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L-BLP25 vaccine plus letrozole for breast cancer
Is translation possible?

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We have recently reported immunomodulatory effects for tamoxifen and letrozole on the L-BLP25 (Stimuvax®)-induced immune response in a MUC1-expressing breast cancer mouse model. While neither tamoxifen nor letrozole appeared to interfere with the Th1-polarized cytokine response induced by L-BLP25, only letrozole increased the survival advantage of L-BLP25.

MUC1 Vaccines

A number of research groups are focused on devising techniques to effectively present mucin 1 (MUC1) as an immunogen to stimulate a strong and highly specific immune response against cells overexpressing MUC1. L-BLP25 (Stimuvax®) is one such innovative liposomal vaccine currently under development.[1] L-BLP25 contains 25 amino acids from the immunogenic variable number of tandem-repeats region (VNTR) of MUC1. By targeting T-cell epitopes from the VNTR region of MUC1 to presentation on MHC Class I molecules, L-BLP25 operates as an active immunotherapy and elicits a cellular immune response. Several MUC1-targeting vaccines other than L-BLP25 are being developed for the therapy of a variety of epithelial cancers.

Mouse Models and MUC1

Development of primary mammary tumors as single lesions on the ducts connected to the nipple is a unique feature of the MTag.Tg-derived MUC1-expressing mammary tumor (MMT) mouse model used in our study. Lin et al. demonstrated that in the polyomavirus middle-T model of breast cancer, four distinct stages of tumor progression occur from premalignant lesions to overtly malignant ones: hyperplasia, adenoma, early carcinoma and late carcinoma.[2] These stages are comparable to those observed in human benign or in situ proliferative and invasive breast cancer. We also documented the hormone responsiveness of our model, as evidenced by the decreased survival of mice treated with estrogen plus vaccine compared with that of animals treated with vaccine alone. This observation makes the MMT model well suited for studying the relationship between the activity of hormones and the immune system.

MUC1 as a Signaling Molecule

The MUC1 cytoplasmic domain (CD) is very active with regard to signaling and interacts with several proteins, including ZAP-70, protein kinase Cδ (PKCδ), glycogen synthase kinase 3β (GSK-3β), c-SRC, LCK, phosphoinositide-3-kinase (PI3K), SHC, phosphoinositide phospholipase C1 (PLC1), growth factor receptor-bound protein 2 (GRB2), p53, IκB kinase β and γ subunits (IKKβ and IKKγ), β-catenin, heat-shock proteins of 70 and 90 KDa (HSP70 and HSP90), and the estrogen receptor α chain (ERα).[3] MUC1 CD interacts with ERα in the nucleus of breast cancer cells and stabilizes it by blocking its ubiquitination-dependent degradation. Thus, MUC1 increases ERα-mediated transcription and contributes to estrogen-mediated growth and survival of breast cancer cells. Furthermore, MUC1 may play a role in the regulation of hormone receptors by inactivating p53 and targeting NFκB to the nucleus.

Hormonal Therapy for Breast Cancer and the Immune System

Selective estrogen receptor modulators (SERMs) such as tamoxifen and nonsteroidal aromatase inhibitors (AIs) such as letrozole and anastrozole interfere with estrogen signaling through different mechanisms. In the breast tissue, tamoxifen principally acts as a competitive antagonist of estrogen receptors. In contrast, nonsteroidal AIs function by interrupting the biosynthesis of estradiol from androgen precursors through competitive inhibition of aromatase, also known as cytochrome P450 19 (CYP19), eventually resulting in reduced levels of circulating estradiol. Such a difference in the mechanisms of action of SERMs and AIs may be important in relationship with the immune system. Tamoxifen is indeed capable of inducing a shift from cellular (Th1) to humoral (Th2) immunity,[4] while anastrozole has been shown to increase the levels of the proinflammatory cytokines interferon γ (IFNγ) and interleukin (IL)-12 (Th1) and decrease the levels of IL-4 and IL-10 (Th2) cytokines. Anastrozole also suppresses the

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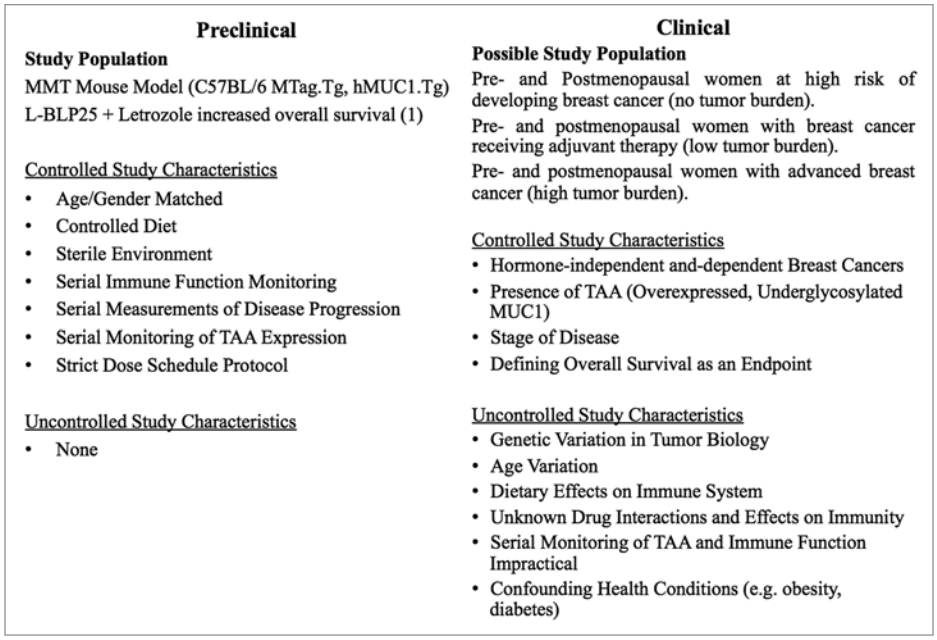

Figure 1. Translational barriers to consider when designing a breast cancer clinical trial based on preclinical data.

differentiation of naïve T cells into Tregs, which are known to produce immunosuppressive cytokines in the tumor microenvironment.[5] It is also possible that cytotoxic T lymphocytes (CTLs) that target MUC1 at the membrane level may sterically interfere with tamoxifen's ability to bind estrogen receptors, thus explaining the reduced effectiveness of tamoxifen seen in our study. Nevertheless, differences in the mechanisms of action of these hormonal agents may help explain why the vaccine/letrozole but not the vaccine/tamoxifen combination exerted additive antitumor activity.

Tumor Burden and Tregs

In agreement with previous studies, we demonstrated that vaccination with L-BLP25 does not produce a durable antitumor response when administered to mice with large tumor burdens. High tumor burdens are indeed associated with increasing Treg populations and an overall immunosuppressive tumor microenvironment which can affect vaccine-induced immune responses. It is well known that the elicitation of cancer-specific immune responses is best when tumor burden is low. Furthermore, it is becoming more common to integrate strategies that enhance T-cell responsiveness while quenching immunosuppression to augment the efficacy of cancer immunotherapies. For instance, low-dose cyclophosphamide is a well established method for reducing the activity of Tregs and other immunosuppressive cell populations.[6] More specific approaches under investigation in the setting of breast cancer include the blockade of CD25 with daclizumab.[7]

The Future of L-BLP25 Combination Therapy

Although chemotherapy has the potential to interfere with immunotherapy, it is now becoming apparent that the immunological effects of cytotoxic chemotherapy are strictly related to dosage. In general, cell cycle-independent cytotoxic chemotherapeutics (e.g., doxorubicin, idarubicin, mitoxanthrone, oxaliplatin) can induce apoptotic cell death, leading to increased antigen presentations by dendritic cells and in situ immunization against tumor antigens. Another common chemotherapeutic agent, cisplatin, has been shown to induce tumor cell death while activating CTLs and/or the secretion of lymphokines.[8,9] Taxanes, such as paclitaxel, can induce a decline in the numbers and activity of Tregs, while promoting the production of Th1 cytokines as well as $CD8^+$ T-cell responses. In summary, commonly used chemotherapeutic agents have immunomodulatory properties that can be taken advantage of for augmenting the immune response to vaccines such as L-BLP25.

Conclusion

The translational potential of our research will depend on a well-designed clinical trial considering factors that are typically controlled in preclinical settings (**Fig. 1**). Preclinical immunological studies are conducted in a pathogen-free environment, adhering to a rigid dose schedule and strict diet, serial monitoring of immune functions, and confirmation of the presence of tumor-associated antigens (TAAs) during therapy. The successful translation of our preclinical study to the clinic will depend on a number of different elements, including the age and tumor burden of patients at inclusion, the continuous monitoring of their immune system, the presence of TAAs, as well as the diet (which can affect immune responses) and possible co-medications (which also may alter the activity of L-BLP25). Clinically relevant endpoints such as overall survival vs. traditional measures of cytotoxic or hormonal drug activity need to be clearly defined for assessing the benefits of cancer vaccine immunotherapy.[10] Finally, the goal of curing cancer with traditional therapy have virtually reached an impasse. The logical

and potentially achievable goal of immunochemotherapy is to turn cancer from a lethal disease into a chronic condition.

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