Co-Development of DELFIA Technology

PerkinElmer - Wallac Inc & Karyon Ltd

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Assessment of DELFIA technology in drug biodistribution in small animals

Project Report

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BACKGROUND

Dissociation of Europium from DTPA & DOTA chelates

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Comparative study

Early findings

BACKGROUND

DISSOCIATION OF EUROPIUM FROM CHELATES

DTPA[Eu] ITC chelate vs. peptide-DOTA[Eu] (comparison done to Eu-control)

Comparison of the signal of Eu-DTPA ITC chelate to three different DOTA-coupled peptides:

1nM solution of Eu-control ←→ 1 000 000 counts

- **1nM content of measured compounds**
- **Compounds diluted to Delfia Enhancement solution**
- **Total volume 200ul (in Delfia Enhancement solution)**

Two identical sets (six parallel wells) **1) direct measurement**
2) 40 min pre-incubation at 37C

BACKGROUND

DISSOCIATION OF EUROPIUM FROM CHELATES

Results

	Eu-control	Eu-DTPA ITC	RGD-DOTA[Eu]	TCTP-DOTA[Eu]	AETP-DOTA[Eu]	
Counts	1004497	652124	32619	127	1182	RT
% of Eu-control		65	3,25	0,01	0,12	
Counts	987427	627531	31788	369	1631	37°C
% of Eu-control		64	3,22	0,04	0,17	
Pep-DOTA / DTPA (%)			5,00	0,02	0,18	
Pep-DOTA / DTPA (%)			5,03	0,06	0,26	

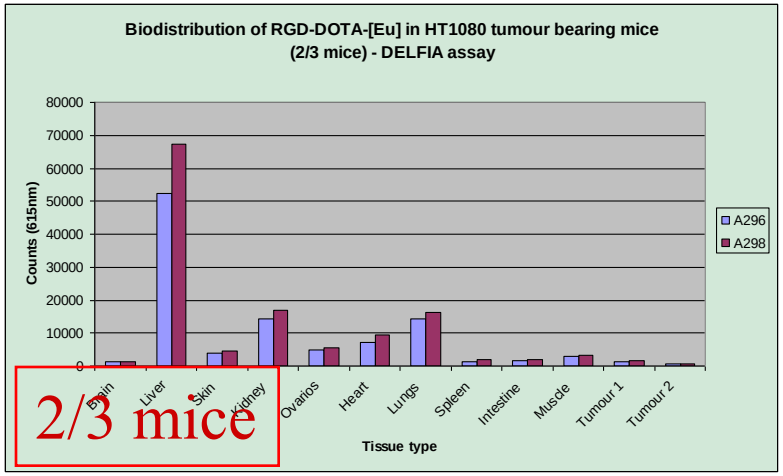
DOTA-coupled peptides gave significantly lower signals compared to Eu-DTPA ITC chelate → from 0,02 % to 5%

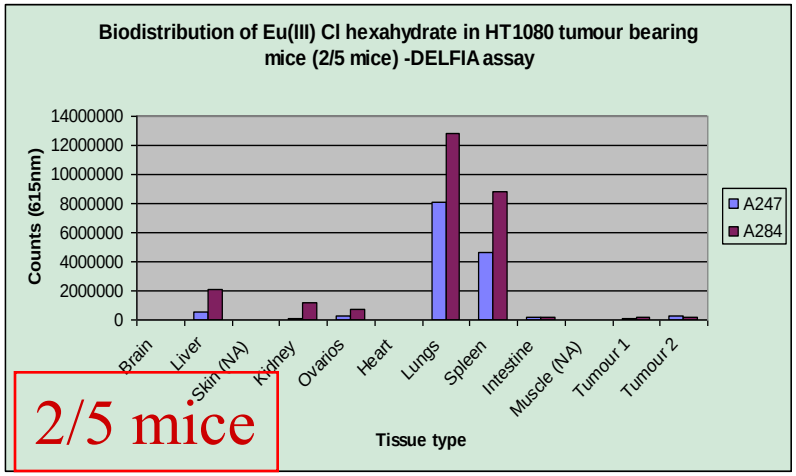
→DOTA chelate is not useful with DELFIA technology without a separate dissociation step (2 M HCl)

BACKGROUND

TAKE HOME: In vivo injected Europium-labelled peptides can be detected from the lysates of mouse organs

Biodistribution profiles:
RGDfK-DOTA[Eu] vs. Eu(III)Cl hexahydrate





RGDfK-DOTA-[Eu] shows identical biodistribution profiles in mice A296 & A298 verifying the potential of DELFIA technology in biodistribution analysis.

Eu(III)Cl hexahydrate shows identical biodistribution profiles in mice A247 & A284 verifying the potential of DELFIA technology in biodistribution analysis.

The determination of biodistribution profile of DTPA[Europium] labelled compounds using DELFIA technology

Aims:

1) Compound specific biodistribution patterns.

2) Reliability – comparison of results to ICP-MS results.

In vivo biodistribution with DELFIA technology

Repetition of the biodistribution study using DELFIA technology with higher number of mice, and comparison the result with those of Inductively coupled plasma mass spectrometry (ICP-MS)

<u>Wallac - contribution</u>

1) Eu-DTPA-iodoacetamido chelate and consultation for chemistry.

2) VICTOR fluorometer and reagents

<u>Karyon - contribution</u>

1) Test compound (Thx1-targeting peptide) and a control compound DTPA[Eu].

2) Cell lines and animal models (20 mice)

3) Development of process for biodistribution assesment

In vivo biodistribution with DELFIA technology

MATERIALS AND METHODS

CHEMISTRY

Tested Compounds

1) **Thx-DTPA[Eu]**

   Coupling of Thx-peptide* having sulphydryl group at its carboxy terminus to Eu-DTPA-iodoacetamido

   ***Karyon™ Targeting Unit (KTU) specific for Lung cancer**

2) **DTPA[Eu]**

   Europium (III) Cl-hexahydrate was chelated to DTPA

In vivo biodistribution with DELFIA technology

MATERIALS AND METHODS

Biology - Thx testing in Non-small cell lung cancer mouse models.

Cell lines:

A549: human adenocarcinoma cell line

NCI-H520: Human epidermoid carcinoma cell line

Tumour model:

Mouse bearing both A549 and NCI-H520 tumours on their flanks (n=20).

In vivo biodistribution with DELFIA technology

MATERIALS AND METHODS

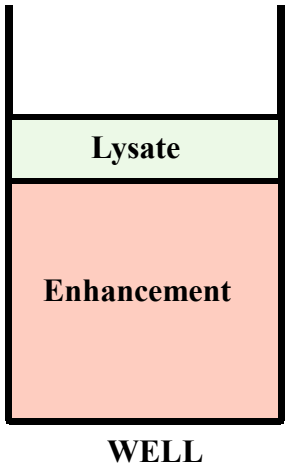
Biology - Thx testing in Non-small cell lung cancer mouse models.

COMPOUND	AMOUNT INJECTED	MICE (n)
Thx –DTPA[Eu]	0,275 umol	7
DTPA[Eu]	0,275 umol	5
DTPA[Eu]	0,550 umol	5
DTPA[Eu]	2,20 umol	5

Tested compounds were injected i.v. into tail vein of mice circulation time being 15 min before perfusion.

MATERIALS AND METHODS

Preparing of samples with minimized lysate proportion



SAMPLES (0,275umol, 0,55umol, 2,2umol)
5 µl of 1:5 to 1:50 diluted tissue lysate (dilution to lysis buffer)
195 µl of DELFIA Inducer solution

TISSUE SPECIFIC STANDARDS FOR COMPOUDS
5 µl of Tissue Lysate (Eu-free lysate from blank mouse)
195 µl of DELFIA Inducer solution containing appropriate amount of Eu-labelled peptide

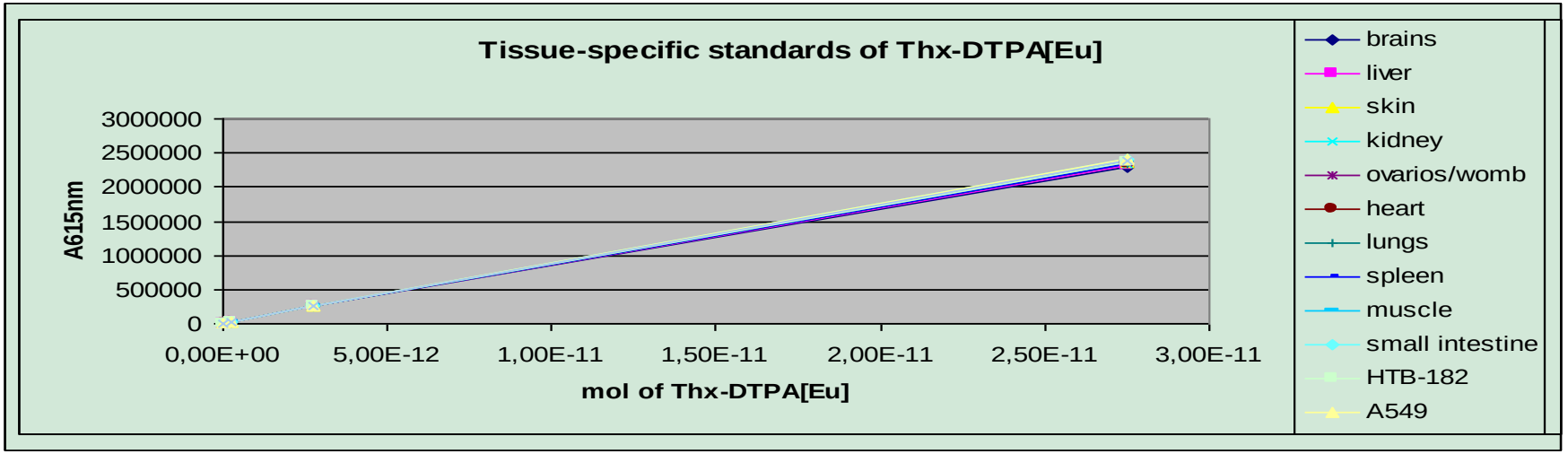
BLANK 5 µl of tissue lysate + 195 µl of DELFIA Inducer

In vivo biodistribution with DELFIA technology

Process Development

Tissue specific standards

Tissue specific standards were analysed → to determine differences of fluorescence signal from lysates of different tissue types.



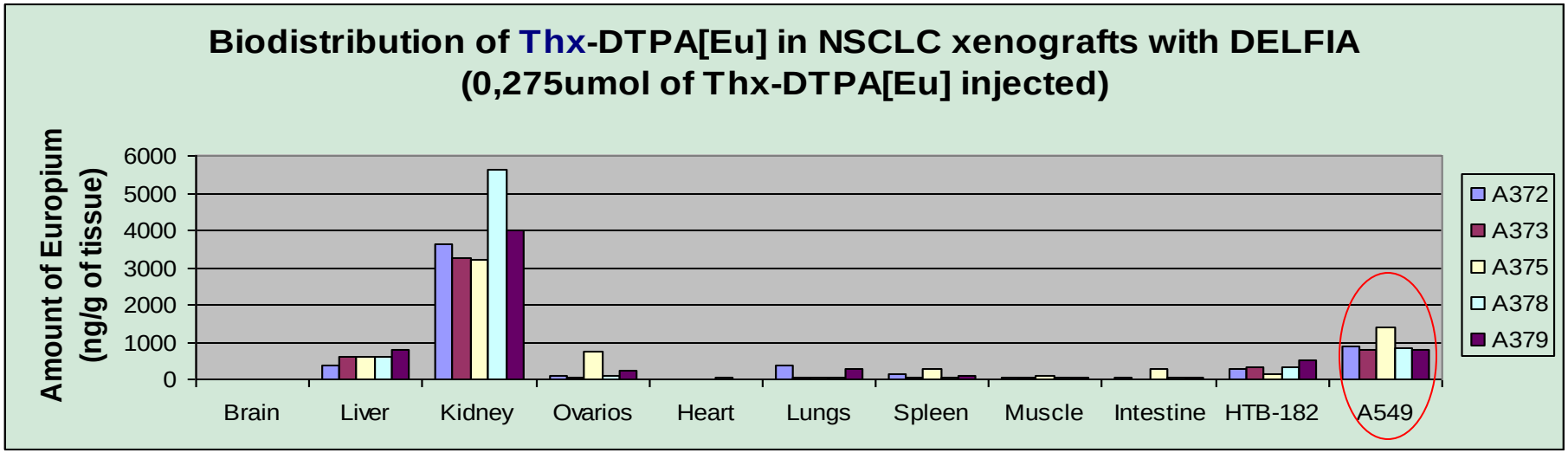
Minimised lysate proportion in DELFIA assay
→ ”no need for tissue-specific standards”

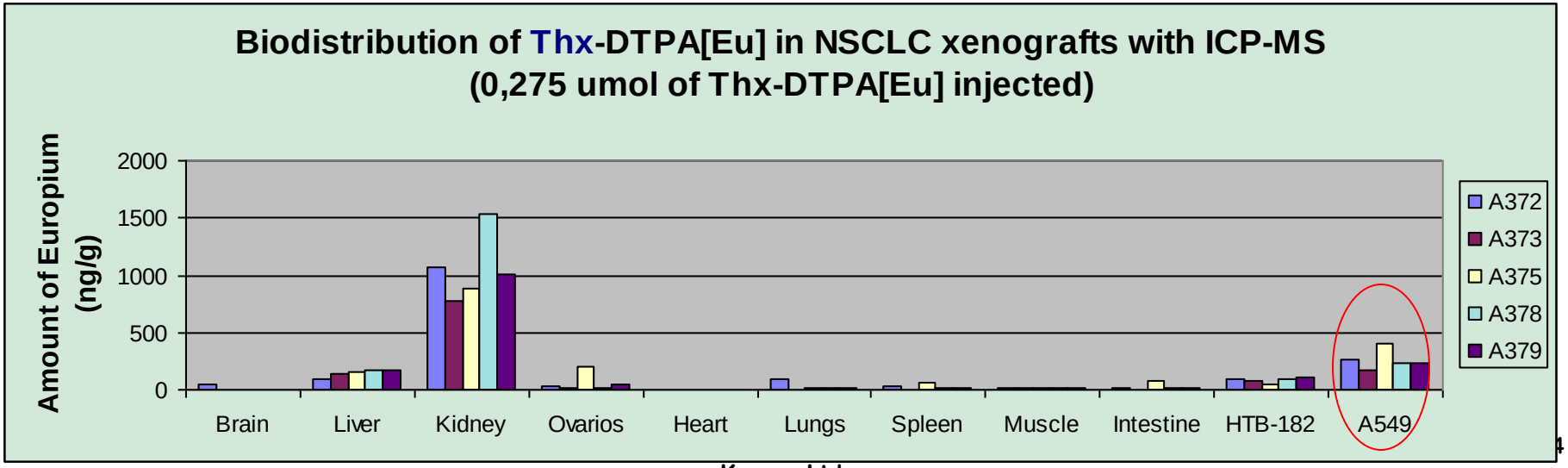
The determination of biodistribution profile of Thx-DTPA[Eu] using DELFIA technology

Results

Biodistribution of Thx-DTPA[Eu] with DELFIA

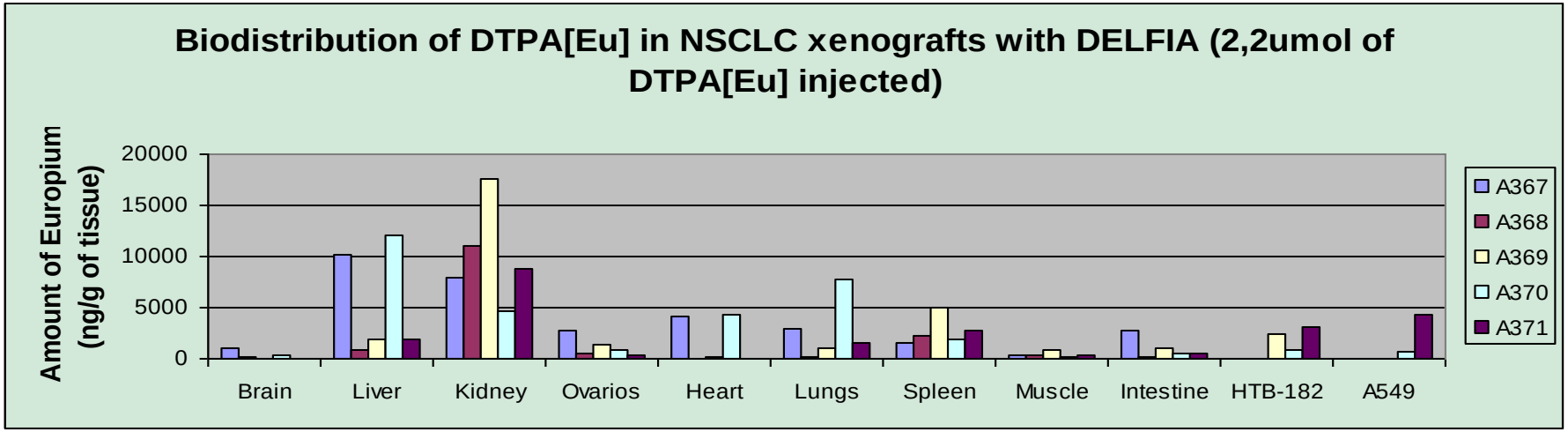
ICP-MS confirms the reliability of DELFIA technology

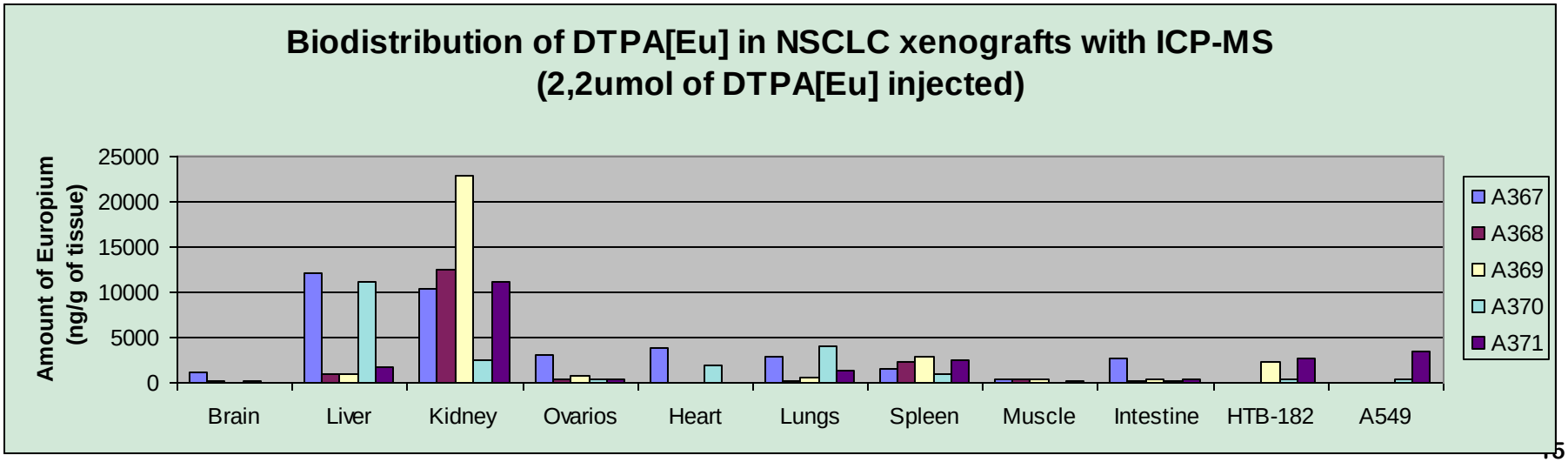


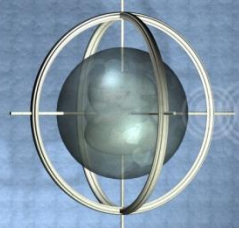


Biodistribution of DTPA[Eu] with DELFIA

ICP-MS confirms the reliability of DELFIA technology







Conclusions

DELFIA appears to be reliable method for biodistribution tests

Detection sensitivity as good as for radionuclides

Safe to use and stable (excellent alternative for radionuclides)

DELFIA is a good alternative for biodistribution studies