

# L-DOS47 a lung adenocarcinoma specific immuno-conjugate

Axel Wellmann<sup>1</sup>, Wah Wong<sup>2</sup>, Baomin Tian<sup>2</sup>, Carl De Luca<sup>2</sup>, Till Braunschweig<sup>1</sup>, Jianbing Zhang<sup>3</sup>, Roger MacKenzie<sup>3</sup>, Donald Segal<sup>2</sup>, Heman Chao<sup>2</sup>



1. University Hospital Aachen, RWTH Aachen Germany  
 2. Helix BioPharma Corp., Aurora Ontario Canada  
 3. NRC Institute for Biological Sciences, Ottawa Ontario Canada

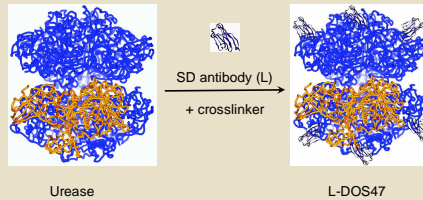
## Summary

Lung cancer with more than 1.1 million deaths annually is the most frequent and most deadly malignant tumor. Recently, adenocarcinoma has overtaken squamous carcinoma as the most common histological subtype in most countries. Differential diagnosis includes metastatic adenocarcinoma, mesothelioma, AAH and atypical reactive pneumocytes due to inflammatory processes. Prognosis depends on histological subtype but is generally poor. The availability of antibodies or agents that distinguish the various subtypes will greatly aid diagnosis and perhaps improve prognosis. L-DOS47 is an immuno-conjugate that is being developed to treat lung cancer. The molecule is composed of a urease enzyme conjugated with single-domain antibodies. Normal and cancer tissue screening studies demonstrated that L-DOS47 recognized the adenocarcinoma subtype almost exclusively. Of the over 400 tissue samples screened, which represent 42 groups consisting of various cancerous and matched normal tissues, only lung adenocarcinoma tissues showed considerable staining with over 80% of the cells being recognized. Corresponding age-matched normal lung tissues were negative with only hints of focal staining in a few activated pneumocytes. Another tissue group that showed significant positive but weak staining was colon adenocarcinoma; an observation that probably relates to the suspected expression of the putative antigen recognized by L-DOS47 in this tumor type. In summary, the study shows that L-DOS47 may be useful as a diagnostic agent in addition to its potential for therapeutic application in lung adenocarcinoma. Additional studies are being conducted to verify the ability of L-DOS47 to recognize metastatic tissues of lung origin.

## Outline

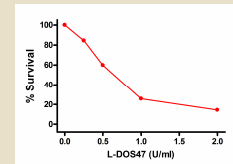
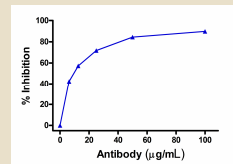
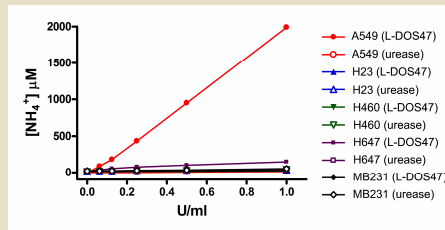
1. L-DOS47 construct
2. Effect of L-DOS47 on tumor cells
3. L-DOS47 binding to lung adenocarcinoma tissues
4. L-DOS47 binding to normal and cancer tissues
5. L-DOS47 binding to colon tissues
6. L-DOS47 binding to lymph node metastases from lung

## L-DOS47 construct



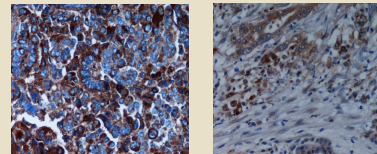
Schematic diagram showing the conjugation of single domain (SD) antibody (L) to urease. The antibody is conjugated to urease using *N*-Succinimidyl[4-iodoacetyl]aminobenzoate as crosslinker. On average, seven antibodies are conjugated per urease molecule.

## Effect of L-DOS47 on tumor cells



Five cell lines (A549, H23, H460, H647, and MDA-MB231) were seeded in 96-well culture plates to test for the binding specificity of L-DOS47 (top). L-DOS47 binds specifically and significantly only to A549 cell – a human lung adenocarcinoma line. The immuno-conjugate also generates ammonia in the presence of urea. Specificity of the binding is confirmed by competitive displacement with the antibody alone in a dose dependent manner (bottom left). L-DOS47 is able to kill A549 cells in a dose dependent manner (bottom right).

## L-DOS47 binding to lung adenocarcinoma tissues



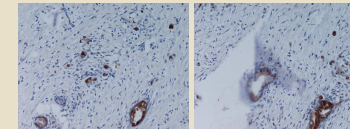
Human lung adenocarcinoma tissue biopsies were sectioned and prepared into slides. After blocking in serum at 4°C overnight, the slides were incubated with L-DOS47 (20 U/ml) at 37°C for 1.5 hours. After washing, the slides were incubated with mouse anti-urease antibody at 37°C for 1 hour, followed by diluted biotinylated secondary antibody solution for 30 min. After incubation, the slides were treated with Vectastain Elite ABC Reagent for 30 min. Color was developed in fresh DAB solution at RT for 2 minutes. Positive binding of L-DOS47 is revealed by brown staining with blue counter stain.

## L-DOS47 binding to normal and cancer tissues

Samples	Tumor Tissue		Age-matched Normal Tissue	
	Positive	Negative	Positive	Negative
Kidney carcinoma	1/112	1/112	0/112	0/112
Parathyroid adenoma	1/1	0/1	0/1	0/1
Pancreas, umbilical cord, atlantis	0/1	0/1	0/1	0/1
Myxofibrosarcoma	1/1	0/1	0/1	0/1
Prostate carcinoma	4/4	0/4	0/4	0/4
Thyroid carcinoma	2/2	0/2	0/2	0/2
Pancreas adenocarcinoma	7/67 weak	4/257	26/26	0/26
Neuroendocrine tumors	0/9	0/9	0/9	0/9
Brain, heart muscle, testis, spleen	0/0	0/0	0/0	0/0
Testis - seminoma and seminoma	3/3	0/3	0/3	0/3
Parasitic tumor	1/1	0/1	0/1	0/1
Cervix squamous carcinoma	2/2	0/2	0/2	0/2
Thymoma	2/2	0/2	0/2	0/2
Colon adenocarcinoma	14/24 weak	10/24	24/24	0/24
- lymph node metastasis	3/3	0/3	0/3	0/3
Breast adenocarcinoma	13/13	0/13	0/13	0/13
- lymph node metastasis	2/2	0/2	13/13	0/13
- lymph node metastasis	1/1	0/1	0/1	0/1
Ovary carcinoma	4/4	0/4	0/4	0/4
Bladder carcinoma	4/42	0/42	36/36	0/36
- lymph node metastasis	1/1 strong	2/2	0/2	0/2
- squamous carcinoma metastasis	1/1	0/1	0/1	0/1
Lung, small cell carcinoma	1/1	0/1	0/1	0/1
- adenocarcinoma	6/5 strong	0/5	0/5	0/5
- squamous carcinoma	4/4	0/4	0/4	0/4
Liver carcinoma	4/4	0/4	0/4	0/4
Soft tissue tumors	4/3	0/3	0/3	0/3
- leiomyosarcoma	4/4	0/4	0/4	0/4
- metastasis	18/18	0/18	0/18	0/18

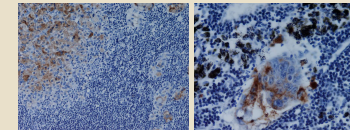
Immunohistochemical staining for L-DOS47 was performed on human tissue biopsies. A total of 449 tissue samples were screened. Immunopositivity is revealed by brown staining. All 5 lung adenocarcinoma and 1 bladder carcinoma metastasized to lymph node show strong positive staining pattern (over 80% of cells are positively stained). Weak positive staining (less than 5%, primarily at invasion front) is also observed in colon adenocarcinoma (14 out of 24). However, no age-matched normal tissue shows any sign of positive staining. The data are presented as number of occurrence per total number of samples in each group, where n/a denotes sample not available.

## L-DOS47 binding to colon tissues



Human colon adenocarcinoma tissue biopsies were sectioned and prepared into slides. After blocking in serum at 4°C overnight, the slides were incubated with L-DOS47 (20 U/ml) at 37°C for 1.5 hours. After washing, the slides were incubated with mouse anti-urease antibody at 37°C for 1 hour, followed by diluted biotinylated secondary antibody solution for 30 min. After incubation, the slides were treated with Vectastain Elite ABC Reagent for 30 min. Color was developed in fresh DAB solution at RT for 2 minutes. Positive binding of L-DOS47 is revealed by brown staining with blue counter stain.

## L-DOS47 binding to lymph node metastases



Immunopositive staining for L-DOS47 in human lung adenocarcinoma metastasized to lymph node. Positive binding of L-DOS47 is revealed by brown staining with blue counter stain. The black pigment is typical for pulmonary or mediastinal lymph node.

Helix BioPharma Corp.

3-305 Industrial Parkway South Aurora Ontario Canada L4G 6X7 <http://www.helixbiopharma.com>