

L-DOS47 – a novel lung adenocarcinoma specific therapeutic candidate with tumour alkalization effect

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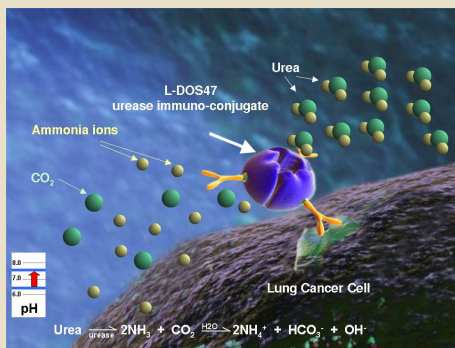
Summary

It has been observed that many solid human tumours generate an acidic and hypoxic microenvironment as a result of altered metabolic pathways and aberrant tumour vasculature. This phenomenon can impact the efficacy of certain chemotherapeutics by altering their partitioning coefficient between extracellular and intracellular compartments. This metabolic condition can also induce metastasis and confer a growth advantage to certain cancers. An immunocjugate cancer therapeutic has been developed to target this unique tumour microenvironment feature. The molecule, L-DOS47, is composed of a lung adenocarcinoma specific single domain antibody and a urease enzyme. The antibody serves as a targeting agent to deliver the enzyme to the affected site while the urease enzyme converts urea, an abundant metabolite, into ammonia and generate a local pH increase. The combined effect of ammonia toxicity and pH increase is cytotoxic to cancer cells shown both in culture and in xenograft models. In vitro experiments showed the L-DOS47 also potentiated the cytotoxicity of a number of chemotherapeutics dramatically. L-DOS47 binds specifically to human lung adenocarcinoma biopsy samples as shown by tissue specific staining. Imaging studies using A549 xenografts and intravenously-given labelled L-DOS47 showed the drug molecule preferentially accumulated and persisted at the tumour site well over 72 hours, consistent with a strong binding antibody. L-DOS47 has also been tested in preliminary rodent and primate toxicological trials as part of the preclinical preparation for human studies. In summary, L-DOS47 is a novel immunocjugate that preferentially targets lung tumours and generates cytotoxicity directly through its enzymatic function. Furthermore, its ability to potentiate certain chemotherapeutics suggested that L-DOS47 may be useful in both mono and combination therapies.

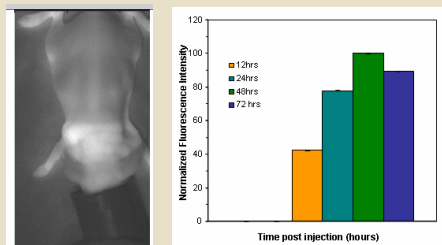
Outline

1. Proposed mechanism of action
2. L-DOS47 effect on tumour cells
3. L-DOS47 binding on human normal and cancer tissue
4. L-DOS47 enhances the effect of weakly basic drugs
5. L-DOS47 fluorescent imaging study
6. L-DOS47 xenograft study

Mechanism of Action

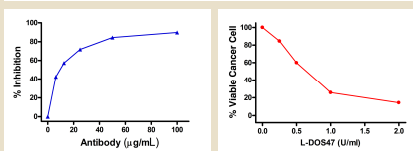
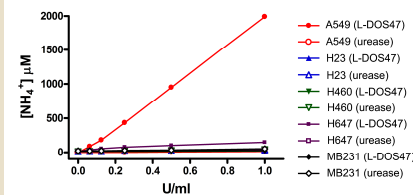


L-DOS47 fluorescent imaging study



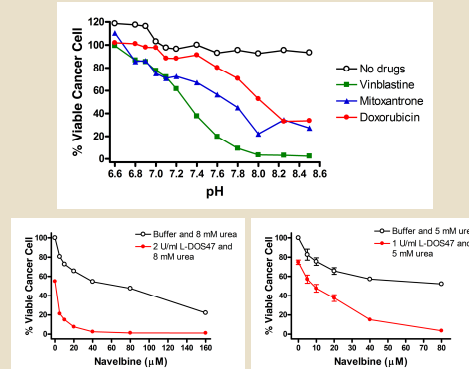
Inactivated L-DOS47 labelled with Cy5.5 was injected in nude mice bearing A549 xenograft. The tumour and major organs were harvested for imaging. Relative signal intensities were used to measure bio-distribution, half life and accumulation. A representative picture of a tumour bearing mouse 72h post injection of the labelled material is presented. Summary result showing signal accumulation in tumour (ex vivo) as a function of time is shown. Detectable L-DOS47-Cy5.5 began at 12 hours and persisted beyond 72 hours.

L-DOS47 effect on tumour 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 cells – a human lung adenocarcinoma line. The immunocjugate also generates ammonia in the presence of urea showing L-DOS47 is active on the tumor cell. 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 effect on tumour cells



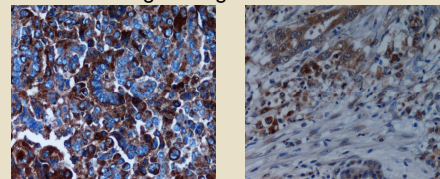
The cytotoxic effect of weakly basic drugs is directly related to the solution pH (top panel). At an acidic tumour pH (<6.8), the effectiveness of these drugs is significantly reduced. L-DOS47 can dramatically raise the effectiveness of these drugs (e.g. navelbine, bottom panels). This synergistic effect is directly related to its enzymatic properties of generating ammonia from urea and raising solution pH. Depending on the dosages and available urea, a 2–10 fold drug effect enhancement can be observed.

L-DOS47 binding to normal and cancer tissues

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

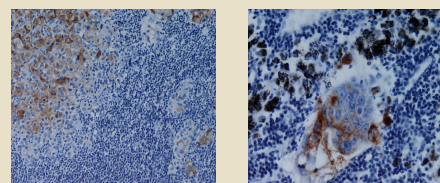
Immunohistochemical staining for L-DOS47 was performed on human tissue biopsies. A total of 449 tissue samples were screened. Immunopositivity is revealed by brown staining. All 5 lung adenocarcinomas and 1 bladder carcinoma metastasized to lymph nodes showed strong positive staining pattern (over 80% 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 lung adenocarcinoma tissues



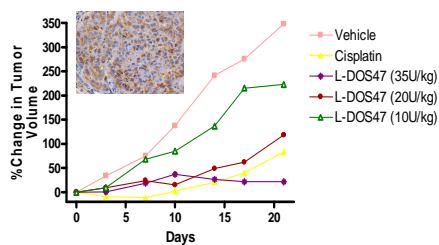
Human lung adenocarcinoma tissue biopsies were sectioned and prepared into slides. Positive binding of L-DOS47 is revealed by brown staining with blue counter stain.

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.

L-DOS47 xenograft study



Previous work has shown that antigen density on commonly used lung cells such as A549 is poor. A corresponding tumour slice stained with the same antibody (inset) revealed about 20-40% of the cells stained positively (brown stain). Despite this lack of antigen on the A549 cells, L-DOS47 is able to reduce tumour growth in a dose dependent manner. The effective doses are well within the tolerable doses observed in preliminary rodent and primate studies.

Conclusion

L-DOS47 is an immuno-conjugate designed to attack solid tumours by either the direct cytotoxic effect of ammonia or in combination with other weakly basic chemotherapeutic agents whose efficacies are hindered by a higher intracellular/lower extracellular pH gradient in solid tumours.

- L-DOS47 is shown to have the following activities:
- Specific binding to human adenocarcinoma tissues
 - Able to detect lung metastasis to lymph node
 - Cytotoxic to cell lines that are recognized by the antibodies
 - Significantly enhance the effect of drugs such as navelbine
 - Fluorescently labelled L-DOS47 accumulated specifically in A549 xenograft tumours persistently over 72h
 - L-DOS47 reduces tumor growth in A549 xenograft study

Future direction

Ongoing animal experiments to measure tumour pH changes *in vivo*, drug dose response and weakly basic drug potentiation effects. Continuing preclinical studies to evaluate the toxicological and pharmacological profiles of L-DOS47 in preparation for clinical studies.