

# Anti-CD33 single chain antibody for radionuclide therapy of acute myeloid leukaemia.

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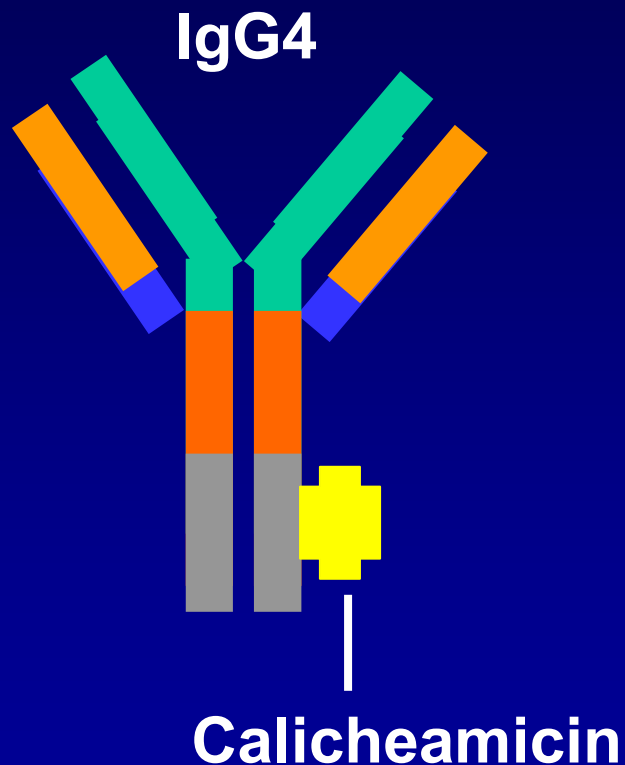
# Acute Myeloid Leukaemia (AML)

- Characterised by proliferation of myeloid cells in the bone marrow.
- In the USA, average annual incidence of the disease is 2.4 per 100,000 people, increasing to 12.6 per 100,000 at >65 years of age.
- Prior to 1970, 5 year survival rates <15%
- Recent refinements in diagnosis/therapy have improved outlook for AML patients.
- Nevertheless, the 5 year survival rate for patients <65 years old is just 40%.

# Current therapies for AML

- Chemotherapy
- Bone marrow transplantation
  - allograft or autograft
- Mylotarg™
  - an antibody-toxin conjugate

# Features of Mylotarg®<sup>®</sup>, a novel reagent for the treatment of relapsed AML



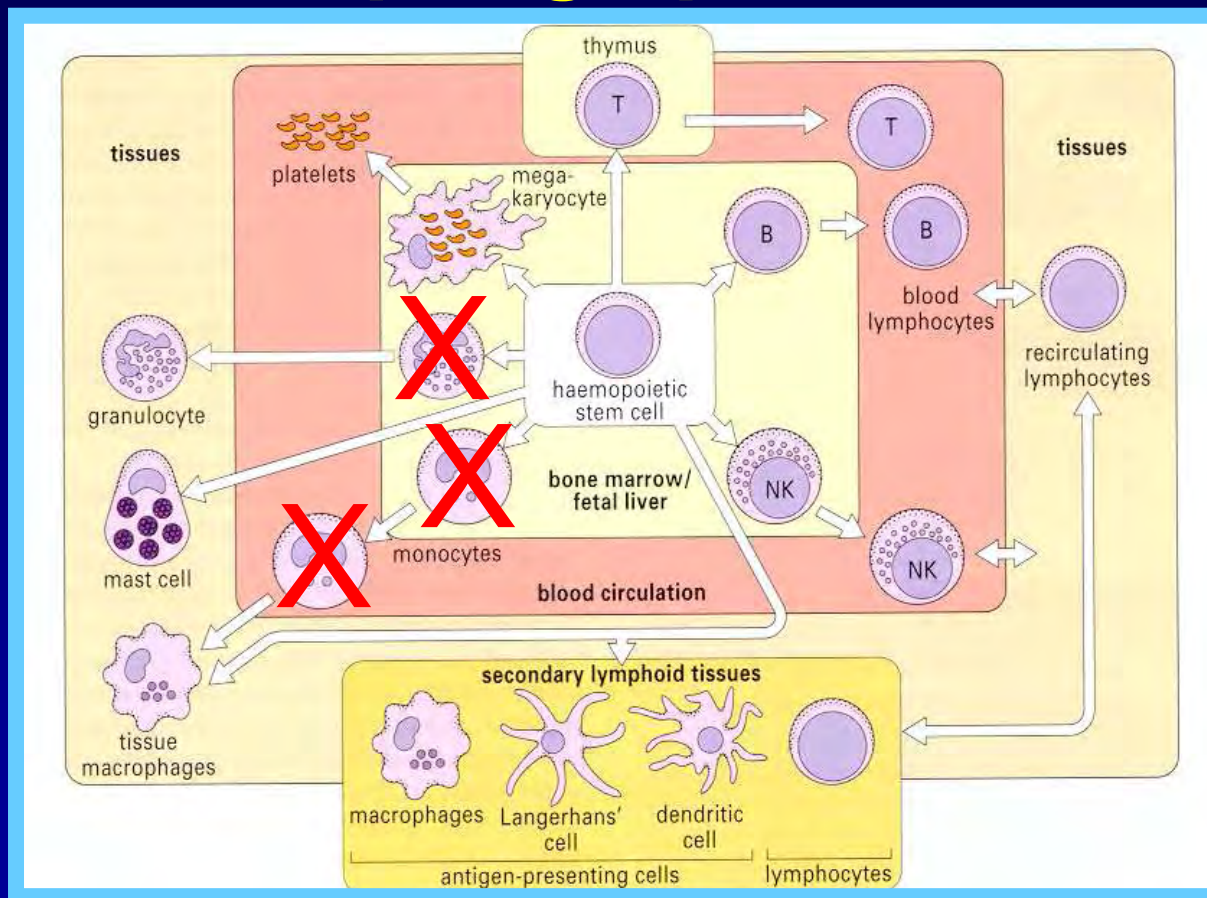
Consists of a **humanised IgG4** capable of binding to CD33 covalently coupled to semi-synthetic **calicheamicin**.

Calicheamicin is a potent **cytotoxic enediyne antibiotic** isolated originally from the actinomycete *Micromonospora echinospora*. It induces **double strand breaks** in DNA, and results in **apoptosis** of target cells.

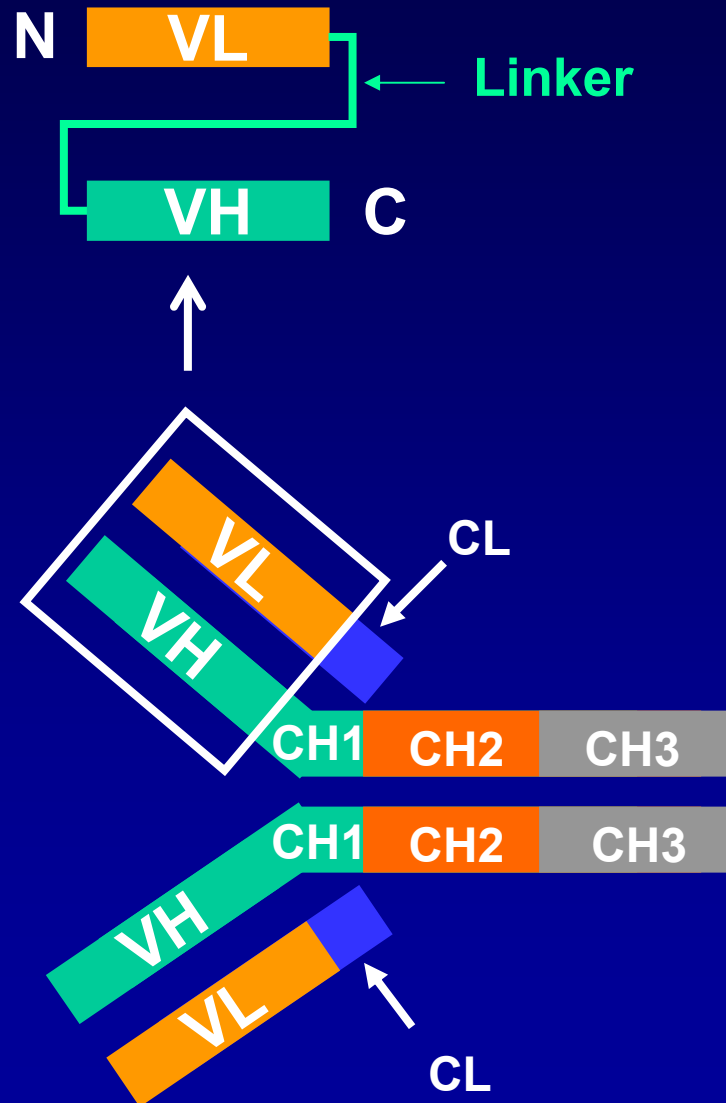
# Disadvantages of Mylotarg® as a therapeutic reagent

- Approved by the FDA in 2000 for treatment of adult patients in first relapse who are not suitable for transplantation.
- Sub-optimal **biodistribution** and **pharmacokinetics**.
- Calicheamicin-induced **liver toxicity** as a result of *in vivo* degradation of the conjugate.
- 26% remission rate, median survival just 6.4 months.
- Excessive **cost** of production.

# Anti-CD33 therapies specifically destroy granulocyte and macrophage precursors



# Features of a single-chain antibody (sFv)



Consists of the variable light (VL) chain of an antibody joined via a *linker* to the variable heavy (VH) domain.

The linker typically consists of a flexible/soluble peptide (for example,  $[GGGGS]_6$ )

The sFv maintains the antigen binding *specificity* (but not always the *affinity*) of the parent antibody.

## Attractive features of the *Pichia pastoris* expression system.

- **Strong inducible expression** from the AOX1 (alcohol oxidase) promoter. Addition of methanol allows simple, complete induction.
- **High levels of expression** of intracellular and secreted proteins in the range of grams/liter has been reported.
- **Eukaryotic post-translational modifications**— Processing and modification of proteins is similar to higher eukaryotes (disulphide bond formation, glycosylation).
- **Facilities available to us for the production of clinical grade antibody**  
Collaboration with Kerry Chester (Royal Free, London).

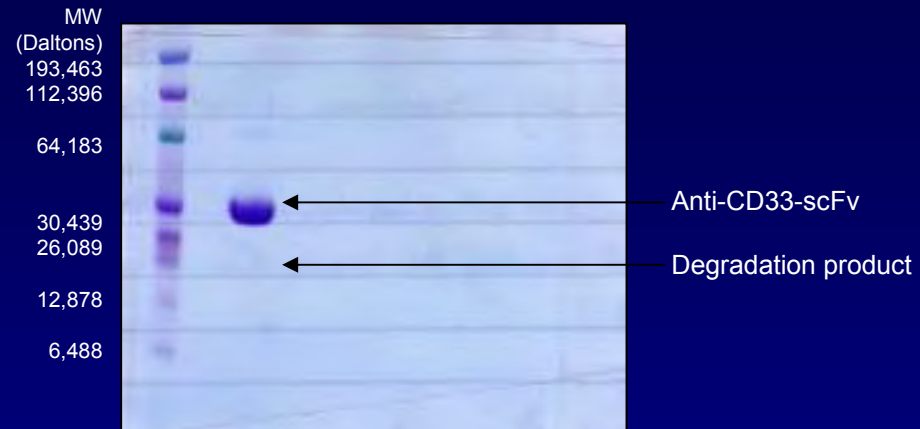


# Purification of Anti-CD33-scFv

## Two Step purification protocol: His-tag purification followed by Size exclusion purification

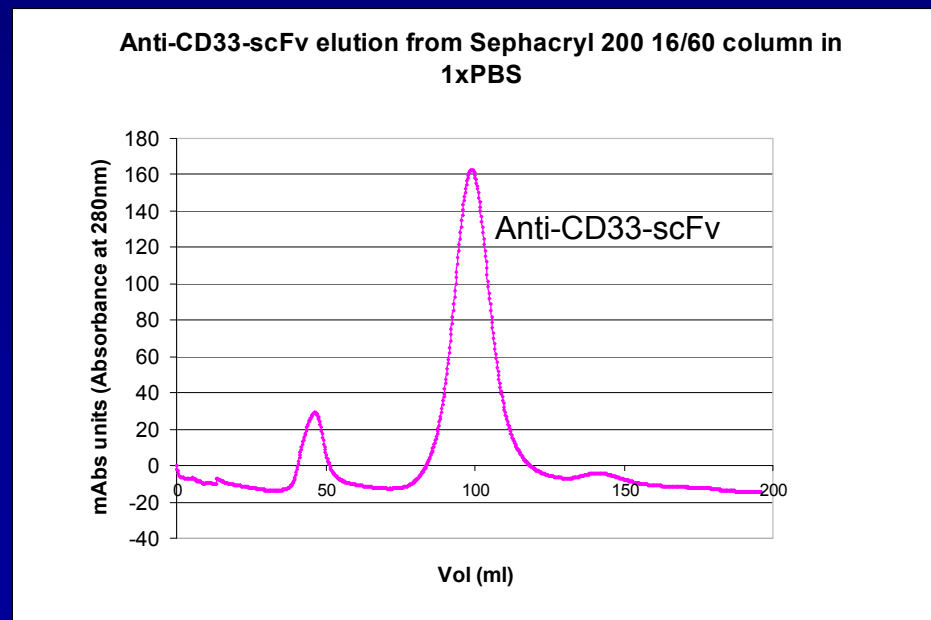
### His-tag purification

- HiTrap Sepharose Fast Flow Ni<sup>2+</sup> preloaded column
- Equilibrate > apply sample > wash > Imidazole elute His-tagged protein
- Concentrate elutions containing protein



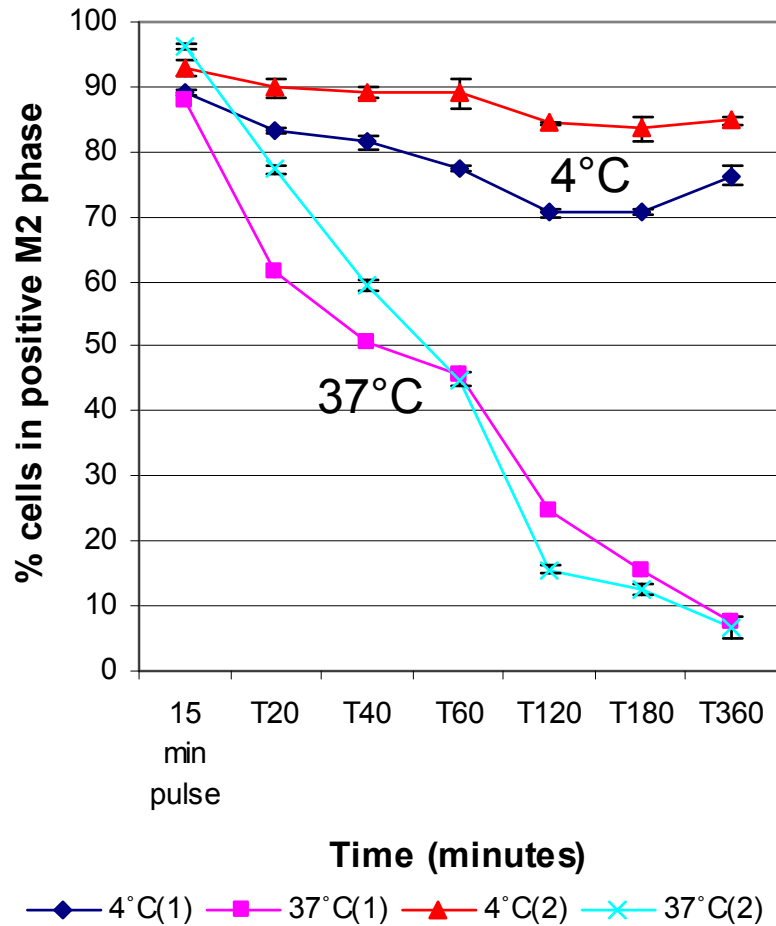
### Size exclusion purification

- Purify scFv using HiTrap 16/60 Sephacryl 200 column with AKTA
- Elute using 1xPBS

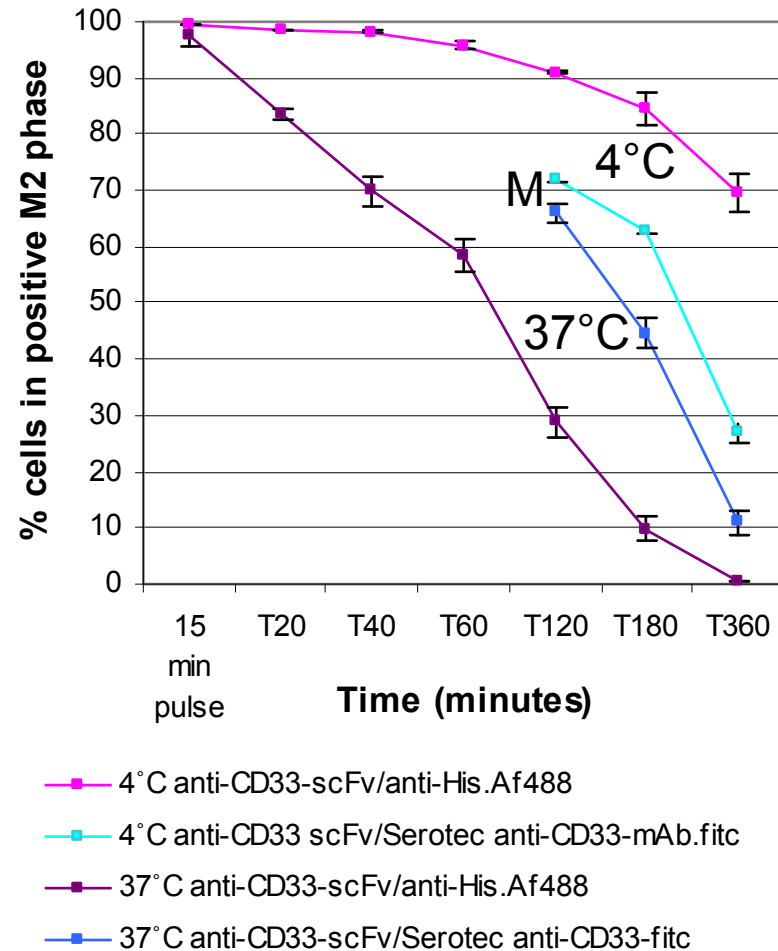


# scFv binding to CD33 triggers internalisation

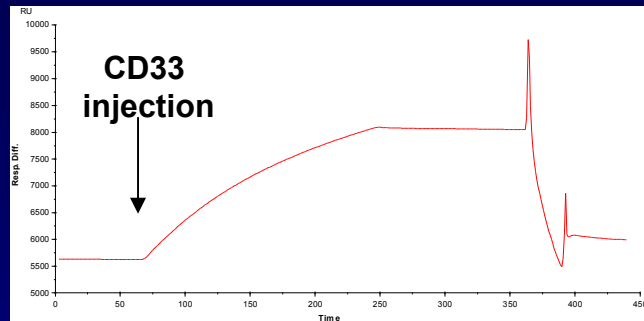
Internalisation of anti-CD33-mAb-FITC into U937 Cells Assayed using Flow Cytometry



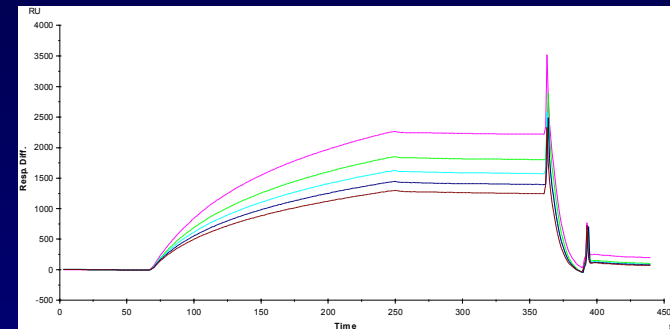
Internalisation of Anti-CD33-scFv and Modulation of CD33 using U937 cells Assayed using Flow Cytometry



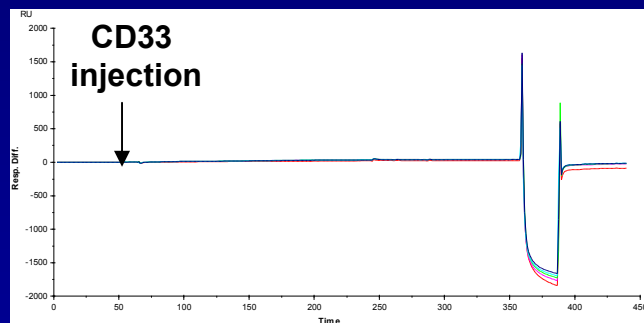
# Biacore analysis of the interaction of immobilised scFv with soluble CD33



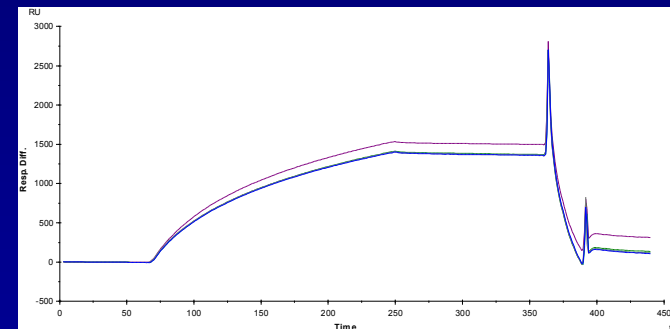
**scFv immobilised**



**5 regeneration cycles pH 1.5**



**Anti- 2 microglobulin  
antibody immobilised**



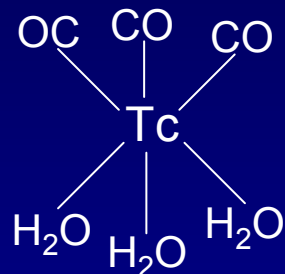
**5 regeneration cycles pH 2.5**

Kinetic analysis (low level of immobilised ligand, high analyte flow rate) indicates a  $K_D$  of  $5 \times 10^{-9}$  M

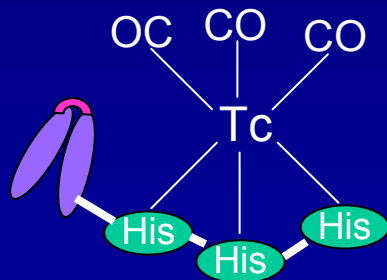
# Radiolabelling of anti-CD33-scFv with $^{99m}\text{Tc}$

Label the scFv via the His-tag using Mallinckrodt Isolink™ Carbonyl labelling Agent

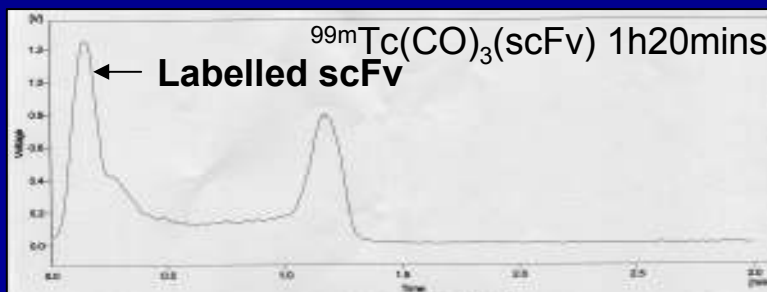
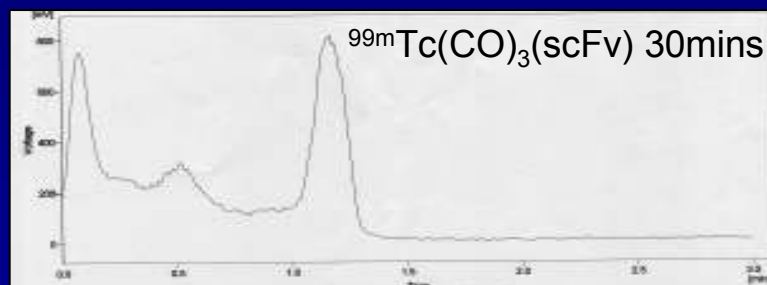
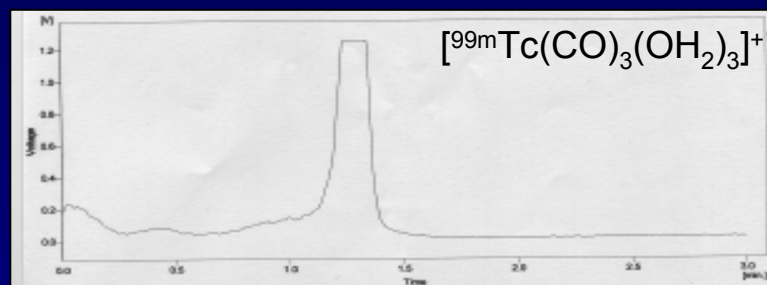
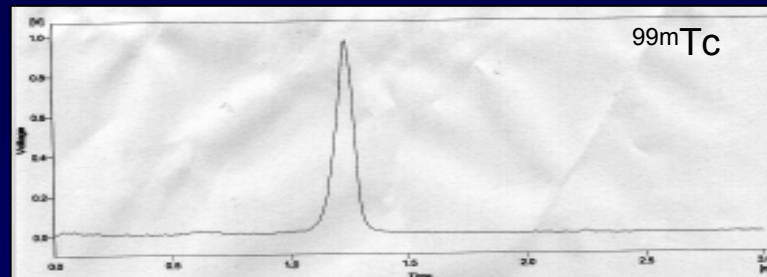
1) Prepare  $[\text{}^{99m}\text{Tc}(\text{CO})_3(\text{OH}_2)_3]^+$



2) Preparation of  $[\text{}^{99m}\text{Tc}(\text{CO})_3(\text{scFv})]$



3) Analyse using ITLC chromatography



# Progress to date

- Optimised production/purification protocols for anti-CD33 scFv with good yields and very low costs (yields >100 mg/l, <£1 per mg). *J. Immunological Methods* 305, 135-151 (2005).
- Developed a battery of SOPs for evaluating antigen binding and stability (flow cytometry, Biacore, light/confocal microscopy).
- Demonstrated internalisation of scFv on antigen binding with kinetics similar to those of the parent MoAb.
- Developed protocols for labelling scFv with <sup>99m</sup>-technitium using Tc(I)-carbonyl complex, and confirmed retention of antigen binding.
- Initiating animal studies using Nano-SPECT to determine biodistribution (*collaboration with Steve Mather, St. Bartholomew's, London*).



# Radiolabelling of anti-CD33-scFv

Aim: to match the characteristics of the scFv with that of a radionuclide to optimise the effectiveness of the radioimmunoconjugate

Develop a range of antibody/linker/radionuclide combinations to facilitate selection of the most appropriate reagents for future imaging and therapeutic studies

Isotope	$t_{1/2}$	Emissions	Linking chemistry	Ab	Purpose
$^{99m}\text{Tc}$	6 hr	$\gamma$	DMSA (non-specific via lysines) Via His-tag (specific)	scFv, MAb  scFv	Imaging/dosimetry
$^{188}\text{Rh}$	17 hr	$\gamma$	DMSA (non-specific via lysines) Via His-tag (specific)	scFv, MAb  scFv	Imaging/dosimetry + Toxicity
$^{211}\text{At}$	7.2 hr	$\alpha$	Astatobenzoic acid (non-specific via lysines)	scFv, MAb	Toxicity
$^{111}\text{In}$	2.8 days	Auger electrons $\gamma$	DOTA (non-specific via lysines)	scFv, MAb	Imaging/dosimetry + Toxicity

# Measuring cellular responses to radiopharmaceuticals

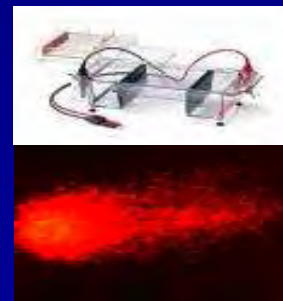


Treat cells with  $^{111}\text{In}$  labelled fusion protein



Clonogenic survival assay

Measure cellular uptake using phosphor-imager



Single cell gel electrophoresis (Comet) assay

# Rational design of the delivery vehicle for improving therapeutic properties of radioconjugates

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## Nuclear Localizing Sequences Promote Nuclear Translocation and Enhance the Radiotoxicity of the Anti-CD33 Monoclonal Antibody HuM195 Labeled with $^{111}\text{In}$ in Human Myeloid Leukemia Cells

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## $^{111}\text{In}$ -Labeled Trastuzumab (Herceptin) Modified with Nuclear Localization Sequences (NLS): An Auger Electron-Emitting Radiotherapeutic Agent for HER2/neu-Amplified Breast Cancer

Danny L. Costantini<sup>1</sup>, Conrad Chan<sup>1</sup>, Zhongli Cai<sup>1</sup>, Katherine A. Vallis<sup>2</sup>, and Raymond M. Reilly<sup>1,3,4</sup>

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