Academic/translational perspective

Prof Phil Blower, King's College London, UK



Monitoring the fate of administered cells using whole body radionuclide imaging

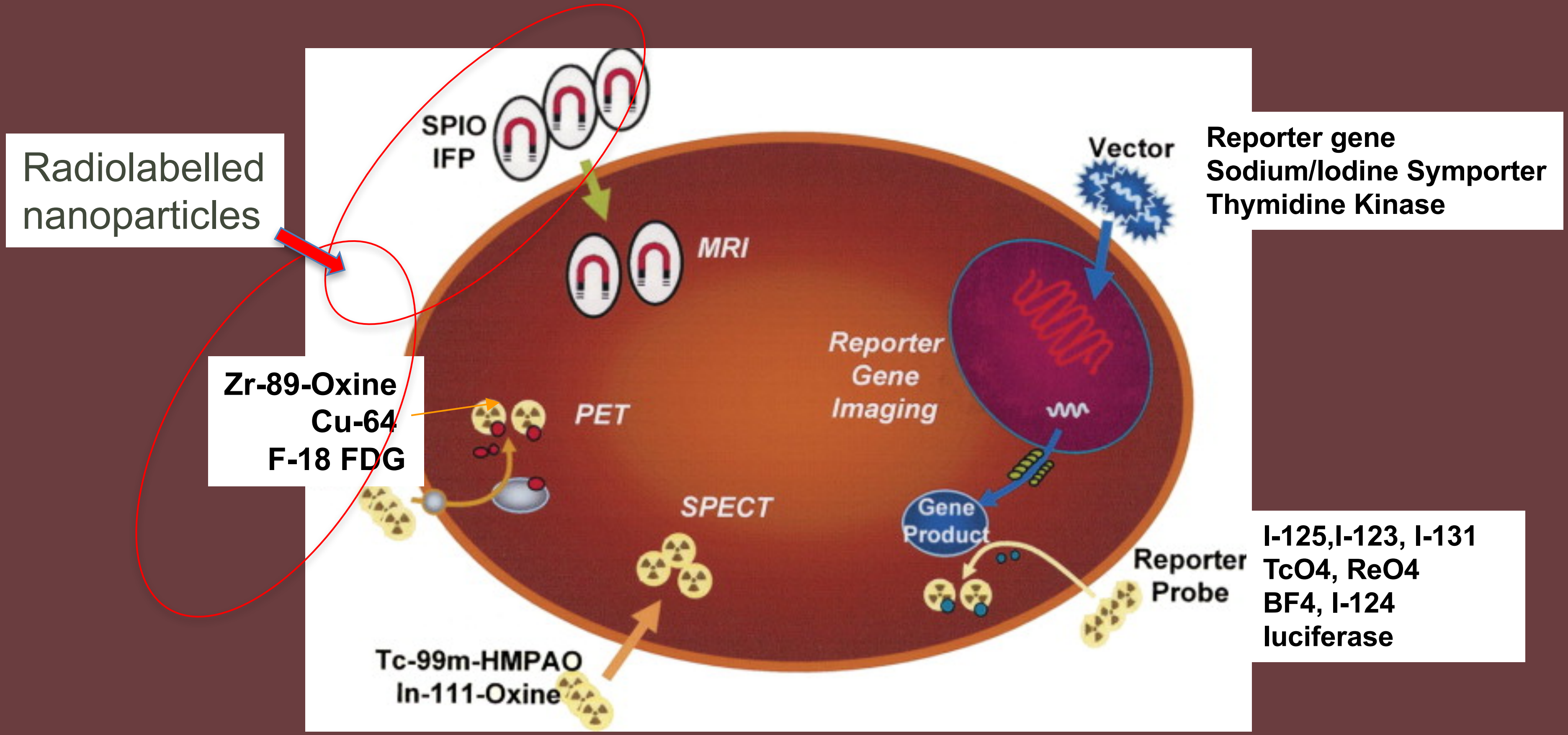
Phil Blower
Professor of Imaging Chemistry
King’s College London

KCL School of Biomedical Engineering and Imaging Sciences:
Dept of Imaging Chemistry and Biology

- Development of whole body imaging tools for…
  - Translation of in vitro research to in vivo setting
  - Clinical diagnosis
- Imaging molecular processes, gene expression
  - Cancer, cardiovascular disease, dementia etc.
  - Including molecular processes associated with immunity
- Cell tracking
  - Immune cell trafficking, transplant medicine, cell based therapy

Imaging Agents for Whole Body Cell Tracking

MR, PET/SPECT, optical...

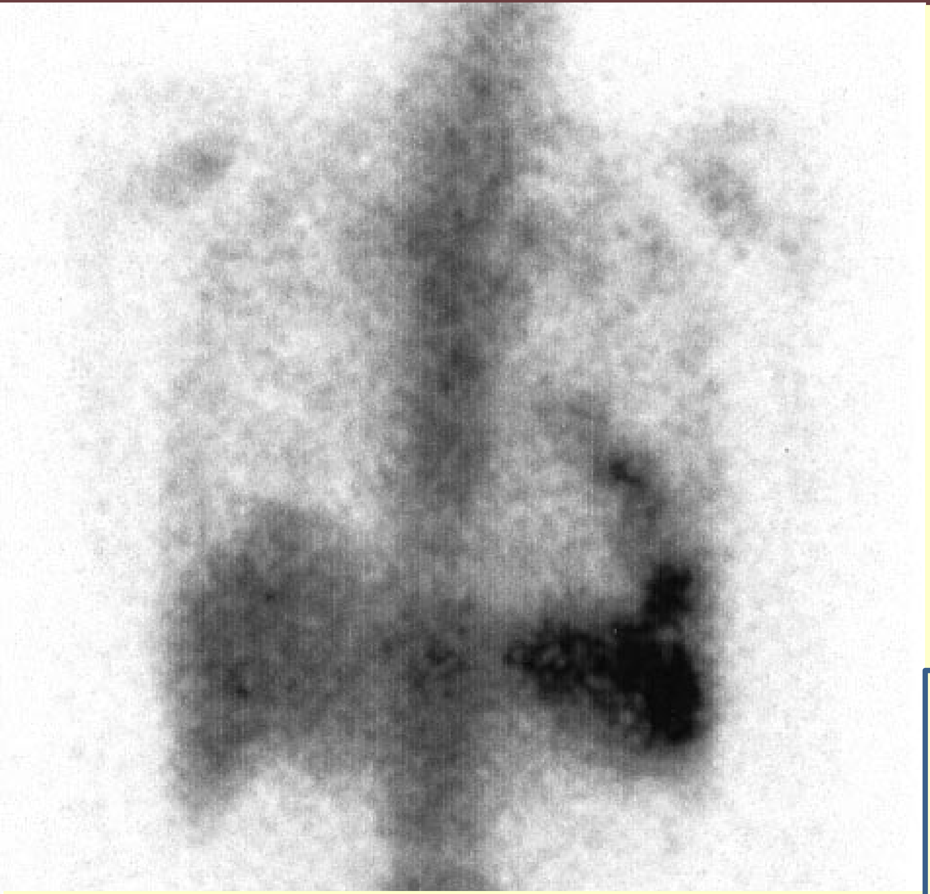


Radionuclides (SPECT, PET) have most utility for human use

- Sensitivity
- Depth penetration
- Quantification

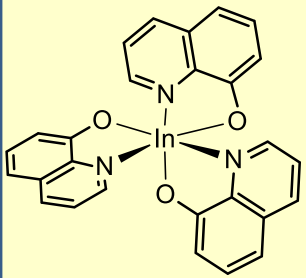
Whole body cell tracking…

- …is not new: routine in nuclear medicine since 1980s: gamma camera imaging of radiolabelled leukocytes to detect inflammation/infection



^{111}In-labelled leucocytes: Focal pulmonary uptake in left lower lung pneumonia. Love et al. Radiographics 2002

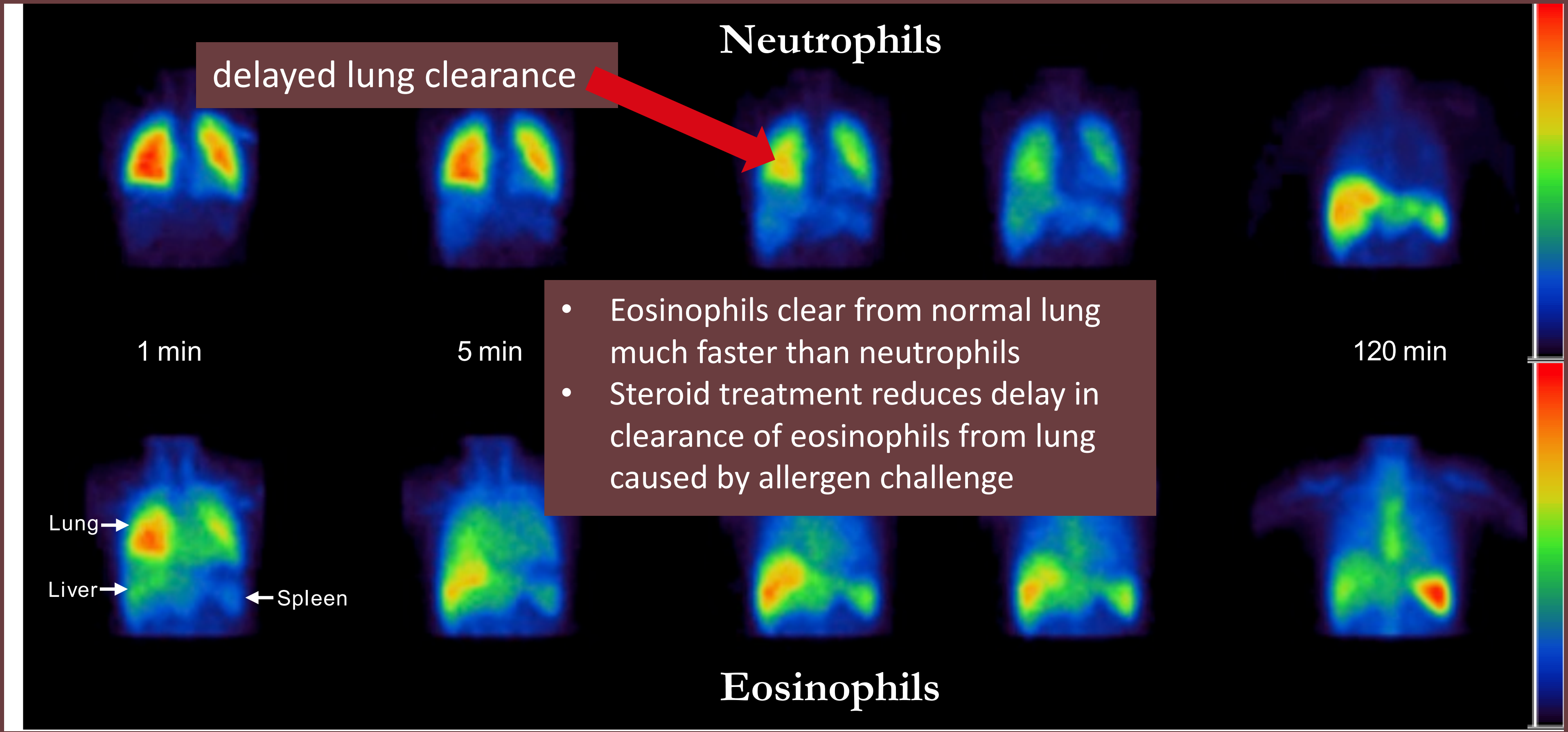
- Autologous leukocytes isolated from patient's peripheral venous blood
- Labelled with ^{111}In (gamma emitter, half life 2.8 days) using oxine complex
- Lipophilic metastable complex enters cells passively, dissociates,
- $^{111}In^{3+}$ binds to intracellular macromolecules
- Cells re-injected
- Image typically 3 h and 24 h



New (human) application of old methods

Imaging human autologous eosinophils in asthmatics

^{99m}Tc-labelled purified eosinophils; 30 min dynamic SPECT scan



- Eosinophils clear from normal lung much faster than neutrophils
- Steroid treatment reduces delay in clearance of eosinophils from lung caused by allergen challenge

Lukawska, Mullen, Corrigan et al JACI, 2013

Similar human study now underway with purified CD4+ T cells

MEDICAL ENGINEERING INITIATIVE wellcome trust EPSRC

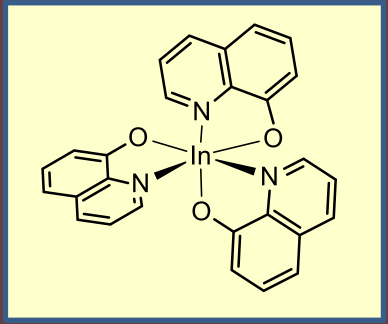
Next generation cell tracking

- *Revolution* in regenerative medicine/cell based therapy – stem cells, immune cells, beta cells… - requires *revolution* in cell tracking:
    - Higher resolution
    - Higher sensitivity (smaller cell numbers)
    - More prolonged (weeks-years),
    - Lower toxicity

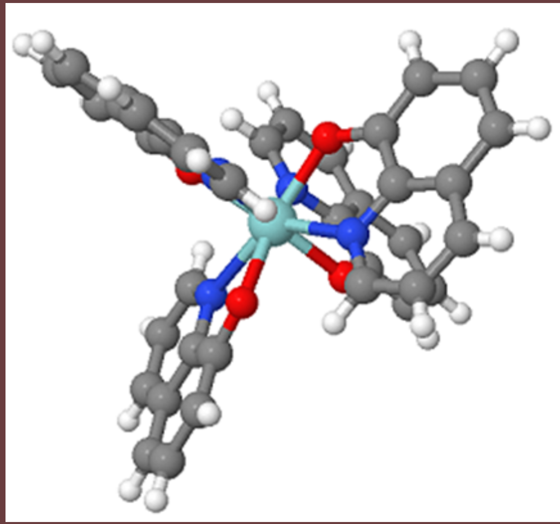
- Strategy for innovation
    - Replace SPECT with new long half-life PET – Zr-89, Mn-52
    - Combine modalities: PET/SPECT, MRI, optical
    - Combine direct labelling (pre-injection) with reporter gene imaging (sodium/iodide symporter, NIS: $[^{18}F]BF_4$, $[^{18}F]SO_3F$)
    - Work towards exploiting the next generation of scanning hardware: PET/MR, whole body PET

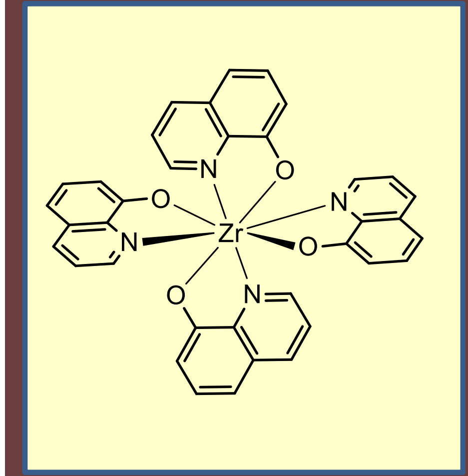
Charoenphun…Blower EJNMMI 2014; Fruhwirth…Blower…Mullen, J Nucl Med 2014; Jauregui-Osoro…Blower, EJNMMI 2010; Khoshnevisan…Blower, J Nucl Med 2016; O'Doherty, Jaruegui-Osoro…Blower…Lewington J Nucl Med 2017

Next generation methods: Zr-89 **PET**

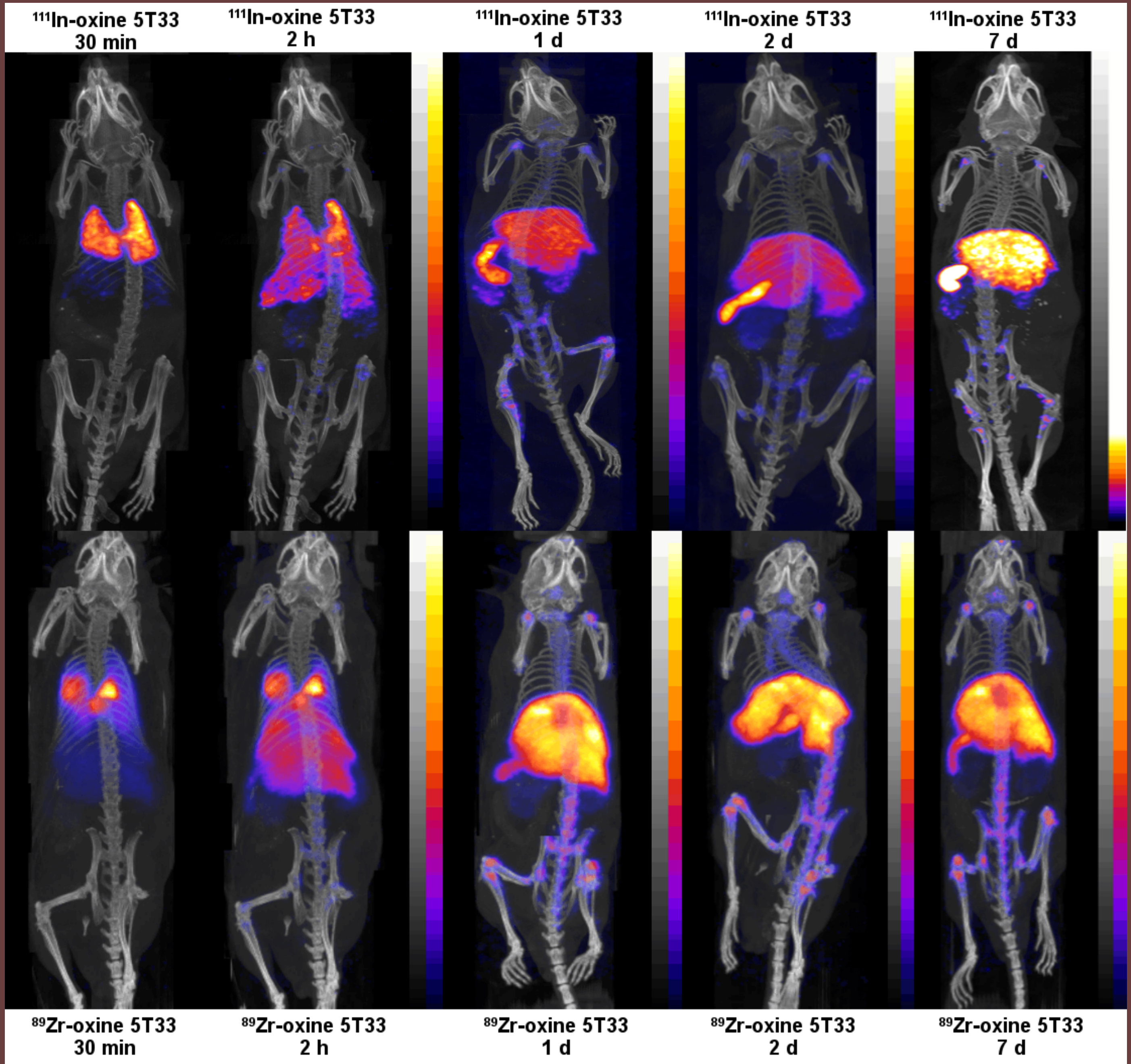


- Longer half life (3.5 days) positron emitter
- Metallochemistry analogous to In-111
- Forms 4+ ions rather than 3+
- Similar ligand preferences
- Could exploit same mechanism as In-111-oxine: metastable lipophilic complex, intracellular dissociation





Myeloma cells labelled with Zr-89 and In-111: in vivo comparison



^{111}In-oxine

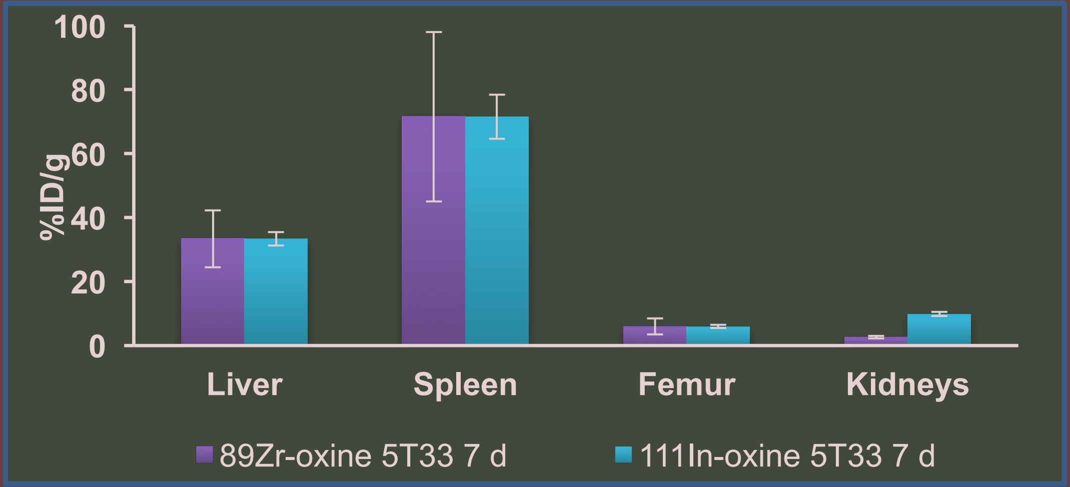
- Kidney uptake signals leakage

^{89}Zr-oxine

- Longer tracking (14 d)
- Better retention
- Lower toxicity
- Better resolution
- Better quantification

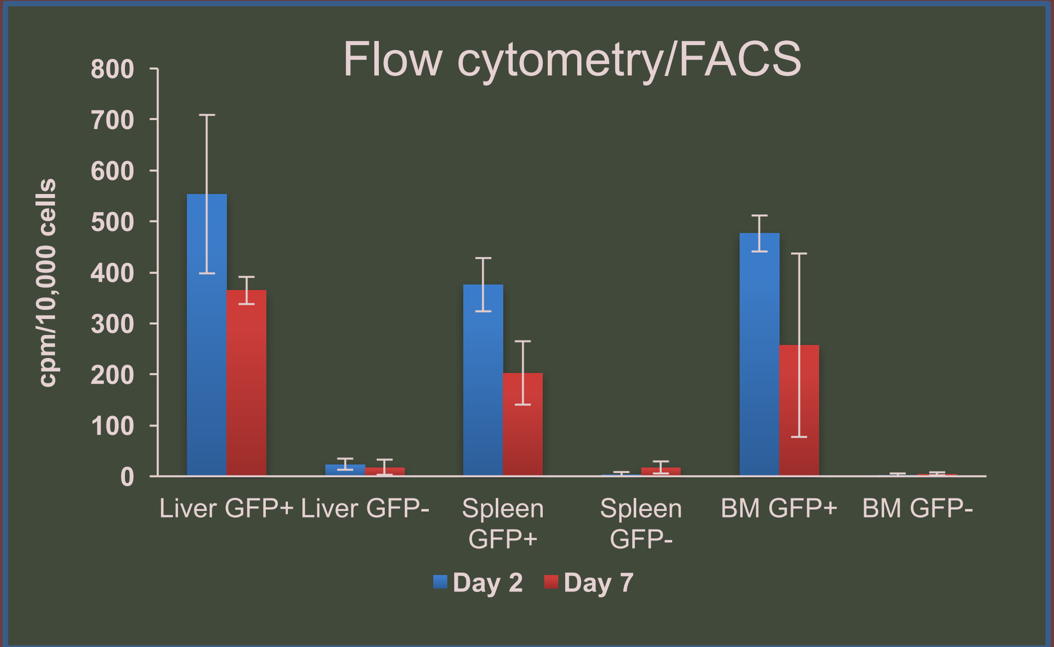
Levente Meszaros, Putthiporn Charoenphun

Zr-89 labelled cells: Comparison with In-111 and validation in vivo



Myeloma cells i.v. injection
7 days p.i.

Less kidney for Zr-89: more stable



Zr-89 labelled myeloma cells: GFP+
Tissues homogenised at 2 d and 7 d
FACS Sorted by green fluorescence
Cells collected and activity counted

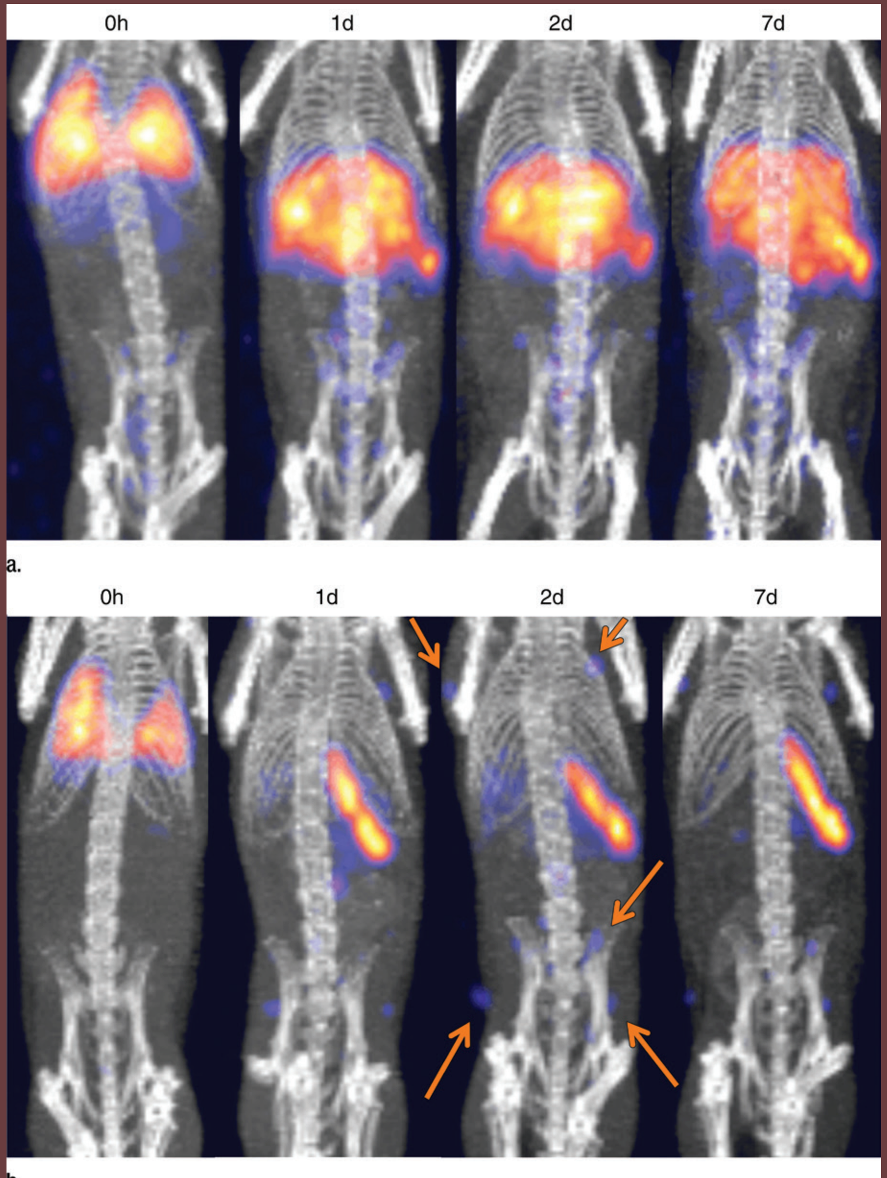
By 7 days:
Activity remains in GFP+ cells
Labelled cells still living

Zr-89 cell labelling: emerging applications

Cytotoxic T lymphocytes…
    Liver, spleen

Dendritic cells…
    Spleen, lymph nodes

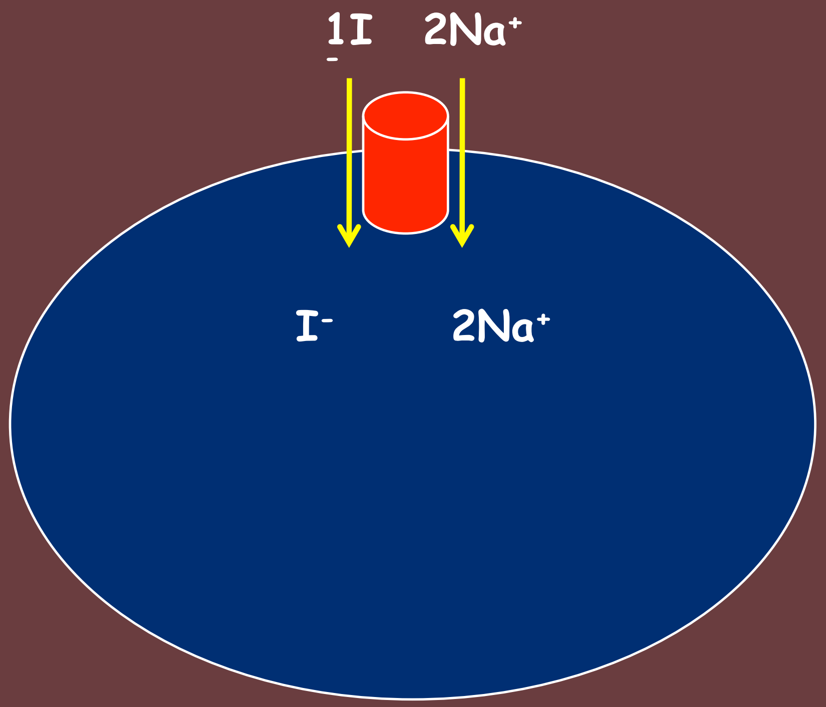
NCI/NIH
Sato et al. Zr-89-Oxine Complex PET Cell Imaging in Monitoring Cell-based therapies. *Radiology* 2015;275:490-500



Reporter genes: imaging repeatedly over a lifetime sodium/iodide symporter

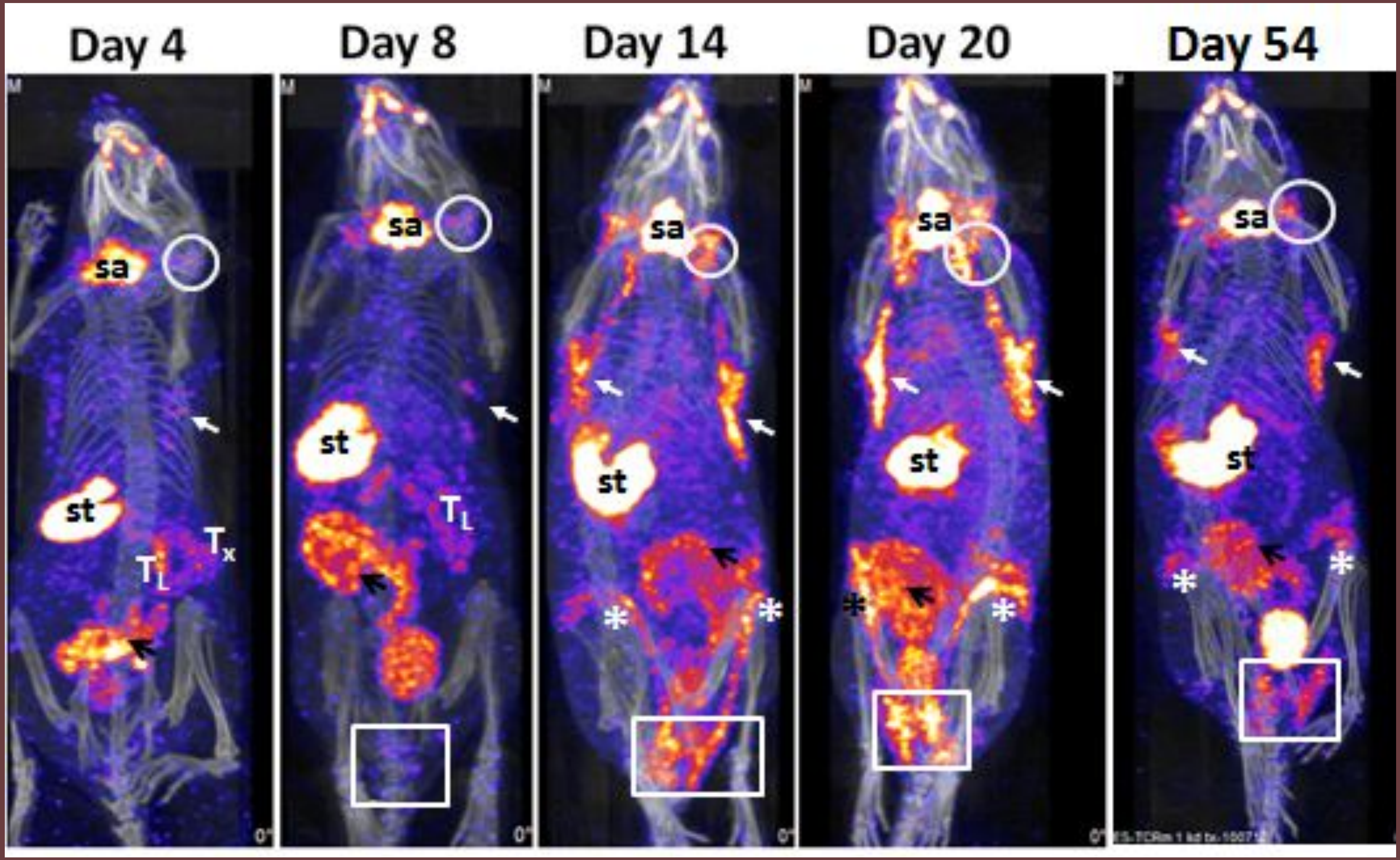
- NIS: imports iodide, couple to 2 x Na^+ import
- Driven by Na^+ gradient maintained by Na^+/K^+ ATPase
- Expressed in thyroid, stomach, salivary glands
- Can be imaged with radioiodine: $^{123}I^-$, $^{125}I^-$, $^{131}I^-$ (gamma), $^{124}I^-$ (positron)
- …and $^{99m}TcO_4^-$ (gamma)

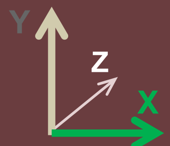
- Other reporter genes feasible



Imaging therapeutic Tregs in skin transplant model

NIS reporter gene approach and SPECT/CT imaging: Mullen, Lombardi PLOS1 2012





O=cervical lymph nodes (LNs), white arrow=axillary LNs, st= stomach, sa = thyroid and salivary glands , Tx=transplanted skin, TL=skin transplant LNs, *=inguinal LNs, black arrow=mesenteric LNs, □=pelvic LNs.

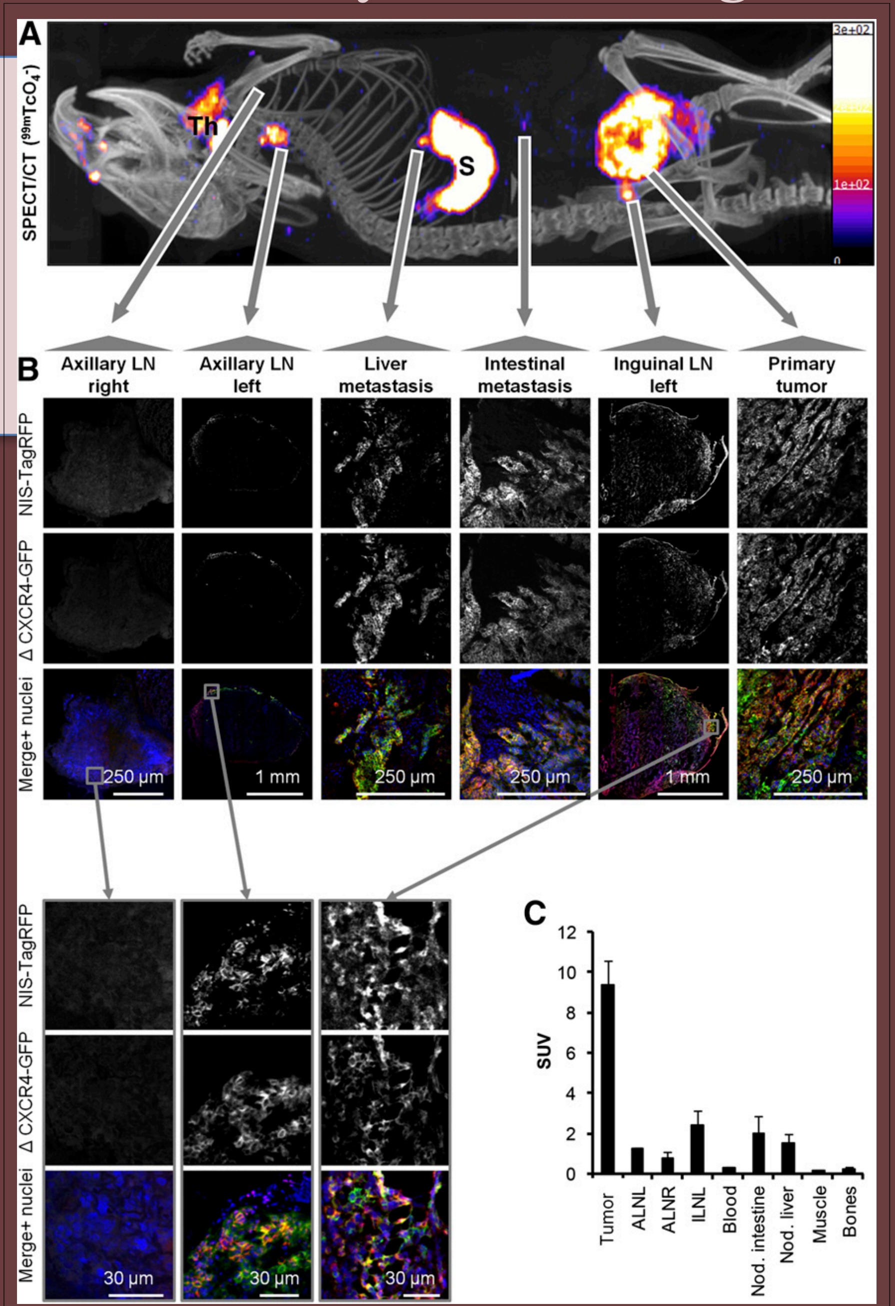
NIS-based reporter gene for combined modality cell tracking

JNM The Journal of NUCLEAR MEDICINE

A Whole-Body Dual-Modality Radionuclide Optical Strategy for Preclinical Imaging of Metastasis and Heterogeneous Treatment Response in Different Microenvironments

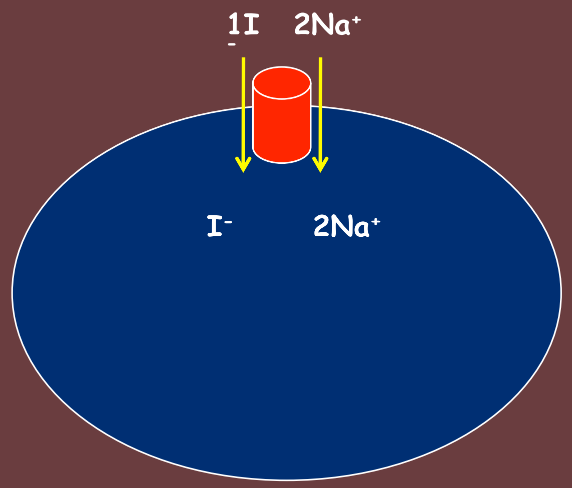
Gilbert O. Fruhwirth, Seckou Diocou, Philip J. Blower, Tony Ng and Greg E.D. Mullen

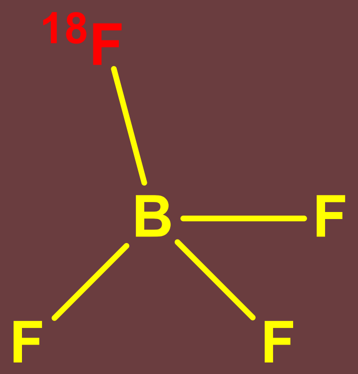
- Orthotopic breast cancer expressing hNIS-tagRFP and CXCR4-GFP
- Imaging tumour development and metastasis – weeks
- Primary and metastases detected with $^{99m}TcO_4^-$ SPECT
- Only primary detected with ^{18}F-FDG
- Cross validation: All metastatic nodes detected by PET/SPECT confirmed by fluorescence microscopy…
- …all nodes negative by SPECT were negative by fluorescence microscopy
- Method for longitudinal *in vivo* study of metastasis and response to therapy



^{18}F-BF_4^-: a PET tracer for NIS reporter gene imaging

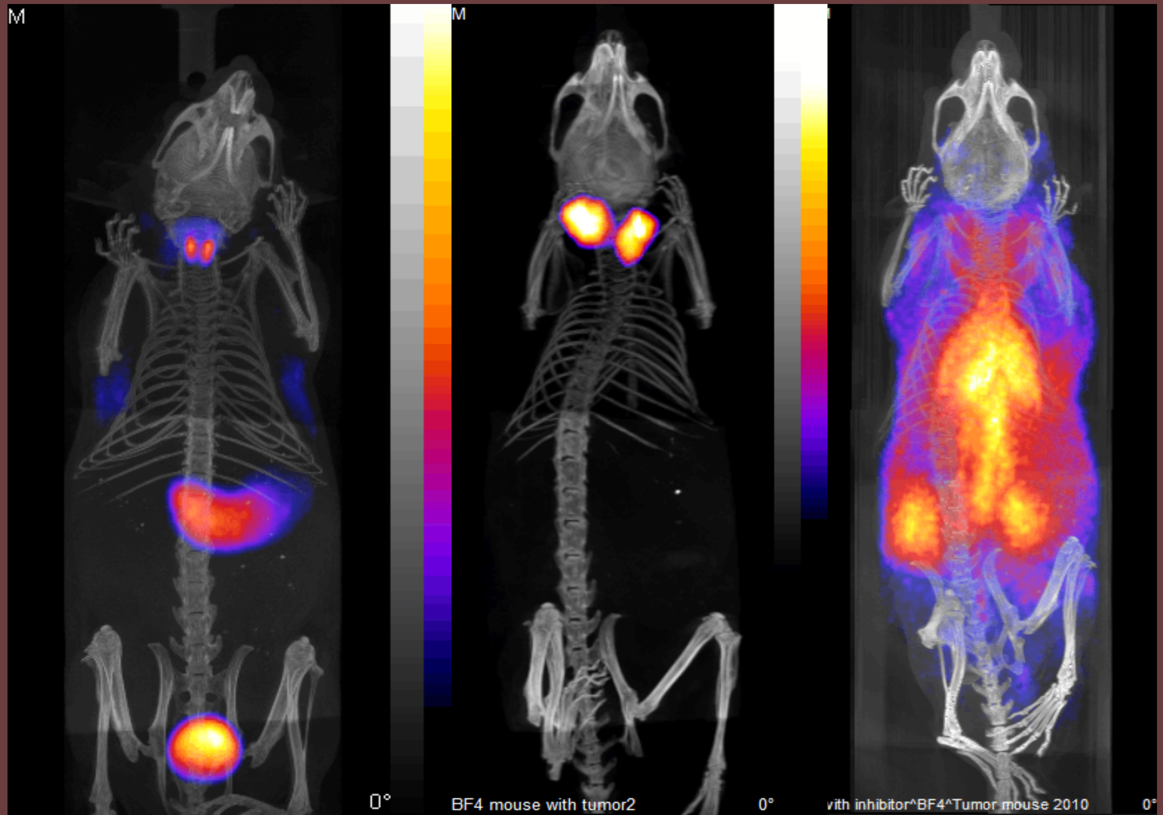
- Sodium/iodide symporter





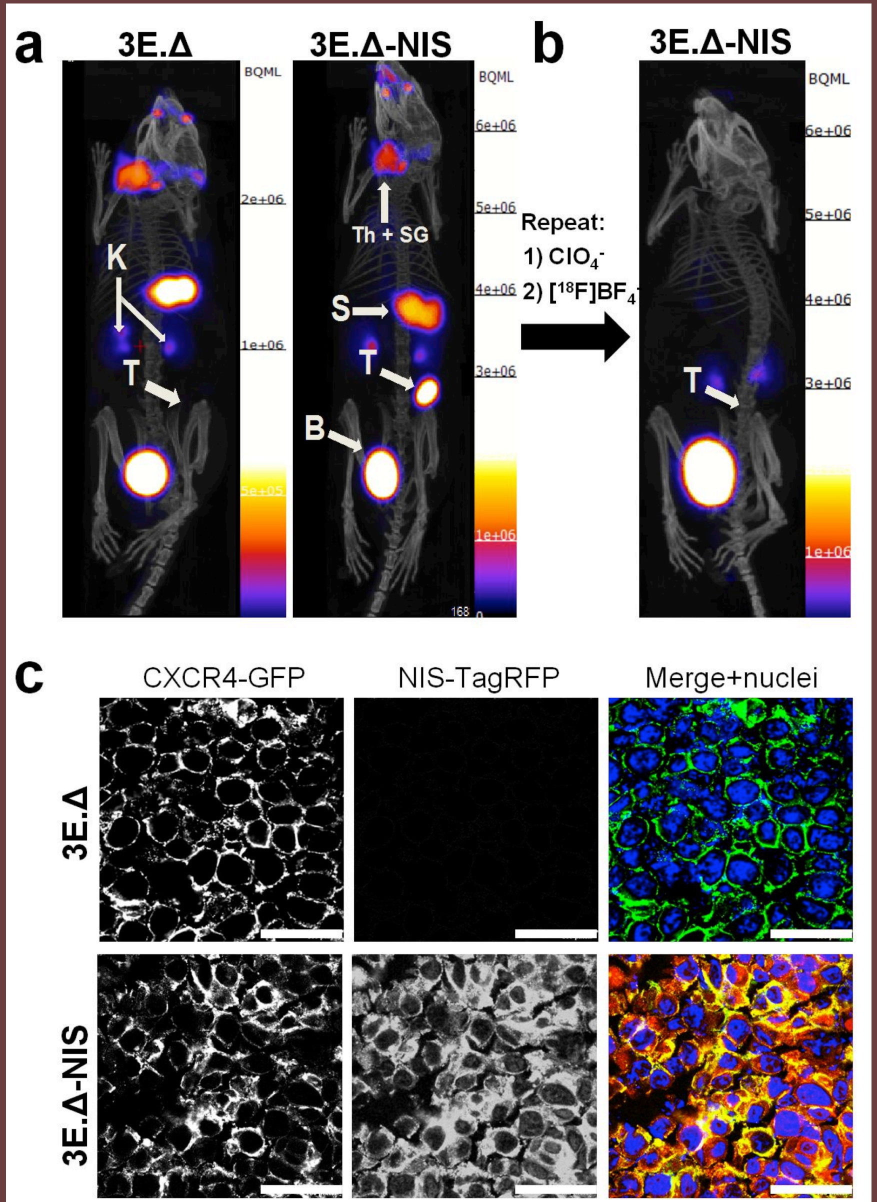
Maite Jauregui-Osoro, Dave Berry, Phil Blower, EJNMMI 2010

- Tracers: $^{123}I^-$, $^{131}I^-$, $^{124}I^-$, metabolised
- $^{99m}TcO_4^-$ $^{188}ReO_4^-$… SPECT, therapy, not metabolised
- ^{18}F-BF_4^-, PET, not metabolised



Multimodality imaging with $^{18}F\text{-}BF_4^-$ PET

Pre-metastatic



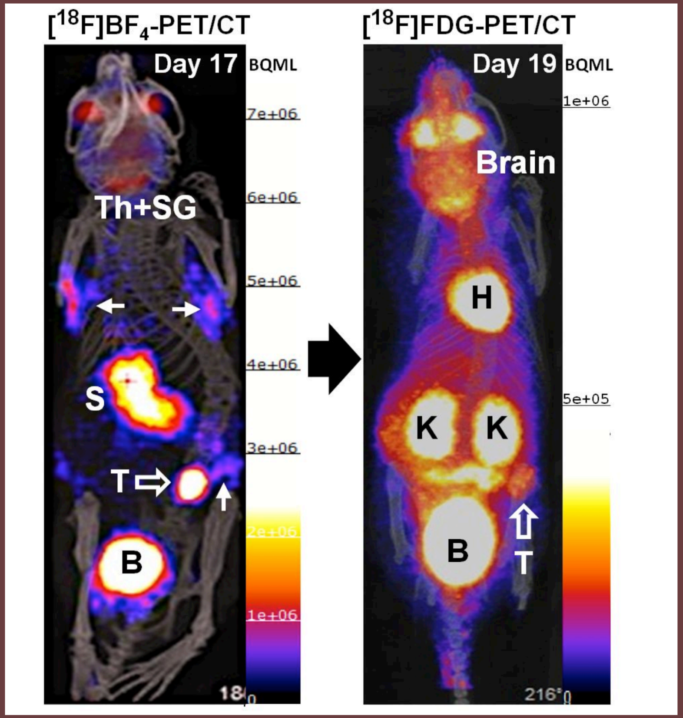
Gilbert Fruhwirth, Seckou Diocou

Orthotopic breast cancer expressing hNIS-tagRFP and CXCR4-GFP (as before)

All metastatic nodes detected by PET

All non-metastatic nodes true-negative

Very sensitive – detects a few hundred cells in a lymph node; ideal for human PET



metastatic

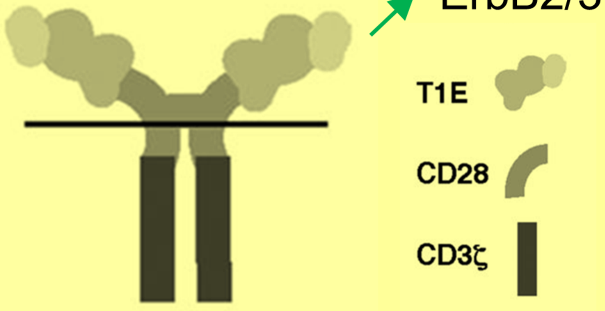
Cell Therapy Development/Monitoring

Implementation of Cell Tracking into CAR T-cells

T1E28z

ErbB1 homo- and heterodimers

ErbB2/3 heterodimers



Phase I Clinical Trial:

Dr. John Maher

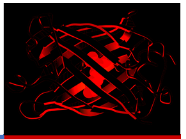
Immunotherapy of head and neck cancer

NCT01818323

King's College London

Guy's and St Thomas' NHS Foundation Trust NHS

Co-expression of CAR and REPORTER → CAR:NIS T-cells



P_{EF1a} — T1E28z — P_{PGK} — hNIS — RFP

Fruhwirth lab / KCL

FP for preclinical setting only

Cell Therapy Development/Monitoring

CAR:NIS T-cells Localize to the Tumour

KING'S College LONDON

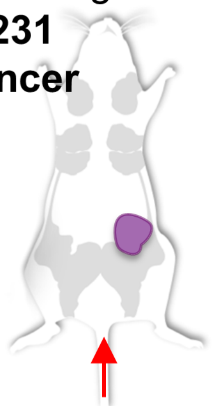
Now by PET
→[^{18}F]BF_4^-

New radiosynthesis:
*High radiochemical purity
*Specific activity **~5 GBq/µmol**

Khoshnevisan, A. et al. EJNMMI Res., Feb 2016

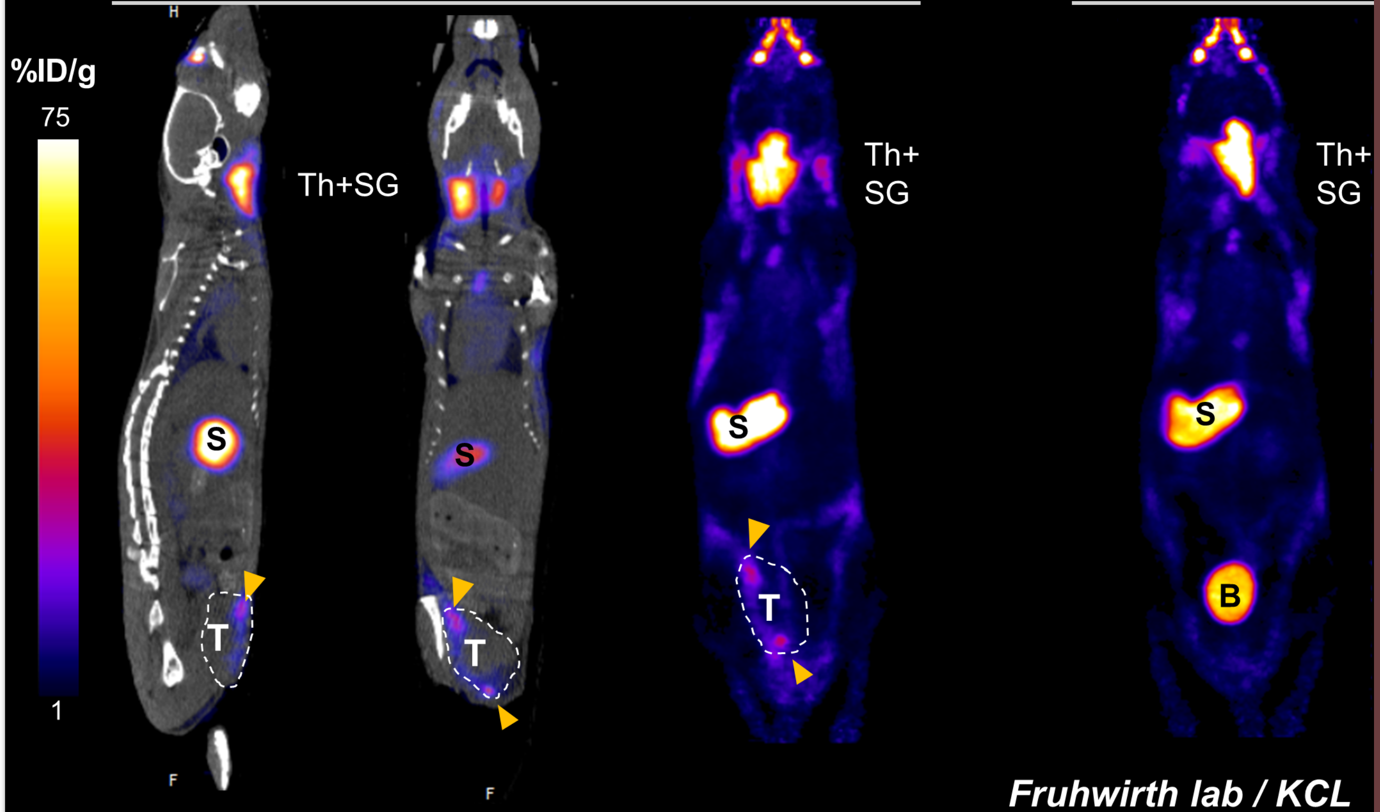
Model: ♀ NSG
Orthotopic xenograft
MDA-MB-231
Breast Cancer

i/v of $1{\cdot}10^7$
CAR:NIS
T-cells



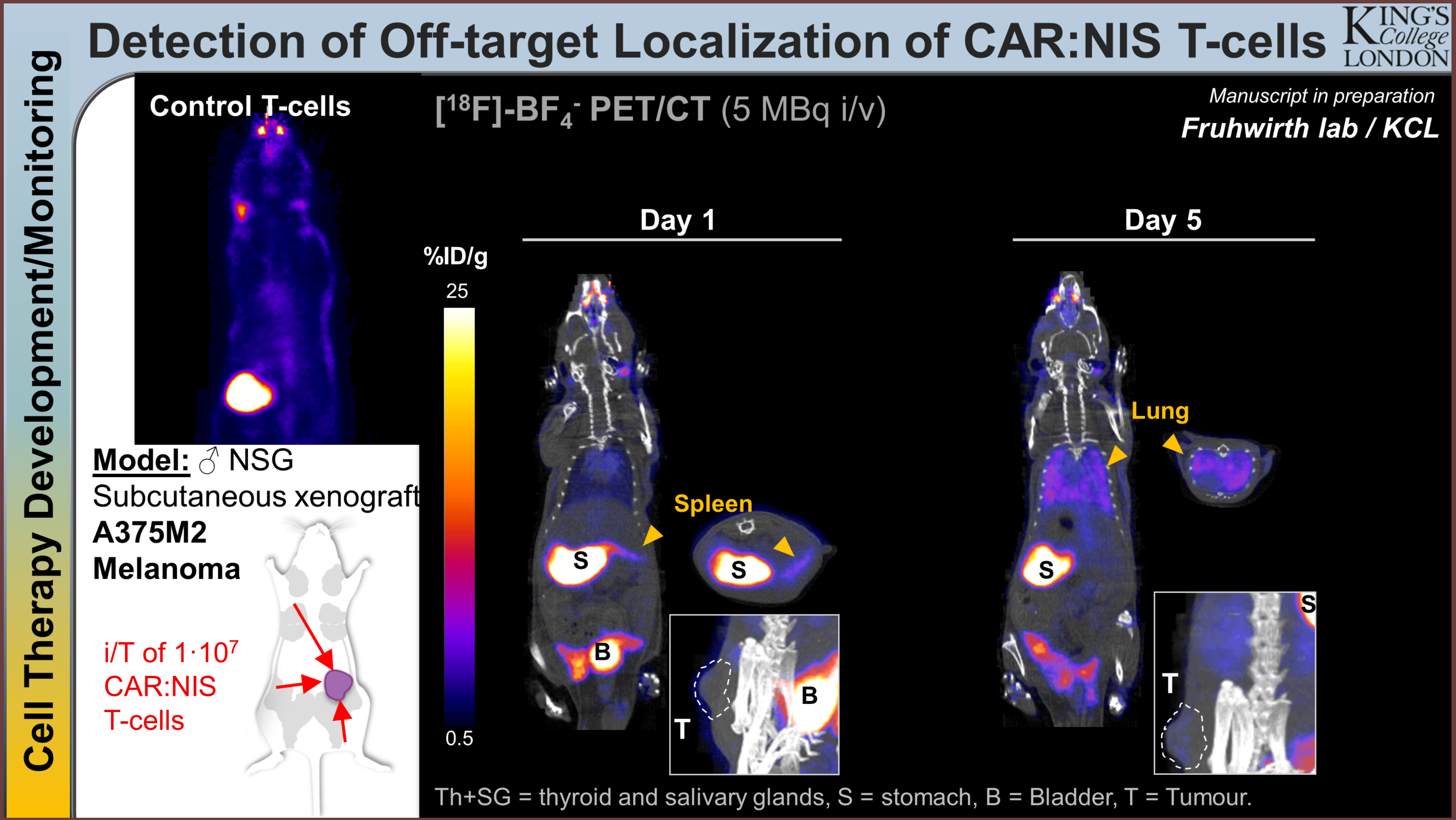
[^{18}F]-BF_4^- PET/CT (5 MBq i/v), 24h post administration.

Tumour bearing mouse | **Naïve mouse**



Th+SG = thyroid and salivary glands, S = stomach, T = Tumour.

Fruhwirth lab / KCL
Manuscript in preparation



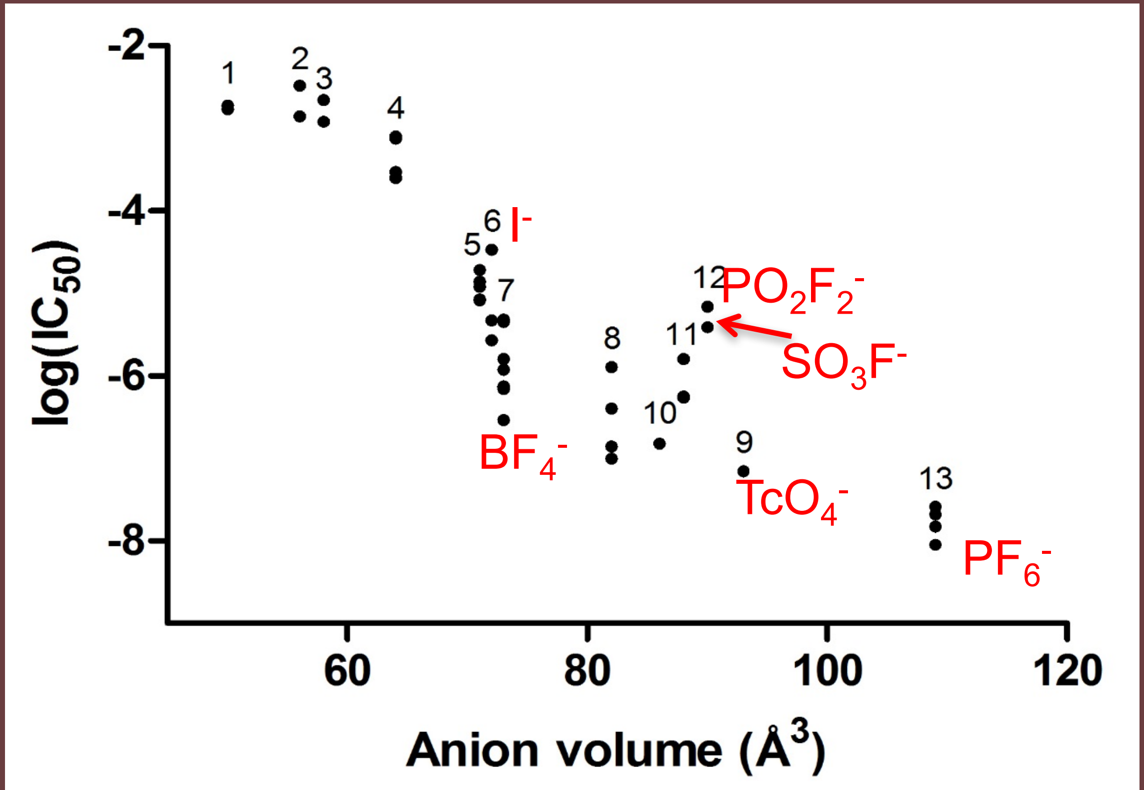
Detection of Off-target Localization of CAR:NIS T-cells
KING'S College LONDON
Cell Therapy Development/Monitoring
Control T-cells
[18F]-BF4- PET/CT (5 MBq i/v)
Manuscript in preparation
Fruhwirth lab / KCL
Day 1
Day 5
%ID/g
25
0.5
Spleen
Lung
S
S
S
S
B
B
T
T
Model: ♂ NSG
Subcutaneous xenograft
A375M2
Melanoma
i/T of 1·10^7
CAR:NIS
T-cells
Th+SG = thyroid and salivary glands, S = stomach, B = Bladder, T = Tumour.

Clinical translation:
$[^{18}F]BF_4^-$ clinical trial in thyroid cancer

Lewington et al., St Thomas' Hospital PET Centre, King's College London; J Nucl Med 2017

Son of $^{18}F\text{-}BF_4^-$

- $^{18}F\text{-}PF_6^-$
- $^{18}F\text{-}PO_2F_2^-$
- $^{18}F\text{-}SO_3F^-$

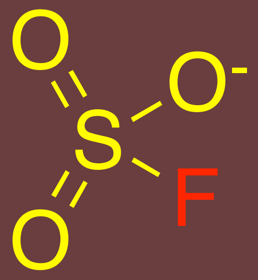


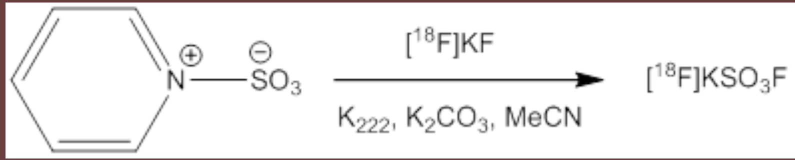
Alex Khoshnevisan, Phil Blower, J Nucl Med 2017

Son of BF_4^-: fluorosulfate first PET tracer with a S-F bond

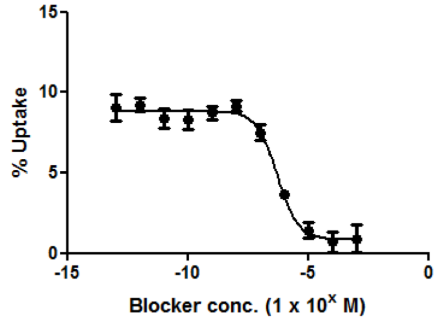
Alex Khoshnevisan

- $^{18}F\text{-}PF_6^-$
- $^{18}F\text{-}PO_2F_2^-$
- $^{18}F\text{-}SO_3F^-$
- Simple synthesis

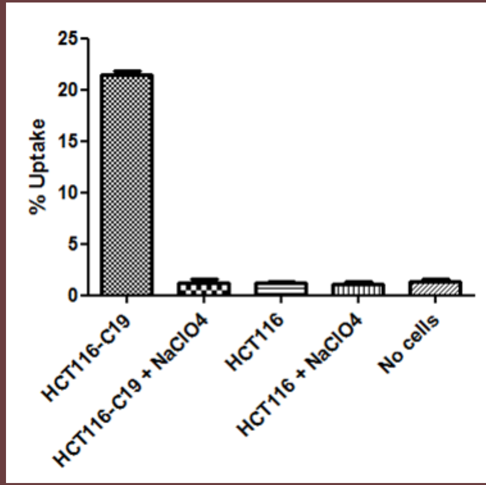




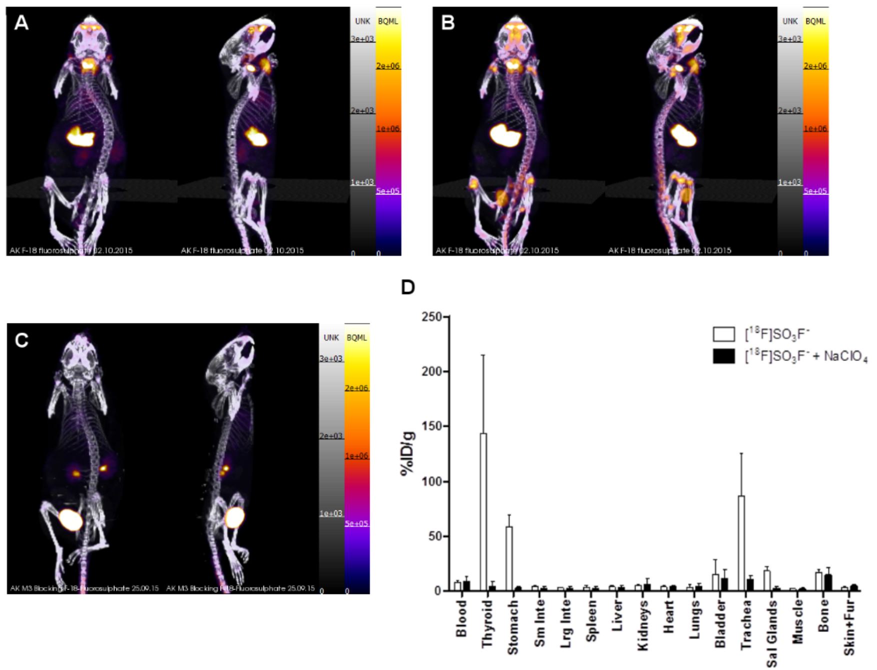
- Spec. ac. > 50 GBq/μmol



Anion	NO_3^-	I^-	BF_4^-	SO_3F^-	$PO_2F_2^-$	PF_6^-
Ionic volume ($Å^3$)	64	72	73	86	85	109
IC_{50} (uM)	755	4.7	4.5	0.55	0.55	0.026



Uptake of $[^{18}F]SO_3F^-$ in HCT116-C19 and HCT116 cells, with and without perchlorate blocking



Curative ex vivo liver-directed gene therapy in a pig model of hereditary tyrosinemia type 1

Hickey et al., Mayo Clinic, Science Translational Med 2016

fumarylacetoacetate hydrolase–deficient (*Fah*$^{-/-}$) pigs

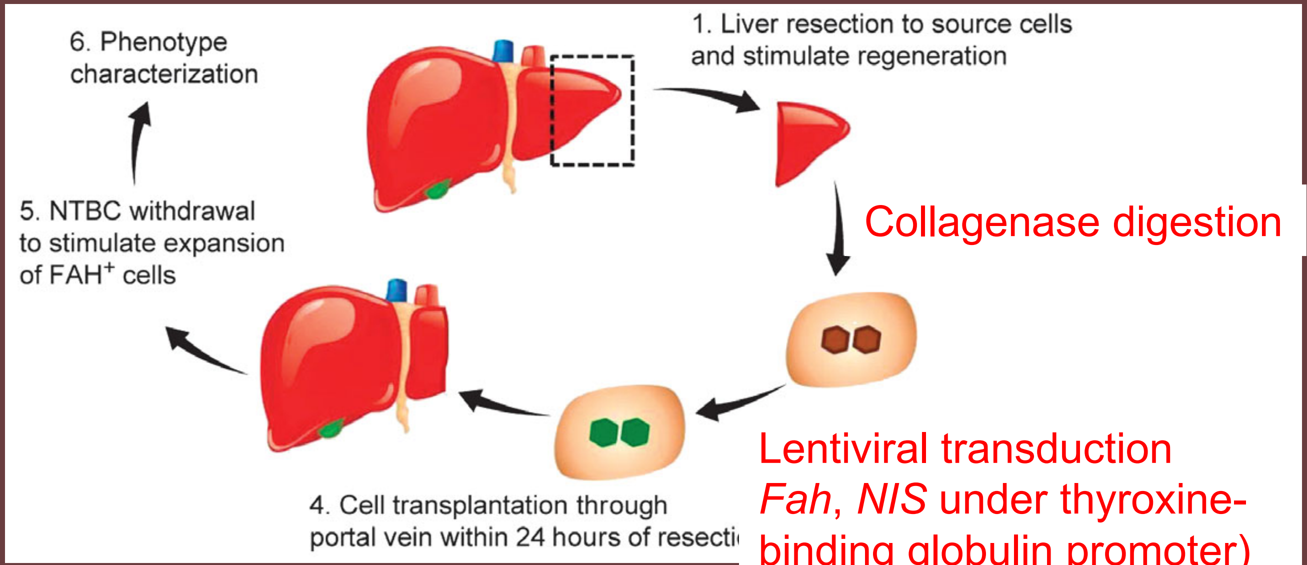
Partial liver resection

Gene replacement/ transplant therapy

Portal infusion of modified cells

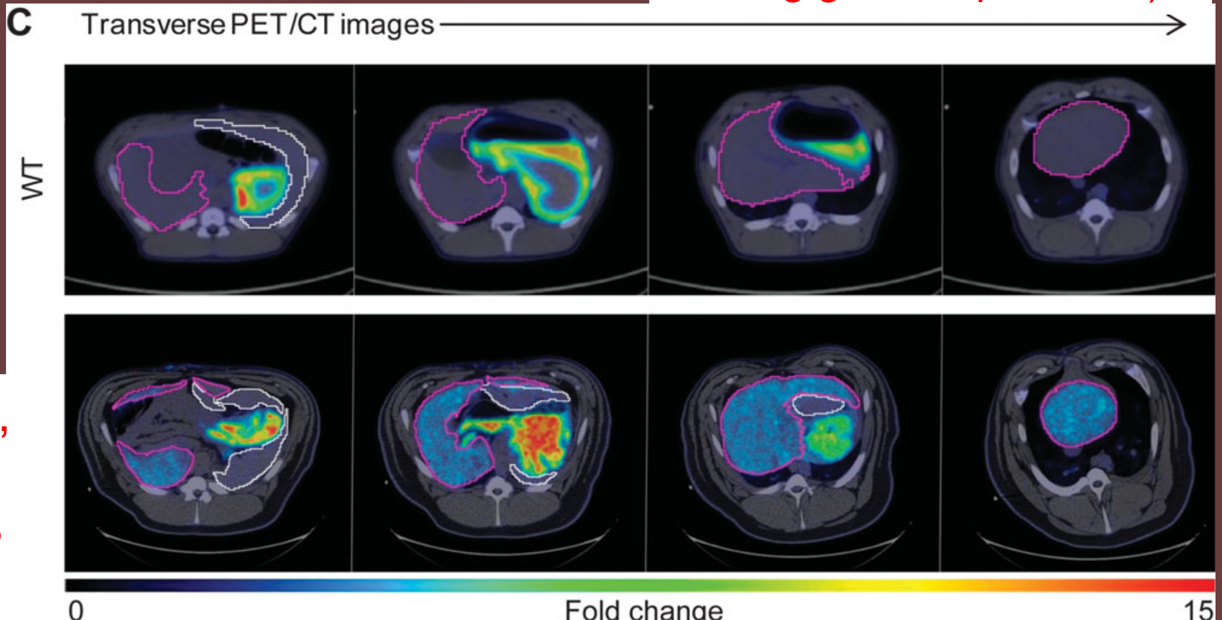
Withdraw protective drug

Monitor regeneration by PET



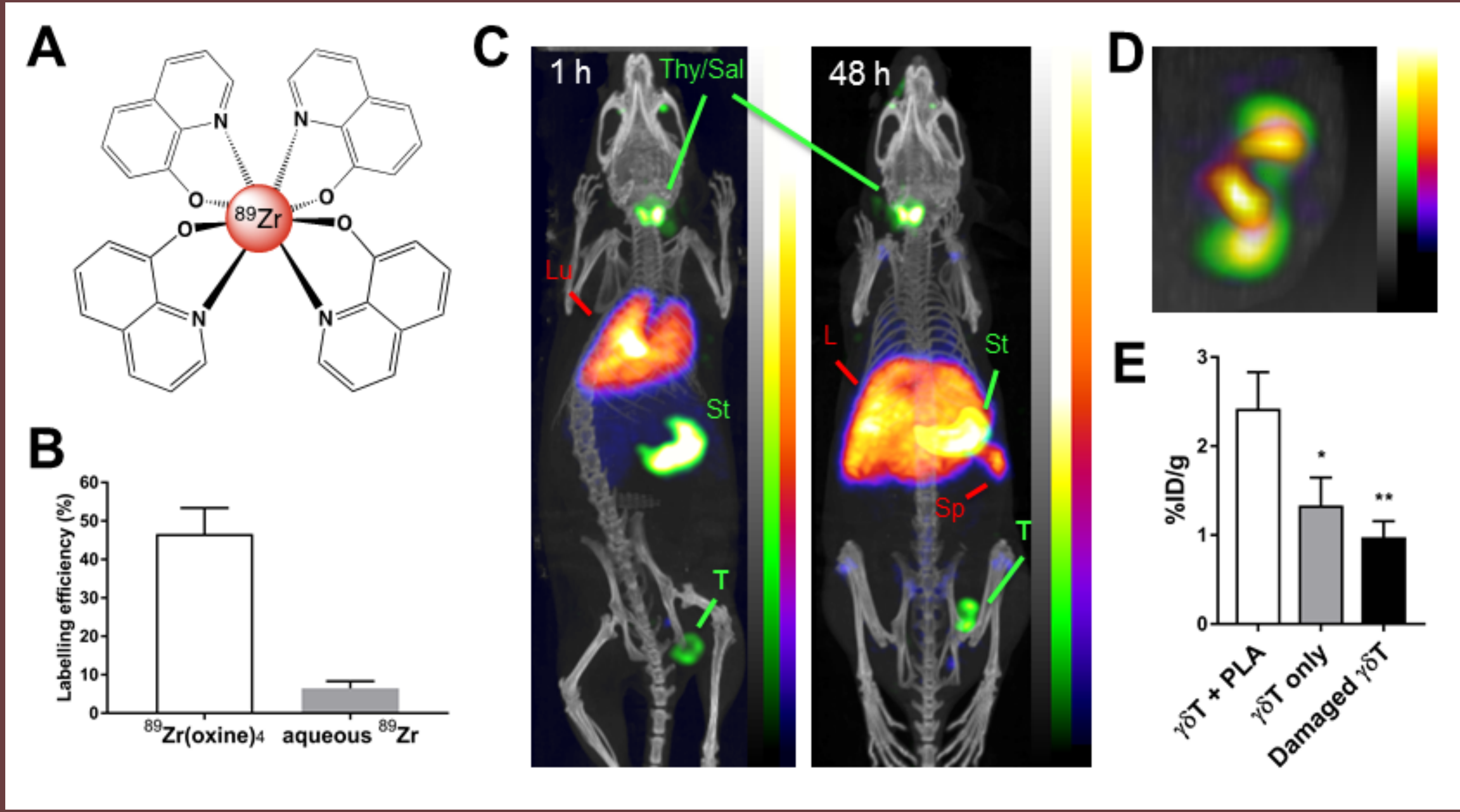
Collagenase digestion

Lentiviral transduction
Fah, *NIS* under thyroxine-binding globulin promoter)



PET imaging, 8 months
[^{18}F]BF_4 (NIS substrate)

Combined direct and reporter gene labelling:
Tracking immune cell tumour therapy with γδ-T cells



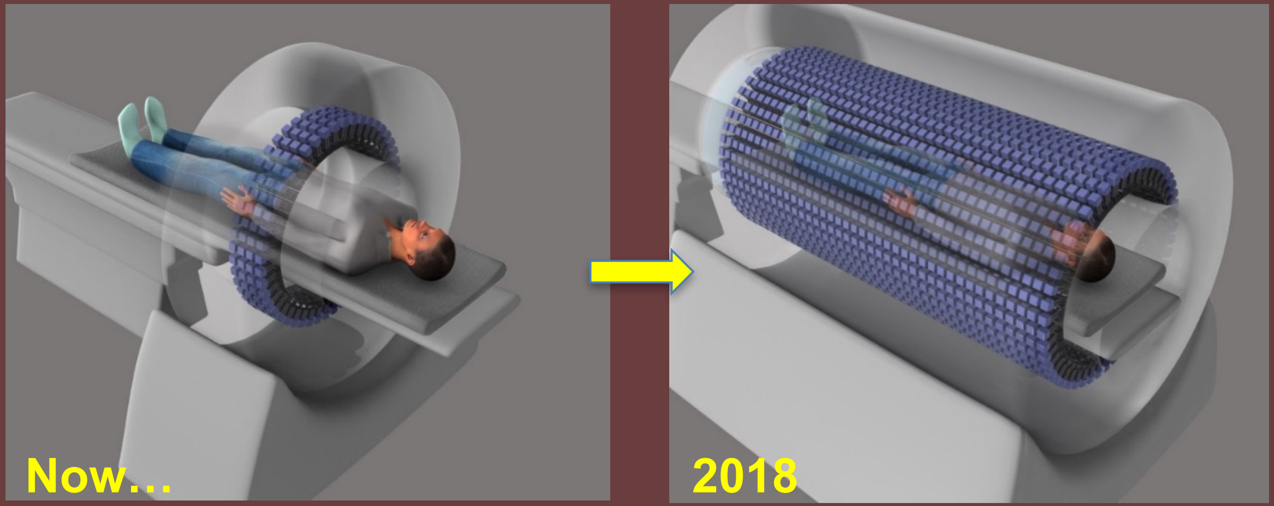
- Gamma-delta (γδ) T cells: subset of immune cells with potent anti-tumour activity used successfully in early clinical trials in cancer immunotherapy
- Direct cell labelling with $^{89}Zr(oxine)_4$ in a breast cancer model shows effect of liposomal Alendronate in improving tumour accumulation of γδ-T cells

Man, Lim, Shmeeda, Draper, Parente-Pereira, Gabizon, Blower, Fruhwirth, de Rosales 2017

Next steps..

- Improve Zr-89 user-friendliness, labelling efficiency; find the right opportunity to translate to first in human study
- NIS reporter gene PET imaging – first in human?
- Mn-52 (PET, >5 day half life) (new chemistry needed – Blower, Long, Torres)
- New Tc-99m direct labelling (wider availability) (Ma)
- Cell surface labelling (Yan, Mullen, Ma)
- New reporter genes (Fruhwirth, Blower, Gee)
  - Non-NIS: PSMA, dopamine transport (BBB)
- Nanoparticles (Torres)
  - Combined modality – MR, PET/SPECT, optical;
- Radiobiology (Terry)
  - Effects of labelling on survival/phenotype/trafficking; intracellular vs cell surface, radionuclide type
- EXPLORER project – whole body PET
- Applications, collaborations and clinical translation…

New engineering/physics



- Whole body PET (EXPLORER) (Cherry et al.)
  - 40x increase in sensitivity, 6x S:N ratio, (Resolution?) (at 5-6x cost!)
  - Smaller tumours
  - Dynamic imaging of whole body at once
  - Longer dynamic imaging (e.g. weeks with Zr-89 cell tracking; several hours for C-11)
  - Lower doses (to whole body and to tracked cells)
  - Multiple scans with different tracers in one session – O-15, Rb-82, N-13, Cu-62, C-11 – complex phenotyping of tumours, heterogeneity, metabolic profiling, systems biology
  - Foetal/neonatal/paediatric PET
  - High throughput scanning: 30 sec for FDG scan! (c.f. 20 min conventional PET)

Technology development by…

- Rafael Torres
- Ran Yan
- Greg Mullen
- Gilbert Fruhwirth
- Lefteris Livieratos

- Levente Meszaros
- Putthiporn Charoenphun
- Truc Pham
- Dave Berry
- Maite Jauregui-Osoro
- Jim O’Doherty
- Kavitha Sunassee
- Amanda Weeks
- Seckou Diocou
- Alex O’Neil
- Alex Khoshnevisan
- Francis Man
- Lindsay Lim

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  - CRUK
  - Rosetrees
  - KHP
  - Wellcome
  - EPSRC
  - MRC