radicals and other molecules
- Complex process with product spectrum dependent on type of radiation, dose rate, trace components (O_2, H_2O)
- Can alter redox conditions

Implications for ^{211}At chemistry:

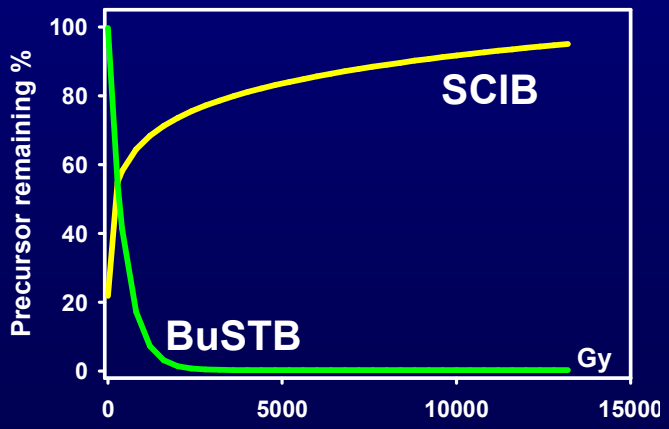
- Decomposition of reaction components
- Reaction of ^{211}At with other species
- Alteration in ^{211}At oxidation state

Effect of Solvent on Radiolysis Induced Loss of Tin Precursor

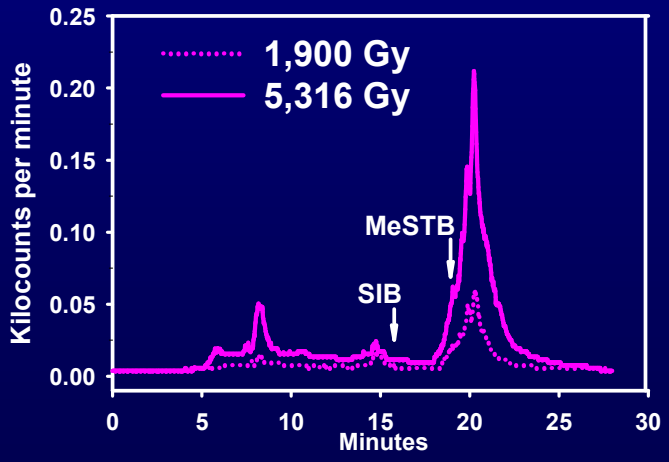


chloroform

benzene



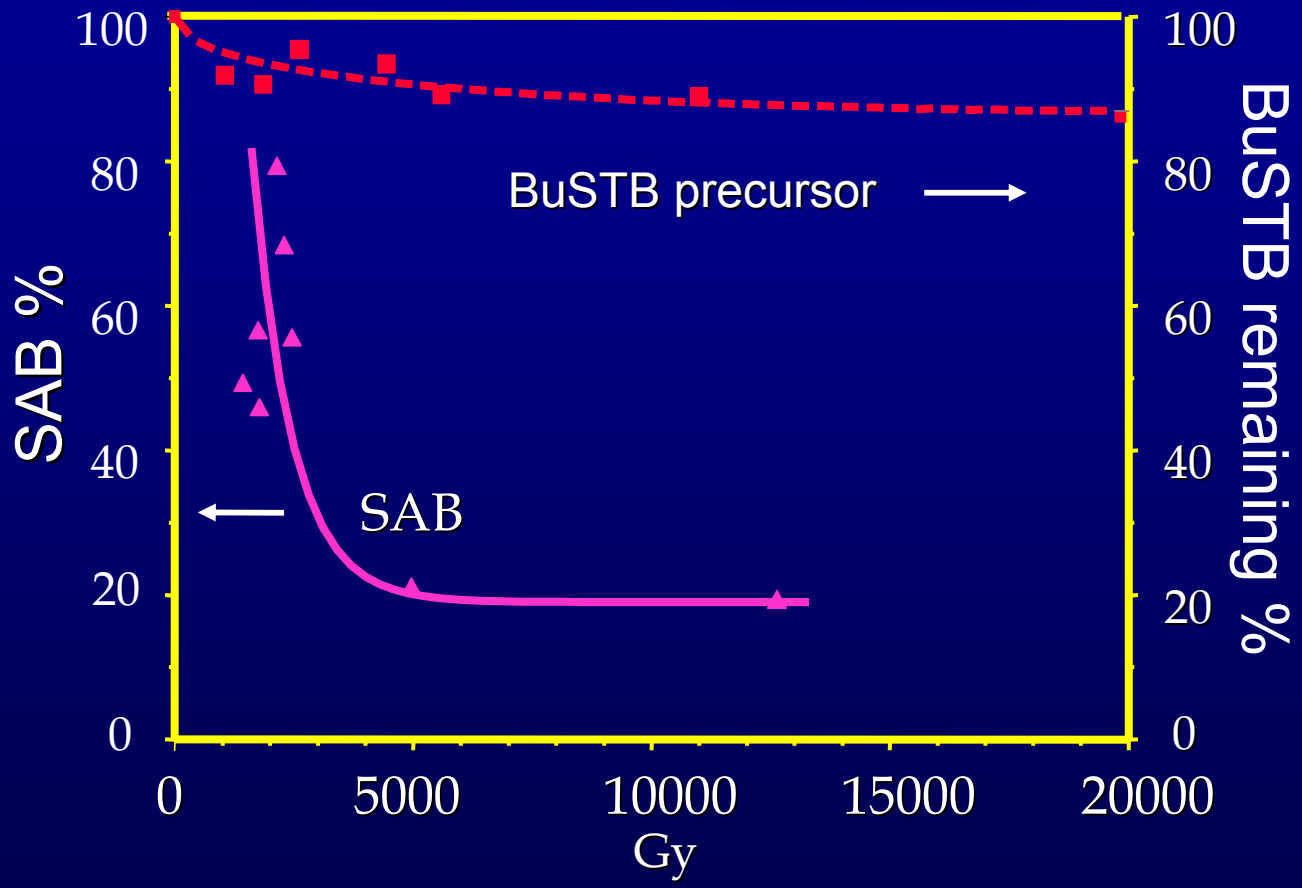
Methanol is the best solvent



In chloroform, a cold byproduct is formed

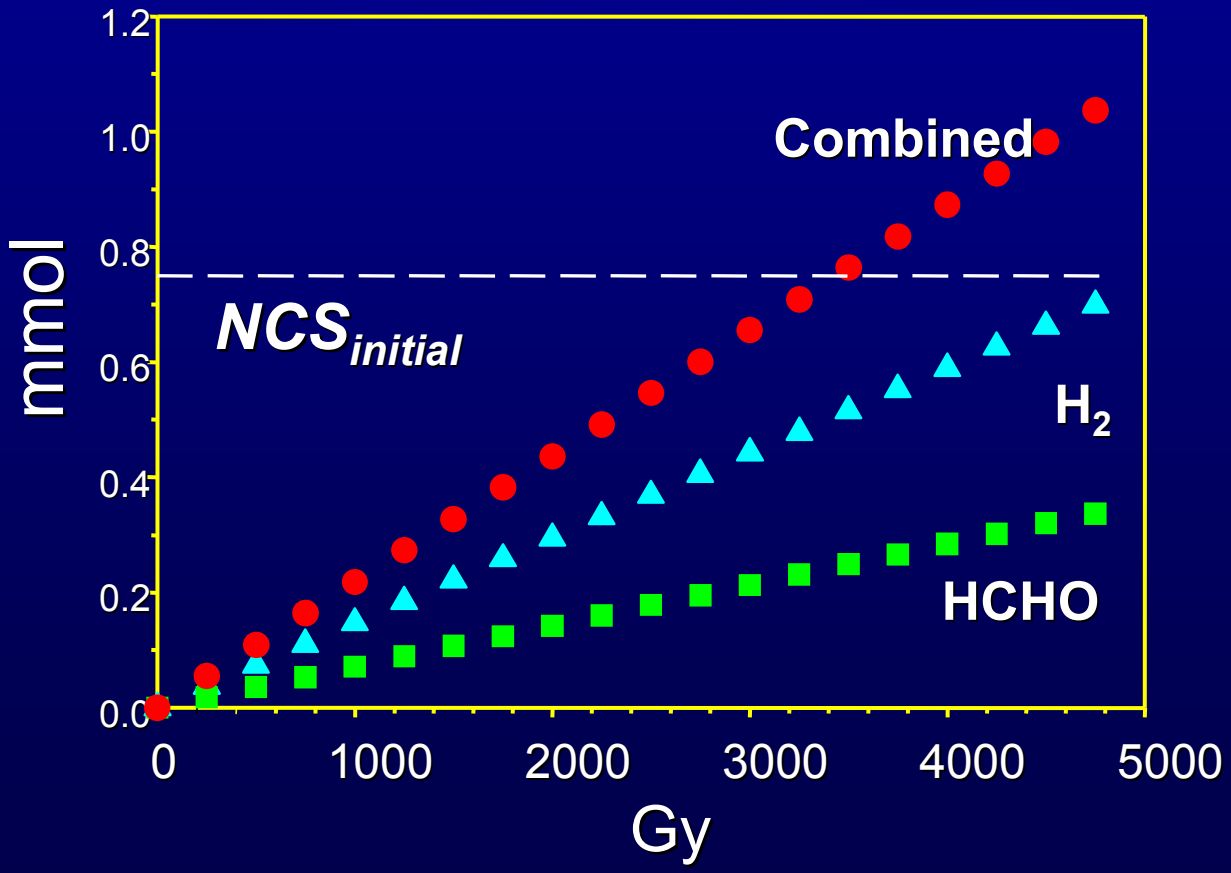
BuSTB is stable in benzene but SAB could not be synthesized; instead lipophilic ^{211}At-labeled species was formed .

Even in Methanol, SAB Yields Decline at Higher Radiation Doses



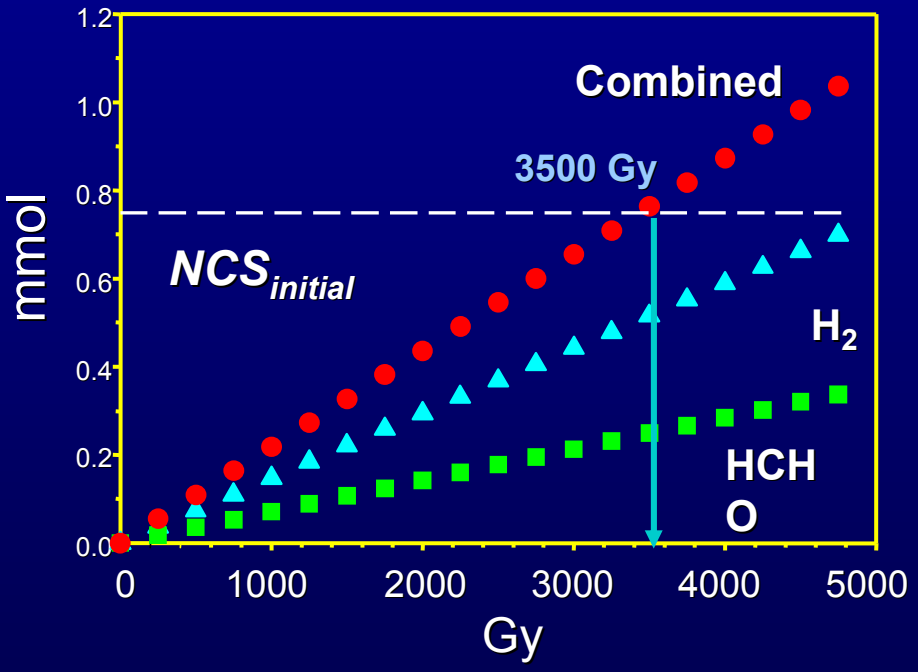
If the decline in SAB yield can not be attributed to degradation of the tin precursor, what factors are responsible?

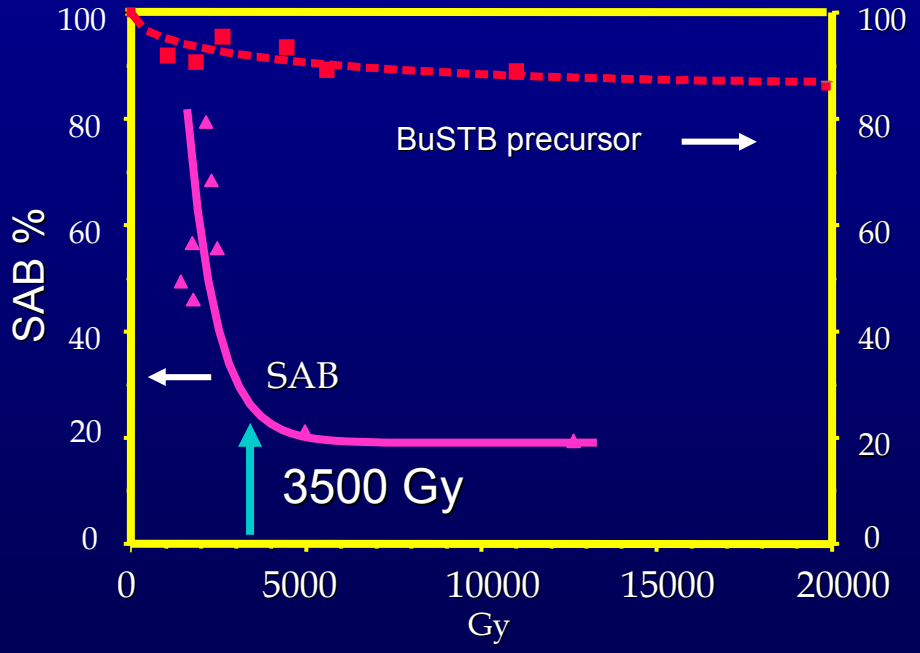
Hypothesis: Radiation-Induced Alterations in Astatine Oxidation State is the Cause of Reduced SAB Yields at High Activity



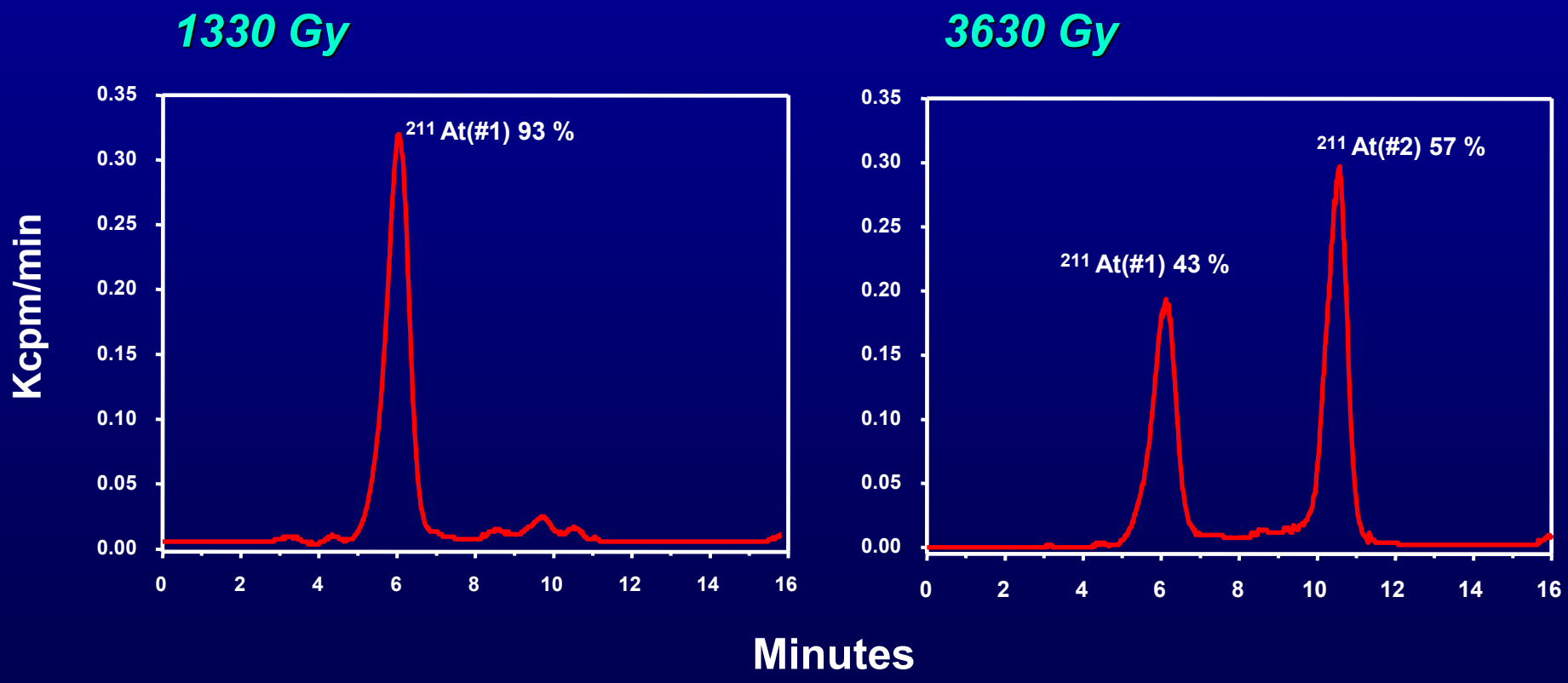
Calculated production of hydrogen and formaldehyde by radiolysis during 20-min reaction in MeOH

Hypothesis: Radiation-Induced Alterations in Astatine Oxidation State is the Cause of Reduced SAB Yields at High Activity

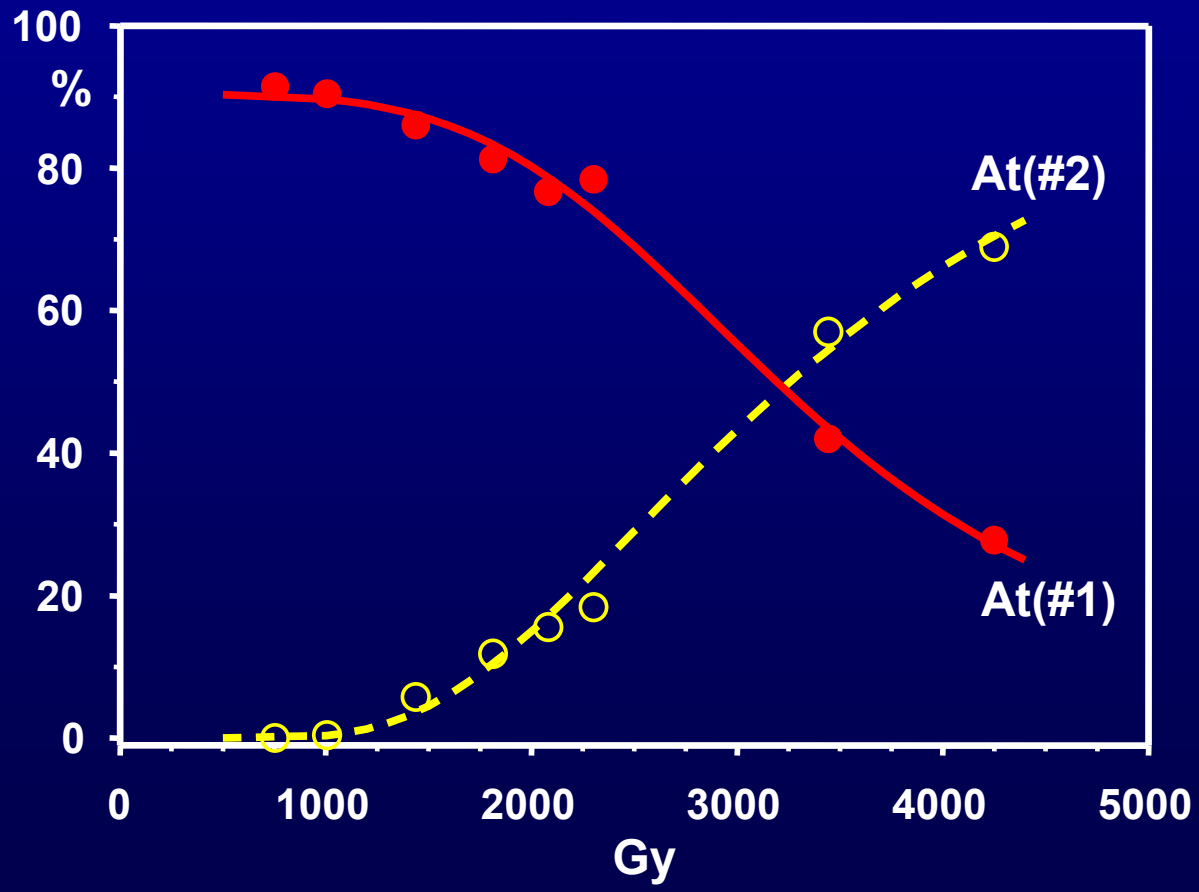




At-211 Species Present in Methanol at Different Radiation Doses



Astatine Species in MeOH as a Function of Radiation Dose

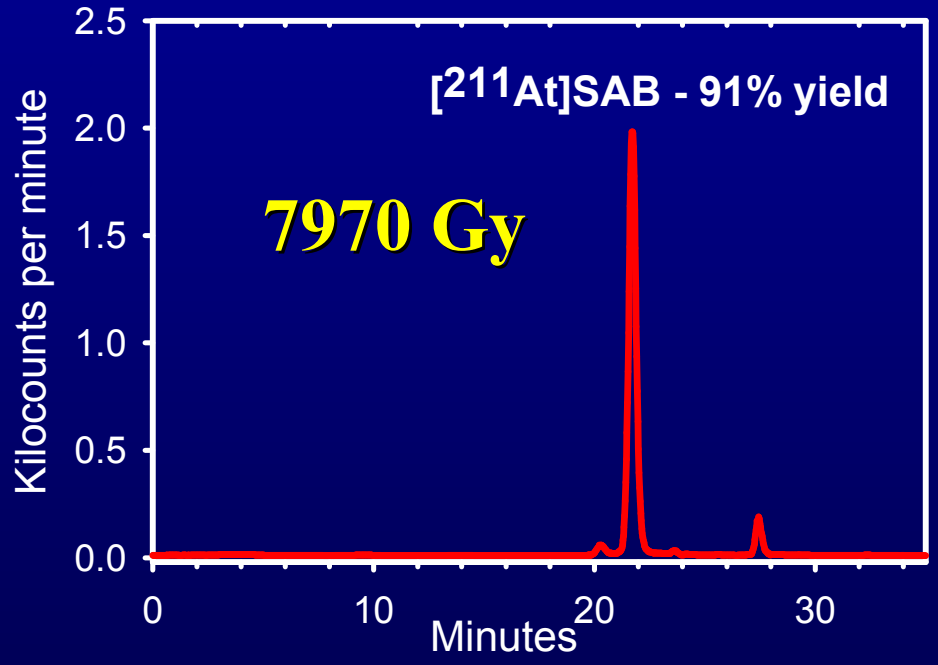


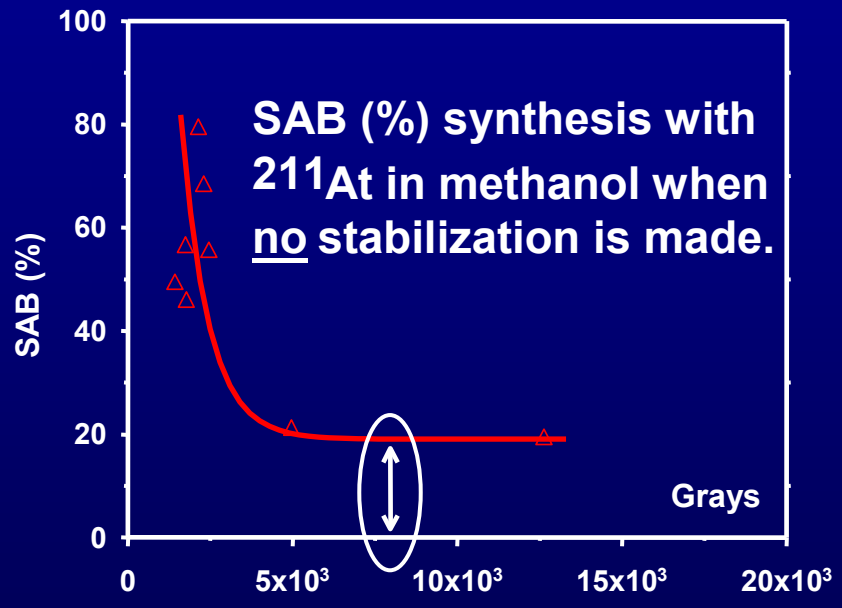
In MeOH under acidic conditions, ^{211}At(#2) predominates (>95%) even at doses below 1000 Gy

^{211}At(#2) was identified as ^{211}At^{-} (astatide)

Synthesis of SAB with Stabilized ^{211}At

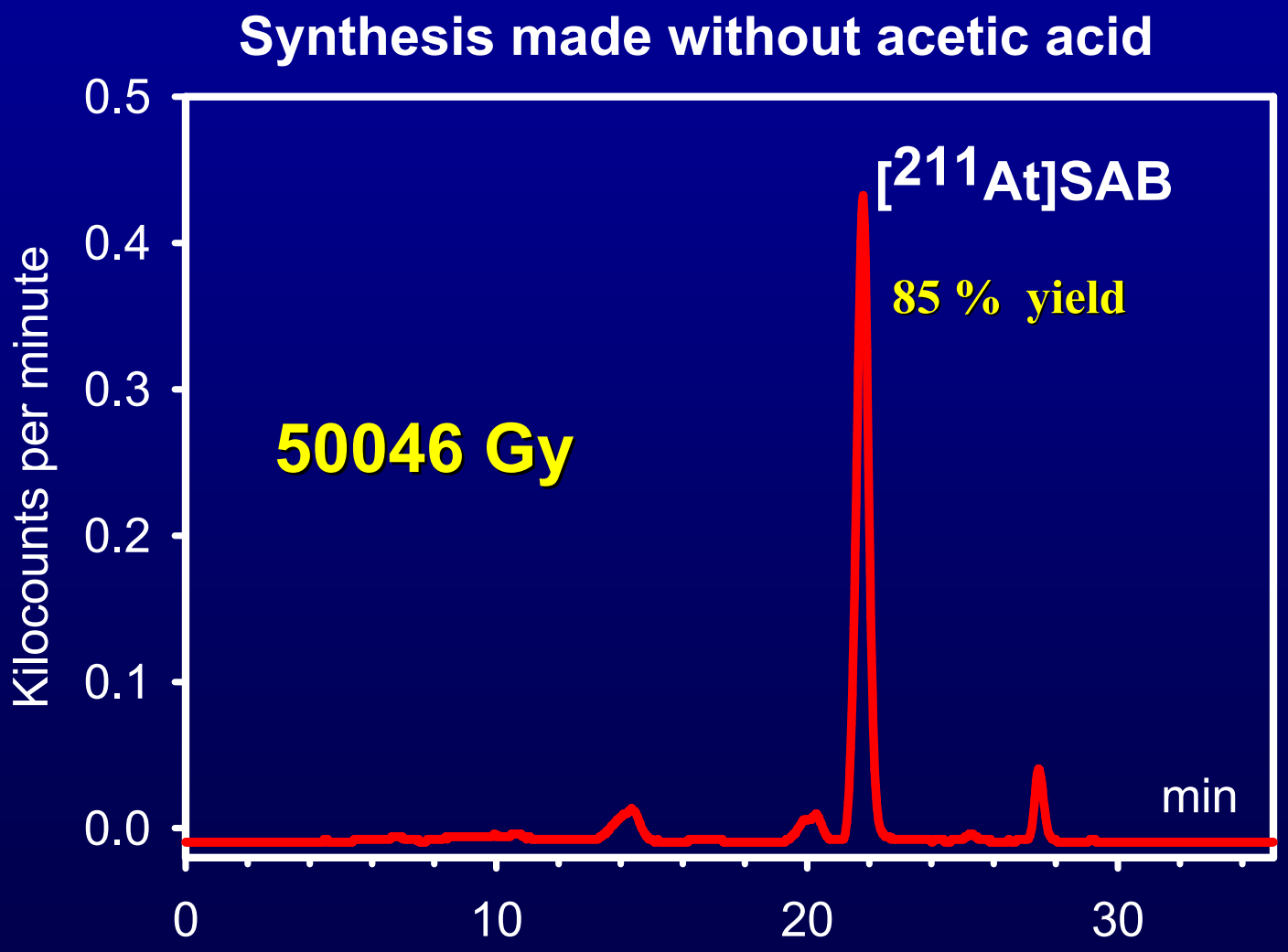
Stabilization with 200 µg NCS





Stabilized ^{211}At permits much high SAB yields at elevated radiation doses: 91% vs < 20%

SAB Synthesis with Stabilized ^{211}At at Radiation Dose Equivalent to 280 mCi Initial ^{211}At activity



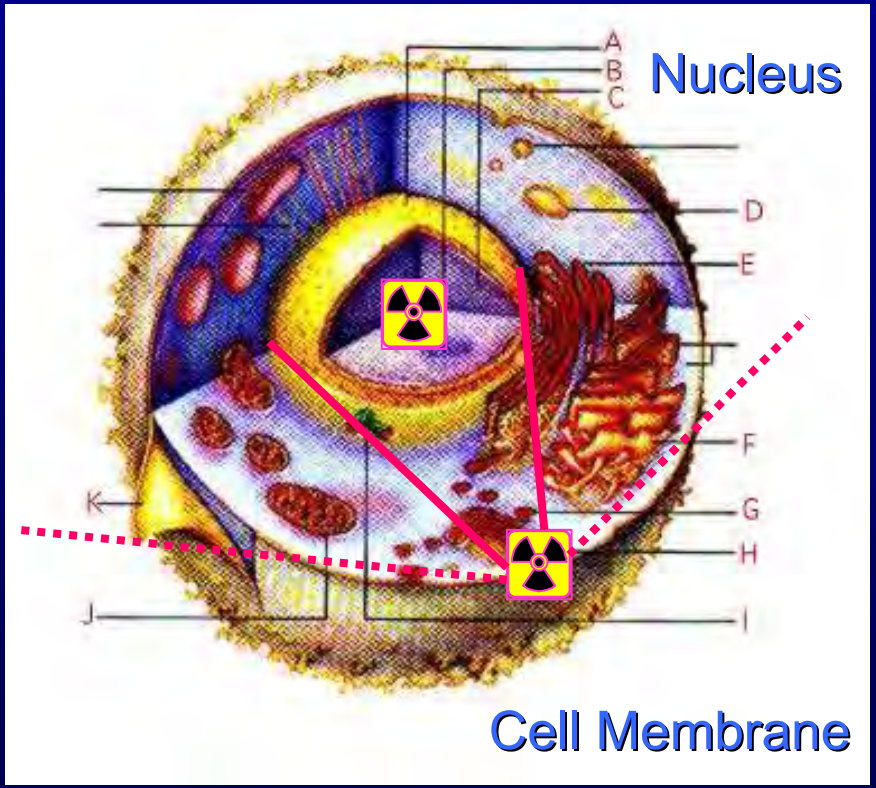
Implications of ^{211}At stabilization

	Cyclotron irradiation time (hr) @55 μA	GBq on target	GBq distilled	GBq of SAB obtained	SAB % yield	GBq of mAb obtained	Coupling % yield
Previous (2001)	4.5	6.44	3.74	0.784	21	0.444	80
With stabilized ^{211}At	1	1.29	0.8	0.67	84	0.439	73
Total time for procedure: **3¼ hours**							

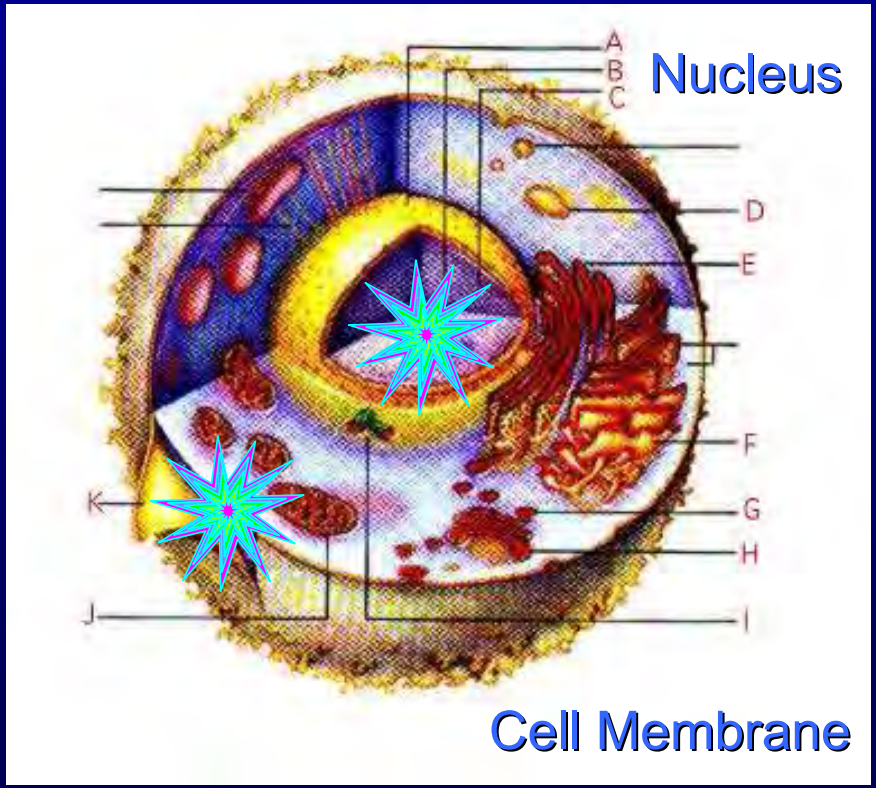
- With ^{211}At stabilization, only 1 hr cyclotron irradiation needed to produce clinical dose of ^{211}At-labeled mAb
- The production cost is reduced by almost a factor of five
- This approach is independent of the molecule to be labeled. Multiple molecules can be labeled with At^{+} from the same batch
- The astatine remains reactive for at least 24 hr so shipping to remote sites is feasible

Rationale for Nuclear Site of α-Particle Decay

Solid Angle Effect

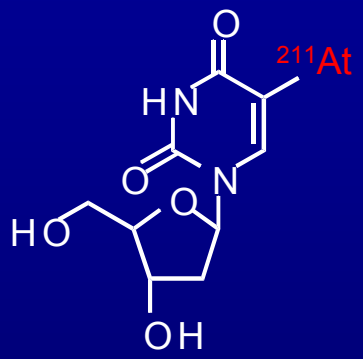


α-Recoil Nucleus Effect



α-Recoil Nucleus: range <100 nm
LET ~ 1000 keV/μm

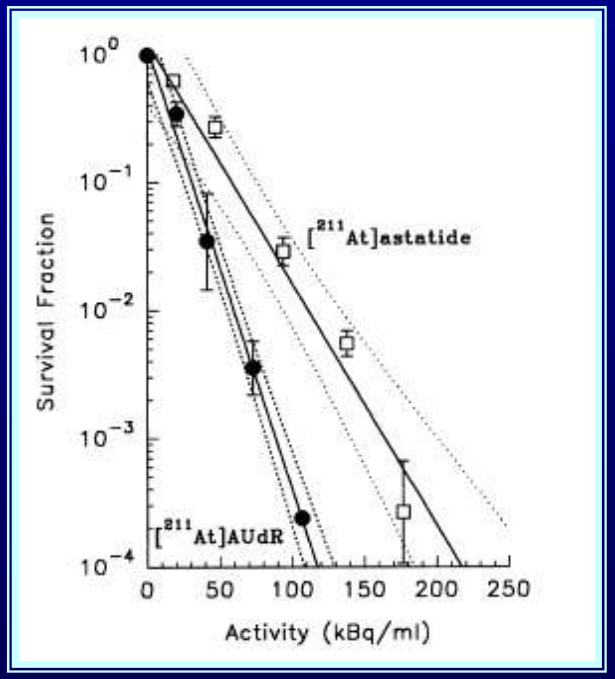
5-[^{211}At]astato-2'-deoxyuridine



[^{211}At]AUdR

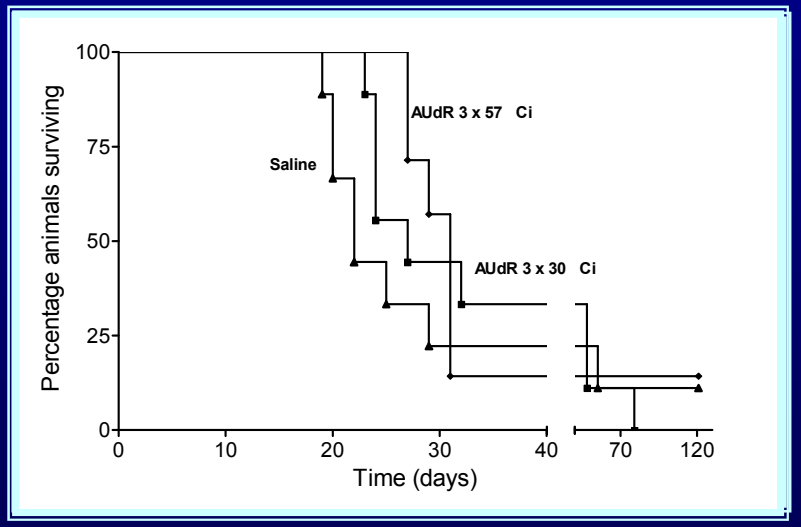
- Synthesized in 80-90% yield from trimethyl-stannyl precursor
- Undergoes DNA incorporation

D247 MG glioma
20 hr incubation



D_0 1-3 α traversals/cell

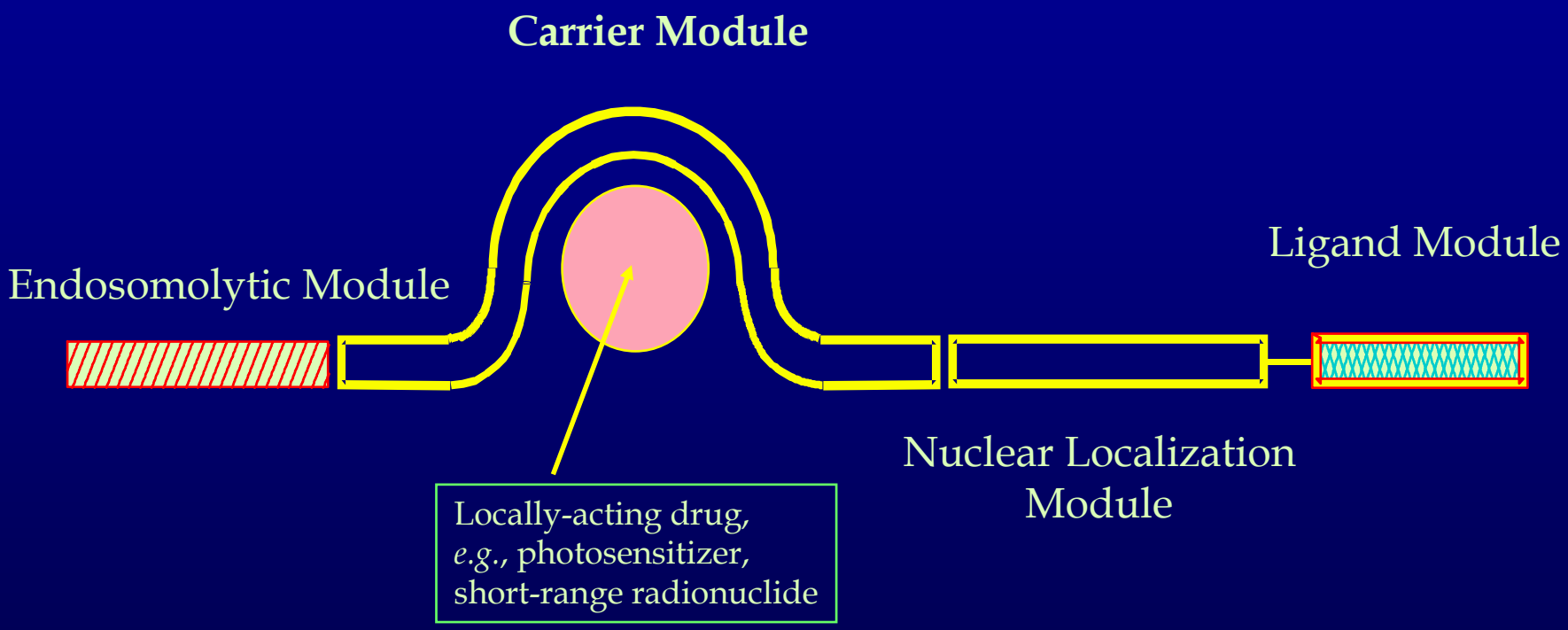
- Intrathecal therapy of D341 Med medulloblastoma
- 17% S-phase fraction *in vitro*



Median Survival

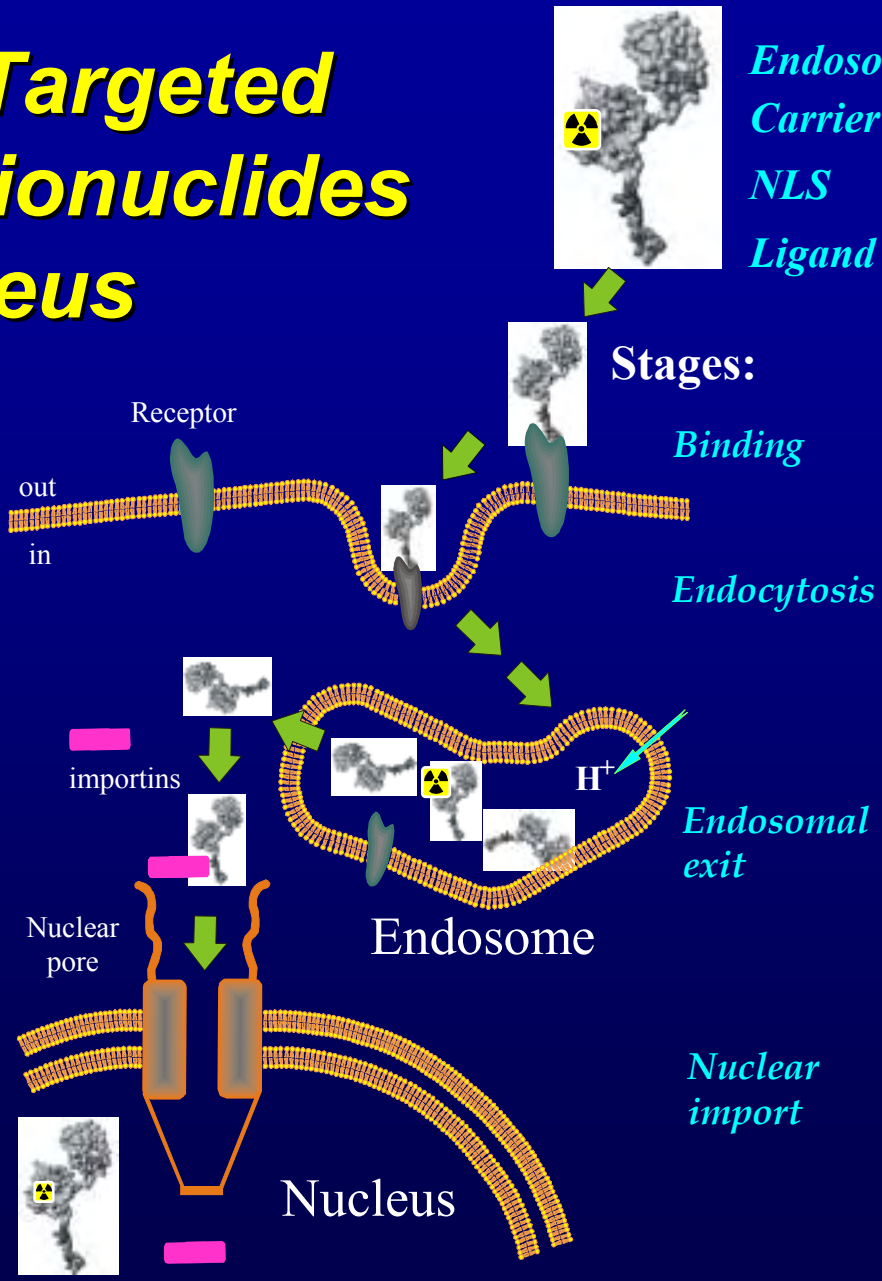
saline	(3×)	22 d
AUdR	(3× 30 Ci)	27 d
AUdR	(3× 57 Ci)	31 d

A Scheme for Modular Recombinant Transporter (MRT) for Delivery of "Locally Acting" Drugs

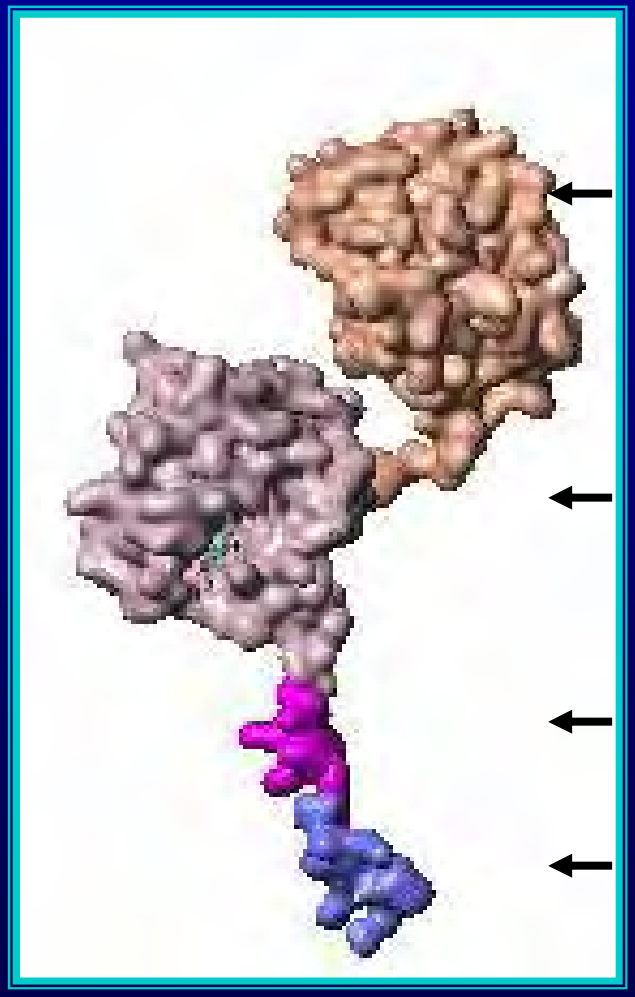


Sobolev and Rosenkranz, Institute of Gene Biology, Moscow

MRT Mediated Targeted Delivery of Radionuclides to the Cell Nucleus



MRT for Targeting EGFR Expressing Malignancies



Endosomolytic: Translocation domain of diphtheria toxin

Carrier: E. coli hemoglobin-like protein HMP

Nuclear Localization: optimized NLS of SV40 large T-antigen

Ligand: epidermal growth factor

Protein Radioiodination Agents

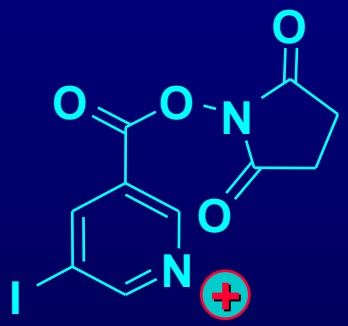
Non-Internalizing mAbs

GOAL:
Dehalogenation resistance

SIB

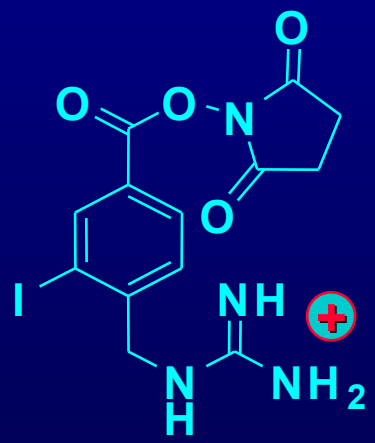
Internalizing mAbs

GOALS:
Dehalogenation resistance
Trapping of labeled catabolites



SIPC

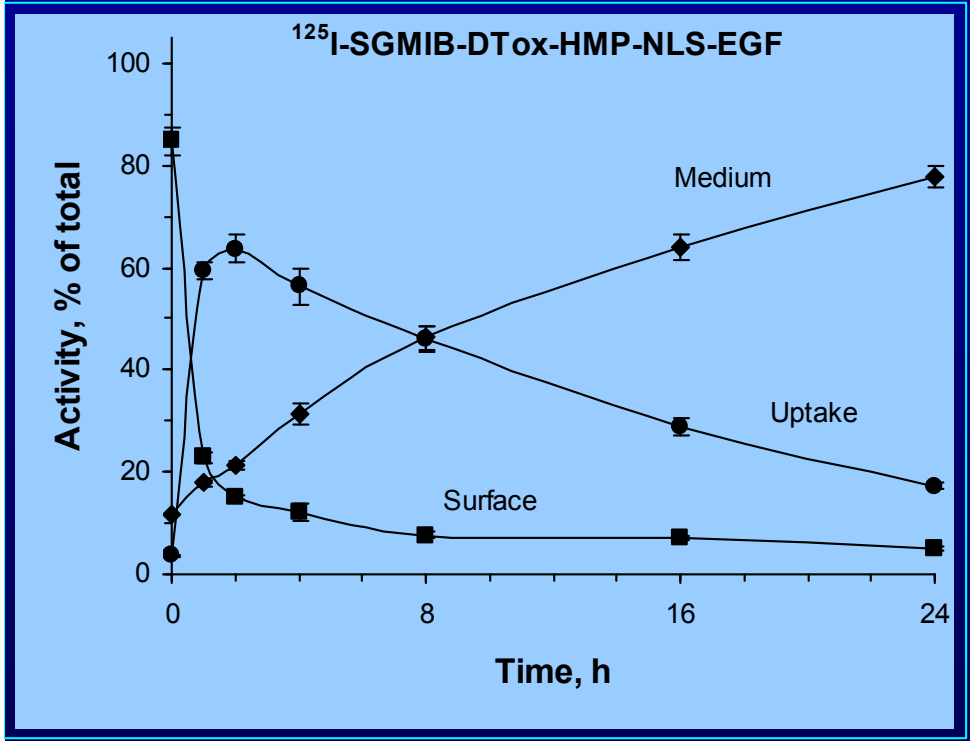
Pyridine nitrogen

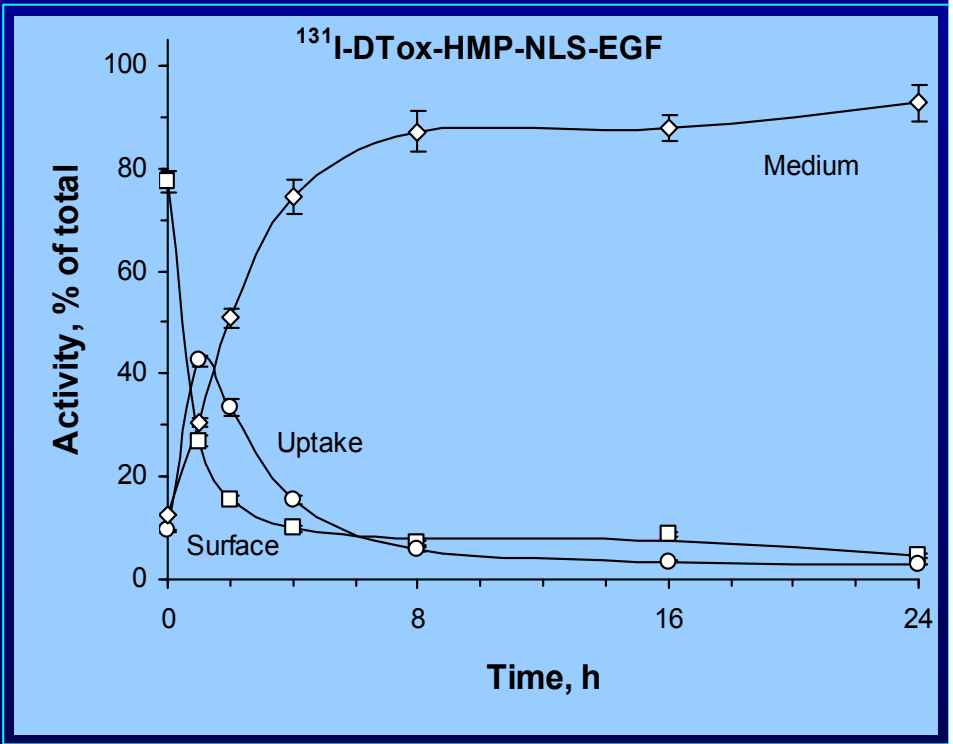


SGMIB

Guanidino nitrogen
more basic

Paired Label Internalization Assay on A431 Cells with MRT Labeled using SGMIB and Iodogen



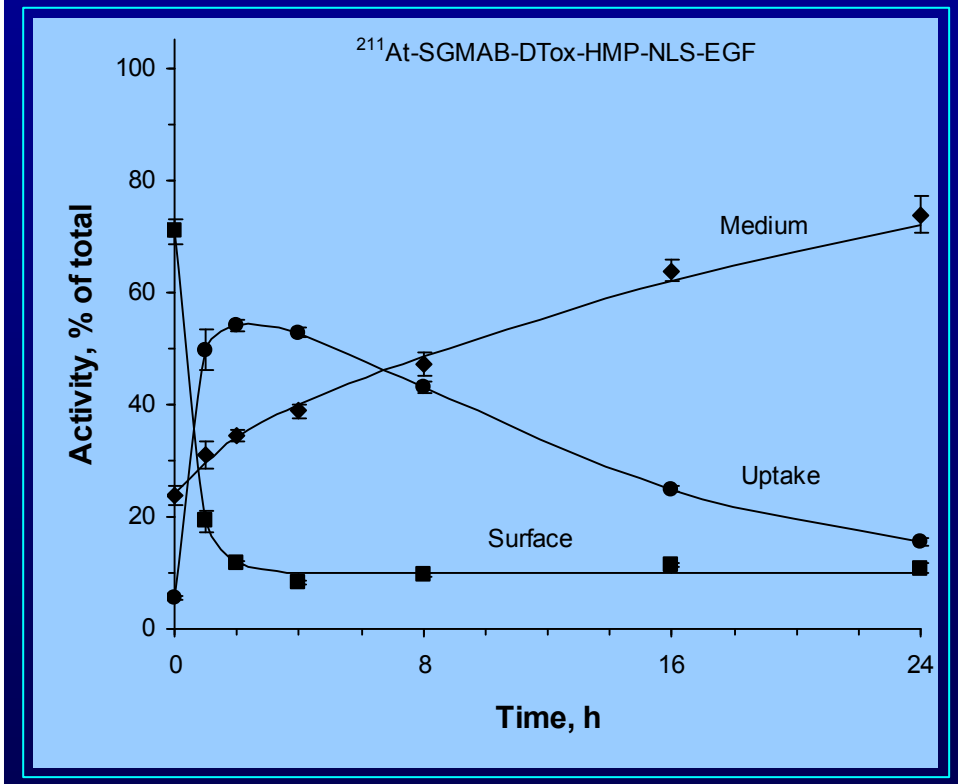


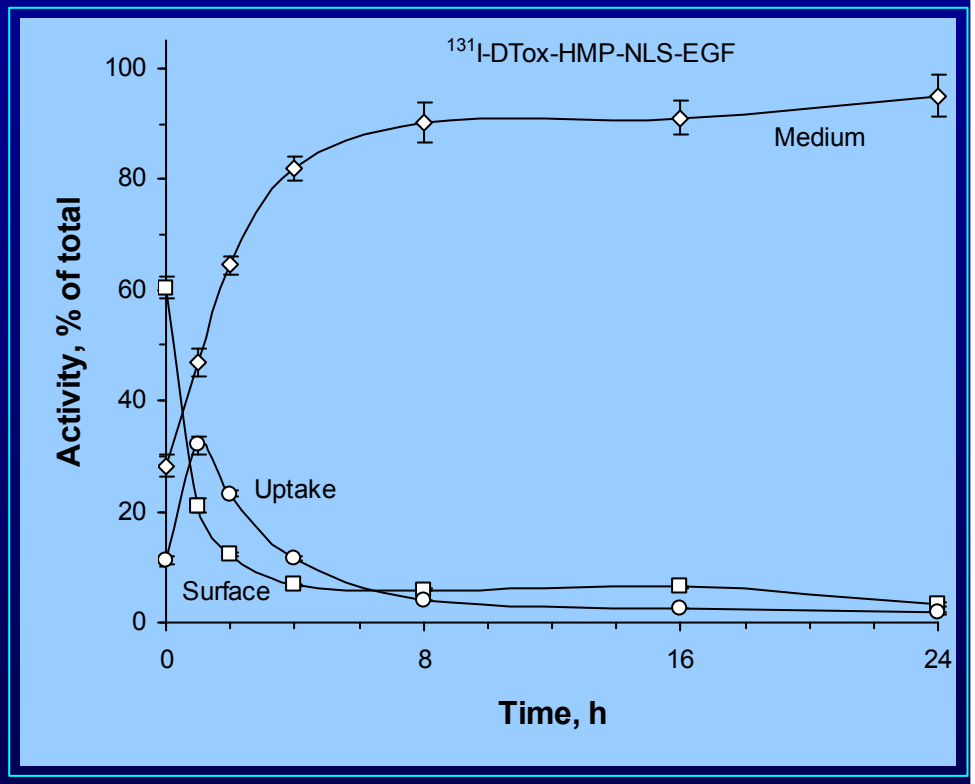
Internalized Activity Present after 4 h:

Iodogen: 15.5 ± 0.8%

SGMIB 56.4 ± 3.6%

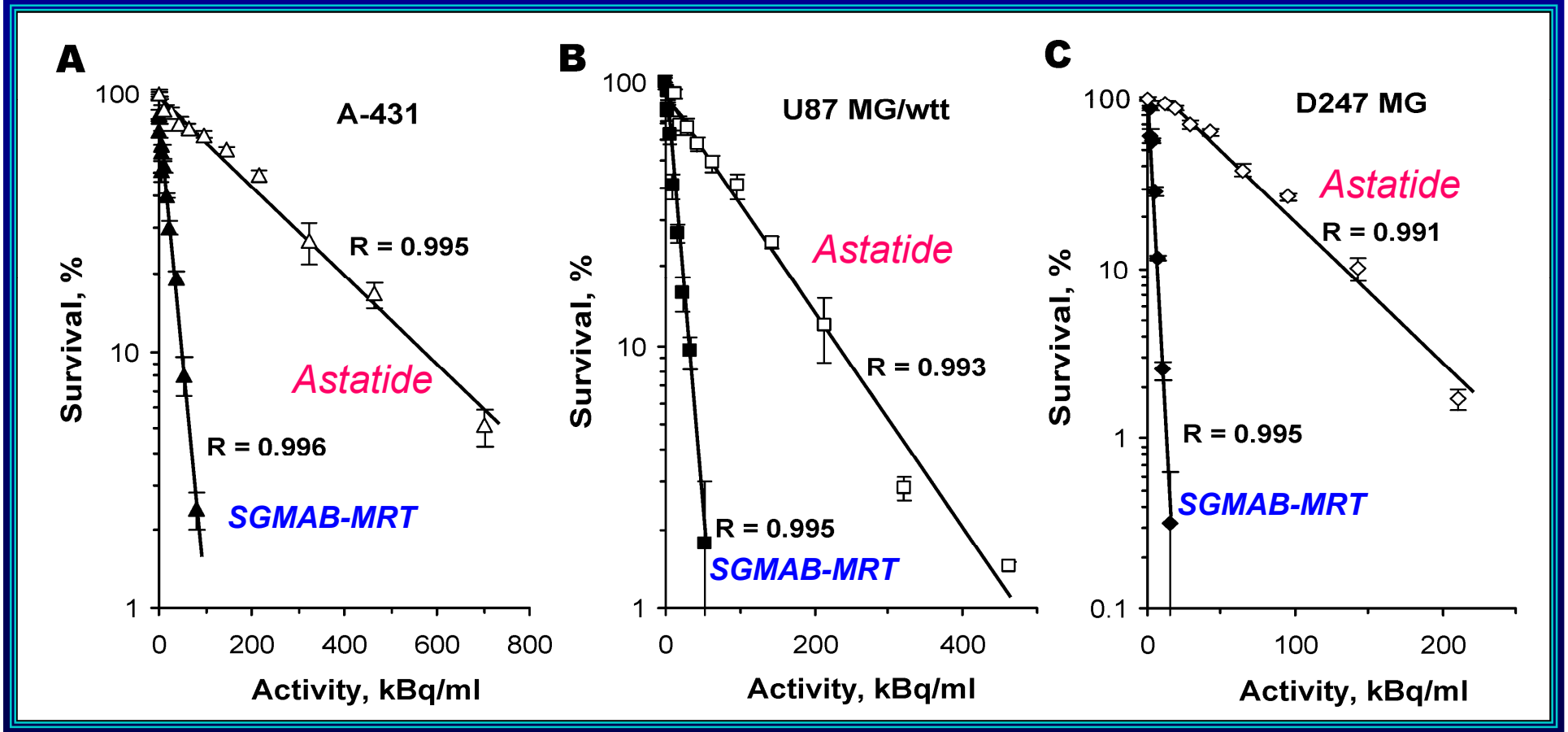
Paired Label Internalization Assay on A431 Cells with MRT Labeled using SGMAB and Iodogen





Internalized Activity Present after 4 h:
SGMAB 52.8 ± 1.5%

Clonogenic Survival



Radiolabeled EGFR Targeted MRT: Summary

- Labeling MRT radioiodine and ^{211}At with retention of EGFR binding capacity
- Labeling method influences intracellular retention
- Specific and efficient killing of EGFR expressing tumor cells (A431, U87 MG, D247 MG) can be achieved with [^{211}At]SGMAB-DTox-HMP-NLS-EGF

Next steps:

Tissue distribution

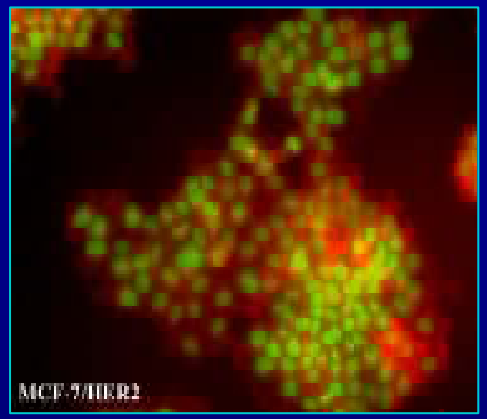
Efficacy in neoplastic meningitis model

Heterogeneity is the Bane of Targeted Radiotherapy

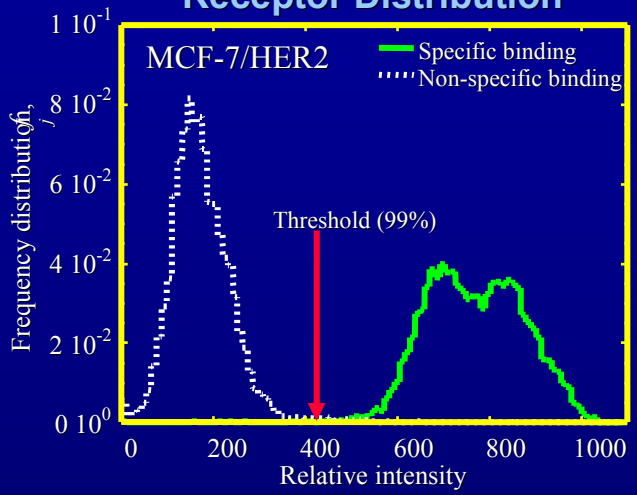
- Target molecule expression can vary considerably and include a significant null population
- Binding site barrier can limit binding to interior regions
- Tumor interstitial pressure can impede tumor penetration
- Variations in blood flow, permeability, and diffusion can lead to non-homogeneous delivery

Heterogeneity of HER2 Expression Effects ^{211}At-Herceptin Cytotoxicity

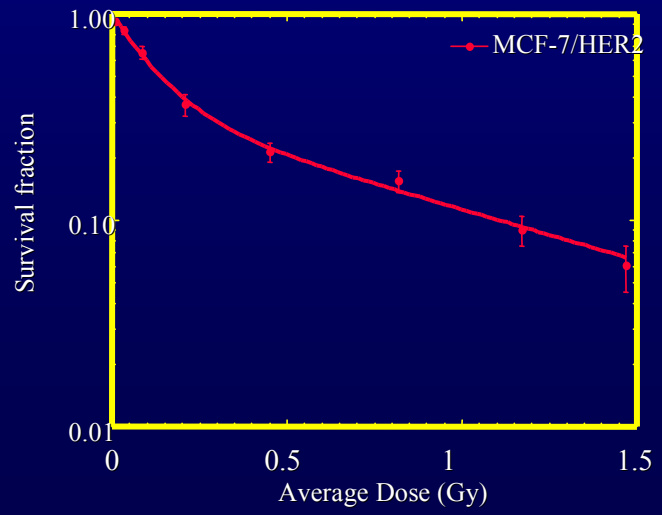
FACS Analysis of mAb Binding



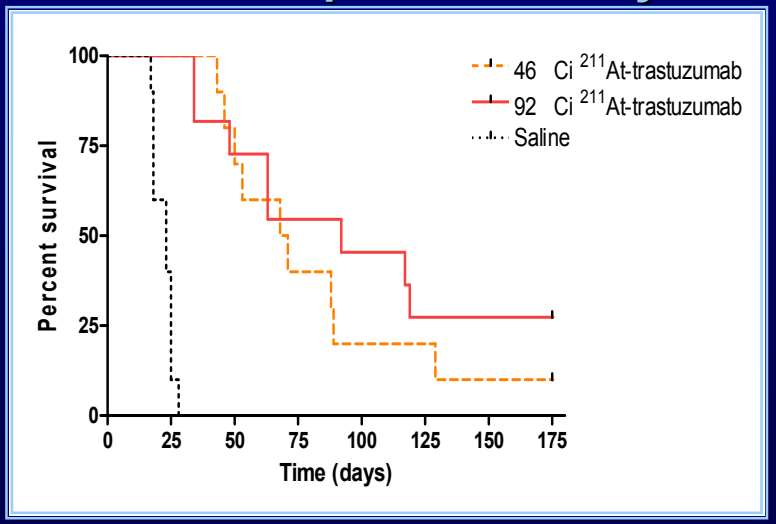
Receptor Distribution



Cytotoxicity

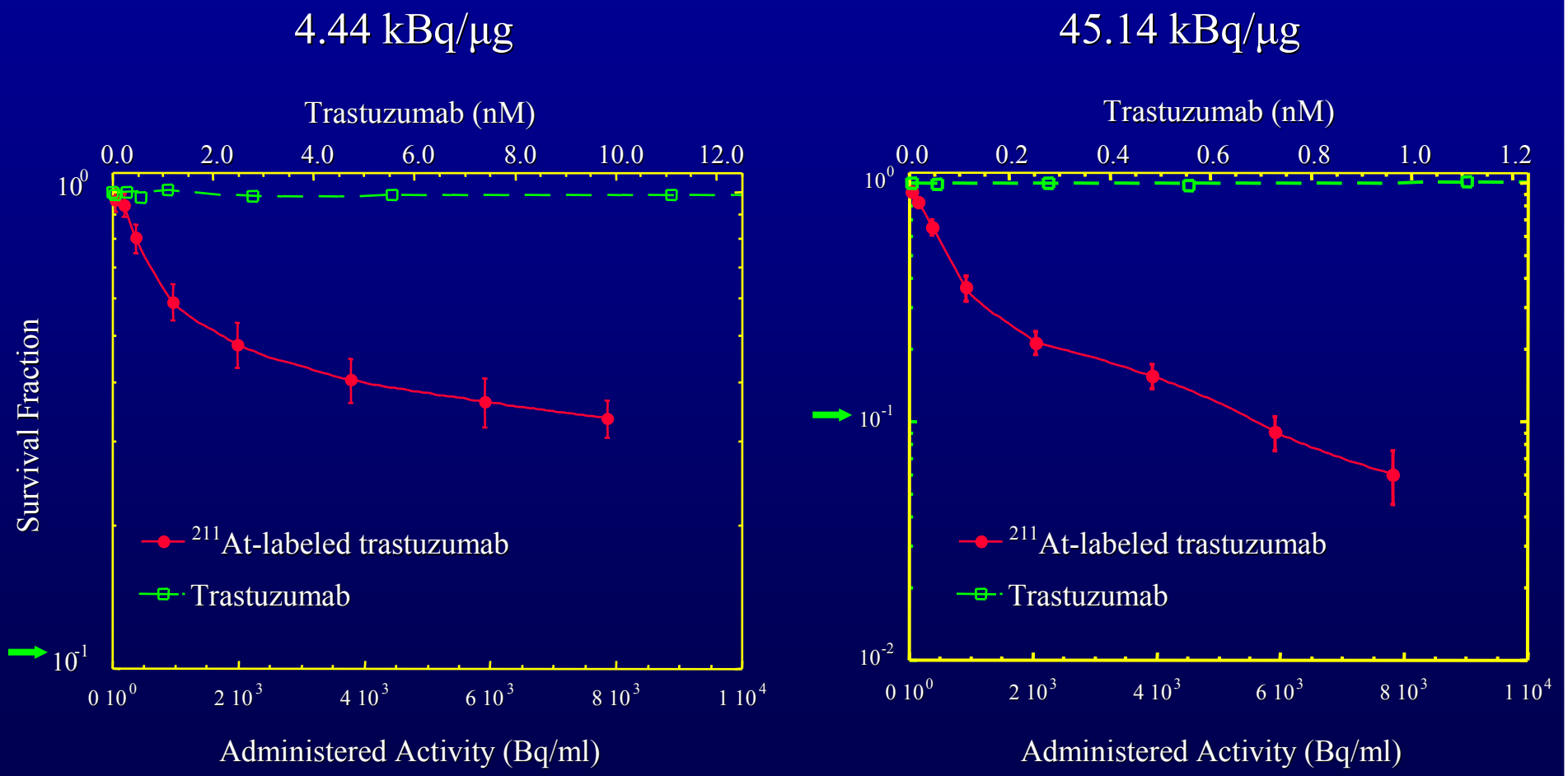


Therapeutic Efficacy

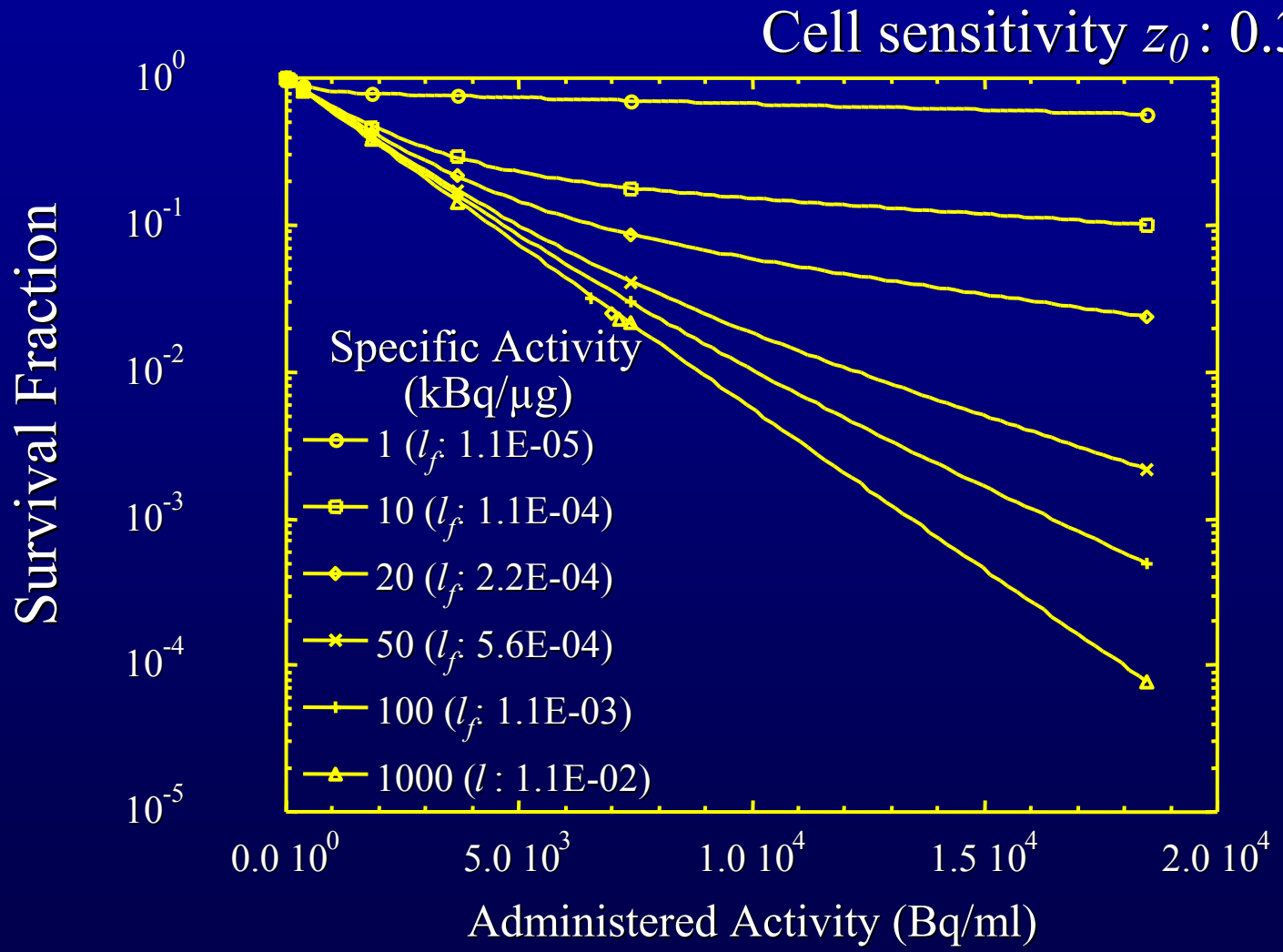


Akabani et al., Nucl Med Biol, 2006

Effect of Specific Activity on Survival of MCF7/HER2 Cells



PK Model: Effect of Specific Activity on Survival Fraction as a Function of Media Activity



Compensating for Heterogeneous Dose Deposition

- Minimum residual disease settings
- Radiotherapeutic cocktails
  - Carrier type (protein, peptide)
  - Molecular target (receptor, enzyme)
  - Radionuclide (alpha, beta, Auger)
  - ❖ Modular Recombinant Transporter
- Vascular Targeting
- Combination with other therapies
  - Example: Gene therapy
- Radiation Induced Biological Bystander Effect

Targeted Radiotherapy with Alpha Particle Emitting Radionuclides: The Problems

- Radionuclide availability is limited
- Release of long lived daughters
- Specific activity effects on targeting
- Radiolysis effects on labeling chemistry (and integrity)
- Heterogeneous dose deposition
- Calculation of radiation dosimetry is complicated

Targeted Radiotherapy with Alpha Particle Emitting Radionuclides: The Promise

- Demonstration of more efficient cell kill in compared with beta emitters or XRT
- Radiotoxicity nearly independent of oxygen concentration and cell cycle stage
- Particle range well matched to treatment of minimum residual disease
- Clinical trials are underway in leukemia, ovarian carcinoma, glioma and other cancers
- These trials have demonstrated feasibility with acceptable toxicity and in some patients, encouraging responses have been observed.