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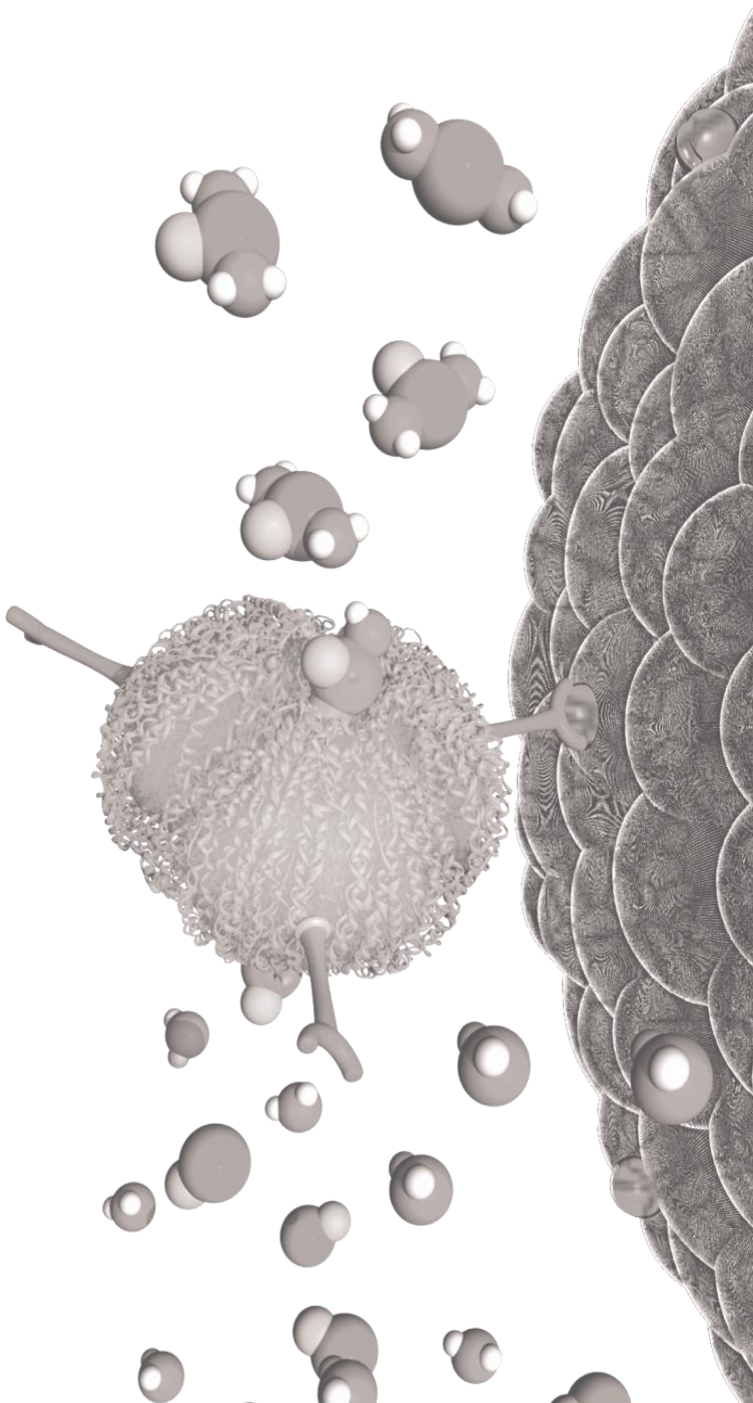
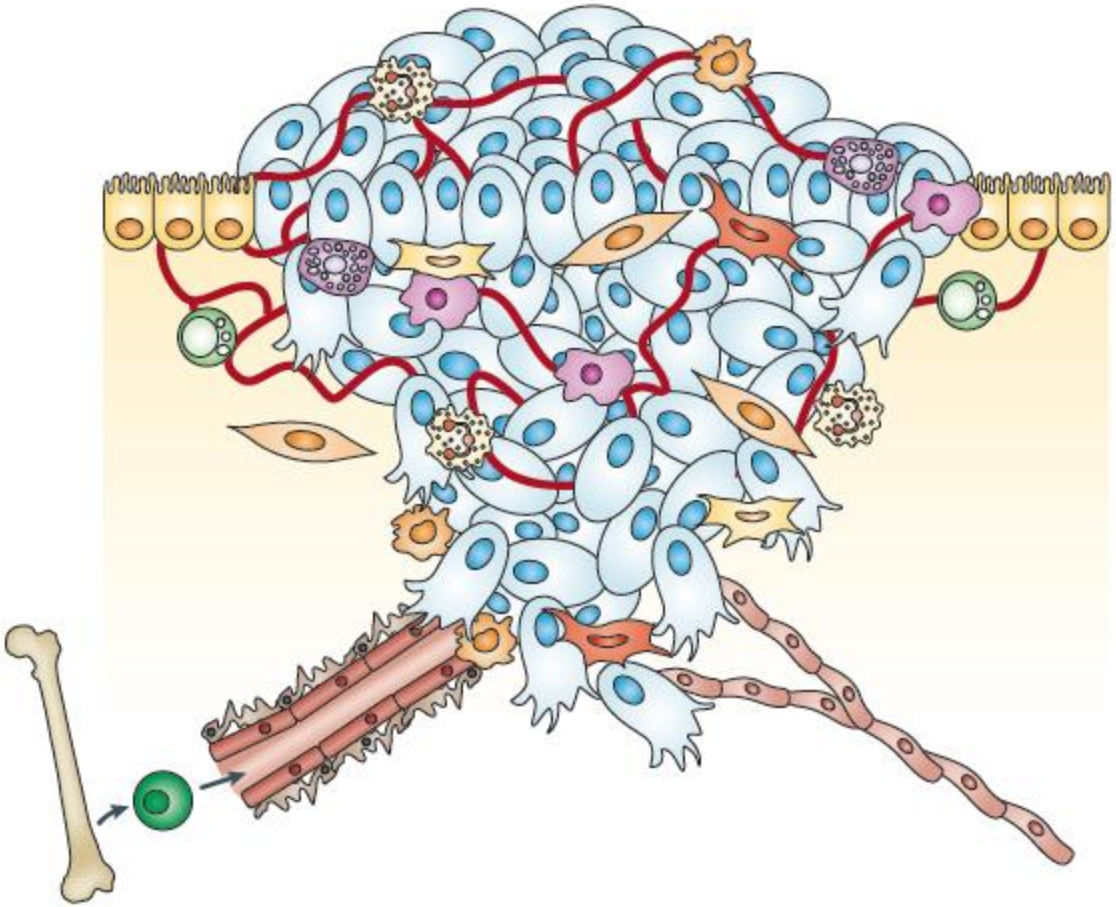
L-DOS47

Tumor Microenvironment



Tumour Microenvironment



Joyce and Pollard, Nature Reviews Cancer 9: 239 (2009)

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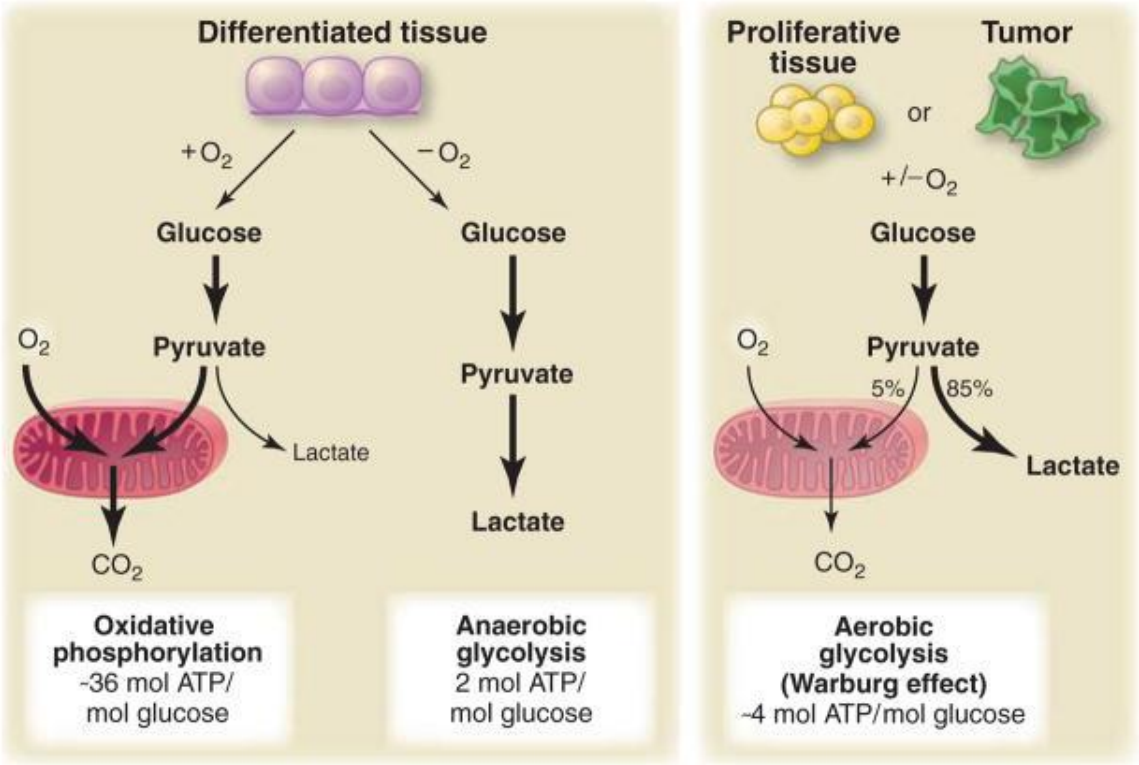
Acidosis – Warburg effect



Vander Heiden et al. Science 324: 1029 (2009)

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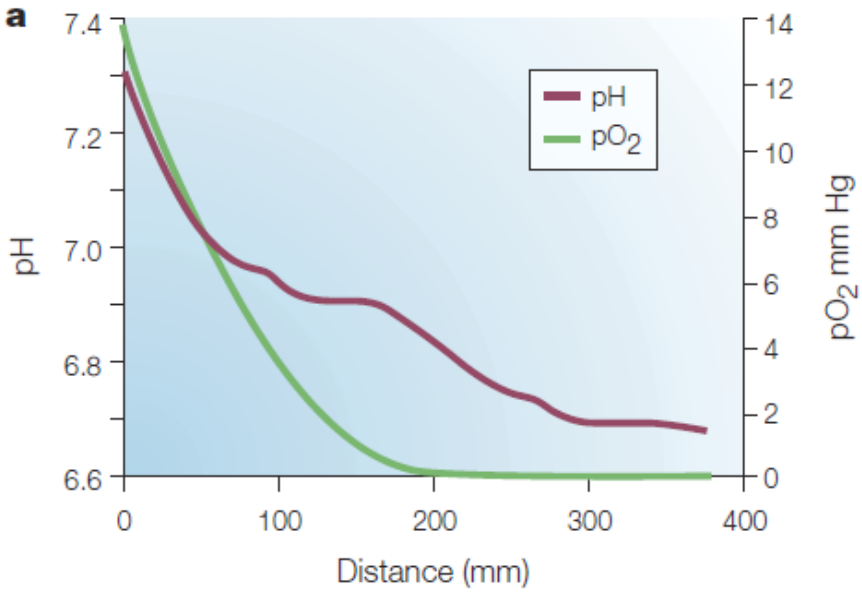
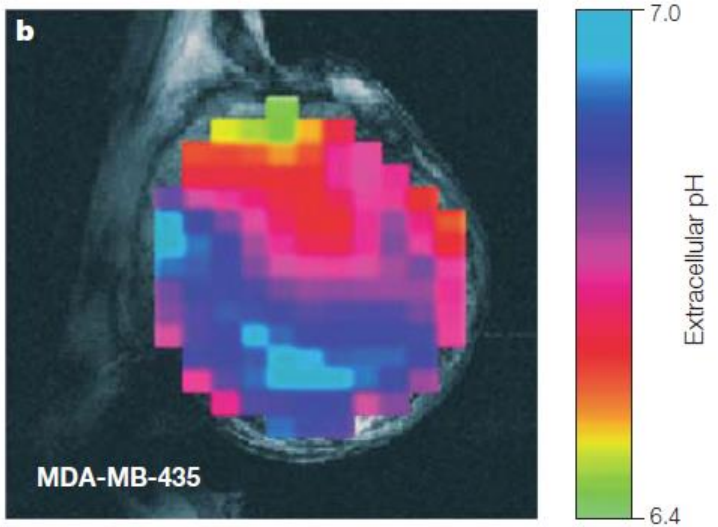
Acidic and Hypoxic Environment



MCF-7 fluorescent ratio imaging



^{1}H MRS

Gatenby and Gillies, Nature Reviews Cancer 4:891 (2004)

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Acidosis and Hypoxia

Hypoxia	Acidosis
Radioresistance	Increased radioresistance
Drug resistance	Resistance to anthracyclines
Metastasis and Invasion	Increased metastases
Increased mutation rate	Increased migration and invasion
Gene expression induced hypoxia-inducible factor	Mutagenesis / clastogenesis
Apoptosis	Apoptosis

Gatenby and Gillies, Nature Reviews Cancer 4:891 (2004)

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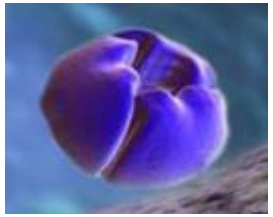
DOS47 – Proposed MOA

- Reverse Tumour Acidity
- Apply Natural Metabolic Toxin
- Induce Chemo-optimized Environment



Urease

$$\text{Urea} \xrightarrow{3H_2O} 2NH_4OH + CO_2$$

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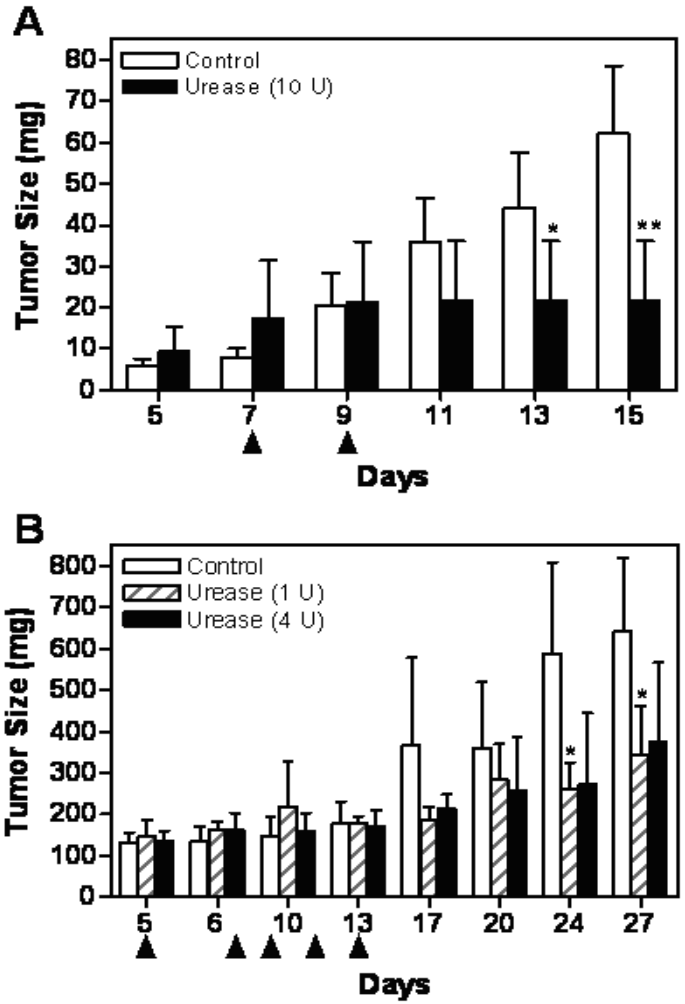
DOS47 MCF7 and A549 Xenograft



MCF-7

A549

Wong et al. Journal of Experimental Therapeutics and Oncology 5:93-99 (2005)

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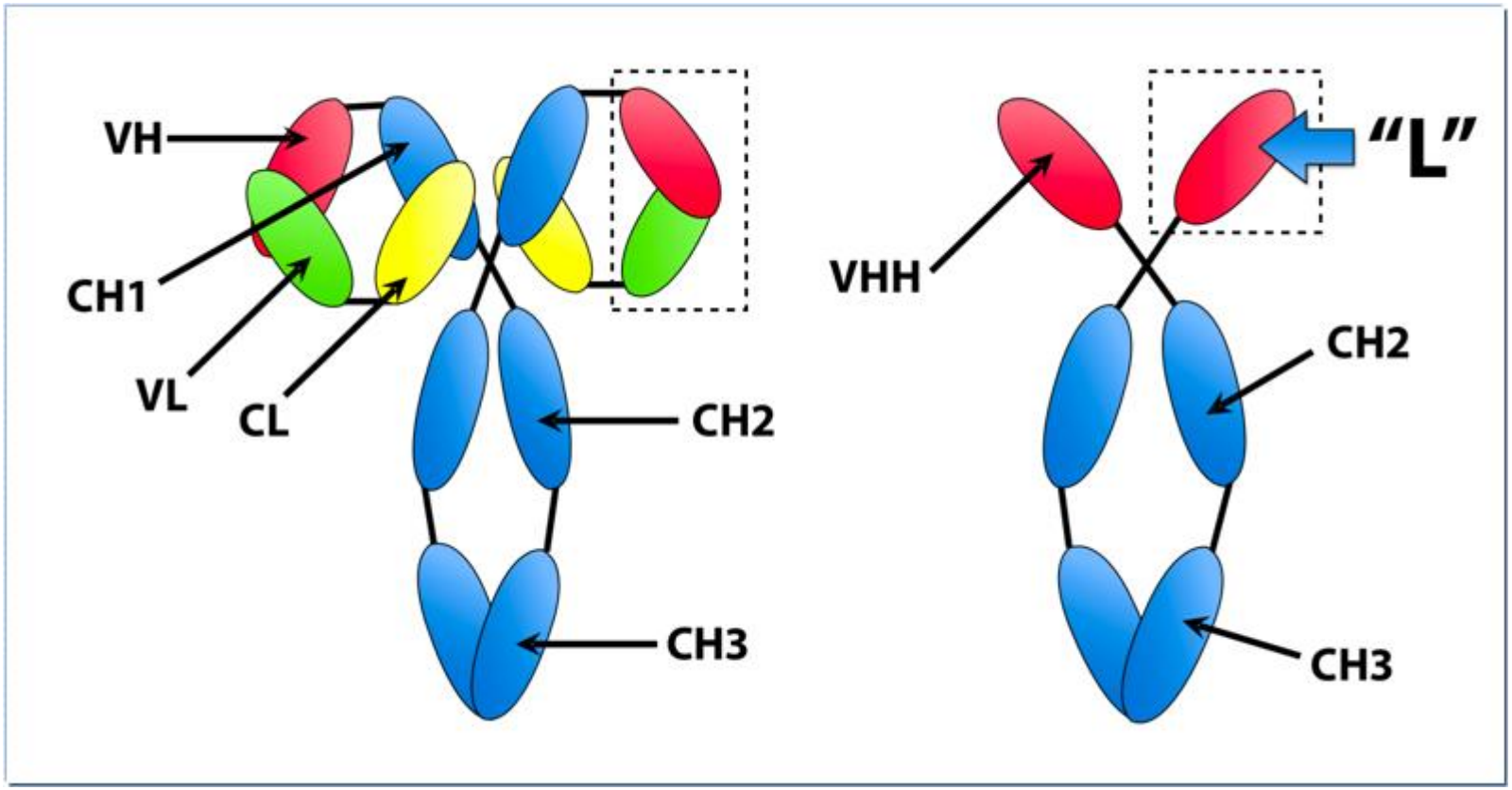
Llama Antibody



Zhang et al, Journal of Molecular Biology 341:161 (2004)

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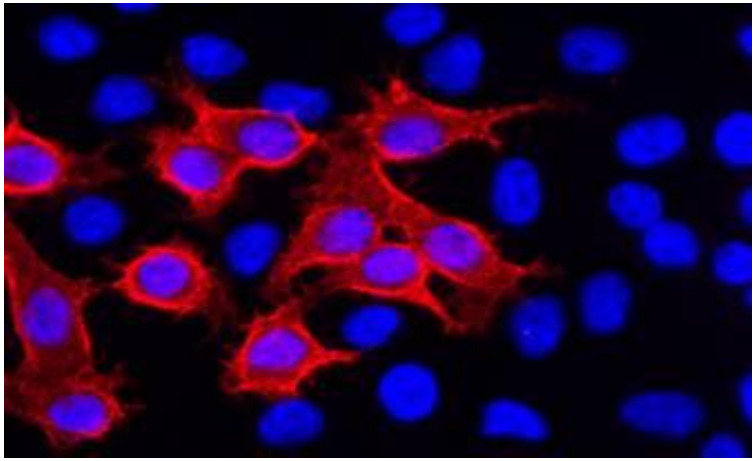
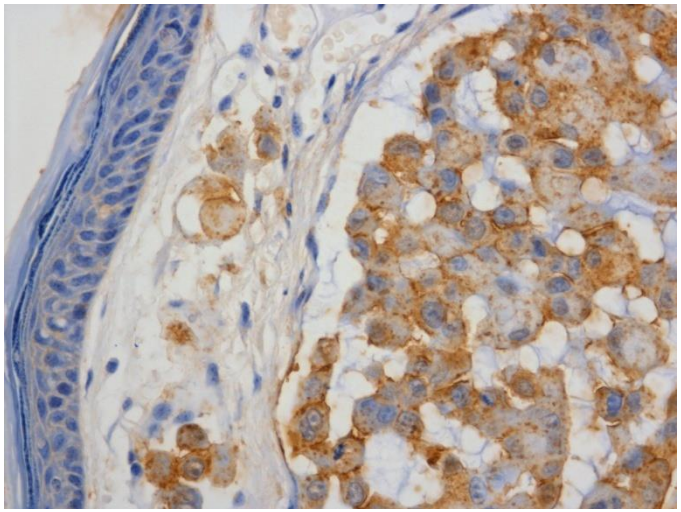
Cell Surface Specific



A549 cell



A549 tumour slide

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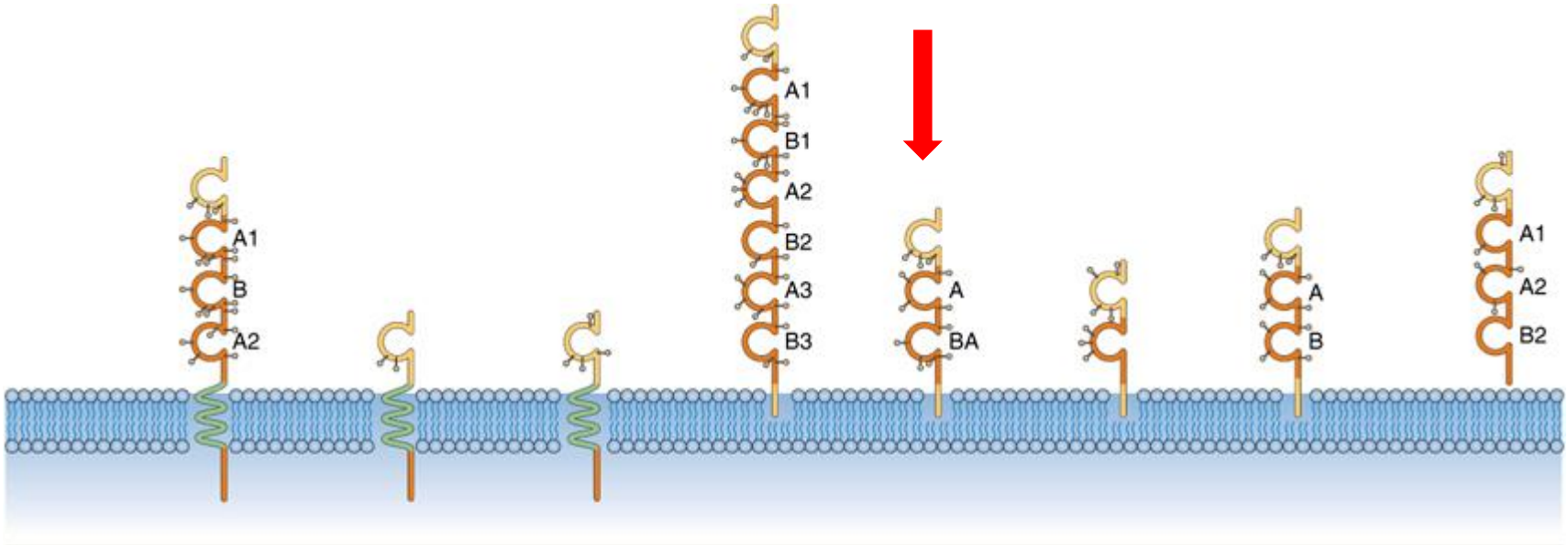
CEACAM Family



New name	CEACAM1	CEACAM3	CEACAM4	CEACAM5	CEACAM6	CEACAM7	CEACAM8	PSG1-11
Alternative names	CD66a, BGP, and C-CAM	CD66d and CGM1	CGM7	CD66e and CEA	CD66c and NCA	CGM2	CD66b and CGM6	CD66f
Tissue expression	Epithelial cells, endothelial cells, lymphocytes, and myeloid cells	Granulocytes	Granulocytes	Epithelial cells	Epithelial cells and granulocytes	Epithelial cells	Granulocytes	Placenta

Nagaishi et al. Mucosal Immunology 1:S39 (2008)

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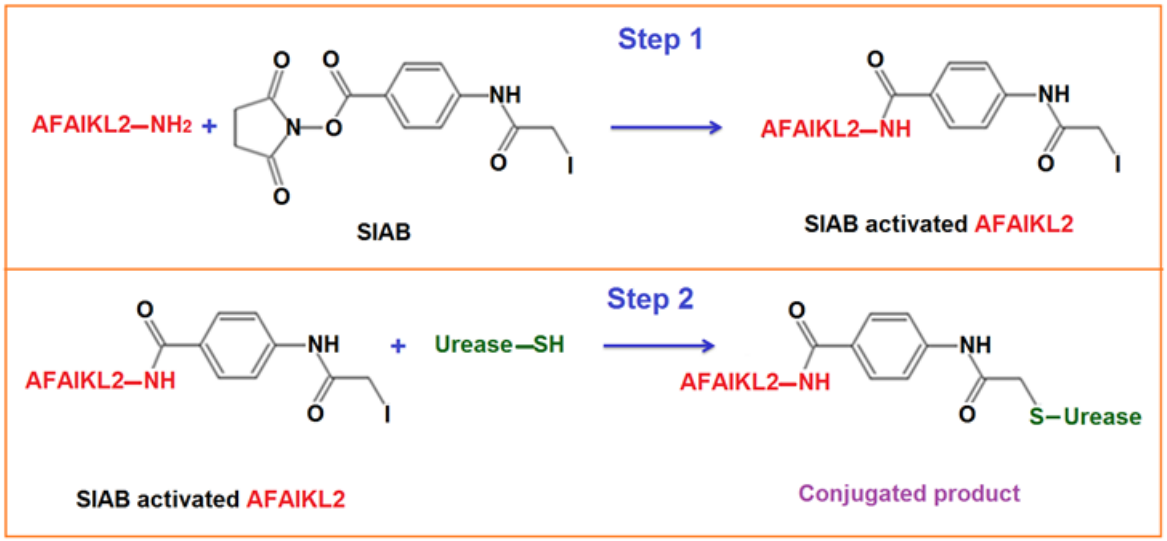
Conjugation

SIAB (N-succinimidyl(4-iodoacetyl]aminobenzoate)



Synthesis of L-DOS47 conjugate product is a two-step reaction. Step 1 is an activation of antibody AFAIKL2 using SIAB and Step 2 involves conjugation of activated antibody with urease enzyme to form the antibody-urease conjugate L-DOS47

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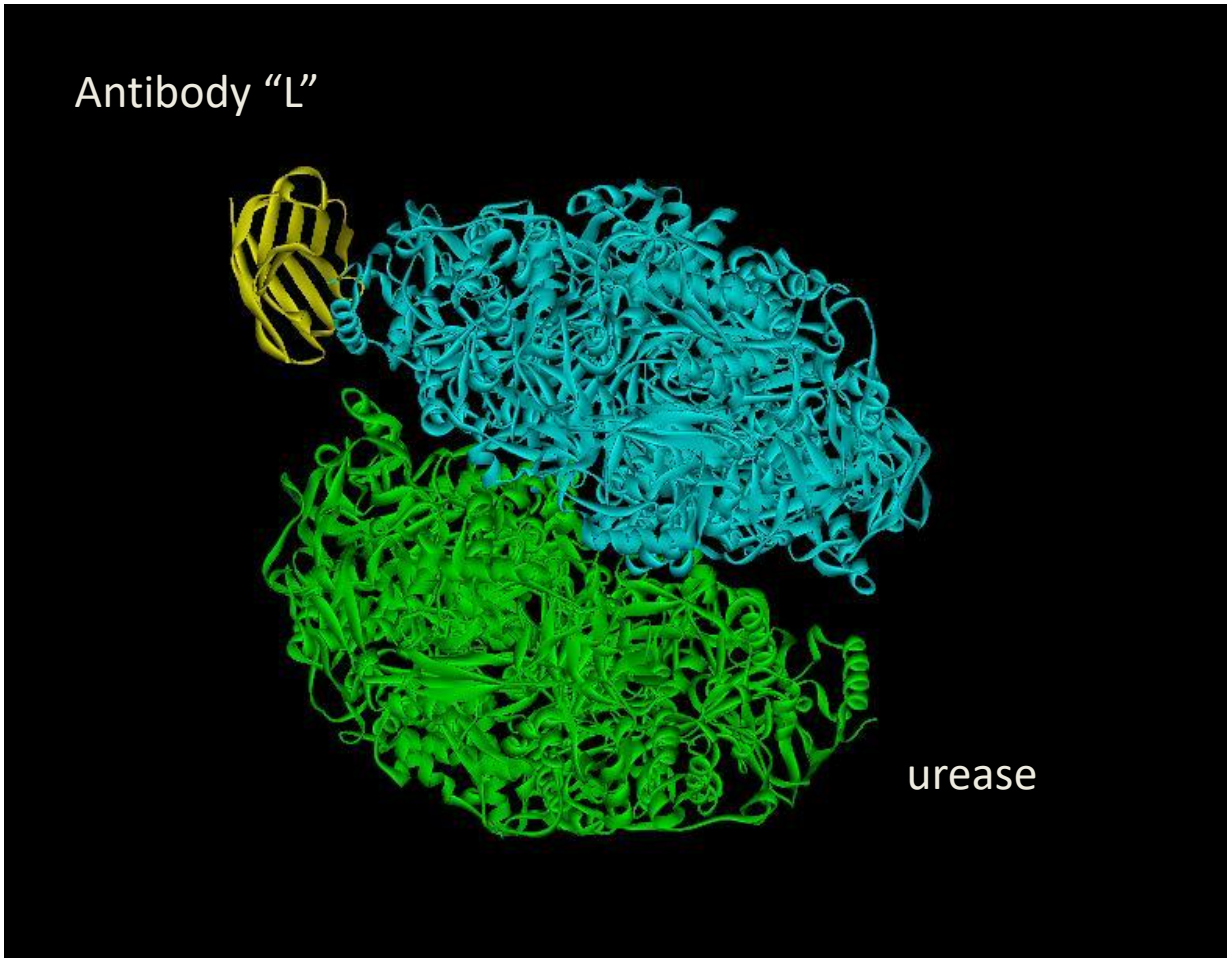
Tian et al. DOI: 10.1021/acs.bioconjchem.5b00237

L-DOS47



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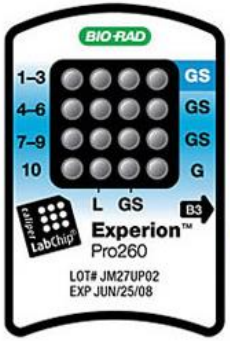
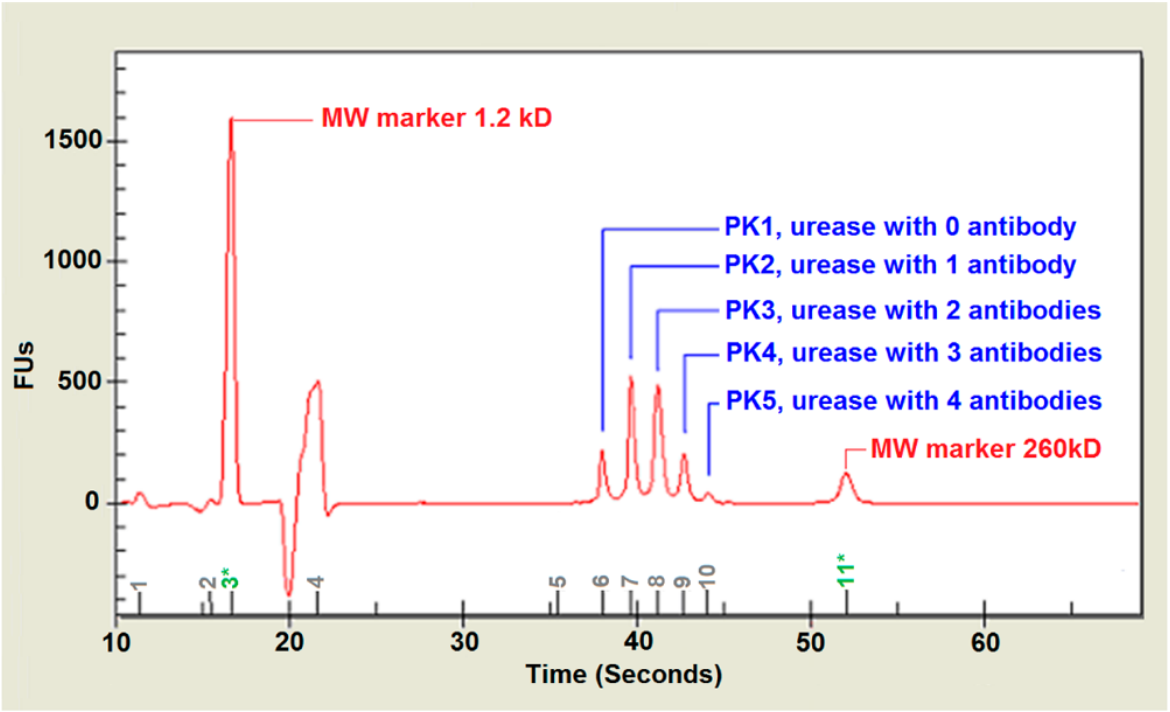
Conjugation





Electropherogram of L-DOS47 (lane 1 from Figure 3) showing the discrete peaks for urease subunits linked with 0-4 antibody molecules. The numbers 1-11 on the x-axis are the peak numbers; 3* represents the lowest MW marker peak and 11* is the highest MW marker peak for the internal MW standard.

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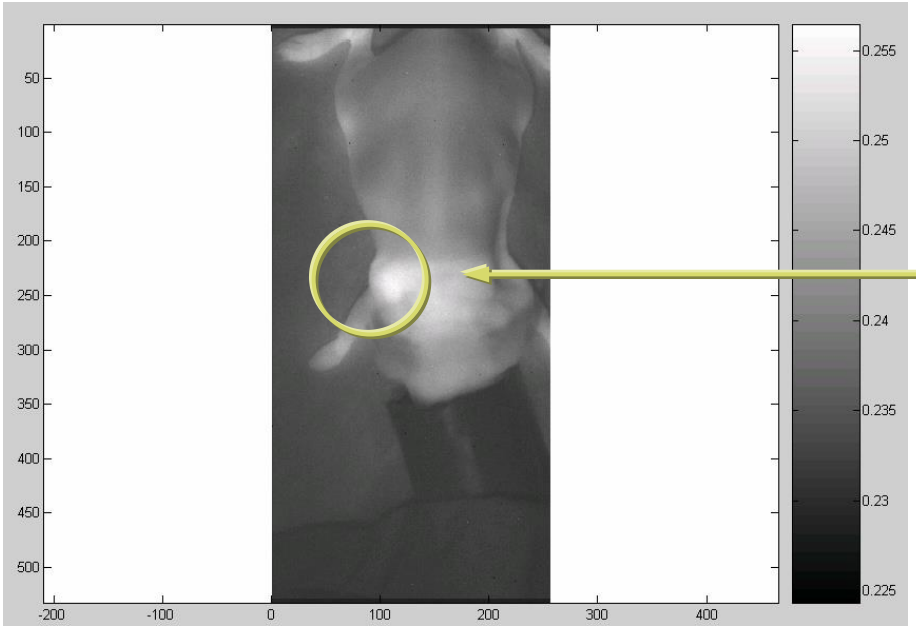
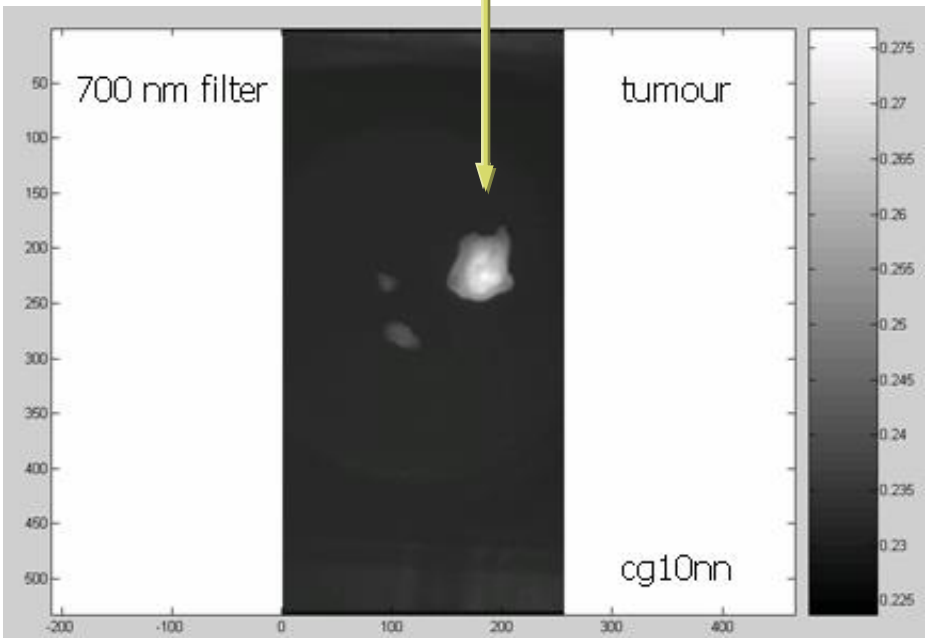
Tian et al. DOI: 10.1021/acs.bioconjchem.5b00237

L-DOS47 Imaging



Tumour specific localization



Full Body Scan

A549 tumour (8 x 7 mm)
L-DOS47-Cy5.5

Filtered Scan

L-DOS47-Cy5.5
Cy5.5 emission max @710nm

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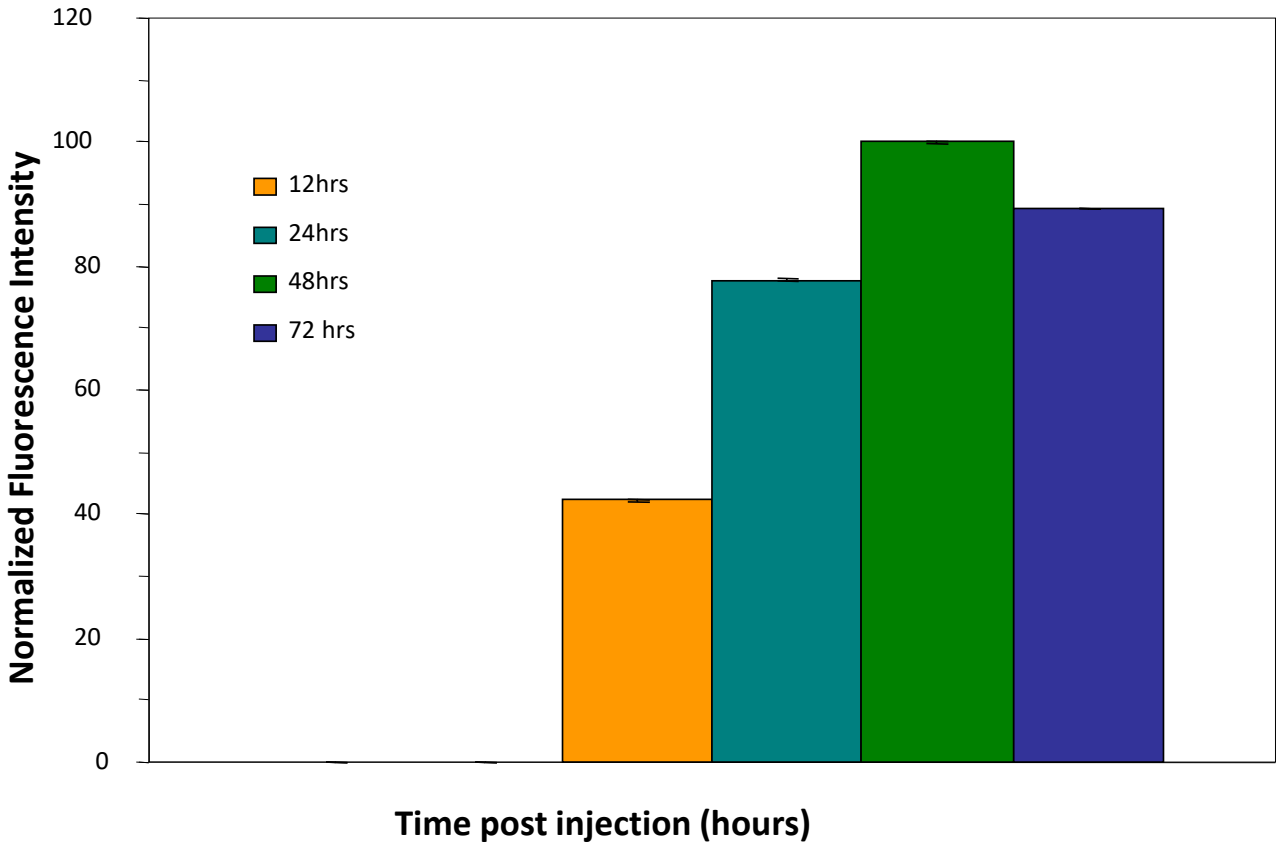
L-DOS47 Imaging



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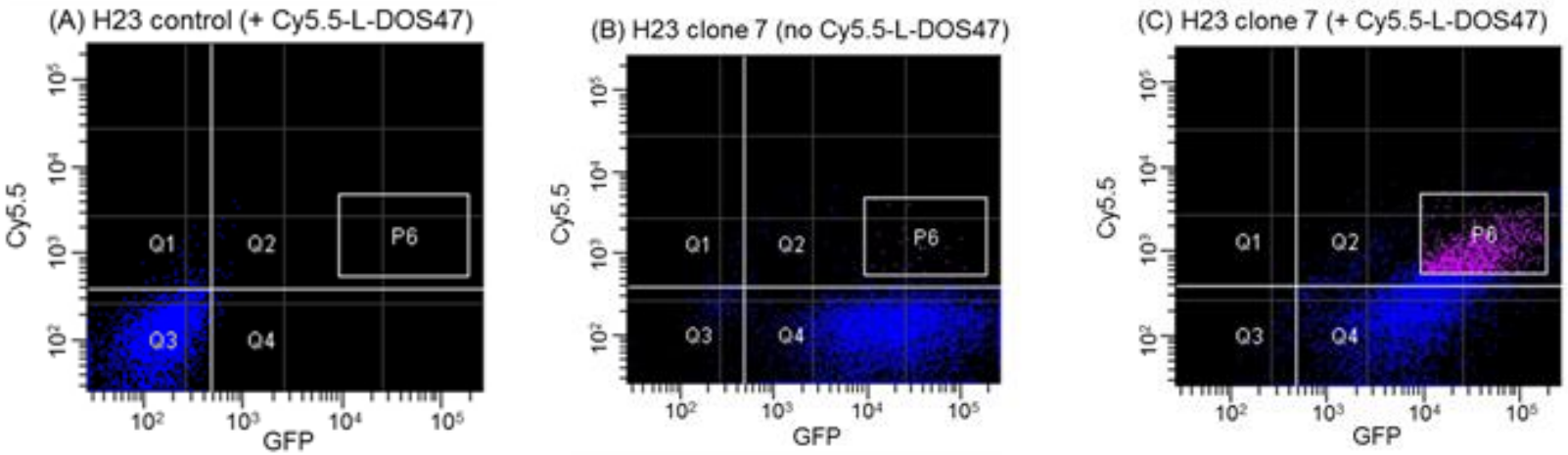
Flow Cytometry



Flow cytometry analysis of CEACAM6 expression in CEACAM6-transfected H23 cell line. The transfected cells (clone 7) co-express GFP from the same mRNA as CEACAM6. Cells were sorted directly after incubation with Cy5.5 labelled L-DOS47. (A) Untransfected H23 control cells incubated with Cy5.5-L-DOS47 (B) CEACAM6-transfected cells (C) CEACAM6-transfected cells after incubation with Cy5.5-L-DOS47. P6 represents transfected cells with Cy5.5 fluorescence, corresponding to CEACAM6 expression on the cell surface.

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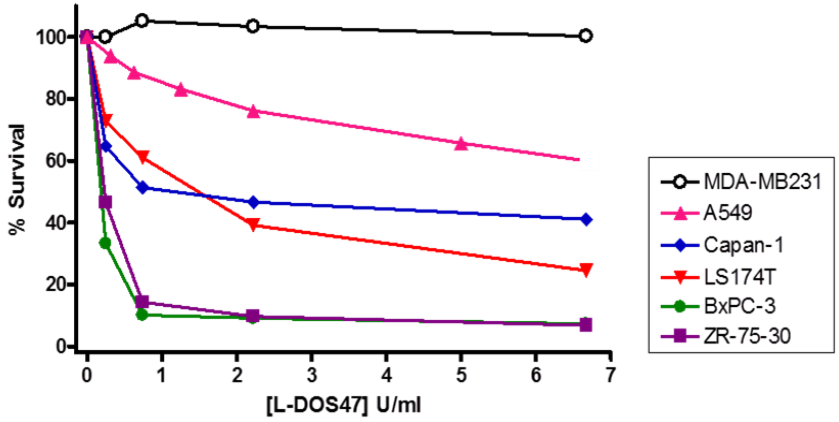
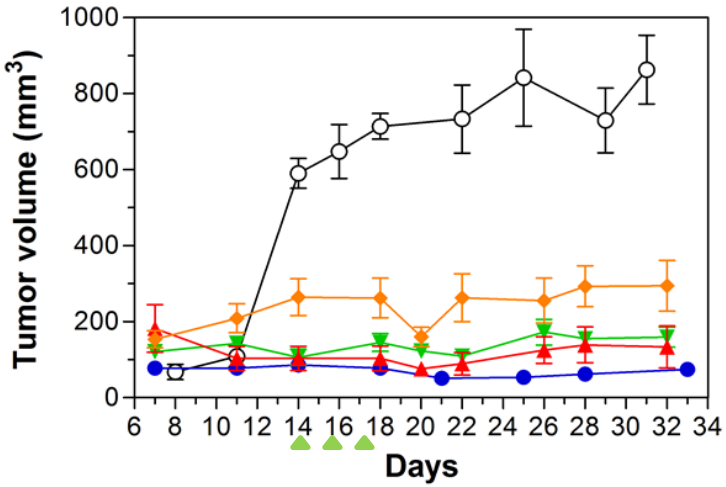
Tian et al. DOI: 10.1021/acs.bioconjchem.5b00237

Bioactivity





Cell lines		Binding assay	Cytotoxicity assay
A549	Lung carcinoma	++	+
H23	Lung adenocarcinoma	-	+
BxPC-3	Pancreatic adenocarcinoma	+++	+++
Capan-1	Pancreatic adenocarcinoma	+++	++
MIA PaCa-2	Pancreatic carcinoma	+	+
MDA-MB231	Breast adenocarcinoma	-	-
MCF-7	Breast carcinoma	-	-
ZR-75-30	Breast ductal carcinoma	+++	+++
LS174T	Colon adenocarcinoma	++	++

L-DOS47 inhibits BxPC-3 xenograft tumor growth in nude mice. Significant inhibition of tumor growth was observed in all three L-DOS47 treatment groups (7 (green), 35 (orange), and 175 (red) µg/kg) and Paclitaxel control (blue) as compared to the vehicle-treated group (open). Values are means (n=5) ± S.E.M.

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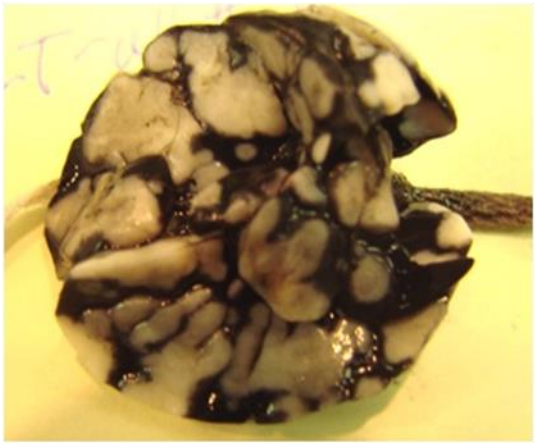
Tian et al. DOI: 10.1021/acs.bioconjchem.5b00237

Bioactivity



Control

Treated

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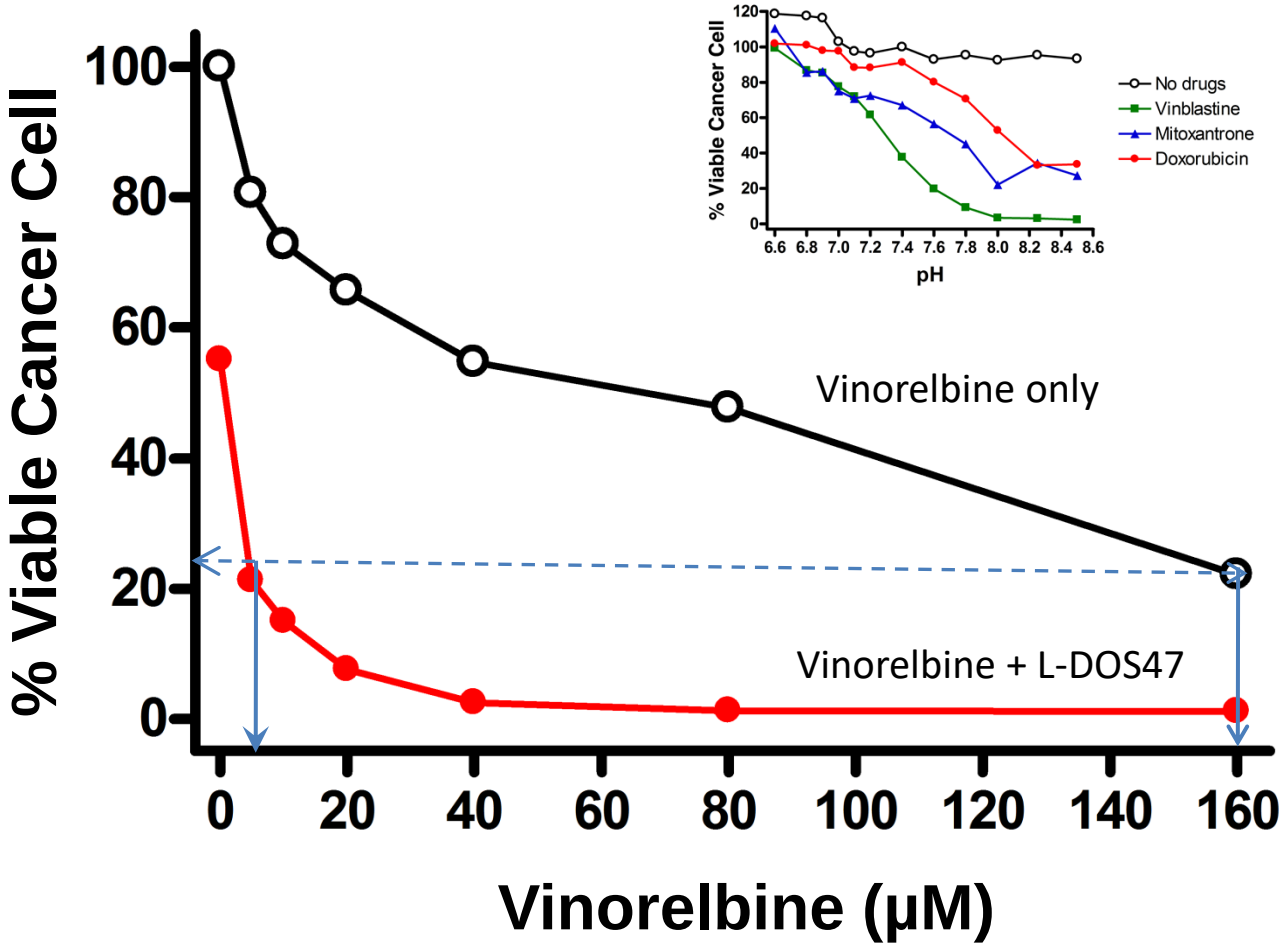
Effect of L-DOS47 and Vinorelbine on A549



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Human Cancer Tissue Screening

Samples	Tumour Tissue		Age-matched Normal Tissue
	Positive	Negative	Negative
Kidney carcinoma		12/12	12/12
Parathyroid adenoma		1/1	n/a
Plaenta, umbilical cord, allantois	n/a		1/1
Myofibroblastic tumor		1/1	n/a
Prostate carcinoma		4/4	4/4
Thyroid carcinoma		2/2	2/2
Pancreas adenocarcinoma	7/57 weak 8/57 v. weak	42/57	25/25
Neuroendocrine tumors		9/9	n/a
Brain, heart muscle, testis, spleen	n/a		30/30
Testis - teratoma and seminoma		3/3	3/3
Parotis tumor		1/1	1/1
Cervix squamous carcinoma		2/2	n/a
Thymoma		2/2	n/a
Colon adenocarcinoma	14/24 weak	10/24	24/24
- lymph node metastasis		3/3	
Breast adenocarcinoma		13/13	13/13
- lymph node metastasis		2/2	
Leiomoma - lung metastasis		1/1	n/a
Ovary carcinoma		4/4	n/a
Bladder carcinoma		42/42	36/36
- lymph node metastasis	1/1 strong		
- squamous carcinoma metastasis		2/2	
Lung - small cell carcinoma		1/1	5/5
- adenocarcinoma	5/5 strong		
Stomach adenocarcinoma		3/3	3/3
Liver carcinoma		4/4	4/4
Soft tissue tumors		3/3	n/a
Melanoma		48/48	18/18
- metastasis		18/18	

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Clinical Trials

- Polish Phase I/II, an open-label study to evaluate the safety, tolerability and preliminary efficacy of ascending doses of L-DOS47, initially as a monotherapy, in patients with inoperable, locally advanced, recurrent or metastatic, non-squamous, stage IIIb/IV NSCLC.
  - Prof. Dariusz Kowalski MD, PhD at The Maria Sklodowska-Curie Memorial Cancer Center & Institute of Oncology, Prof. Cezary Szczylik, MD, PhD at the Military Medical Institute, Prof. Elzbieta Wiatr, MD, PhD at the National Tuberculosis and Lung Diseases Research Institute, Dr. Aleksandra Szczensa, MD, PhD at the Mazovian Center of Pulmonary Diseases and Tuberculosis in Otwock, Prof. Rodryg Ramlau, MD, PhD at the Med Polonia Sp. z.o.o., Poznan.

- U.S. Phase I, open label, dose-escalation study to evaluate the safety and tolerability of ascending doses of L-DOS47 in combination with pemetrexed/carboplatin in patients with Stage IV recurrent or metastatic nonsquamous NSCLC
  - Dr. Sarina Piha-Paul at the MD Anderson Cancer Center, and Dr. Chandra Belani at Penn State University and at the Milton S., Hershey Medical Center and Dr. Afshin Dowlati at University Hospitals Case Medical Center

Clinical Trial Updates

- Polish Phase I/II monotherapy
  - Doses of 0.12, 0.21, 0.33, 0.46, 0.59, 0.78, 1.04, 1.38, 1.84, 2.45 and 3.26 ug/kg of L-DOS47 have been successfully administered
  - An interim data review for the first eight dosing cohorts was conducted last September. The review included all available data, including patient demographics, safety assessments, PK data, immunogenicity and radiological tumour assessments.
    - adverse events reported are those expected for investigational product and population under study
    - no dose limiting toxicities have been reported
    - stable disease observed in radiological assessments of 12 of 24 (50%) of patients treated; and two patients completed six cycles of treatment
- US Phase I combo study
  - First patient dosed (April 2015)

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National Research Council of Canada

Helix R/D Team and advisors

Clinical CROs

Helix BioPharma Corp

THANK YOU