Rosalyn Yalow: Contributions and Legacy

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Radioimmunoassay:
A Probe for fine structure of biological systems

Rosalyn Yalow's Nobel lecture on 8th Dec, 1977

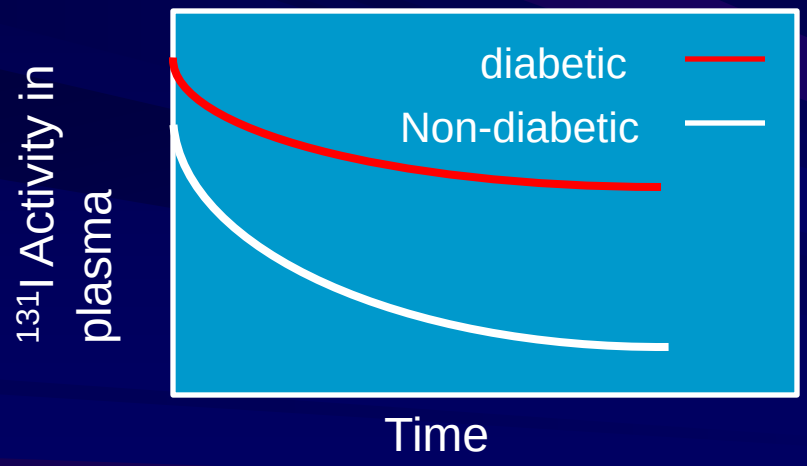
"From 1950 until his untimely death in 1972, Dr Solomon Berson was joined with me in this scientific adventure and together we gave birth to and nurtured through its infancy radioimmunoassay, a powerful tool for determination of virtually any substance of biologic interest."

Radioimmunoassay

“fall out from an seemingly unrelated study”

Dr Arthur Mirsky had hypothesized that maturity-onset diabetes might not be due to a deficiency of insulin secretion but to abnormally rapid degradation of insulin by hepatic insulinase.

To test this hypothesis, Drs. Berson & Yalow studied the metabolism of ^{131}I-insulin to diabetic and non-diabetic subjects.



Their hypothesis was that the retarded rate of insulin disappearance was due the binding of labeled insulin to antibodies, produced in response to the administration of exogenous insulin.

Radioimmunoassay
A new ultra-sensitive method is born

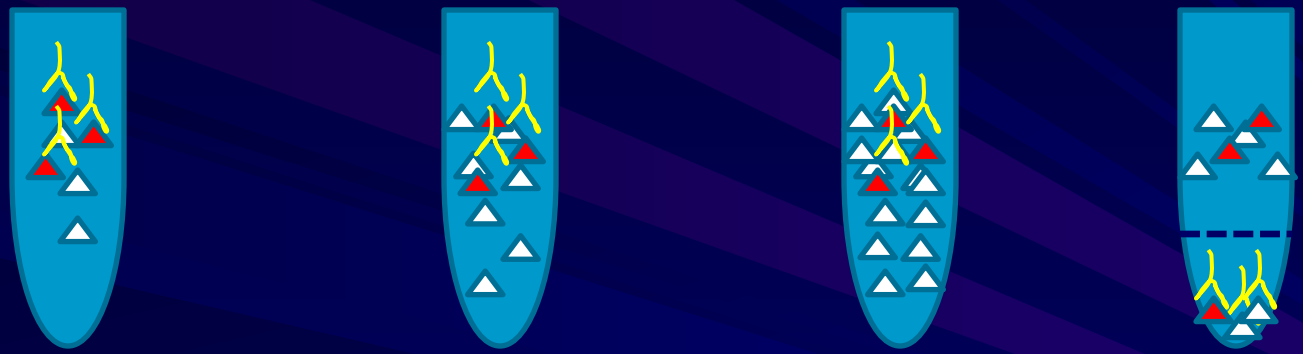
Using a variety of techniques Berson & Yalow were able to demonstrate the ubiquitous presence of insulin binding antibodies in insulin treated diabetic subjects.
This concept was so foreign to immunologists of the time that Dr Yalow's paper was rejected by Science.

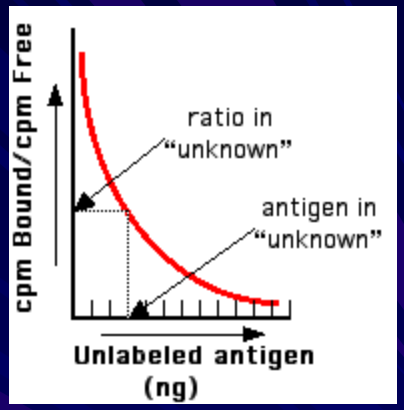
It was then sent to Journal of Clinical Investigation – it was initially rejected there as well.
The journal editor Stanley Bradley said amongst other comments
"The second major criticism relates to the dogmatic conclusion set forth which are not warranted by the data".

Finally the journal accepted this landmark study provided the authors would remove the use of the word insulin antibody from both the paper title and conclusions.

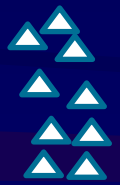
Yalow, RS, Berson, SA. Immunoassay of endogenous plasma insulin in man. *J. Clin. Invest.* 1960. **39:**1157-1175.

Radioimmunoassay
The Technique

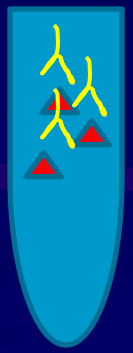




Generate binding curve



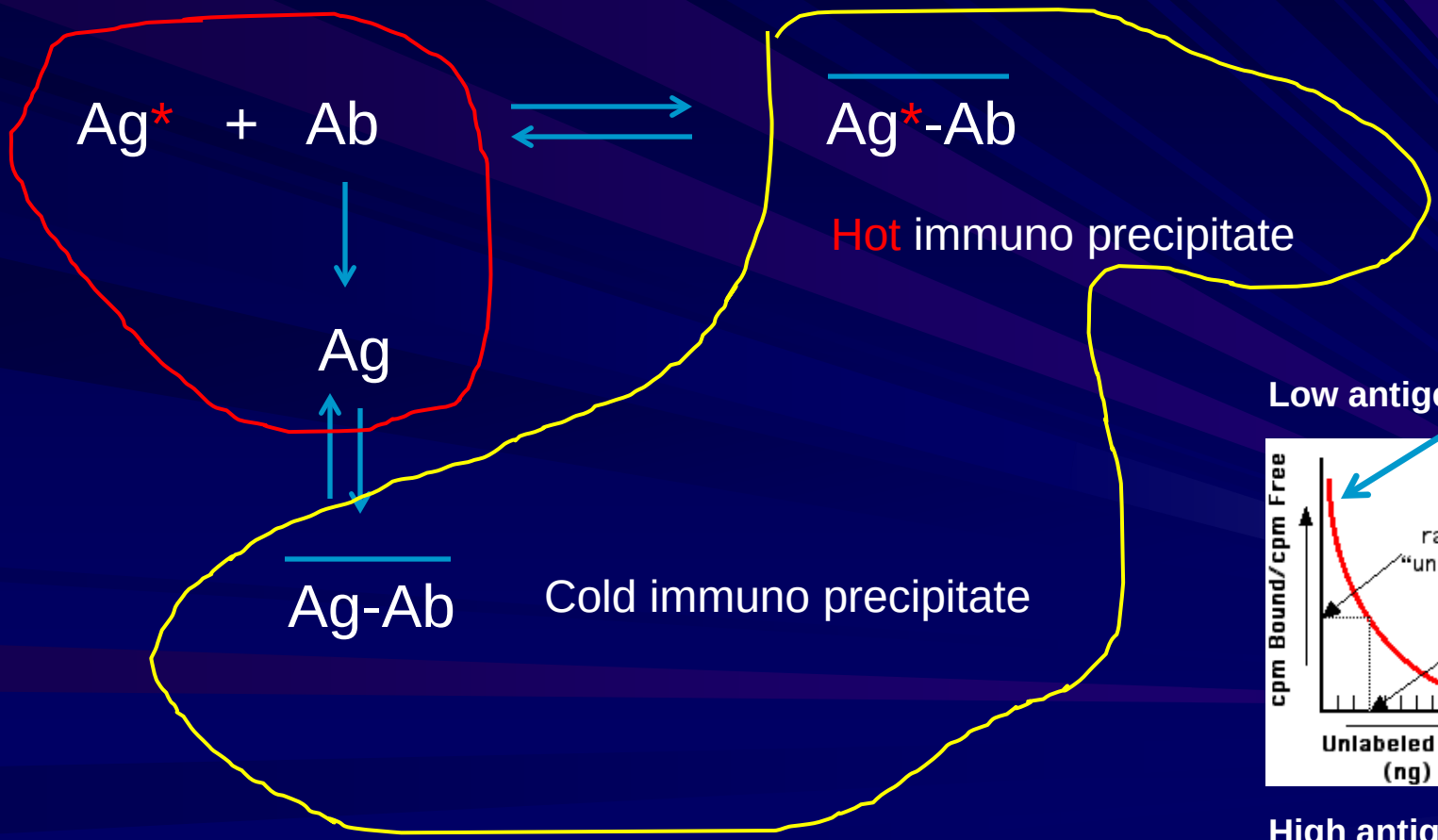
Patient serum with unknown antigen concentration



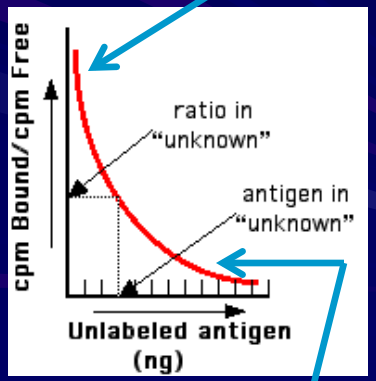
RIA has the ability to measure antigens down to picomolar concentrations

The Formalism

Competing reactions that form the basis of radioimmunoassay



Low antigen conc.

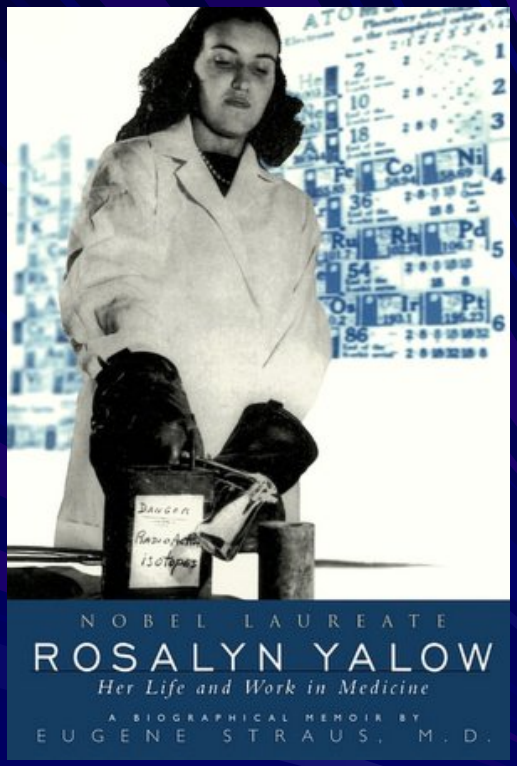


High antigen conc.

The Legacy

“The radioimmunoassay principle is not limited to immune systems.

The specific antibody can be replaced by any specific binding protein in plasma, a specific enzyme or tissue receptor site. “



This is a truly universal vision and shows the importance and enormity of the discovery of Yalow and Berson.

Extrapolating the Legacy

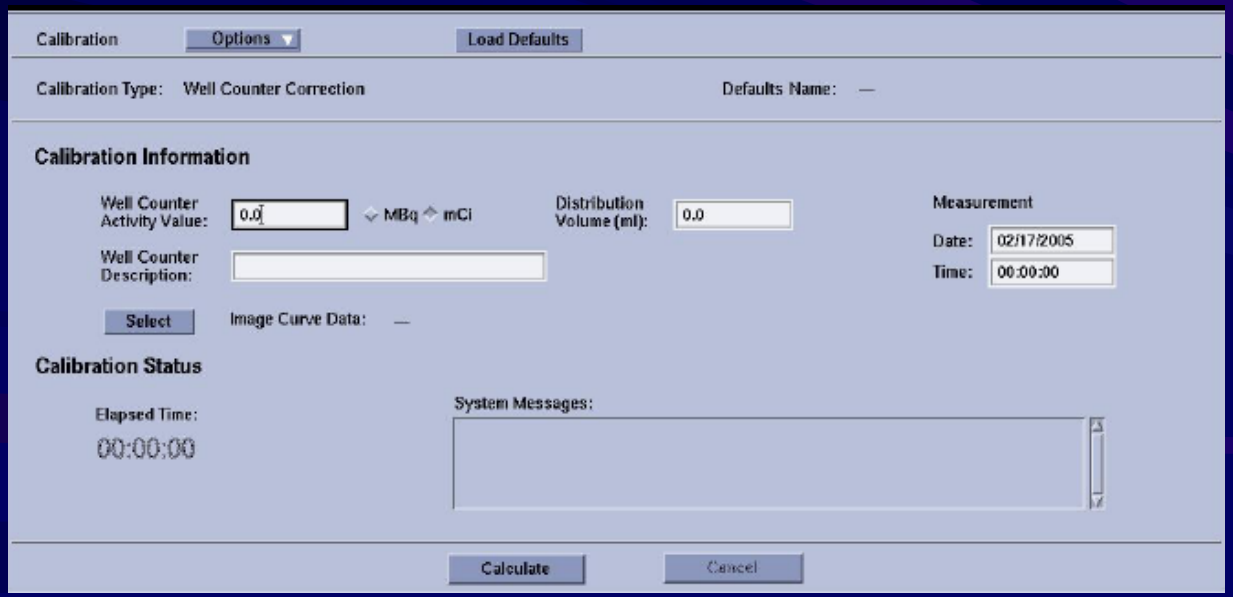
Could these RIA principles based on *ex vivo* measurements be applied to *in vivo* measurements?

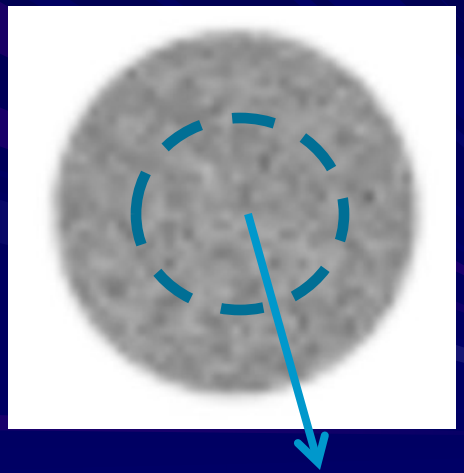
PET scanners can determine the absolute amount of a radiotracer within the body.

PET System Calibration

The PET scanner is calibrated by performing an emission scan with a known specific activity

This provides the scanner with the necessary information to convert count rate into activity.





How Sensitive is PET?

Physics definition of sensitivity

Ratio of the number of photons detected/number of photons emitted by the source (1.8 cps/kBq)

Chemical translation of sensitivity

What typical specific activities of the PET tracer are taken up in the tumor e.g. 1.0 μCi/g translates into ~ **0.4 picogram** of FDG in the lesion. This is ~ 0.6 picomolar concentration compared to 1 mM for the plasma concentration of glucose (a clear demonstration of the tracer principle).

The sensitivity of Radioimmunoassay

Using antibodies of high affinity ($K_0 = 10^8$–10^{11} M^{-1}), it is possible to detect a few picograms of antigen in the tube

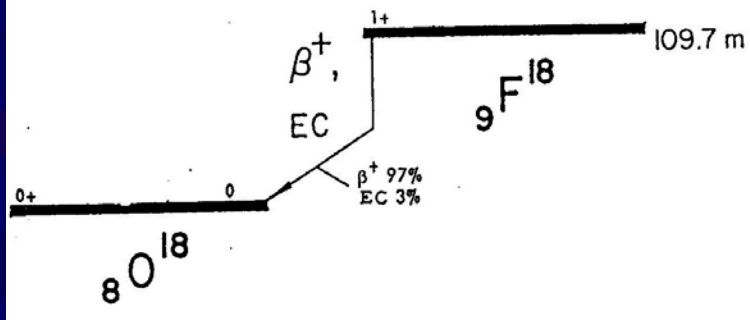
Physical Properties of Positron Emitters

Radionuclide	Half-Life	β^+			"High-Energy" * γ-rays	
		Mean Energy (keV)	Mean Range in Tissue (mm)	Branching Ratio (%)	Energy (keV)	Abundance Prompt γ (%)
Carbon-11	20.4 min	385	1.1	100	N/A	N/A
Nitrogen-13	10 min	492	1.4	100	N/A	N/A
Oxygen-15	2 min	735	2.5	100	N/A	N/A
Fluorine-18	110 min	250	0.6	97	N/A	N/A
Iodine-124	4.2 d	819	2.8	23	603-1,690	84

Simple vs. Complex positron emitting decay schemes

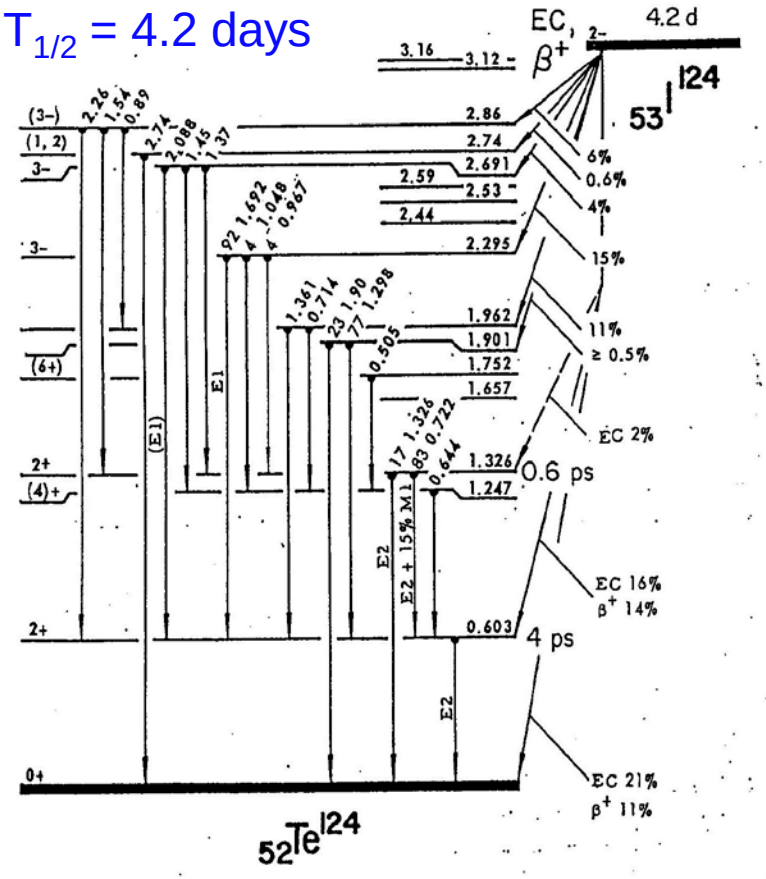
Fluorine-18

$T_{1/2}$ = 110 minutes

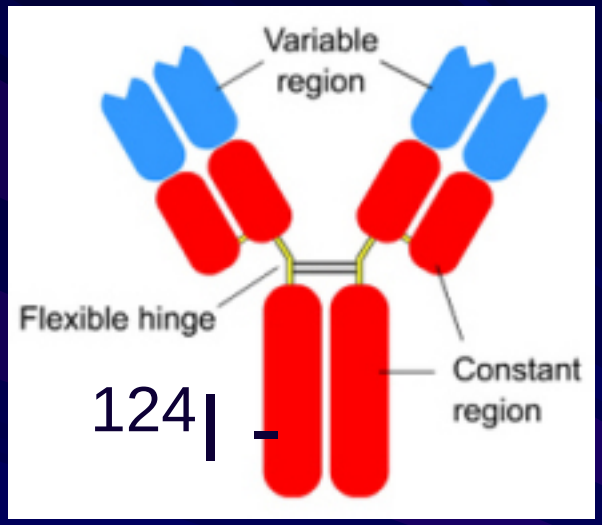


Iodine-124

$T_{1/2}$ = 4.2 days



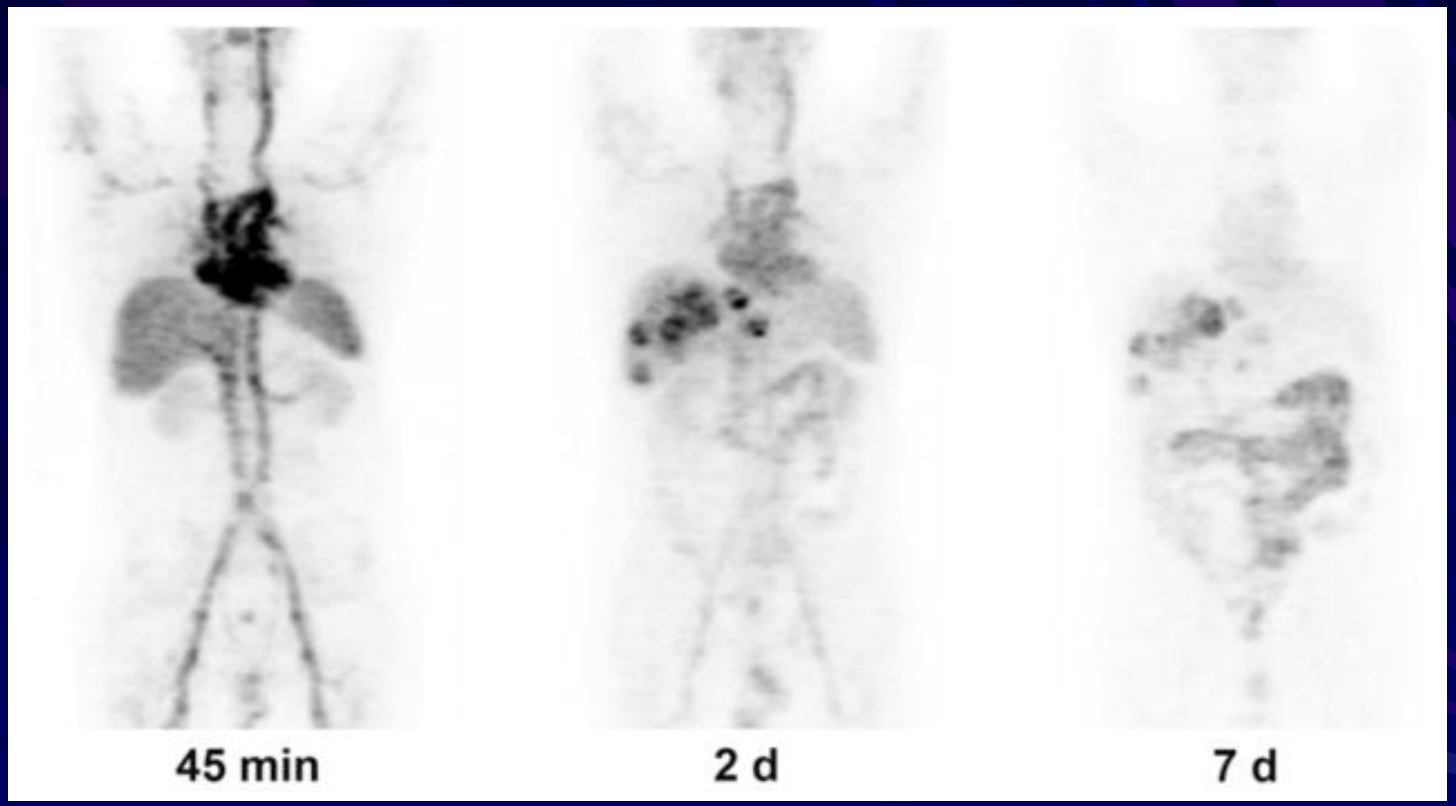
RadioimmunoPET



A33 – is a transmembrane glycoprotein antigen that is expressed on >95% of human colon cancers. It is also expressed in normal human colonic and small bowel epithelium but absent from all other human tissues.

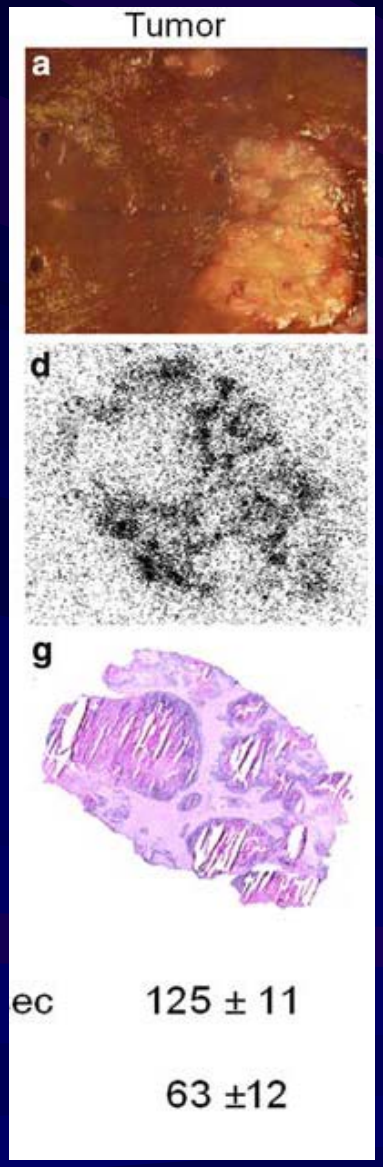
The A33 antibody that binds to this antigen has an affinity of between 10^{-8} and 10^{-9} M (comparable to those used for RIA).

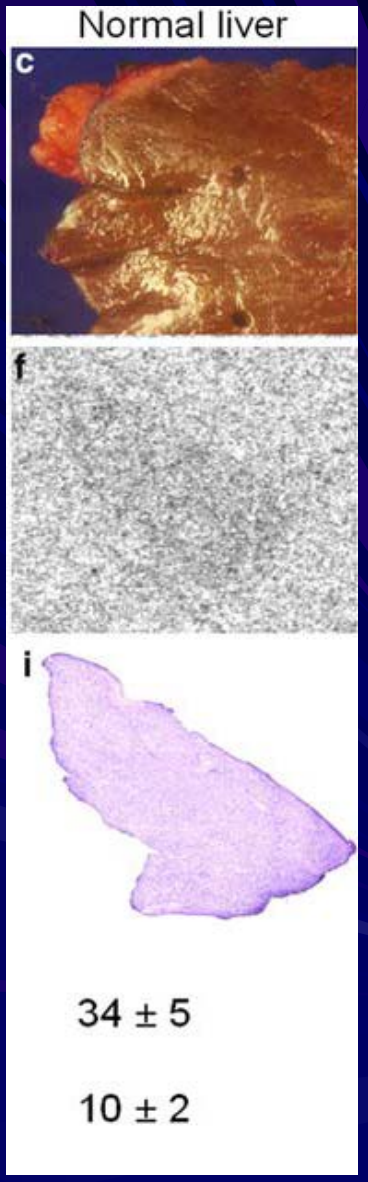
When to Image?
^{124}I-huA33 serial PET Images



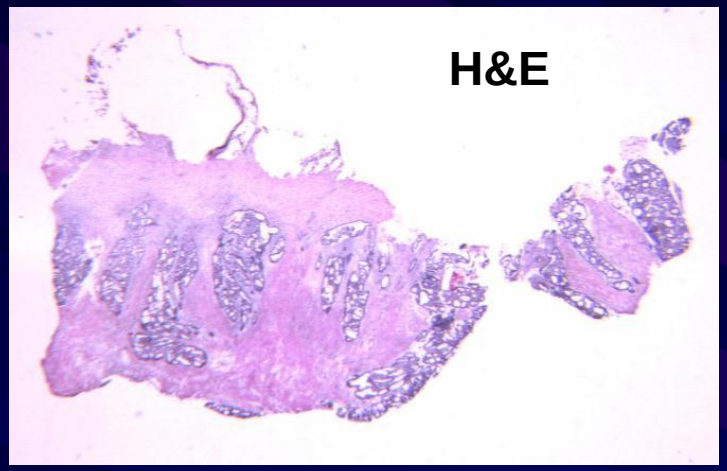
Carrasquillo et al, ^{124}I-huA33 antibody PET of colorectal cancer. J Nucl Med. 2011 Aug;52(8):1173-80.

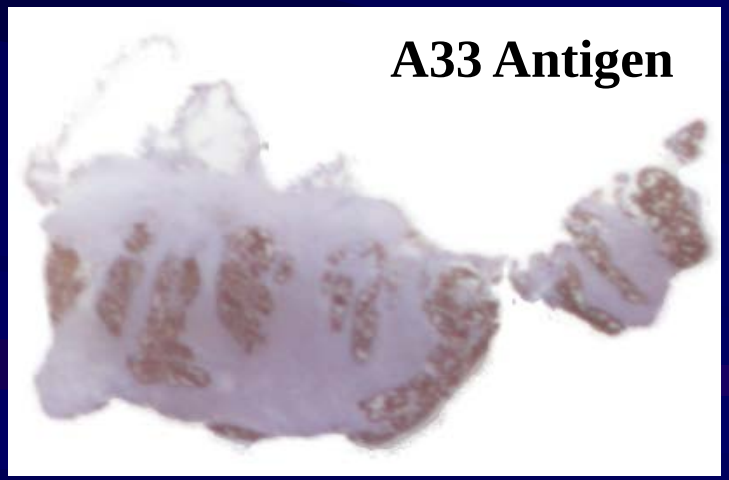
Lets surgically check if ^{124}I-huA33 binds to tumor antigen

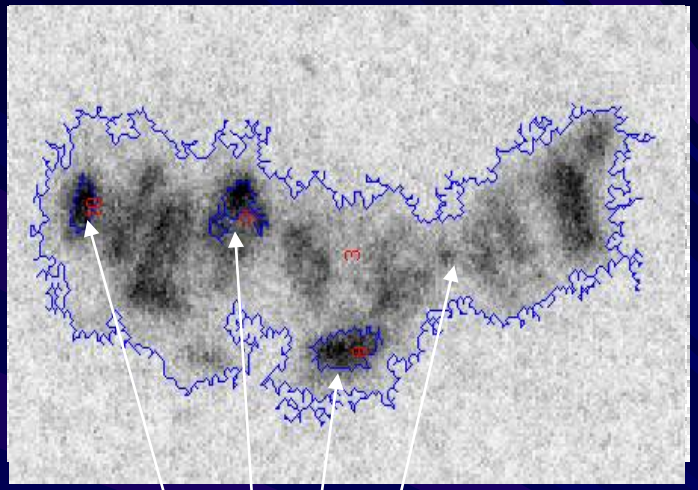




Histology, DAR and IHC

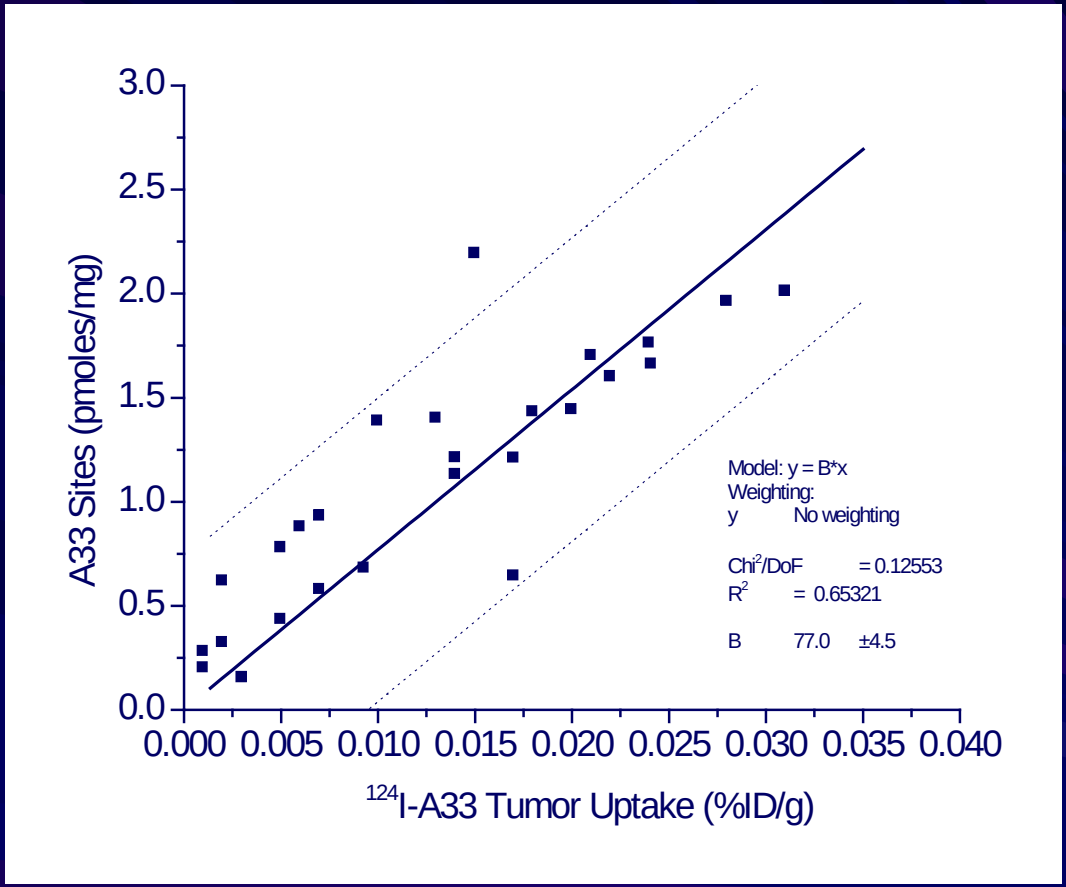






ROI	PSL/mm^2	uCi hr/g	uCi/g	%ID/g
Average:	37.73	8.28	0.64	0.017
H1:	79.52	19.52	1.50	0.039
H2:	98.56	24.19	1.86	0.049
H3:	102.54	25.17	1.94	0.051

Correlation between ^{124}I-A33 uptake and antigen levels



O'Donoghue JA et al , ^{124}I-huA33 antibody uptake is driven by A33 antigen concentration in tissues from colorectal cancer patients imaged by immuno-PET. J Nucl Med. 2011 Dec;52(12):1878-85.

Non-invasive imaging of antigen concentration

Imaging after several days using ^{124}I – antibodies may provide a non-invasive signal intensity that is directly proportional to the antigen concentration within a tumor voxel.

The result would be a non-invasive in-vivo radioimmunoassay.

If the number of antigens per cell are known, this might yield the number of tumor cells per voxel (a radiation oncologist's dream)

CONCLUSION

“Only if we can detect and measure can we begin to understand.”

“Herein lies the major contribution of radioimmunoassay as a probe for insight into the function and perturbations of the fine structure of biologic systems.”