Targeted Radionuclide Therapy: Practicalities and Potentials

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Radium Remedies

RADITHOR—PERPETUAL SUNSHINE

These Radium Rays

have proved highly valuable in the treatment of the following conditions:

Anemia
Arteriosclerosis
Arthritis
Catarrhal conditions
Diabetes
Dental conditions
General debility
Goitre
High blood pressure
Menopause and
Menstrual disorders
Nephritis
Neuralgia
Neurasthenia
Neuritis
Nervous conditions
Obesity
Prostatitis
Rheumatism
Senility
Sexual conditions
Skin disorders

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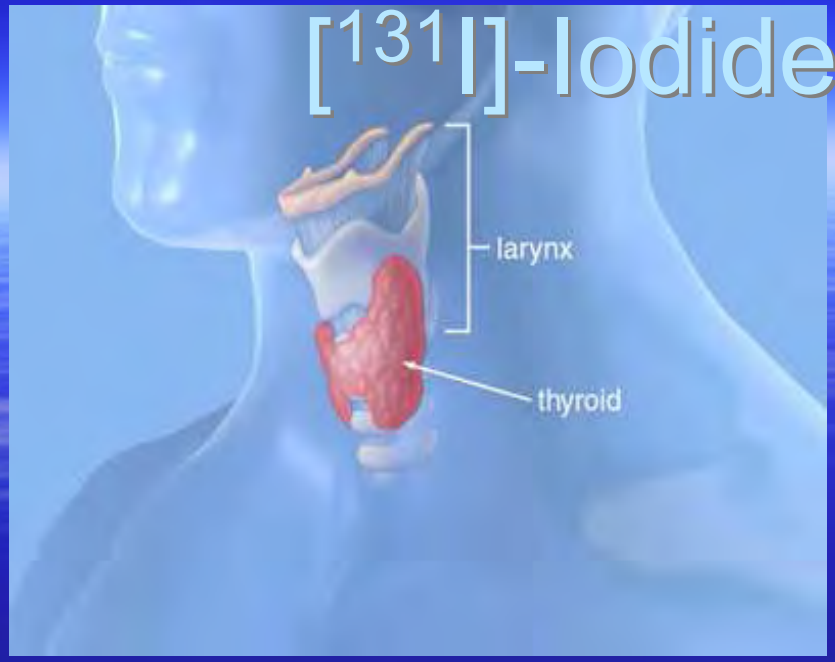
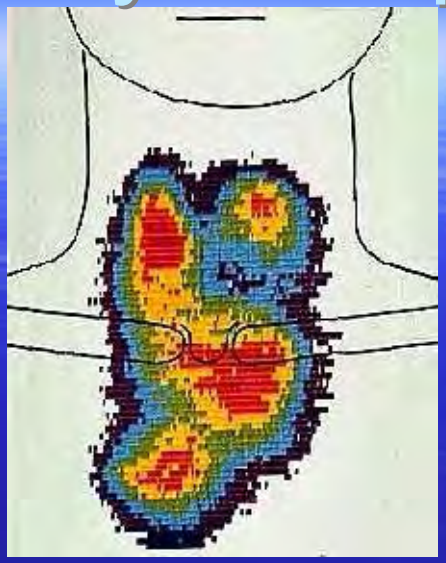
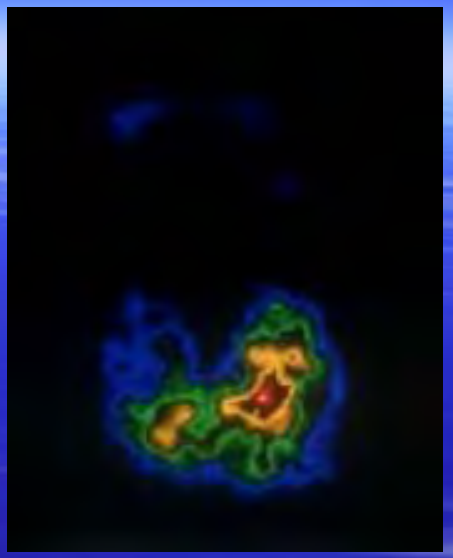
[^{131}I]-Iodide Thyroid uptake







- First developed in the 1950s
- Unique in nuclear medicine
- ^{131}I-sodium iodide uptake by differentiated follicular thyroid cells

Proven efficacy, safety & cost
Stands as a marker against which new forms of targeted therapy can be judged.

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UK Liceneced Therapeutic Radiopharmaceuticals

- NaI
- MIBG
- Metastron
- Quadramet
- Zevalin

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Global Perspective

- Different perception of value of nuclear medicine therapy.
- Vast discrepancy in resources between different countries.
- Difference in priorities between different countries.
- Complex relationships between practitioners, researchers, industry and regulatory authorities.
- Variable standards (efficacy/safety).

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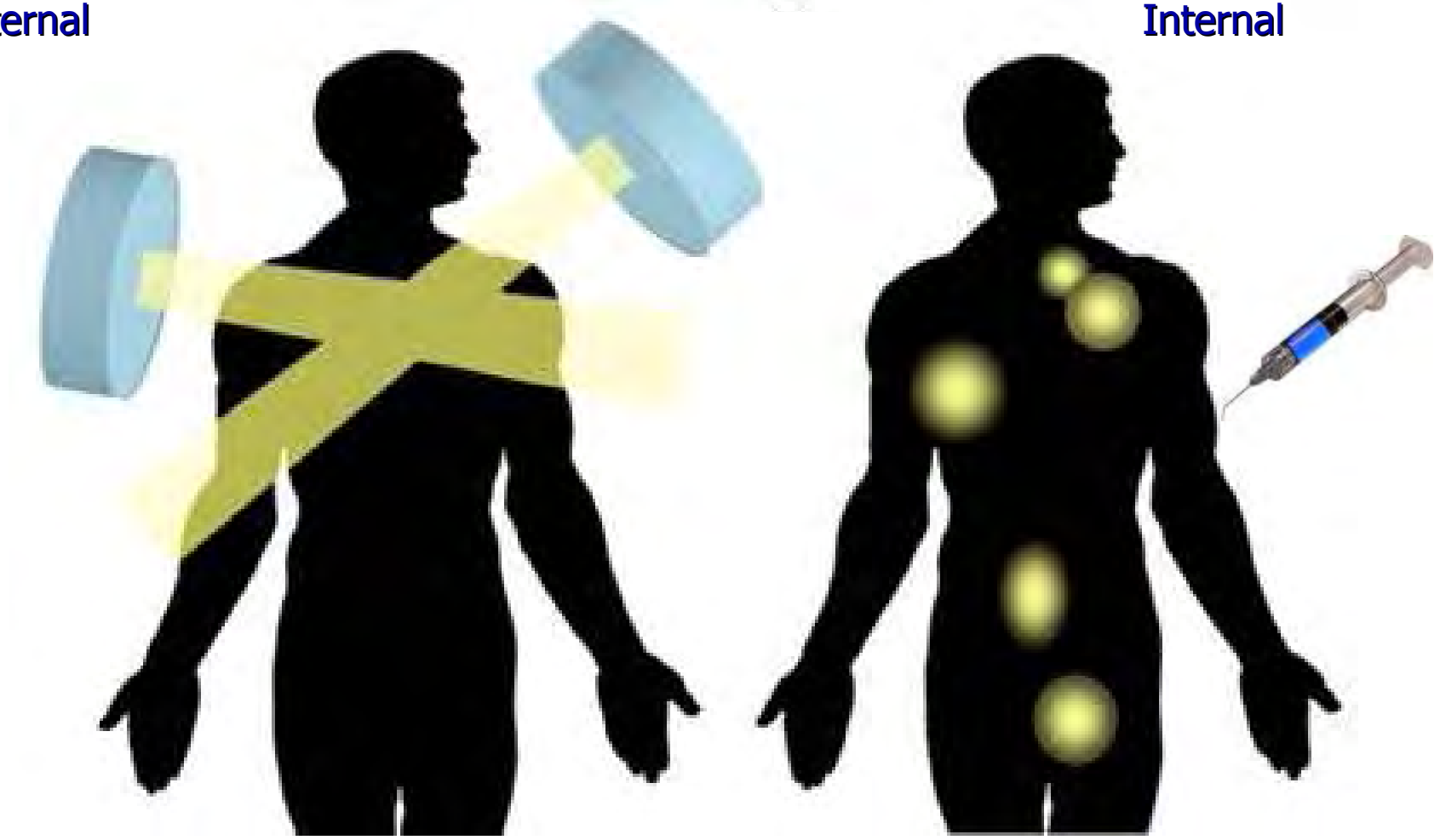
The promise of targeted molecular radiotherapy

External

Internal



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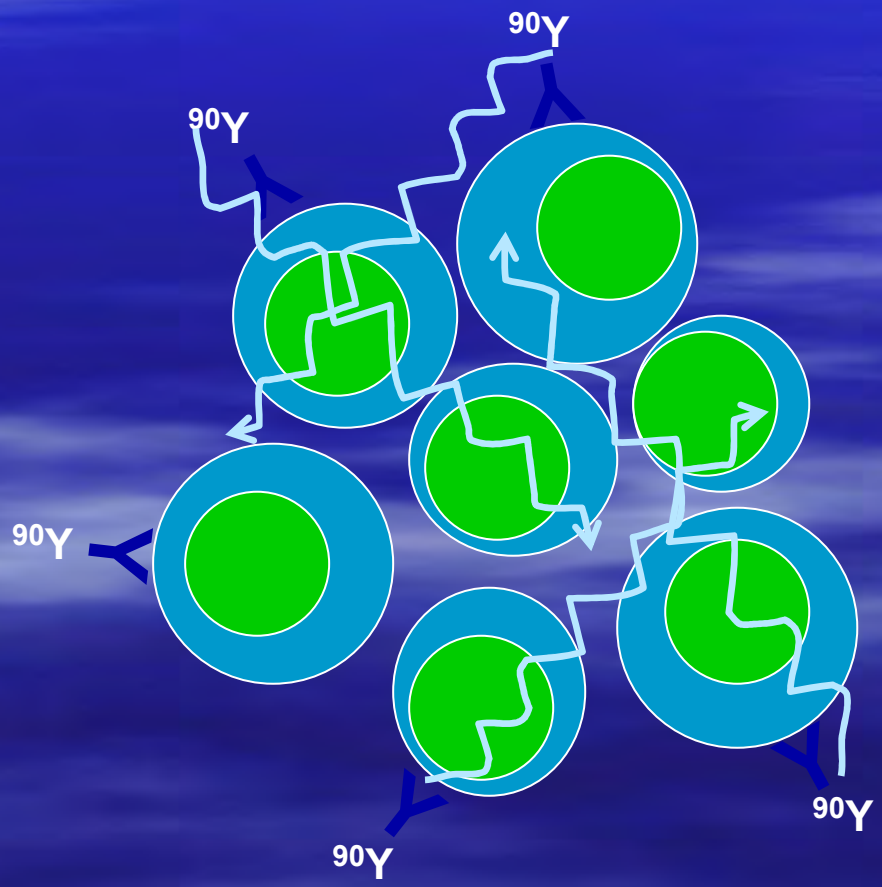
The Bystander effect : Penetrating radiation minimises the problem of limited access in bulky or poorly vascularized tumours.



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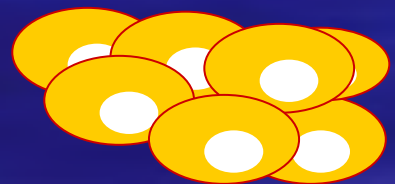
Choice of Radionuclide

Nuclide	T1/2	emission	mean path length
I-125	60.0d	auger	10nm
At-211	7.2h	alpha	65nm
Lu-177	6.7d	beta/gamma	0.7mm
Cu-67	2.58d	beta/gamma	0.7mm
I-131	8.04d	beta/gamma	0.9mm*
Sm-153	1.95d	beta/gamma	1.2mm
Re-186	3.8d	beta/gamma	1.8mm
P-32	14.3d	beta	2.9mm
Re-188	17h	beta/gamma	3.5mm
In-114m	50d	beta/gamma	3.6mm
Y-90	2.67	beta	3.9mm*

*^{131}I ---> 3mm dia. ^{90}Y ---> 2cm dia.

Wheldon et. al. Radiother. Oncol. 1998



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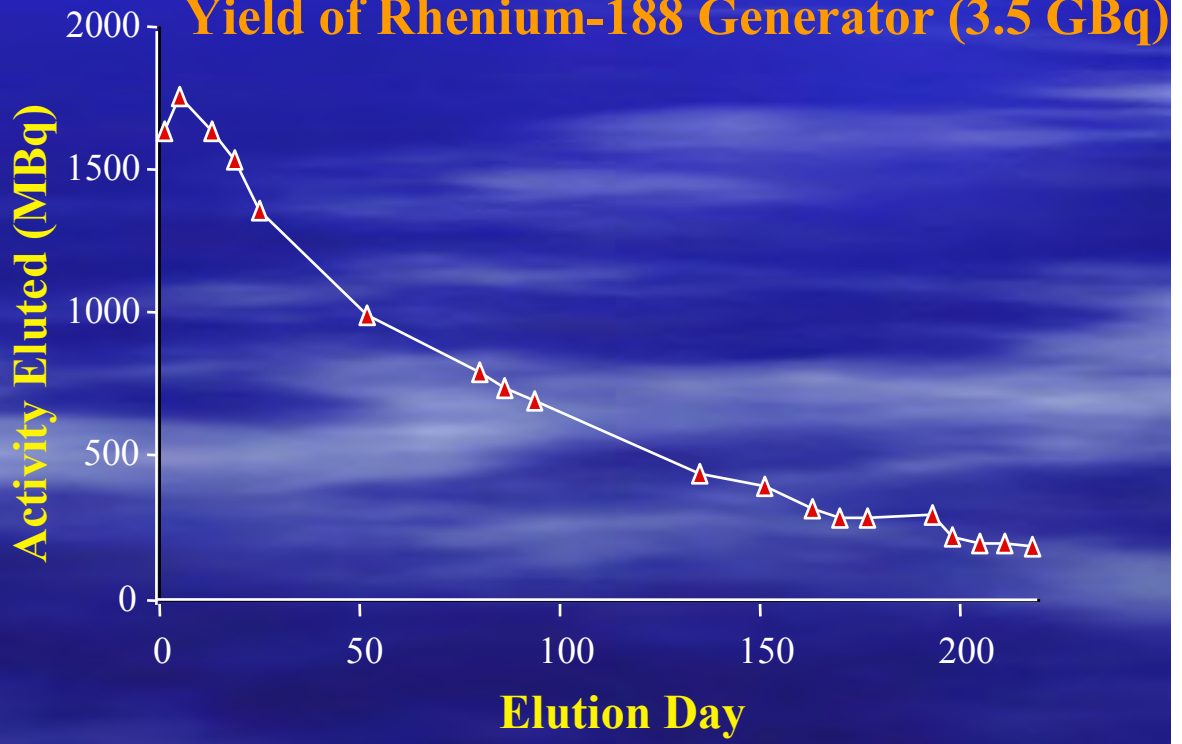
Rhenium-188

- Generator produced ($^{188}W/^{188}Re$ generator).
- ^{188}Re obtained as sodium perrhenate.
- Carrier-free.
- Same periodic group as technetium therefore Tc & Re complexes have similar chemical properties.
- Re chemistry requires validation.

$^{188}W/^{188}Re$ generator

^{188}Re carrier free sodium perrhenate



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Re-188 therapy conjugates

Re-188-antibody
Re-188-lipodol for hepatoma
Re-188-HEDP

Clinical

British Journal of Cancer (2003) **89,** 625 629.
doi:10.1038/sj.bjc.6601158

Therapeutic efficiency of rhenium-188-HEDP in human prostate cancer skeletal metastases

K Liepe[1], J Kropp[1], R Runge[1] and J Kotzerke[1]
[1]Department of Nuclear Medicine, University Hospital Dresden, Fetscherstr. 74, 01307 Dresden, Germany

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RHENIUM-188 ANTIBODIES FOR CANCER RADIOIMMUNOTHERAPY

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University of Groningen NL
J HARVEY TURNER
University of Western Australia

^{188}Re - RITUXIMAB ANTI CD 20

^{188}Re - BASILIXIMAB ANTI CD 25

^{188}Re - TRASTUZUMAB ANTI HER neu 2

^{188}Re - CAMPATH ANTI CD 52

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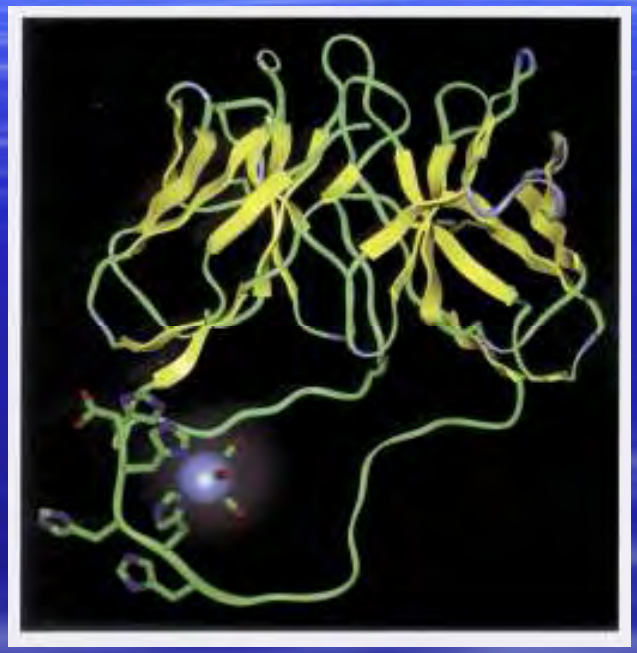
Targeting mechanisms

1. Metabolic process
   - Radioiodine
   - Radiophosphorus
   - Meta-iodobenzlguinadine
2. Extracellular mechanisms
   - Bone seeking agents
   - Radiolabelled cells
3. Cell surface receptors
   - Hormones
   - Peptides
   - Antibodies
   - Aptamers
4. Directed administration
   - Intralesional
   - Intra-arterial
   - Intracavitary

Molecular carriers?



Antibodies

Peptides

Aptamers

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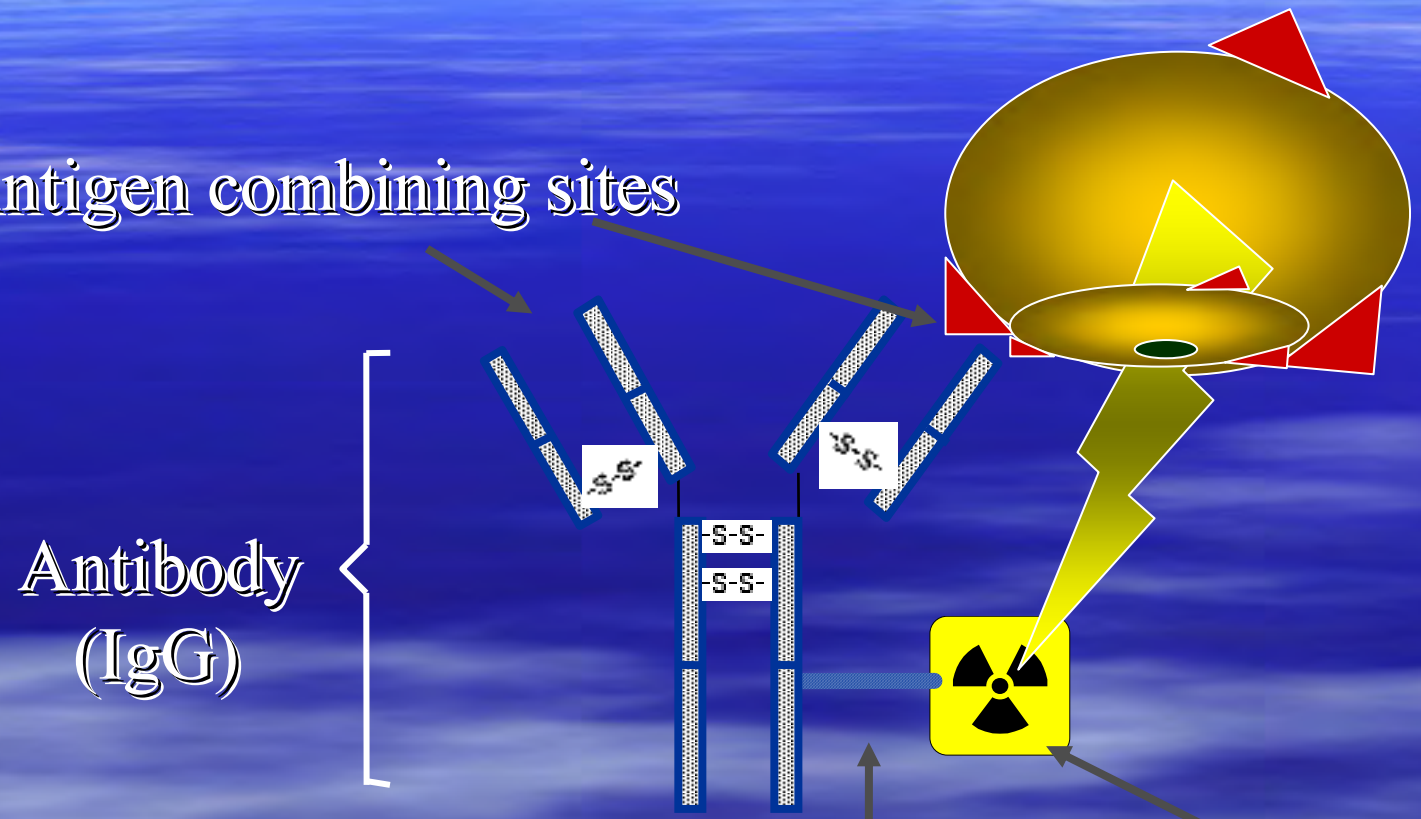
Antibody Targeting



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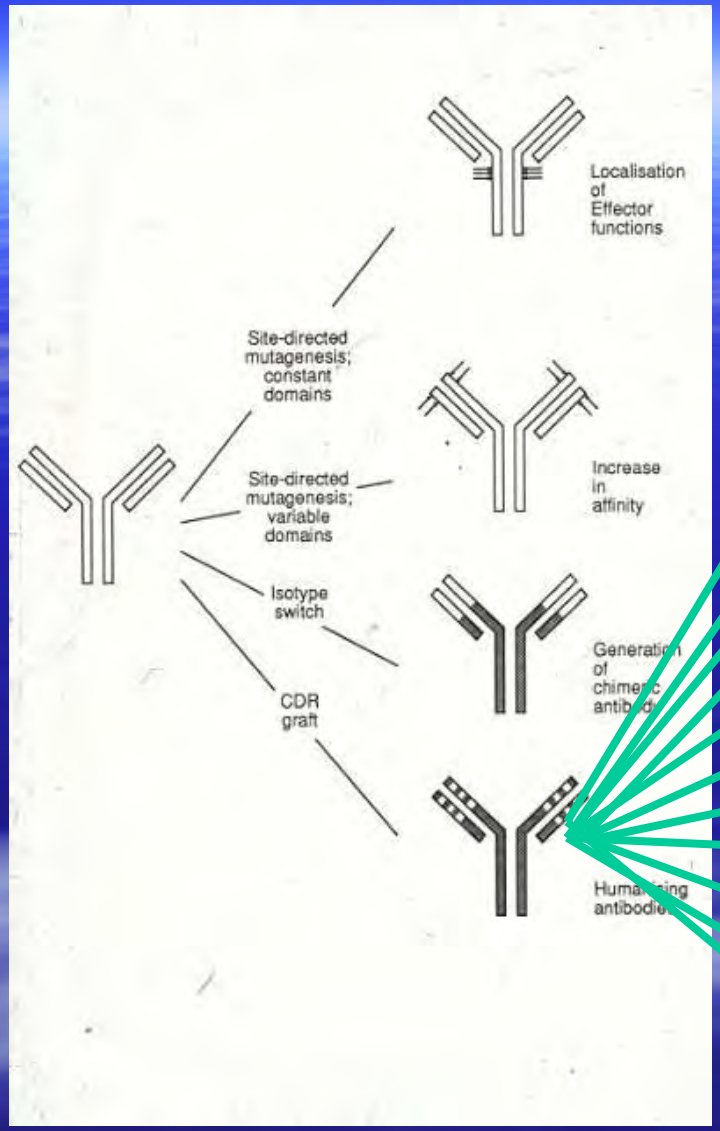

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Re-188	17h	beta/gamma
In-114m	50d	beta/gamma
Y-90	2.67	beta

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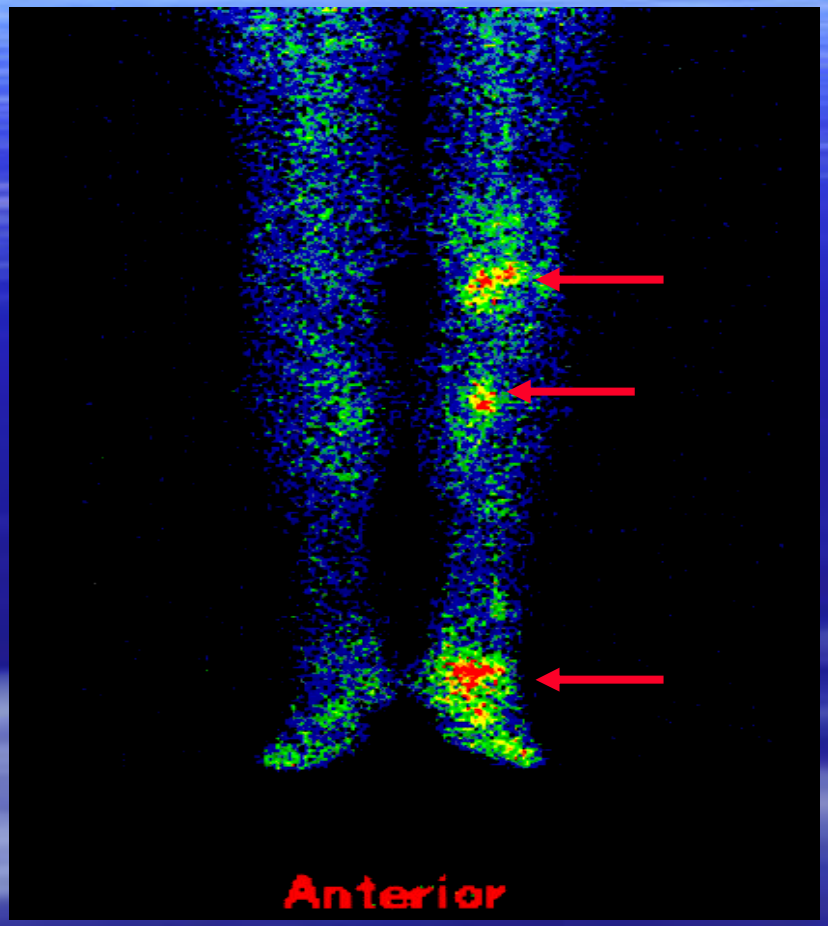
Table 1. New CD designations

CD designation	Name	Section	Locus link
CD15u	Sulphated CD15	Carbohydrate structures	
CD60a	GD3	Carbohydrate structures	
CD60b	9-O-acetyl-GD3	Carbohydrate structures	
CD60c	7-O-acetyl-GD3	Carbohydrate structures	
CD75	Lactosamines	Carbohydrate structures	
CD75s	Alpha-2,6-sialylated lactosamines (formerly CDw75 and CDw76)	Carbohydrate structures	
CD85	ILT/LIR family (see Table 2)	Dendritic cells	
CD110	MPL, TPO R	Platelets	4352
CD111	PRR1/Nectin1	Myeloid cells	5818
CD112	PRR2	Myeloid cells	5819
CD133	AC133	Stem/progenitor cells	8842
CD156b	TACE/ADAM17	Adhesion structures	6868
CD158	KIR family (see Table 2)	NK cells	
CD159a	NKG2A	NK cells	3821
CD160	BY55	T cells	11126
CD162R	PEN5	NK cells	6404
CD167a	Discoidin domain R (DDR1)	Adhesion structures	780
CD168	RHAMM	Adhesion structures	3161
CD169	Sialoadhesin	Adhesion structures	6614
CD170	Siglec-5	Adhesion structures	8778
CD171	L1	Adhesion structures	3897
CD172a	SIRP alpha	Adhesion structures	8194
CD173	Blood group H type 2	Carbohydrate structures	
CD174	Lewis y	Carbohydrate structures	
CD175	Tn	Carbohydrate structures	
CD175s	Sialyl-Tn	Carbohydrate structures	
CD176	TF	Carbohydrate structures	
CD177	NB1	Myeloid cells	
CD178	Fas ligand	Cytokine/chemokine receptors	356
CD179a	Vpre-B	B cells	7441
CD179b	Lambda 5	B cells	3543
CD180	RP105	B cells	4064
CD183	CXCR3	Cytokine/chemokine receptors	2833
CD184	CXCR4	Cytokine/chemokine receptors	7852
CD195	CCR5	Cytokine/chemokine receptors	1234
CDw197	CCR7	Cytokine/chemokine receptors	1236
CD200	OX2	Non-lineage molecules	4345
CD201	EPC R	Endothelial cells	10544
CD202b	Tie2 (Tek)	Endothelial cells	7010
CD203c	NPP3/PDNP3	Myeloid cells	5169
CD204	Macrophage scavenger R	Myeloid cells	4481
CD205	DEC205	Dendritic cells	4065
CD206	Macrophage mannose R	Dendritic cells	4360
CD207	Langerin	Dendritic cells	50489
CD208	DC-LAMP	Dendritic cells	
CD209	DC-SIGN	Dendritic cells	30385
CDw210	IL-10 R	Cytokine/chemokine receptors	3587; 3588
CD212	IL-12 R	Cytokine/chemokine receptors	3594
CD213a1	IL-13 R alpha 1	Cytokine/chemokine receptors	3597
CD213a2	IL-13 R alpha 2	Cytokine/chemokine receptors	3598
CDw217	IL-17 R	Cytokine/chemokine receptors	23765
CD220	Insulin R	Non-lineage molecules	3643
CD221	IGF1 R	Non-lineage molecules	3480
CD222	Mannose-6-phosphate/IGF2 R	Non-lineage molecules	3482
CD223	LAG-3	Non-lineage molecules	3902
CD224	Gamma-glutamyl transferase	Non-lineage molecules	2678
CD225	Leu13	Non-lineage molecules	8519
CD226	DNAM-1 (PTA1)	T cells	10666
CD227	MUC.1	Non-lineage molecules	4582
CD228	Melanotransferrin	Non-lineage molecules	4241
CD229	Ly9	Non-lineage molecules	4063

Antibody
~~Structure and~~
Function

Do antibodies work in vivo?

Imaging metastases



Patient with bone metastases from bladder cancer.

Gamma camera image of the lower legs, 5 hours following injection of Tc-99m-C595 anti MUC1 antibody.

AC Perkins QMC Nottingham

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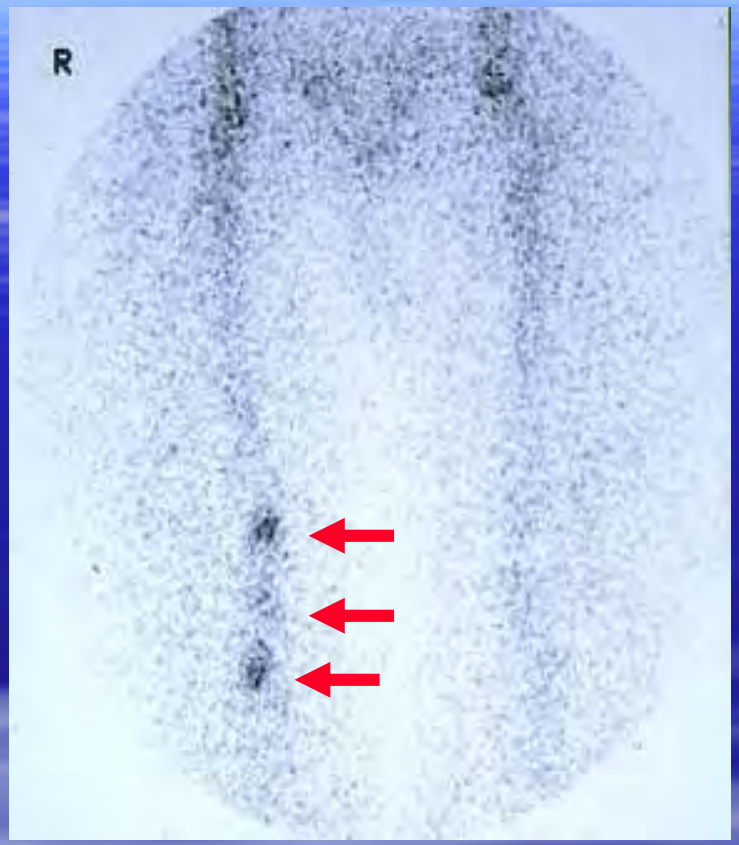
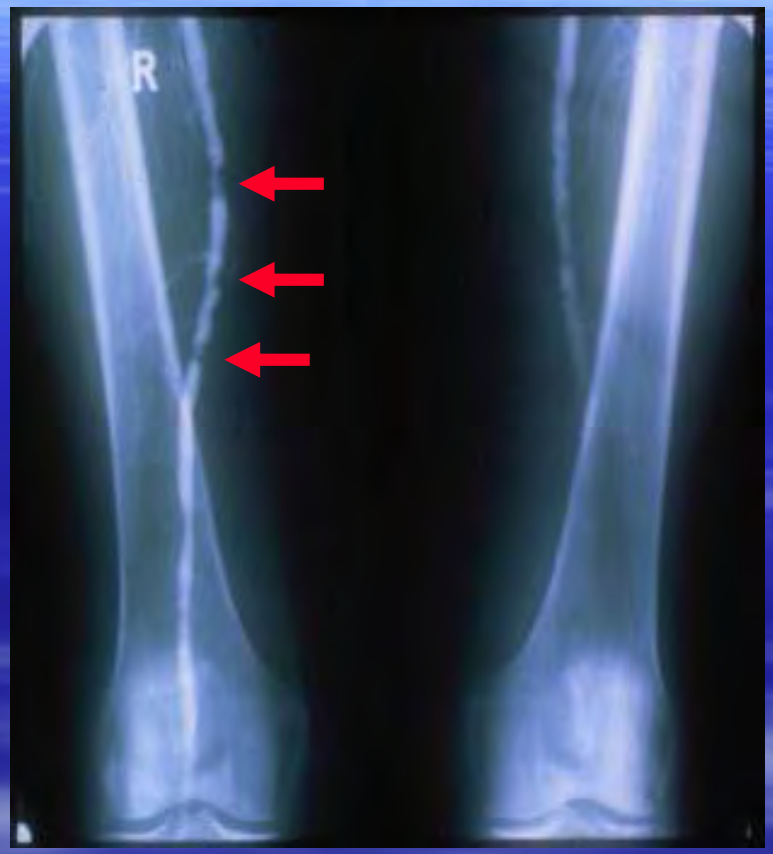
Antibody uptake in thrombus





Patient with arterial thrombus in the right femoral artery.

Right: In-111-P256 Fab' platelet specific antibody showing focal uptake at 3 sites.

Left: X-ray contrast angiogram confirming sites of thrombus in femoral artery.

A.C. Perkins, QMC Nottingham

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Haematological malignancy

Review TRENDS in Molecular Medicine Vol.8 No.2 February 2002

Antibody-directed therapies for hematological malignancies

Michael L. Linenberger, David G. Maloney and Irwin D. Bernstein

British Journal of Haematology, 2000, 108, 679–688

THE EMERGING ROLE OF RADIOIMMUNOTHERAPY IN HAEMATOLOGICAL MALIGNANCIES

T. M. Illidge
P. W. M. Johnson

Cheson BD - J Clin Oncol 2001;19:3908-3911

Press OW, Rasey J. Semin Oncol 2000 ;27(6 Suppl 12):62-73.

Rationale for Radioimmunotherapy in NHL

- NHL is inherently sensitive to radiation.
- Radiotherapy *can* be curative in early stage NHL but is less easily applied to advanced stage disease.
- Synergistic activity between naked Mab and radionuclide.

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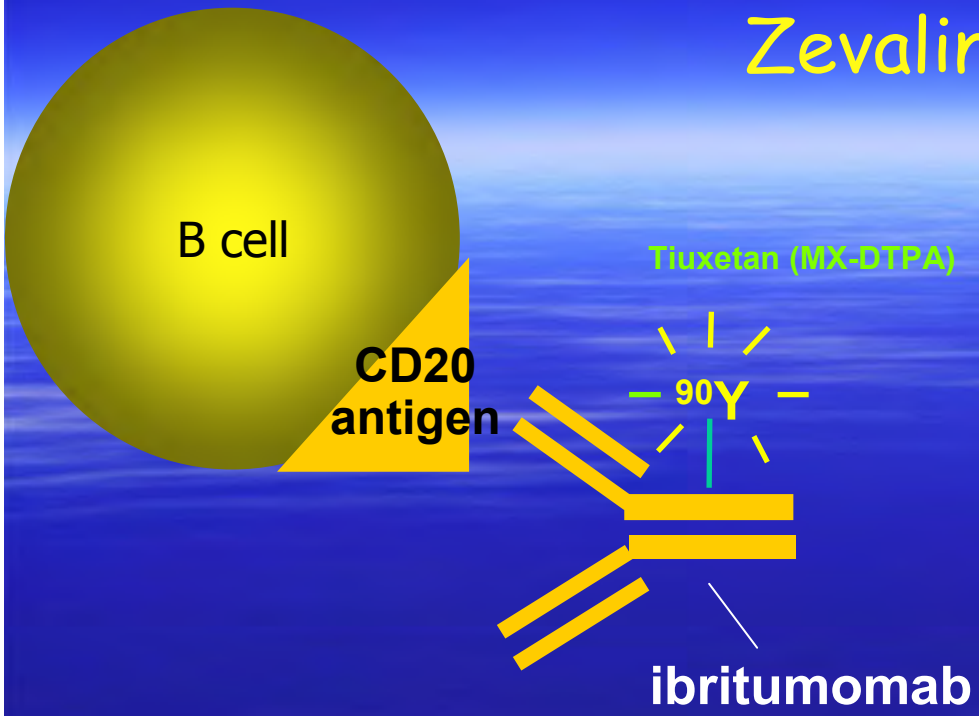
Zevalin (Ibritumomab Tiuxetan)

Murine parent of rituximab



Suitable for outpatient administration

CD20 antigen

- Hydrophobic, 35 kD phospho-protein
- Expressed only on B lineage cells
- Important for cell cycle initiation and differentiation
- Does not shed, internalize or modulate

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Bexxar - Iodine-131 Tositumomab

Tositumomab

- murine $IgG2_a$ anti-CD20 MAb
- B-cell specific
- triggers apoptosis
- antibody-dependent cellular cytotoxicity

Iodine-131

- gamma emission - allows individual dosimetry
- restricts outpatient use.

Antibody Targeted Therapy

Systemic administration

Largely limited to diffuse refractory lymphomas

Design of treatments can be improved

Pretargeting strategies

Intracavitary administration

Suitable for solid tumours

Direct intralesional injection e.g.glioma

IP administration for ovarian carcinoma

Inravesical administration for superficial bladder cancer

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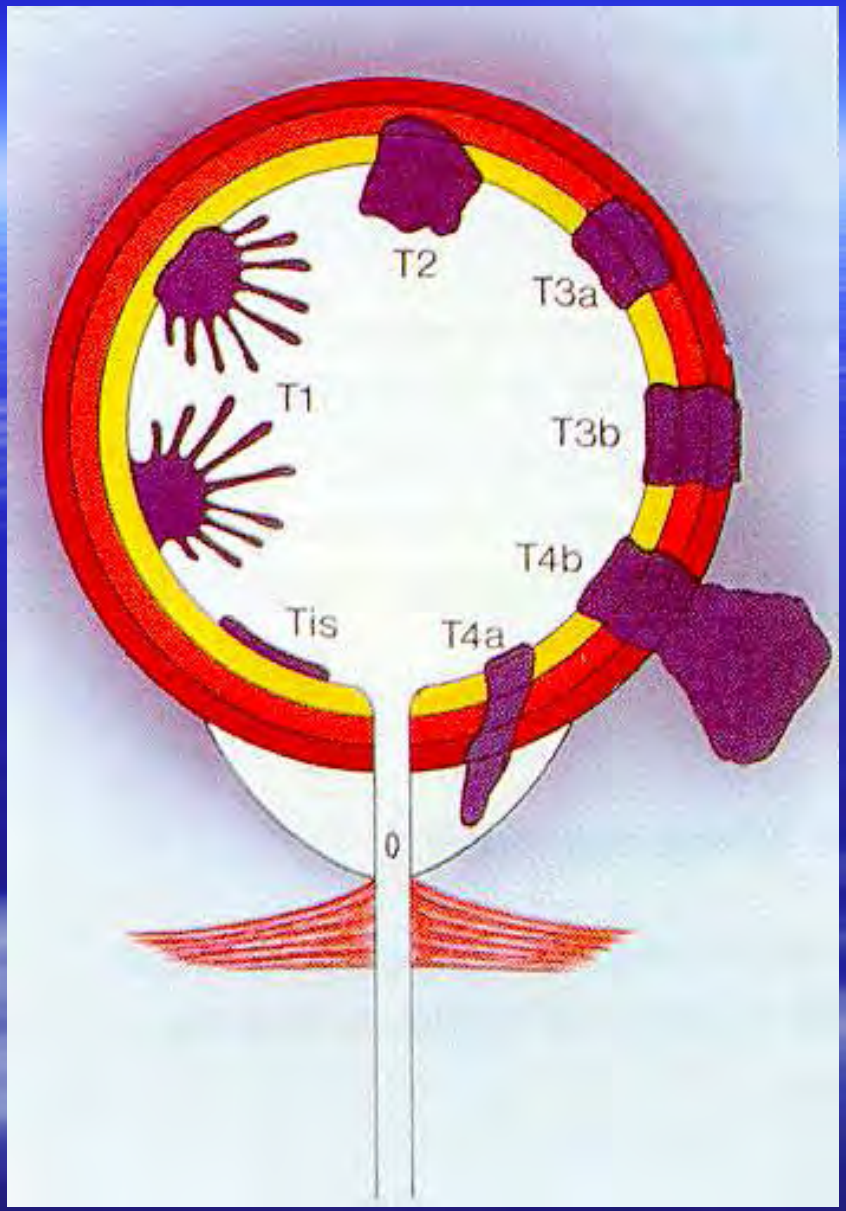

Intravesical Therapy

- Simple procedure
- Well tolerated
- No systemic effects
- Can be easily repeated

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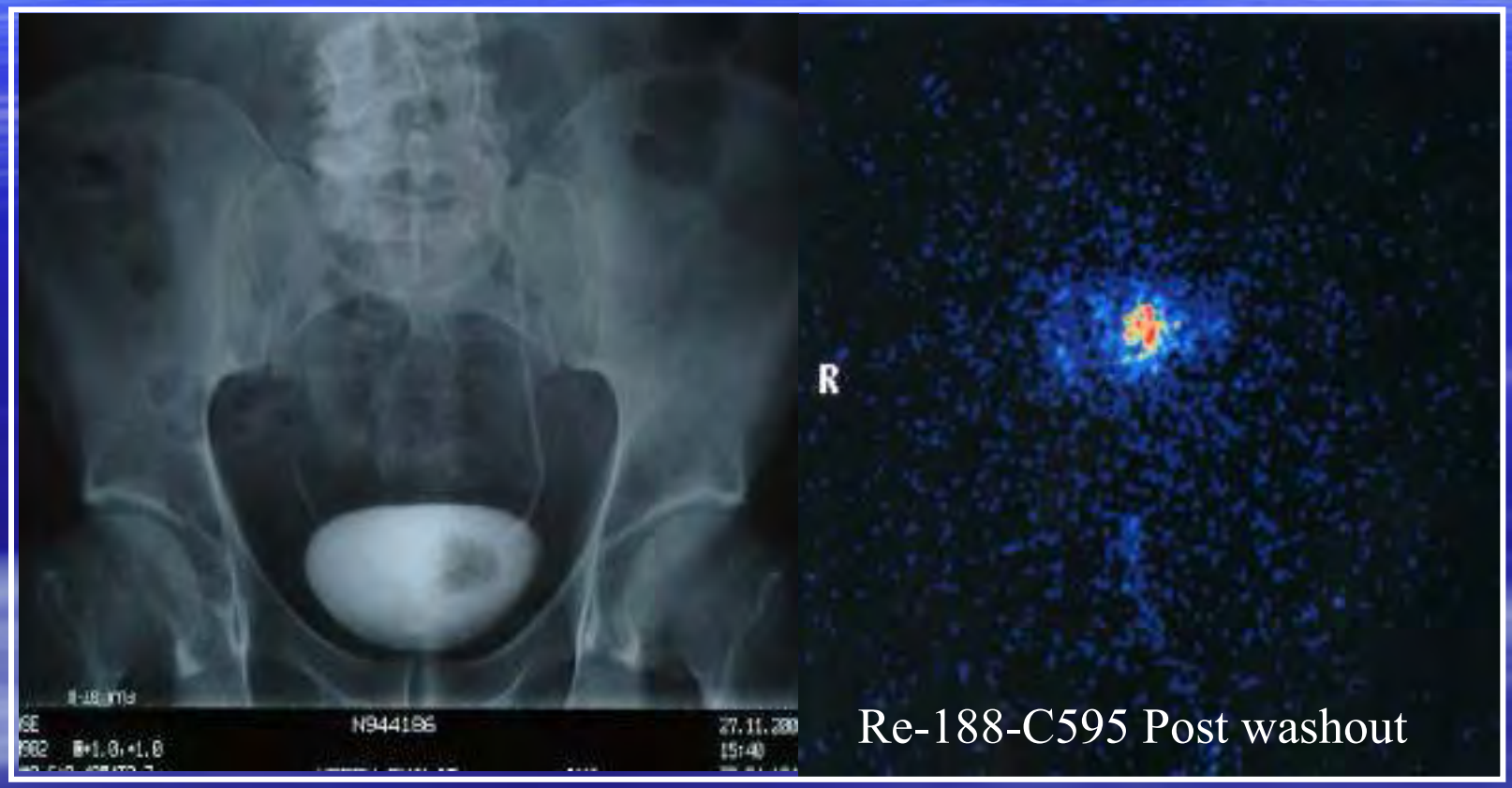
Patient 10 (PK): 40MBq Re-188-C595



Large superficial TCC
Tumour to normal tissue ratio = 79:1

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What are aptamers ?

- Aptamers derived from the Greek word *aptus*, meaning "to fit"

- Aptamers are single or double stranded RNA or DNA oligonucleotide ligands selected for high affinity and the specific molecular fit with targets of interest.

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Molecular size

Intact antibody	150kDa
Fab fragment	50kDa
Single chain fragment	27kDa
Aptamer	8-12kDa
Small peptide	0.5-2kDa

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Aptamer production

Systematic Evolution of Ligands by EXponential enrichment SELEX

- SELEX is a method of combinatorial chemistry employing libraries of nucleic acids for the recognition of a variety of biological and chemical targets
- It is a technology for the identification of high affinity and specificity oligonucleotide ligands (aptamers) based on consecutive rounds of selection and amplification

What is SELEX made of ?

SELEX Libraries

- Libraries may be:
  - Synthetic oligonucleotides (ds or ss, RNA or DNA)
  - Digested genomic DNA

- Library size:
  4^n where n=number of degenerate bases, eg for an oligonucleotide with 25 degenerate bases:

  $4^{25} = 10^{15}$ different ligands

  25 degenerate bases will allow formation of all common secondary structural motifs

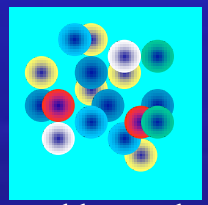
Selection Procedure

Add Library

(1)

(2) Selection

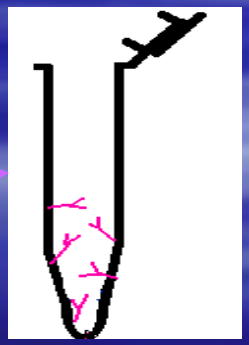
(3) all unbound species are washed away



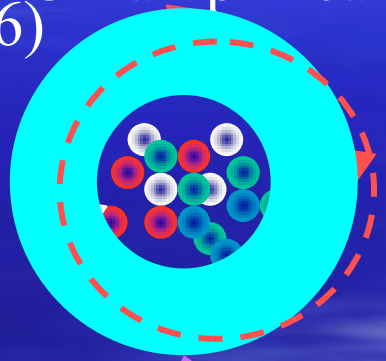
(4) Elution

Desalting

(5) Freeze-drying



(6) PCR amplification



Successive Selection rounds

Cloning

(7)

(8)

Sequencing

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Aptamer binding

Aptamers have proved to be highly selective high affinity-binding ligands

The binding characteristics of aptamers can be influenced by the experimental system used for their selection (modified bases, pH changes, or salt concentrations).

- Affinity of aptamers for:
  - Antibodies: Kd = 1nM
  - Growth Factors Kd = 0.2nM
  - Hormones Kd = 60nM
  - Enzymes Kd = 10nM
  - Amino acids Kd = ~10nM

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Why use Aptamers ?

- Cheap, efficient, reproducible and rapid production
- Stable: - long term storage
  - transportation in ambient temperatures
- Versatile and easy to modify
- Small size - less immunogenicity,
  - good tumour penetration
- Good as inhibitors, antagonists or regulators of pathways
  (VEGF, NX1838)
- Carrier/reporter molecules
  (Fluorophores, radionuclides etc)
- Excellent molecular probes and sensor recognition units

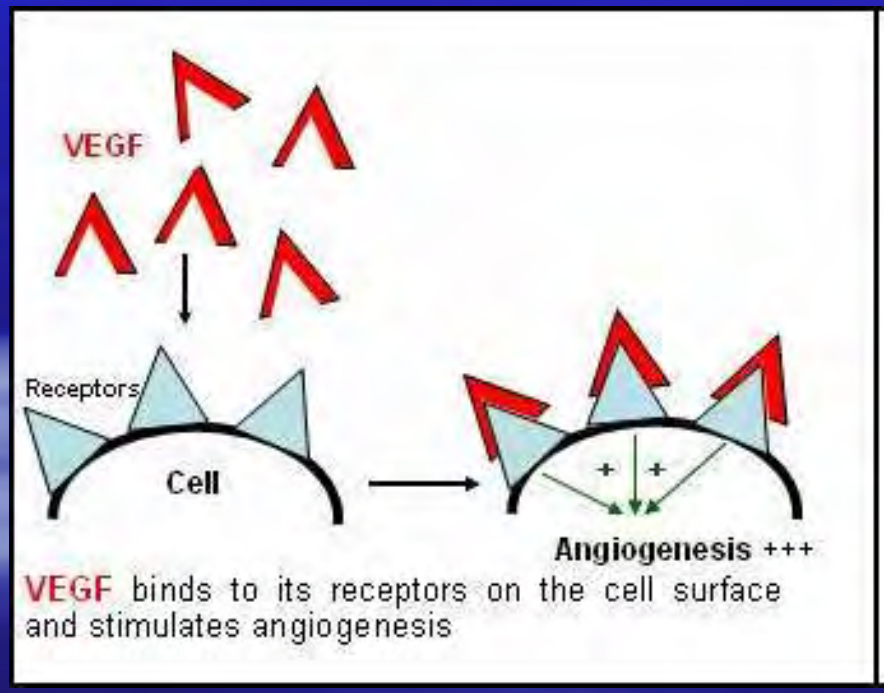
Applications

- DNA ligands as inhibitors or antagonists
- Diagnostic assays
- Sensors (biosensors/chemosensors)
- Targeted therapeutics :
  - Delivery system for non-specific inhibitors
  - Drug attached to aptamer
  - Radionuclide attached to aptamer

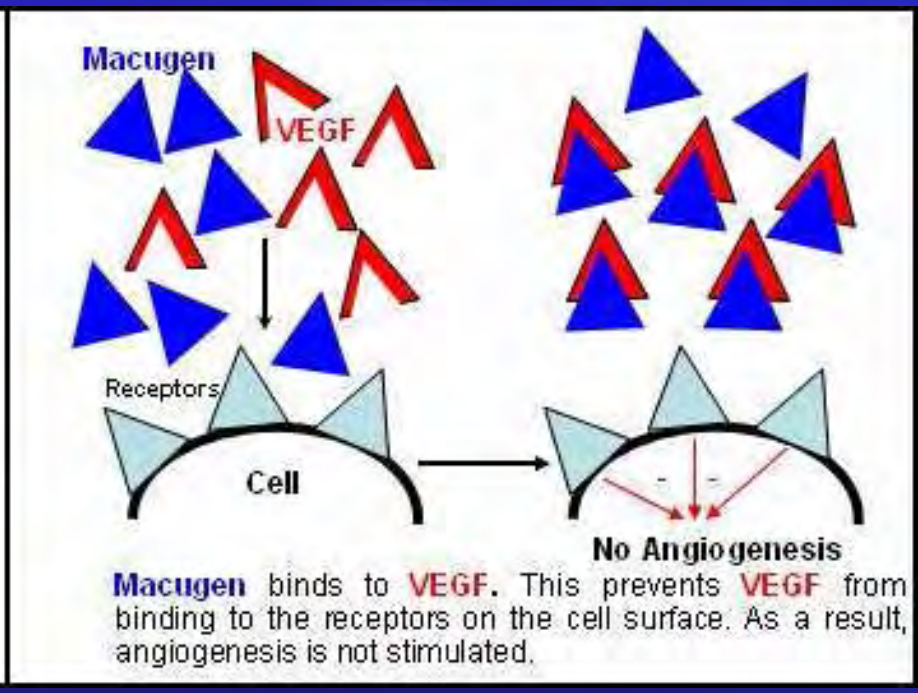
Macugen™

- The first pharmaceutical aptamer formulation, Macugen® (pegaptanib sodium injection) was approved in the United States in 2004.
- Anti-VEGF aptamer formulation.
- Used for the treatment of Neovascular age-related macular degeneration.
- Pegaptanib sodium is a covalent conjugate of twenty-eight nucleotides in length terminating in a pentylamino linker, to which polyethylene glycol (PEG) is attached via the two amino groups on a lysine residue.
- It is formulated as a sterile, aqueous solution for intravitreous injection.

Macugen™ Mode of action



VEGF binds to its receptors on the cell surface and stimulates angiogenesis



Macugen binds to VEGF. This prevents VEGF from binding to the receptors on the cell surface. As a result, angiogenesis is not stimulated.

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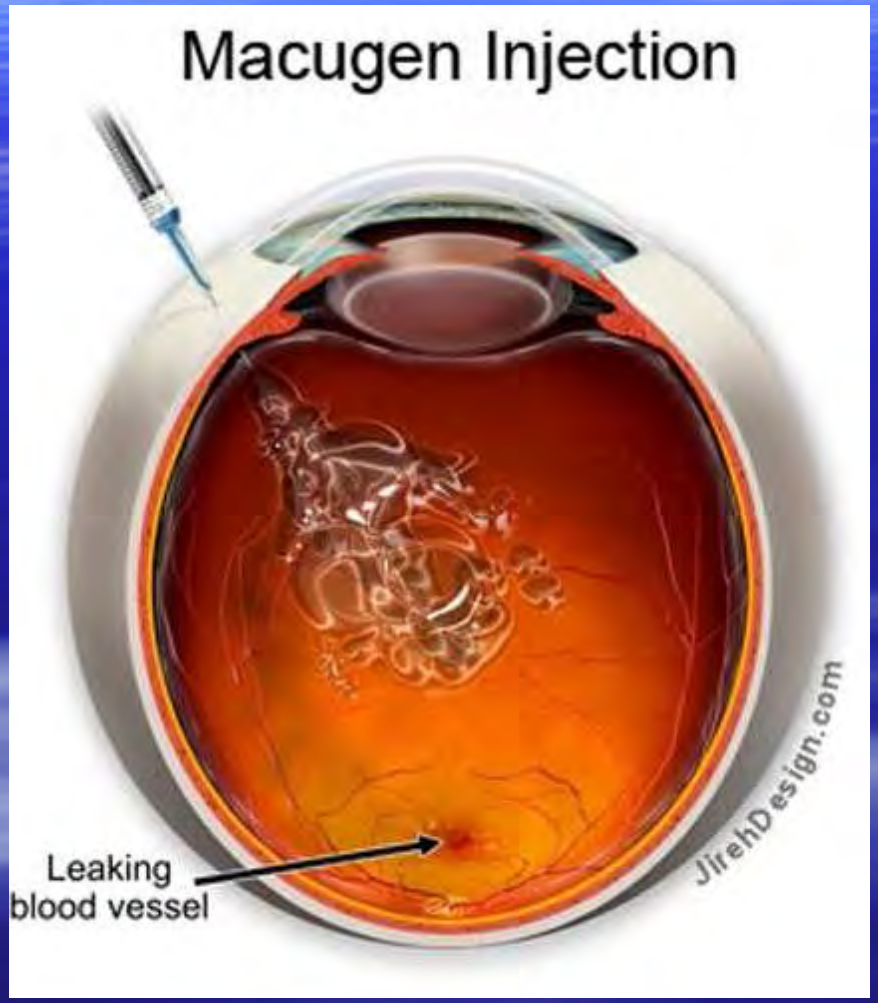
intravitreous injection



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Aptamers as imaging agents

1. Thrombus

Dougan et. al. Evaluation of DNA aptamers directed to thrombin as potential thrombus imaging agents. Nuclear Medicine and Biology 2003; 30:61-72.

2. Inflammation

Charlton et. al. In vivo imaging of inflammation using an apatamer inhibitor of human neutrophilo elastase. Chem Biol 1997;4:809-816.

3. Alzheimer's Disease

Ylera et. al. Selection of RNA aptamers to the Alzheimer's disease amyloid peptide. Biochemical and Biophysical Research Communications 2002; 290:1583-1588.

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Hicke et. al. Tumour targeting by an aptamer. J. Nucl. Med. 2006;47:668-678.

- In 2000 Hicke and Stephens used the term "escort aptamers" indicating that aptamers offered a delivery service for diagnosis and therapy.

- Aptamer (TTA1) produced against extracellular matrix protein Tenascin-C.
- Radiolabelled with ^{99m}Tc using mercapto-acetyl glycene (MAG_2) and DTPA.
- Biodistribution studies were undertaken in nude mice bearing either U251 glioblastoma or MDA-MB-435 breast tumour xenografts.

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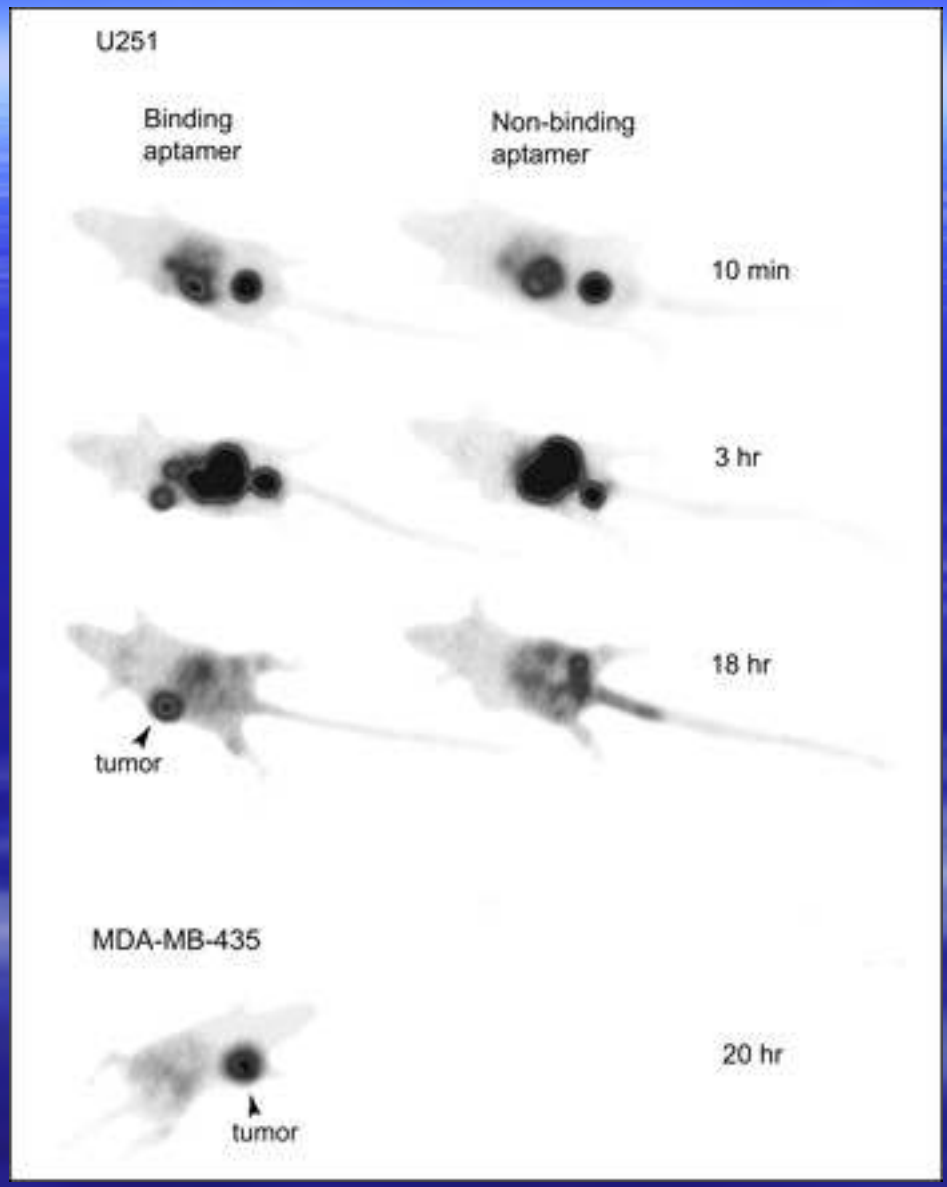
Hicke et. al. JNM. 2006;47:668.

Tc-99m-TTA1 aptamer directed against Tenascin-C

U251 glioblastoma xenografts



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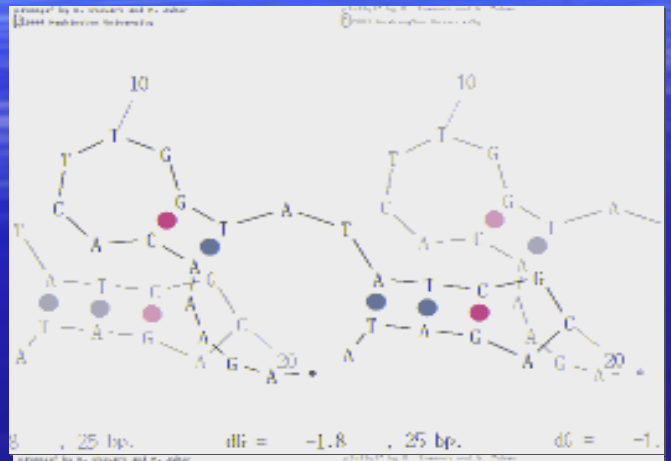
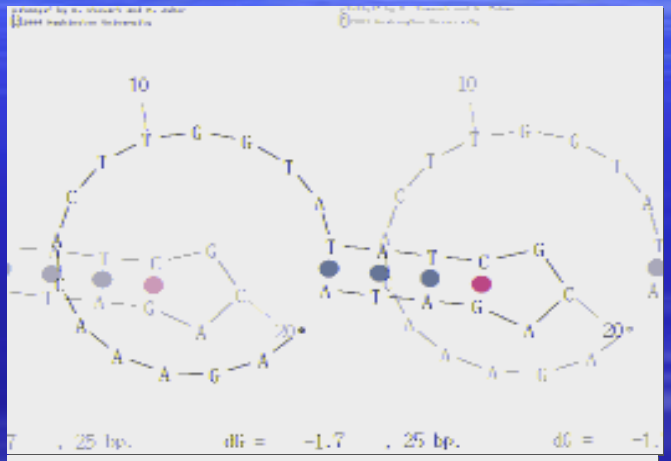
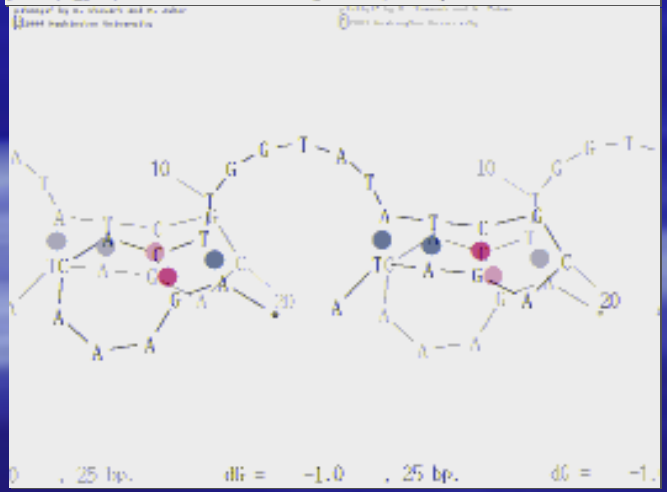
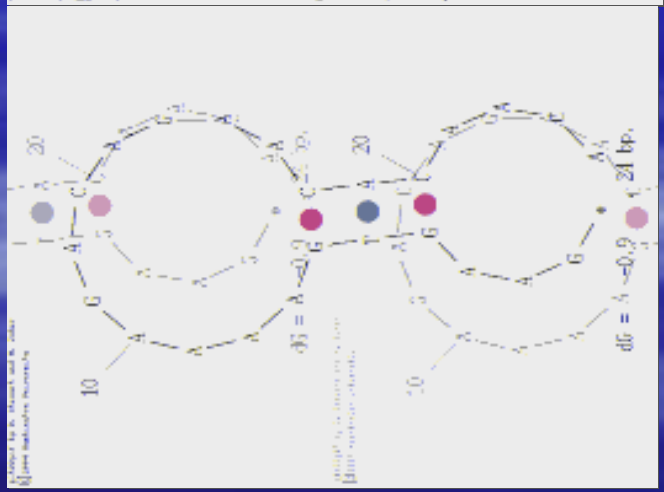
Selected aptamers against MUC1









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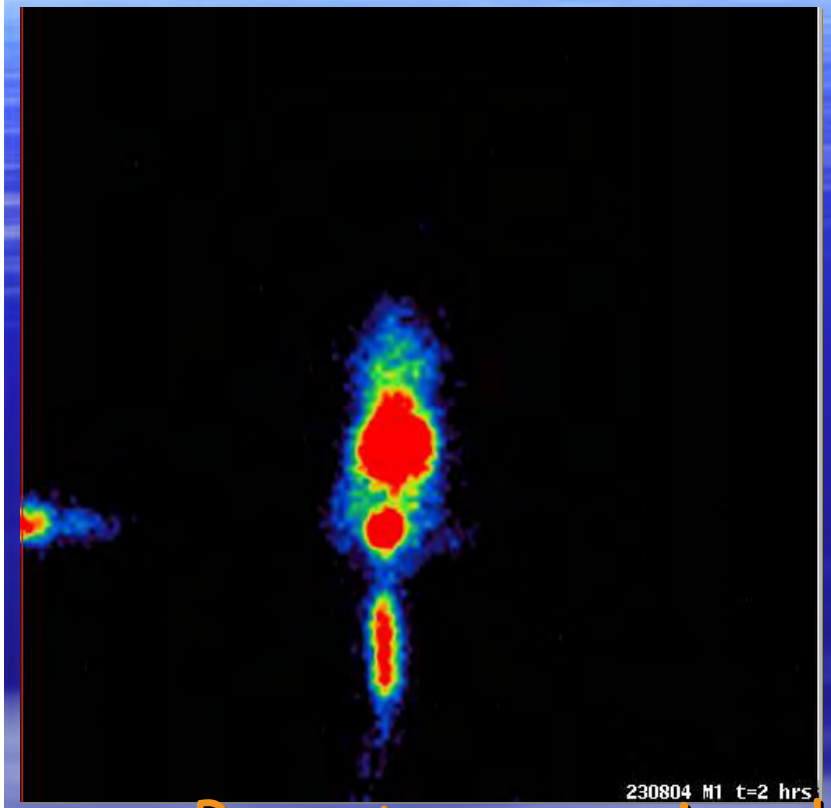
MCF-7 tumour imaging



Poor tumour visualisation due to high amount of activity in the kidneys and bladder resulting from the rapid clearance of the aptamers due to their small size.

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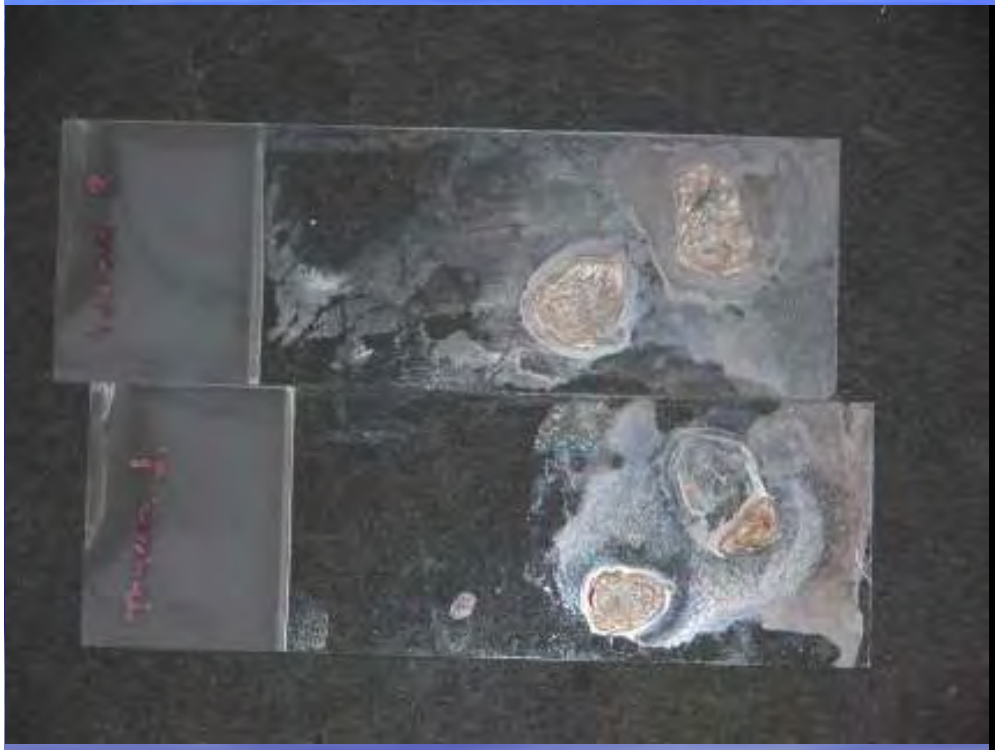
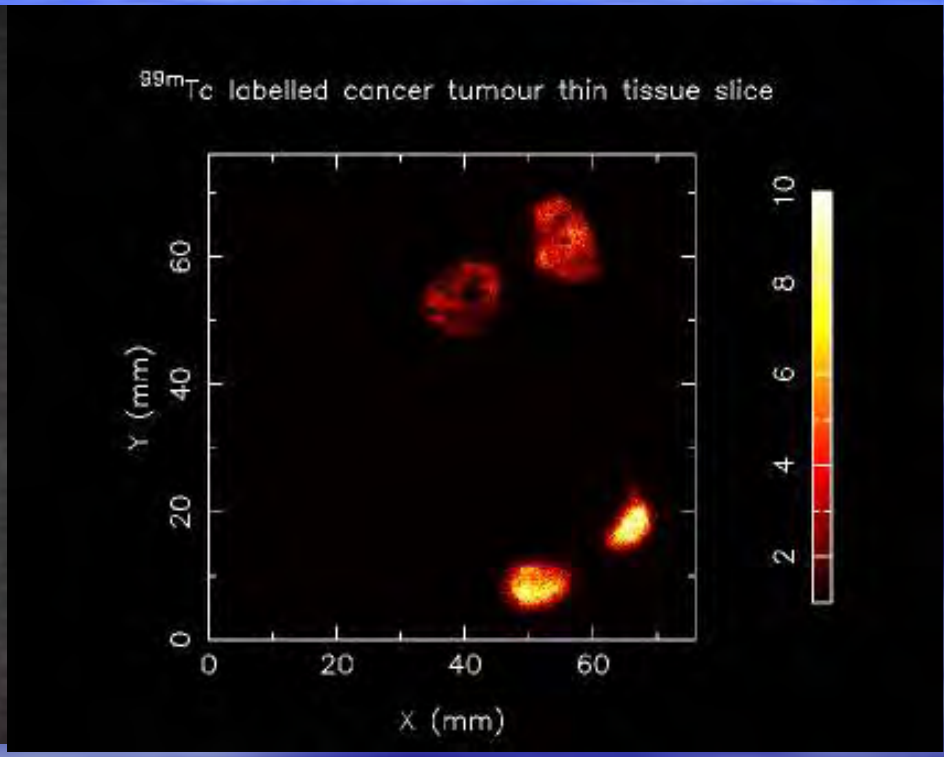
Electronic autoradiography



MCF-7 Tumour Slides



Imaging of tumour slides in a microchannel plate detector

Tumour penetration of aptamers superior to Mab !

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Tumor Therapy with Targeted Atomic Nanogenerators

Michael R. McDevitt,[1] Dangshe Ma,[1] Lawrence T. Lai,[1]
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A single, high linear energy transfer alpha particle can kill a target cell. We have developed methods to target molecular-sized generators of alpha-emitting isotope cascades to the inside of cancer cells using actinium-225 coupled to internalizing monoclonal antibodies. In vitro, these constructs specifically killed leukemia, lymphoma, breast, ovarian, neuroblastoma, and prostate cancer cells at becquerel (picocurie) levels. Injection of single doses of the constructs at kilobecquerel (nanocurie) levels into mice bearing solid prostate carcinoma or disseminated human lymphoma induced tumor regression and prolonged survival, without toxicity, in a substantial fraction of animals. Nanogenerators targeting a wide variety of cancers may be possible.

SCIENCE VOL 294 16 NOVEMBER 2001 1537

Merits of and radiation

- The mass is 7000 x that of (4 mass units versus 1/1800)
- The 's energy is 30 x that of (typically 6 MeV versus 200 keV)
- The electric charge is double (+2 versus -1)
- LET ~100 times greater (range 50-90 m)
- Typically 0.25Gy in 10 m cell diameter
- The effective range of particles in tissue is approx 5 cell diameters compared with hundreds/thousands for particles.

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Some -emitting radionuclides of interest to nuclear medicine

Radionuclide	$T_{1/2}$	energy (MeV)	Range (m)
^{149}Tb	4h	3.97	26
^{211}At	7.21h	6	65
^{212}Bi	1h	8.79	87
		6.0	65
^{213}Bi	45.6min	8.4	80
		5.9	58
^{225}Ac	10d	6.0	65
		7.0	75
		8.4	85
		5.9	58

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Radium-223 treatment of skeletal metastases

Cations of the heavy alkaline earth elements naturally seek bone !

^{223}Ra (, $T_{1/2}$ = 11.4d)
--> ^{219}Ra (, $T_{1/2}$ = 3.96s)
--> ^{215}Po (, $T_{1/2}$ = 1.78ms)
--> ^{211}Pb (, $T_{1/2}$ = 36.1m)
--> ^{211}Bi (, $T_{1/2}$ = 2.17min)
--> ^{207}Tl (, $T_{1/2}$ = 4.77min)
--> ^{207}Pb (stable)

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Alpharadin™ Algeta, Oslo

Preclinical studies employing ^{223}Ra ($T_{1/2}$ = 11.4 d) in a skeletal metastases model of human breast cancer revealed a strong affinity for the skeleton and demonstrated significant anti-tumor activity.

Henriksen et al. J Nucl Med 2003 Feb;44(2):252-9.

Phase I clinical trial in patients with skeletal metastases from breast and prostate cancer.

Dose range 46-250kBq/kg

Nilsson et al. Clin Cancer Res 2005:11;4451-4459

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Algeta is conducting trial BC1-02 as part of its Phase II clinical trial of Alpharadin(TM), a novel radiopharmaceutical based on the alpha particle emitter radium-223, which naturally targets and attacks skeletal metastases. The double-blind placebo-controlled trial involves 64 patientswith painful skeletal metastases as a consequence of HRPC and is in its follow-up phase at 11 centers in Norway, Sweden and the UK. The trial wasfully enrolled in May 2005

Algeta believes that Alpharadin may offer an anti-tumor effect and significant advantages over existing palliative treatments, improving life expectancy and quality of life based on the following key properties: Demonstrated anti-tumor effects

Minimal side-effects

Ready-to-use formulation of radium-223 chloride

Administered on out-patient basis

Intrinsic targeting of skeletal tissues

Selective accumulation in skeletal metastases

Optimal half-life of 11.4 days

Photo emission enables concurrent imaging

Safe and easy produce, delivery, handling and disposal

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Practicalities of targeted therapy

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Radiopharmaceutical Laboratory

Sterile pharmaceutical production area

Radiation laboratory

Regulatory certificates and licences

e.g.UK MHRA
Environment Agency
Heath and Safety
Dept of Health.

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Radiation Protection Standards

Registration and Authorisation Certificates
Local rules dated and reviewed
Warning signs for Controlled/Supervised areas
Staff monitoring
Appropriate use of shielding
Calibration of contamination monitors
Storage of radioactive materials
Disposal of radioactive waste
Safe to clean/permit to work
Radiation audit

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References

1. International basic safety standards for protection against ionizing radiation and for the safety of radiation sources. Safety series 117 IAEA Vienna 1996.
2. Applying radiation safety standards in nuclear medicine. Safety reports series 40 IAEA Vienna 2005.
3. Nuclear Medicine Resources Manual. IAEA Vienna 2006.
4. ICRP Publication 94: Release of Patients after Therapy with Unsealed Radionuclides. International Commission on Radiological Protection ISBN 0080445608 2005
5. Medical and dental guidance notes. IPEM York 2002

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Therapeutic radiopharmaceuticals

- Dispensed in the radiopharmacy as sterile products.
- Use licensed products when possible.
- Written procedures for all preparations.
- Batch manufacturing records,

  lot No, staff names etc.
- QC and sterility tests

  Essential for therapeutics

  (some tests may be retrospective)
- Adverse reactions reported.

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Administration to patients

Checklist

1. Full patient history including home circumstances
2. Patient identification

   Name / d.o.b. / address
3. Any patient questions ?
4. Check radiopharmaceutical,

   Radionuclide & chemical form.
5. Amount of radioactivity prescribed for the procedure.

   Administration set

   Gloves and syringe shield.
6. Route and speed of injection.
7. Flushing of the line.

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Breast feeding patients

(Physical and chemical characteristics of emitted radiation)

Interrupt breast feeding and monitor activity in milk.

(*Pharmacokinetics and excretory path of radiopharmaceutical)*

Obtain advice from:

Publications and Notes for Guidance.

(*For table of excretion data see J Nucl Med 2000;41:863-873)*

Nuclear Medicine Department.

RPA/MPE.