

Lixte Biotechnology Holdings Inc.

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New anticancer compounds could boost effectiveness of standard therapies

Lixte is developing new drug candidates that could safely bolster the effectiveness of chemotherapy and radiation and treat several diseases.

Over the past few decades, cancer survival rates have gradually increased, in part owing to advances in treatment strategies. Despite these improvements, more than 7.6 million people die of cancer worldwide each year, with 2.8 million of these in economically developed countries. Cancer is the second most common cause of death in the US, accounting for nearly one-quarter of deaths. These statistics underscore the need for more effective treatment options.

"I've been more than a little discouraged that better drugs haven't come along over the years," said John Kovach, founder, president and CEO of Lixte Biotechnology Holdings. "I have been involved in academic cancer research and the administration of cancer centers for more than 40 years and have a long-standing interest in developing more effective anticancer drugs for clinical evaluation."

Kovach founded Lixte in 2005 to do just that. The clinical stage therapeutics company went public to secure financing in 2006. It is developing new small molecules that could work synergistically with currently available anticancer drugs and radiation therapies to inhibit tumor growth and enhance survival rates for many types of cancer, potentially without increasing toxicity.

The inspiration for Lixte came from Kovach's interest in observations made during studies with collaborators at the US National Institutes of Health. The team discovered a previously unknown signaling abnormality that seemed important in brain tumor development. "I decided to start Lixte to pursue possible new avenues for affecting cell differentiation and develop targets that might lend themselves to improving cancer treatments," said Kovach, who has experience directing US National Cancer Institute-designated Comprehensive Cancer Centers at the Mayo Clinic and the City of Hope National Medical Center.

Lixte's product pipeline comprises two major categories of compounds at various stages of preclinical and clinical development (Table 1). These two types of compounds, known as phosphatase inhibitors and deacetylase inhibitors, target molecular signaling pathways that are altered in a variety of diseases. Thus, they have broad therapeutic potential beyond the treatment of cancer.

"Companies have steered away from pursuing molecules such as the targets we've chosen, largely because they carry out so many different activities in the body. The feeling was that they probably would have a toxicity, which would not be

tolerable," Kovach said. "We believe that that's not the case, and we're in the process of trying to prove it."

First-in-class compound

At the furthest stage of development by Lixte is a compound called LB-100, which has been approved by the US Food and Drug Administration for a phase 1 study in patients with advanced cancers. LB-100 inhibits an enzyme called protein phosphatase 2A (PP2A), which removes phosphate groups from proteins involved in the control of cell growth and cell death. Currently, no PP2A inhibitors are approved for clinical use, although they might improve treatments for a broad range of rare and common cancers.

Phosphatase inhibitors such as LB-100 interfere with the ability of cancer cells to withstand DNA damage caused by chemotherapy and radiation therapy. Whereas normal cells have a range of intact repair mechanisms to resist DNA damage from anticancer drugs and radiation therapy, cancer cells often have mutations that interfere with their ability to recover from DNA damage and survive under stressful conditions. LB-100 seems to enhance the antitumor activity of radiation therapy and chemotherapy in animal models without increasing toxicity.

"Generally, molecules that are used to potentiate radiation therapy are standard anticancer drugs that, similar to radiation, cause DNA damage. For many years, pharmaceutical companies and academic researchers have been looking for compounds that could further increase the effectiveness of radiation without increasing toxicity," Kovach said. "We believe that our phosphatase inhibitors are one of the few true radiation sensitizers. If so, these compounds would be of great clinical benefit. By increasing the effectiveness of radiation, they could potentially offer the possibility of decreasing the amount of radiation needed to treat tumors."

Lixte's animal studies have demonstrated that LB-100 can be given intermittently or daily for five days at effective anticancer doses without increases in toxicity. Just a 20% inhibition of PP2A in tumors can potentiate the effects of radiation therapy and anticancer drugs, suggesting that the amount of LB-100 needed therapeutically is probably clinically tolerable. Moreover, LB-100 does not increase toxicity in normal cells or in animals when combined with radiation therapy.

Although LB-100 has modest anticancer activity when used alone, in vivo studies show that it significantly enhances the effectiveness

of not only radiation therapy but also standard anticancer drugs, including temozolomide, cisplatin, doxorubicin and taxotere. LB-100 is now undergoing a phase 1 trial in patients with advanced solid tumors to determine the maximum tolerable dose and assess its efficacy when given intravenously daily for three days alone and in combination with a full dose of taxotere.

By early 2014, results will be available for the clinical trial of LB-100 given alone at doses expected to potentiate the effects of radiation therapy and anticancer drugs. By the end of 2014, the maximum tolerable dose of LB-100 combined with taxotere should be determined, as well as its antitumor response data. Subsequently, depending on financial resources, Lixte plans to conduct single-arm or potentially randomized phase 2 trials of taxotere with and without LB-100. As taxotere alone is approved to treat patients with hormone-resistant prostate cancer, as well as those with recurrent gastric, breast, prostate, stomach and non-small cell lung cancers, there are many opportunities to test the ability of LB-100 to improve the results of therapy in common diseases.

Lixte would also like to conduct phase 1 and 2 studies of LB-100 in combination with chemoradiation therapy to treat localized tumors that cannot be surgically removed, such as stage III lung, stomach, esophageal and pancreatic cancers in the US and potentially in Asia, where gastric and esophageal cancers are very common.

Potential value for acute vascular disease

Because PP2A is involved in diverse cellular processes, Lixte's phosphatase inhibitors could have therapeutic potential for diseases beyond cancer. For example, when cells are deprived of oxygen, the concentration of PP2A increases in blood vessels and other tissues, enhancing tissue damage. LB-100 is at the proof-of-concept stage of preclinical development for treating ischemia—oxygen deprivation caused by reduction in blood supply to tissues—associated with heart attack, stroke and other conditions.

Heart attacks are the leading cause of death worldwide. In most cases, they occur when a clot in the coronary artery blocks the supply of blood and oxygen to the heart. Currently available interventions that reduce the extent of tissue death caused by a lack of oxygen have improved survival rates, but their effectiveness is limited because of treatment delays associated with travel time to the hospital. Of the people who


Table 1. The Lixte drug pipeline

DRUG	MECHANISM OF ACTION	INDICATIONS	PRE-CLINICAL DEVELOPMENT			CLINICAL DEVELOPMENT		
			DISCOVERY	PROOF OF CONCEPT	PRE-CLINICAL	PHASE 1	PHASE 2	PHASE 3
LB-100 <i>intravenous</i>	PHOSPHATASE INHIBITION	SOLID TUMORS <i>in combination with cytotoxic drugs/x-ray</i>	█	█	█	█		
		ACUTE ISCHEMIA <i>heart, brain, septic shock</i>	█	█				
LB-201 / LB-205	DEACETYLASE INHIBITION	MISFOLDED PROTEINS <i>Gaucher, neurofibromatosis 2, von Hippel-Lindau, others</i>	█	█				
		NEUROPROTECTION <i>traumatic brain injury</i>	█	█				
LB-201 <i>topical</i>		FUNGAL DERMATITIS	█	█				
LB-300s	Undisclosed	CANCER	█	█				
LB-400s	Undisclosed	CANCER	█					

die from heart attacks, about half die within an hour of the first symptoms and before they reach a hospital.

Other experimental PP2A inhibitors that are not available for clinical use reduce the extent of heart tissue damage in animal models when given promptly after the onset of heart attack. Moreover, Lixte's PP2A inhibitors improve survival of experimental tissue grafts in animal models.

On the basis of this evidence, Lixte is seeking a partner to investigate the ability of LB-100 to minimize tissue damage in animal models of heart attack, vascular stroke and septic shock—a condition that occurs when an infection leads to life-threateningly low blood pressure. PP2A inhibitors might also help reduce tissue damage caused by accidents or surgical procedures that interfere with blood supply.

Deacetylase inhibitors have diverse applications

In addition to phosphatase inhibitors, Lixte is developing deacetylase inhibitors to treat cancer and other diseases. These compounds prevent the removal of acetyl groups from many proteins, including histones, which regulate gene expression. They also interfere by a different mechanism with the degradation of mutant but functional proteins. Because abnormal gene regulation is a hallmark of cancer, deacetylase inhibitors could be effective against many types of cancer. Several of these compounds have already been approved to treat lymphoma of the skin, but Lixte's new molecules seem more stable *in vivo* than the clinically available compounds and could be more suitable for treating a range of diseases.

For example, deacetylase inhibitors may be useful for treating certain inherited metabolic diseases, including Gaucher's disease. This rare condition is caused by deficiency in an enzyme

called glucocerebrosidase, which triggers a buildup of harmful, fatty substances in various organs and tissues. This buildup leads to an enlarged liver and spleen, bone and blood cell abnormalities, lung disease and, in some cases, central nervous system impairment.

By preventing the breakdown of misfolded but functional glucocerebrosidase, Lixte's compounds in the LB-200 series increase the concentration of this enzyme in cells with common Gaucher's disease-associated mutations to levels that may be therapeutically beneficial. These compounds also stabilize mutant proteins that cause enzyme deficiencies in several other severe inherited metabolic diseases, including type 2 neurofibromatosis, von Hippel-Lindau disease, and pheochromocytomas and paragangliomas.

The LB-200 compounds, which are at the proof-of-concept stage of preclinical development, could be a cost-effective enhancement to standard enzyme replacement therapy. Currently there is no effective treatment for brain damage that may occur in individuals with neurological forms of the disease. "A significant number of patients with Gaucher's disease have neurological problems, so a small molecule that can enter the brain, such as LB-201, might be an important treatment on its own in addition to increasing the clinical benefit when combined with enzyme replacement therapy, which helps with the peripheral disease but not the brain disease," Kovach said.

In addition to their potential therapeutic benefit for cancer and metabolic disorders, Lixte's deacetylase inhibitors show promise in treating traumatic brain injury, which affects more than 1 million people each year and causes nearly one-third of all deaths due to trauma in the US. By reducing toxicity caused by various biochemical stresses, LB-201 protects against neuron

injury in cell culture models. In addition, LB-205 reduces the extent of brain injury and markedly decreases anatomic and functional neurologic impairment when given after experimental traumatic brain injury in rodent models. These compounds are at the proof-of-concept stage of preclinical development for the treatment of acute brain trauma.

The molecular signaling pathways involved in adding and removing acetyl groups from proteins also have a key role in regulating growth across a range of organisms, including fungi. Several compounds in Lixte's LB-200 series have been examined for activity in model systems against various fungal pathogens. A topical version of LB-201 cures two of the most common fungal skin infections of humans and animals in a guinea pig model without causing toxicity.

Lixte's product pipeline also includes new agents in the LB-300 and LB-400 series. These compounds, which are at the discovery and proof-of-concept stages, inhibit signaling pathways involved in cell division and other important cellular processes necessary for cancer cell survival. They have shown efficacy against different types of human cancers in cell culture models, and they are currently being evaluated in animal models. "We are seeking to exploit important signal transduction pathways that have been in the shadows as drug targets primarily because of the fear that interfering with the activity of a multi-functional enzyme could not yield therapeutically valuable results without undue toxicity," Kovach said. "This does not appear to be the case, at least in animal models, for our lead compounds. We hope to confirm this in humans."

Lixte has been awarded solely owned composition-of-matter patents in the US, and worldwide patents are pending for its phosphatase and deacetylase inhibitors. The company is collaborating with academic, governmental and contract research organizations to advance its technology. Lixte is now seeking additional strategic partnerships to accelerate clinical development of its phosphatase inhibitors and deacetylase inhibitors. "We think that both classes of compounds offer the possibility of treating several types of cancer and a number of serious non-neoplastic diseases," Kovach said. "I see our portfolio of novel compounds as an attractive opportunity for the development of new drugs for several diseases where more effective treatments are urgently needed."

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