Treating Lung Tumour Through Alkalization

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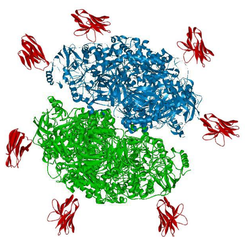
Introduction

Many solid human tumours generate an acidic and hypoxic microenvironment due to altered metabolic pathways and aberrant tumor vasculature. These acidic conditions reportedly activate proteases which digest the extracellular matrix and allow phagocytosis of non-tumor cells, leading to invasiveness and metastatic behaviour. It has been suggested that tumor acidosis affects chemotherapy by promoting resistance to (i) weakly basic chemotherapeutic agents by altering their intracellular/extracellular partition coefficient and (ii) radiation therapy by suppressing early steps of apoptosis and reducing the degree of radiation induced fixation. L-DOS47, an immunoconjugate cancer therapeutic, targets this unique tumor microenvironment by localizing urease to lung tumor using a specific antibody. Urease converts the abundant metabolite urea to ammonia, increasing the local pH and exerting a cytotoxic effect on cancer cells in culture and xenograft models.

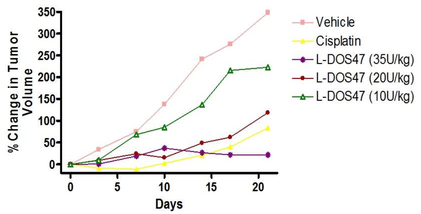
L-DOS47

L-DOS47 is a novel immunoconjugate therapeutic designed for the treatment of lung adenocarcinoma. It comprises of an urease enzyme with multiple copies of a single domain antibody specific for CEACAM6



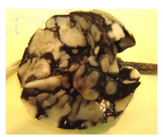
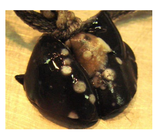
L-DOS47 Xenograft Studies

L-DOS47 suppresses A546 tumour in a dose dependent manner. Effective dose was observed at 35U/kg.



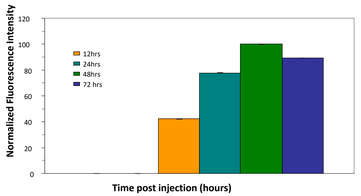
In a separate experiment, L-DOS47 was premixed with A549 and the complex was injected through the tail vein of the mice. Groups of mice were sacrificed at the third and tenth week. L-DOS47significantly reduced the ability of A549 cells to colonize the lungs at week 3. Inhibition is over come by the tenth week if no further treatment is given.

Group	Cell Treatment	Dose U/ml	Mean number 3 weeks	Mean number 10 weeks
1	Untreated	-	103.8 ± 30.0	110.6 ± 50.0
2	Isotype	17.5	44.6 ± 5.1	60.4 ± 14.3
3	L-DOS47	12.5	104.6 ± 35.5	140 ± 52.5
4	L-DOS47	17.5	28.0 ± 7.2	50.0 ± 17.7
5	L-DOS47	25.0	18.2 ± 7.8*	112.2 ± 52.5

Control   Treated

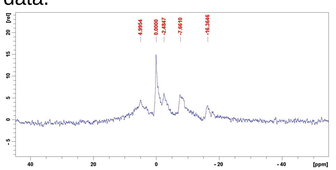
L-DOS47 Imaging

L-DOS47 labelled with Cy5.5 was injected into A549 tumour bearing mice. Fluorescence was induced at 647 nm and the signal at 710nm was imaged. Data were collected at 10 minutes, 2 hrs, 6 hrs, 12 hrs, 24 hrs, 48 hrs, and 72 hrs. Fluorescence peaked at 48 hours post injection.

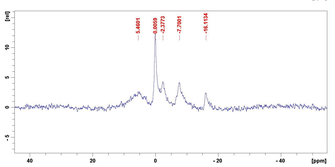


31P-MRS and pH Electrode

Tumour pH of A549 xenograft is measured extracellularly with a micro-pH electrode or intracellularly (chemical shift between Pi and PCr peaks) with a 11.7T magnet using a custom-built coil capable of acquiring 1H-MRI and 31P-MRS data.

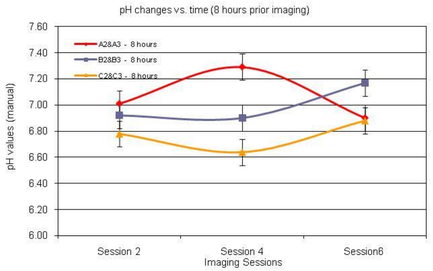


Example of a 31P-spectrum from a A549 tumor bearing control mouse showing a Pi peak near 5.000.



Example of a 31P-spectrum from a mouse treated with L-DOS47. Pi peak broadening as a result of treatment.

pH Calculation



An example of pH data is presented. Extracellular pH data is obtained using a micro-pH electrode. Orange line represents the control group. pH value is taken 8 hours after drug injection through the tail vein. Full set of data is still being analyzed, however, preliminary data suggest the method is suitable to monitor L-DOS47 action.

Summary

1. L-DOS47 is effective in reducing tumour growth in mice
2. L-DOS47 treated tumour showed Pi peak broadening which may indicate cell wall has collapsed
3. 31P-MRS and micro-pH electrode can be used to monitor the action of L-DOS47. Full data set is still being analyzed. Further experiments will be conducted to refine data collection and analysis
4. L-DOS47 has been approved for a phase I clinical study by the FDA recently