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TITLE: Herceptin-resistance and overexpression of anti-apoptotic molecule Bcl-XL: a potential strategy for overcoming resistance to Herceptin

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Herceptin-resistance and overexpression of anti-apoptotic molecule Bcl-XL: a potential strategy for overcoming resistance to Herceptin

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**14. ABSTRACT**

The major goal of this Concept Award project is to investigate whether a small molecule inhibitor of Bcl-xL will be able to overcome the resistance of Her-2/neu-(+) breast cancer cells to Herceptin. (-)-gossypol showed potent anti-tumor activity to human breast cancer cell lines with high levels of Bcl-xL, but has only minimal effect on human normal breast epithelial cells with low Bcl-xL. (-)-gossypol potently enhanced growth inhibition and apoptosis induction by doxorubicin and docetaxel, the currently used chemotherapeutic agents for breast cancer. However, interaction of (-)-gossypol with Herceptin activity in Her-2(+) breast cancer cells are still ongoing. Bcl-xL knockdown by siRNA abolished the tumorigenecity of Her-2(+) MCF-7 cells. The data support that Bcl-xL plays a critical role in breast cancer initiation, progression and chemoresistance, but its role in Herceptin resistance remains to be further elucidated. The study provide us a solid foundation to develop (-)-gossypol as a novel molecular targeted therapy for the treatment of breast cancer with Bcl-xL overexpression.

15. Subject Terms (keywords previously assigned to proposal abstract or terms which apply to this award)

Her-2/neu, Bcl-xL, small molecule inhibitor, Herceptin

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## I. Introduction:

The major goal of this Concept Award project is to investigate whether a small molecule inhibitor of Bcl-xL will be able to overcome the Herceptin-resistance of Her-2/neu(+) breast cancer. Our *hypothesis* is that anti-apoptotic molecule Bcl-xl may play a role in Herceptin resistance, and a potent and specific Bcl-$X_L$ inhibitor might be able to block or even reverse this resistance, thus improving efficacy of Herceptin therapy. This is based on our basic hypothesis that Bcl-xL is the primary molecular target that mediate the anticancer activity of the small molecule Bcl-xL inhibitor, (-)-gossypol, in human breast cancer cells. Our ultimate goal is to develop (-)-gossypol as a novel molecular targeted therapy for the treatment of breast cancer with Bcl-xL overexpression. In this project, we will investigate *in vitro* and *in vivo* anti-tumor activity and the mechanism of action of (-)-gossypol in human breast cancer with Bcl-xL overexpression, and investigate the potential synergistic effects of (-)-gossypol in combination with Herceptin therapy.

## II. Research progress and key research acomplishments:

This project is one-year Concept Award project. Due to the move of the PI's lab from Department of Internal Medicine to Division of Cancer Biology in Department of Radiation Oncology, and the time required to finish the animal study, a 12-month no-cost extension was requested and approved. During the first year period, we carried out the first task proposed in the Statement of Work. Specifically, we carried out the following studies:

**II.1.** To analyze the correlation of the expression levels of Bcl-$X_L$ and HER2 and response to Herceptin, to assess whether there is any link between Bcl-$X_L$ overexpression and Herceptin response. *(Task 1)*

***II.1.1.*** *Using established HER2(+) human breast cancer cell lines with different levels of Bcl-$X_L$, to assess their cellular responses to Herceptin and relation to Bcl-$X_L$ expression.*

We are testing the Herceptin response of the breast cancer cell lines with Her-2/neu overexpression, i.e., BT-474, SK-BR-3, MDA-453, as well as MCF-7 which is Her-2 positive, versus the Her-2 negative MDA-231 cells.

Due to the lab move, as of the end of the first year, 6/30/2005, the studies were still ongoing. The data will be reported in our final report.

***II.1.2.*** *Using a Bcl-$X_L$-transfected HER2(+) cell line to see if Bcl-$X_L$ overexpression renders the cells more resistant to Herceptin.*

Extensive effort was put into culturing the MCF-7-Her-2 (MCF-7-H18) cells which were transfected with human Her-2/neu gene. As of the end of the first year, 6/30/2005, the MCF-7-H18 cells did not grow well as expected. We were trying to obtain a new batch of the cells from the original lab in MD Anderson Cancer Center. The data will be reported in our final report.

## III. Reportable outcomes:

1. Two abstracts funded from this grant were presented in international and national meetings.

- Liang Xu, et al. Discovery and therapeutic potential of novel Bcl-2/Bcl-xL small-molecule inhibitors in human breast and prostate cancer. *International Conference on Tumor Progression and Therapeutic Resistance.* Philadelphia, PA, November 8-9, 2004. (Dr. Xu was awarded **2nd Prize of Poster Award)**.
- Xu L, et al. Therapeutic potential of Bcl-2/Bcl-xL small-molecule inhibitor in human breast cancer in vitro and in vivo. DOD BCRP Era of Hope 2005 Meeting, Philadelphia, PA, June 8-11, 2005. (Poster P67-19)

2. One investigational new drug (IND) application filed in 2004, on (-)-gossypol safety in human beings.

Based on the exciting data obtained partly from this BCRP project, the IND for (-)-gossypol was filed in 2004 and approved by FDA in 2005. (-)-gossypol is now in **Phase I clinical trials**. The **Phase II clinical trial** of (-)-gossypol in combination with chemotherapy will start soon in University of Michigan.

**3.** One US and International Patent application filed in 2005

**Xu L,** Lippman ME, Liu M. *RNA-based therapeutics targeting Bcl-xL*. Provisional United States Patent Application filed on 12/27/2004. Full US patent application and international PCT filed on 12/27/2005.

## IV. Conclusions:

The major goal of this Concept Award project is to investigate whether a small molecule inhibitor of Bcl-xL will be able to overcome the Herceptin-resistance of Her-2/neu(+) breast cancer.

Due to the lab move, as of the end of the first year, 6/30/2005, the studies were still ongoing. The data will be reported in our final report.