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Altering the Tumor Microenvironment

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DOS47 – Killing Cancer by Altering the Tumor Microenvironment

By: Heman Chao, PhD, Chief Scientific Officer, Helix BioPharma Corp.

Introduction

Throughout the past 15 years, the effort in anti-cancer drug research has been directed toward targeted pathway therapeutics. These compounds target one and possibly more intracellular biochemical pathways that have been implicated in tumorigenesis. The rationale for their development is that more targeted therapeutics would provide efficacy while minimizing adverse events. Several compounds have completed clinical testing and have been launched as anti-cancer treatments, including a VEGF inhibitor, bevacizumab (Avastin®), an EGFR antagonist, cetuximab (Erbitux®) and a bcr-abl inhibitor, imatinib (Gleevec®).[1-3] However, although most of these compounds generate measurable benefit, their efficacy is generally modest or, in the case of imatinib, require chronic therapy. New treatment paradigms are needed, especially for solid tumors, such as non-small cell lung cancer (NSCLC).

Most solid tumors arise in a microenvironment that has been altered to enable tumor cells to thrive and proliferate. The changes to the microenvironment are varied and complex and include suppressed host immunity, deregulated inflammation and increased production of cellular growth and survival factors that induce angiogenesis and inhibit apoptosis, as well as a lowering of pH.[4,5] Such changes in the tumor cell microenvironment could be a target for therapeutic intervention.

$$\text{Urea}[(NH_2)_2CO] + 3H_2O \xrightarrow[\text{Urease}]{} 2NH_4^+ + 2OH^- + CO_2$$

Figure 1. Chemical Pathway of Urease Activity

The following will describe an approach to chemically alter a part of the tumor microenvironment with the goal of developing a cancer therapeutic that can be either directly cytotoxic to the tumor cells and/or act synergistically with other

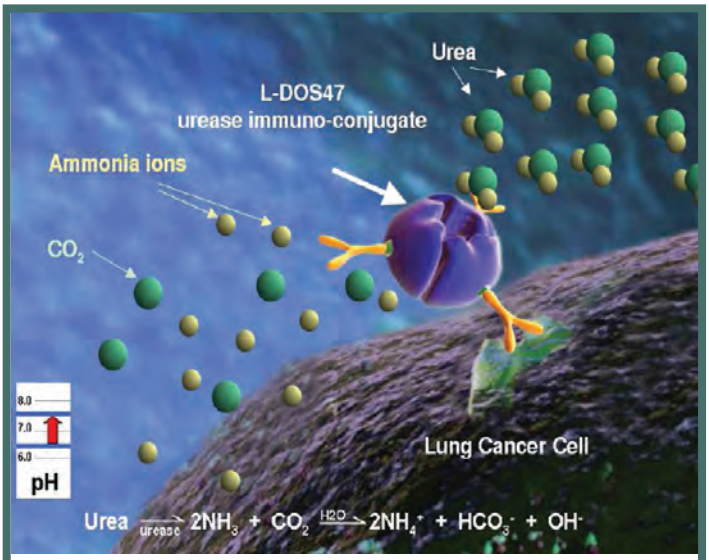


Figure 2. L-DOS47 Mechanism of Action

chemotherapeutic agents to enhance efficacy and/or tolerability.

Altering the Microenvironmental pH as an Approach to Cancer Therapy

Tumor cells create an external microenvironment that is more acidic and hypoxic than the microenvironment surrounding normal tissues.[6] This is a result of altered metabolic pathways and abnormal tumor vasculature. Tumor cells appear to thrive in this environment. In addition, the efficacy of weakly basic chemotherapy drugs is adversely affected by the acidic conditions surrounding tumor cells.[7] It is possible that changes to the microenvironment of tumor cells may actually affect their sustainability and could potentially offer a target for cancer drug development.

DOS47 - Enabling Targeted Alteration of Microenvironmental pH

DOS47 is an oncology platform technology developed by Helix BioPharma, Aurora, Ontario, that offers a new approach to the debilitation and destruction of cancer cells. DOS47 is designed to offer a means of deriving a therapeutic pharmacological effect by acting upon a naturally occurring substance in the body. The technology is based on the principle of using an enzyme to catalyze the metabolism (catabolism) of an endogenous substrate molecule in order to yield metabolites of therapeutic benefit.

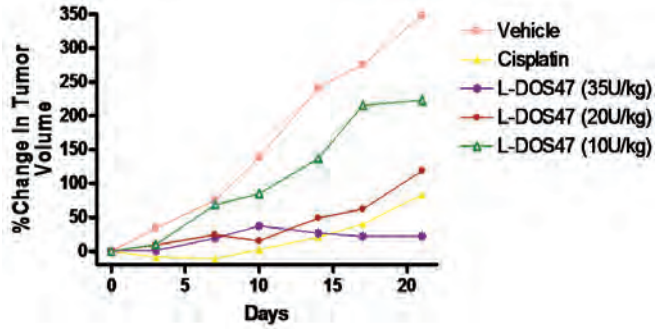


Figure 3. **L-DOS47 In Vivo A549 Xenograft Efficacy**
L-DOS47 doses were injected every other day X 5. 35 U/kg p = 0.0158 significant, 20 U/kg p = 0.0417 significant, 10 U/kg p = 0.3943 not significant.

Cancer cells generally exist and proliferate under conditions of abnormally low interstitial pH (ie, high acidity). DOS47 is designed to induce an increase in pH locally at the site of cancerous cells in the body to create an environment inhospitable to their continued growth and survival.

DOS47 is an enzyme called urease derived from the jack bean that catabolizes the naturally occurring substrate, urea. By inducing the catabolism of urea in the interstitial medium surrounding cancer cells, urease action may promote the production of metabolites, including ammonia and hydroxide ions. These metabolic products of urease activity are believed to stress cancer cells by a combination of effects, including direct toxicity and the induction of an alkaline microenvironment (Figure 1).

Ammonia itself is cytotoxic, a behavior thought to stem from its interference with cell processes, many of which are more pronounced in rapidly growing cells. While ammonia is toxic to cancer cells, the catabolism of urea increases the local pH of the surrounding medium. The increased alkalinity may also counteract the adverse effect of the acidic microenvironment on weakly basic drugs and enhance the uptake of these chemotherapeutics.

Following in vitro studies to understand urease's cytotoxic mechanism of action, in vivo studies were carried out in mouse models of lung and breast cancer to assess the anti-tumor activity of urease. It was demonstrated that urease injections significantly inhibited tumor growth in both models. When combined with weak-base anti-cancer drugs, the increased pH resulting from the catabolism of urea enhanced the activity of the chemotherapeutic agents.[8]

Because DOS47 activity is potentially toxic to any cell in the body, the molecule must have a targeting mechanism that very specifically targets the tumor cell type of interest.

Otherwise, unwanted side effects would be generated due to off-target activity.

With the aim of recreating the anti-tumor response in vivo to urease injection, DOS47 was designed to be combined with highly specific antibodies that could effectively deliver the drug to solid tumor cells. Antibodies whose antigens are highly expressed on a particular tumor cell type can be joined to DOS47 by a chemical linker and subsequently serve as a highly-specific targeting agent, whether or not the antibody has any intrinsic therapeutic activity. The local delivery of DOS47 to tumor sites promises to limit the cytotoxicity to the tumor cells themselves. Additionally, DOS47 can be administered simultaneously with other chemotherapeutics with the possibility of enhancing weak-base anti-cancer drugs locally at the tumor site.

L-DOS47 - Altering the Microenvironment of Non-Small Cell Lung Cancer (NSCLC) Solid Tumors

L-DOS47 is a novel immuno-conjugate therapeutic designed for the treatment of lung adenocarcinoma, a sub-type of NSCLC. L-DOS47 is a combination of the DOS47 urease drug compound and a NSCLC-specific single domain antibody, AFAI.

ES1 is a petameric and highly avid form of the AFAI antibody that is highly specific to a variant form of the well-known antigen, carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6 or CEA6) on non-small cell lung adenocarcinomas.[9] ES1, when compared with other forms of the AFAI antibody, was shown to be more lung carcinoma sensitive, particularly those poorly differentiated adenocarcinomas that are usually associated with distant metastasis, and less immunoreactive with normal tissues.[10]

The treatment of NSCLC is a significant unmet medical need, with lung cancer accounting for the most cancer-related deaths in most men and women in the United States. The 5-year survival rate for all stages of lung cancer combined is only about 16%.[11] NSCLC accounts for 85% of lung cancer diagnoses, of which 35% to 40% are hard to treat adenocarcinomas.[12,13]

For most patients with NSCLC, current treatments do not cure the cancer. Despite treatment with new agents like Avastin and Erbitux, patients generally receive the limited benefit of one or two months of additional median survival. The targeted alteration of the NSCLC adenocarcinoma microenvironment to kill cancer cells shows promise as a new paradigm for treating solid tumors and satisfying the unmet medical need.

With its precise targeting, L-DOS47 operates with an unprecedented mode of action, taking advantage of the localized delivery of urease to cause alkalinization of the tumor cell microenvironment,

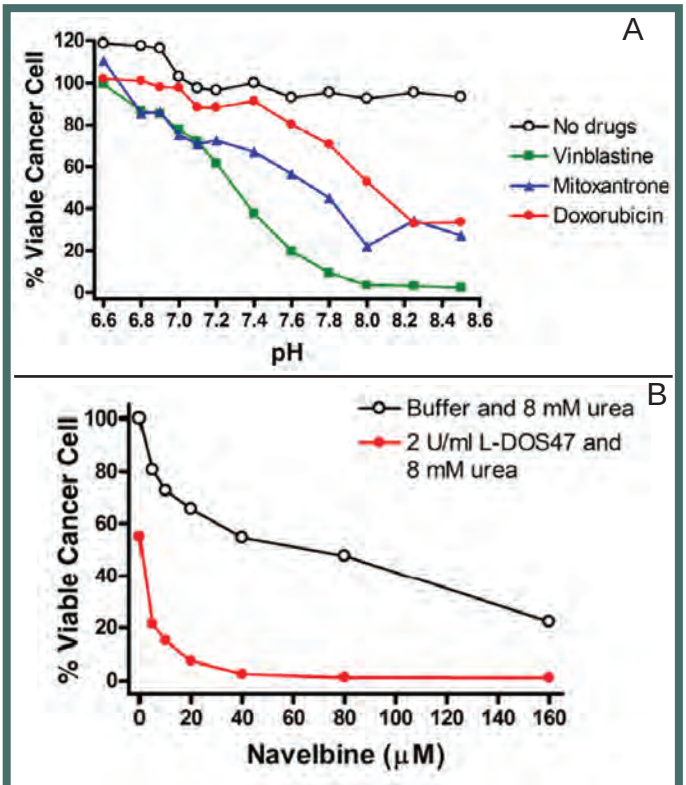


Figure 4 A&B. **The Effect of L-DOS47 on Tumor Cells in Combination With a Chemotherapeutic Agent**
The cytotoxic effect of weakly basic drugs is directly related to the solution pH (Figure 4a). At an acidic tumor pH (< 6.8), the effectiveness of weakly basic drugs is significantly reduced. L-DOS47 can dramatically raise the effectiveness of these drugs (eg, navelbine, Figure 4b). This synergistic effect is directly related to its enzymatic properties of generating ammonia from urea and raising solution pH.

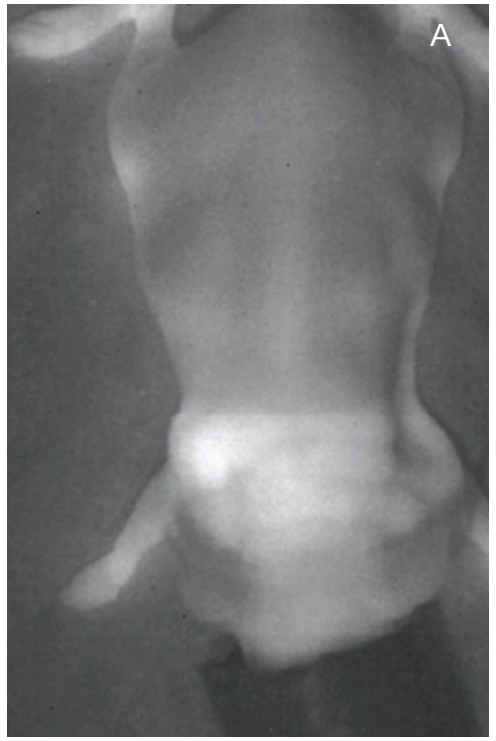
deliver ammonia toxicity to NSCLC tumor cells and, in combination with other anticancer drugs, potentially enhance the effect of chemotherapeutic agents (Figure 2). L-DOS47 has the potential to both stop and reverse the progression of NSCLC adenocarcinomas in those patients with few options.

In preclinical trials, L-DOS47 was tested in vivo in A549 xenograft models of NSCLC (Figure 3). In these studies, the combined effect of ammonia toxicity and an increase in pH was demonstrated to be cytotoxic to cancer cells alone and in combination with chemotherapeutic agents (Figures 4a & 4b). Imaging studies using A549 xenografts and intravenously given labeled L-DOS47 demonstrated the high affinity and high specificity of the AFAI antibody, with histological evidence showing that the drug molecule preferentially accumulated and persisted at the tumor site for well over 72 hours (Figures 5a & 5b). With its ability to preferentially target NSCLC adenocarcinoma cells and to drive local anticancer cytotoxicity, L-DOS47 holds promise as a new treatment as it moves into the clinic.[14]

L-DOS47 is in late preclinical development, and the company's most recent disclosures indicate a US submission of an Investigational New Drug Application (IND) and a European submission of a Clinical Trial Application (CTA) are upcoming. The US Phase I clinical trial is intended to assess the safety of L-DOS47 in patients who have received multiple chemotherapeutic regimens to treat solid tumors. The European Phase I/II clinical trial, to be performed in Poland, is intended to assess the safety and efficacy of L-DOS47 alone and in combination with other drugs in patients with advanced lung adenocarcinoma.

Discussion

Cancer is a highly complex pathology and, unlike other diseases, it is not wholly gene specific. Rather, to manage the



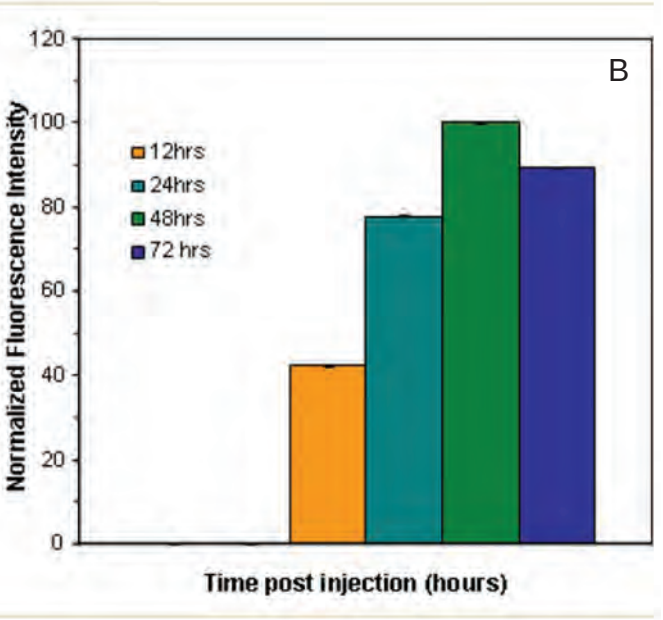


Figure 5 A&B. **L-DOS47 Fluorescent Imaging Study**
Inactivated L-DOS47 labelled with Cy5.5 was injected in nude mice bearing A549 xenograft. The tumor and major organs were harvested for imaging. Relative signal intensities were used to measure biodistribution, half life, and accumulation. A representative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

variety of molecular and cellular processes that contribute to the development of cancer, you need to go after the disease from different fronts. DOS47 is an example of a drug with several approaches in a single molecule. When attached to highly specific antibodies, DOS47 is intended to essentially become a highly targeted sledgehammer that makes the microenvironment of the tumor inhospitable to survival and growth. The urease in DOS47 effectively catabolizes endogenous urea to produce two anticancer agents: a cytotoxic substrate, ammonia, and a change in pH that is hazardous to cancer cells while offering favorable conditions to some chemotherapy drugs.

L-DOS47 is a compelling validation of the novel approach of the DOS47 platform. L-DOS47 is a promising therapeutic for the treatment of NSCLC, a market where there are limited options and where there is a significant need for new agents. Beyond L-DOS47, the DOS47 platform technology is potentially broadly applicable across a variety of solid tumors and, with the right specific antibody, can extend its therapeutic value to other indications. Looking forward, there is now the possibility to build upon the preclinical success of L-DOS47 with other DOS47-based cancer drugs. ◆

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Dr. Heman Chao is currently the Chief Scientific Officer of Helix BioPharma Corp. of Aurora, Ontario. He is a co-inventor of the DOS47 technology. Prior to his current position, Dr. Chao was President of Sensium Technologies, a Helix BioPharma Corp. subsidiary from 2004 until it was incorporated into Helix in April 2008. Before joining Helix, he was a Research Fellow in the Protein Engineering Network of Centres of Excellence in Canada.