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## Lymphocyte transformation test in LA7 rat mammary cancer model administered with MMP-11 based therapeutic chimeric DNA vaccine

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**Abstract**

Matrix metalloproteinase 11 (MMP-11), a member of the matrix metalloproteinase family, is highly expressed in various tumor cells and the surrounding tumor microenvironment. It has been identified as a potential target for breast cancer immunotherapy. This study investigates the efficacy of a chimeric DNA vaccine, pVAX1.MMP-11, targeting MMP-11 in the LA7 rat mammary cancer model. We assessed the immune response induced by the vaccine through lymphocyte transformation tests (LTT), which measure T cell proliferation upon exposure to the MMP-11 antigen. The results revealed a significant increase in T cell proliferation, suggesting that the chimeric DNA vaccine may activate an immune response targeting MMP-11 expressing mammary tumors. These findings provide insight into the potential of therapeutic DNA vaccines in cancer immunotherapy.

**Keywords:** Chimeric DNA vaccine, MMP-11, LA7 rat mammary tumor, cancer immunotherapy, lymphocyte transformation test, T cell proliferation, immune response

**Introduction**

Cancer is a class of diseases characterized by uncontrolled cellular growth, tissue invasion, and metastasis (Kim *et al.* 2020) [2]. Malignant tumors, unlike benign ones, can invade surrounding tissues and spread to other parts of the body via the lymphatic or circulatory systems. Mammary cancer, commonly found in both humans and animals, presents a significant challenge in oncology. In particular, mammary tumors in animals, such as dogs serve as valuable models for studying human breast cancer (Gherman *et al.* 2024) [1]

Matrix metalloproteinases (MMPs) are a family of zinc-dependent enzymes that degrade extracellular matrix components and are crucial in tissue remodeling processes. Among the MMPs, MMP-11 (also known as stromelysin-3) has been linked to various cancers, including breast cancer, due to its role in tumor progression, invasion, and metastasis (Zhang *et al.* 2016) [5]. MMP-11 is overexpressed in tumor cells and stromal cells in the tumor microenvironment, making it a potential therapeutic target (Ma *et al.* 2021) [4]. MMP-11 promotes cancer development by inhibiting apoptosis as well as enhancing migration and invasion of cancer cells (Zhang *et al.* 2016) [5] and inhibiting MMP-11 can reduce tumor growth and promote apoptosis in cancer cells.

Immunotherapy, particularly DNA-based vaccines targeting tumor-specific antigens, has emerged as a promising approach for treating cancer. Chimeric DNA vaccines, which express foreign antigens with high homology to human proteins, have shown greater efficacy in activating immune responses compared to conventional vaccines. This study aimed to evaluate the immunogenicity of a chimeric DNA vaccine expressing MMP-11 in the LA7 rat mammary cancer model using a lymphocyte transformation test (LTT) to measure T cell activation and proliferation. LTT using alamar blue is a highly sensitive assay (Hamid *et al.* 2004) [6] hence chosen for this study.

**Materials and Methods****Experimental Animals**

Female virgin Sprague Dawley rats (6-8 weeks old, ~180 g) were obtained from the

Laboratory Animal Facility, Central Drug Research Institute (CDRI), Lucknow. The rats were housed under controlled conditions, acclimatized for 15 days before the experiment, and fed an autoclaved diet. The experiments were conducted in accordance with institutional ethical guidelines and approved by the Institute Animal Ethics Committee (IAEC) and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

### Vaccine Construction and Administration

The pVAX1.MMP-11 chimeric DNA vaccine was constructed by inserting the gene encoding canine MMP-11 into the pVAX1 vector. This DNA vaccine was administered intramuscularly to the rats in two doses, 2 weeks apart. The rats were divided into the following groups: Control group (no vaccine, no tumor); pVAX1.MMP-11 group (tumor-bearing rats vaccinated with pVAX1.MMP-11); pVAX1 (vector control); pVAX1.MMP-11+IL-2 group (tumor-bearing rats vaccinated with pVAX1.MMP-11 plus interleukin-2 for immune stimulation).

### Lymphocyte Transformation Test (LTT)

The LTT was performed at the end of the trial to evaluate T cell proliferation in response to the vaccine. Blood was collected from each group using heparin as an anticoagulant. Lymphocytes were separated by density gradient centrifugation using HiSep LSM-1077. The lymphocytes were cultured in RPMI-1640 medium, seeded in 96-well plates, and incubated at 37 °C in a CO<sub>2</sub> incubator.

Concanavalin A (Con-A) and cell lysates from transfected cells were added to stimulate T cell proliferation. The proliferation response was measured by adding AlamarBlue® and reading absorbance at 570 nm and 600 nm using an ELISA reader.

### Statistical Analysis

Data were analyzed using appropriate statistical methods, with significance defined as  $p < 0.05$ . Proliferation indices were calculated according to the AlamarBlue® protocol.

## Results

### Lymphocyte Proliferation Response

The lymphocyte transformation test (LTT) results showed a significant increase in T cell proliferation in the pVAX1.MMP-11 and pVAX1.MMP-11+IL-2 groups compared to the control group without any tumors. The pVAX1.MMP-11 group exhibited a 26% increase in lymphocyte proliferation, while the pVAX1.MMP-11+IL-2 group showed a 14% increase in proliferation compared to the control (Figure1). The inclusion of IL-2 in the vaccine formulation resulted in a modest enhancement of T cell activation. The principle of the LTT is based on the fact that lymphocytes, which have been sensitized by a certain antigen ("memory cells"), transform into blasts and proliferate when they are again exposed to this antigen (Klein *et al.* 2004) [3]. This result indicates that to some extent, lymphocyte priming had taken place in MMP-11 vaccinated groups.

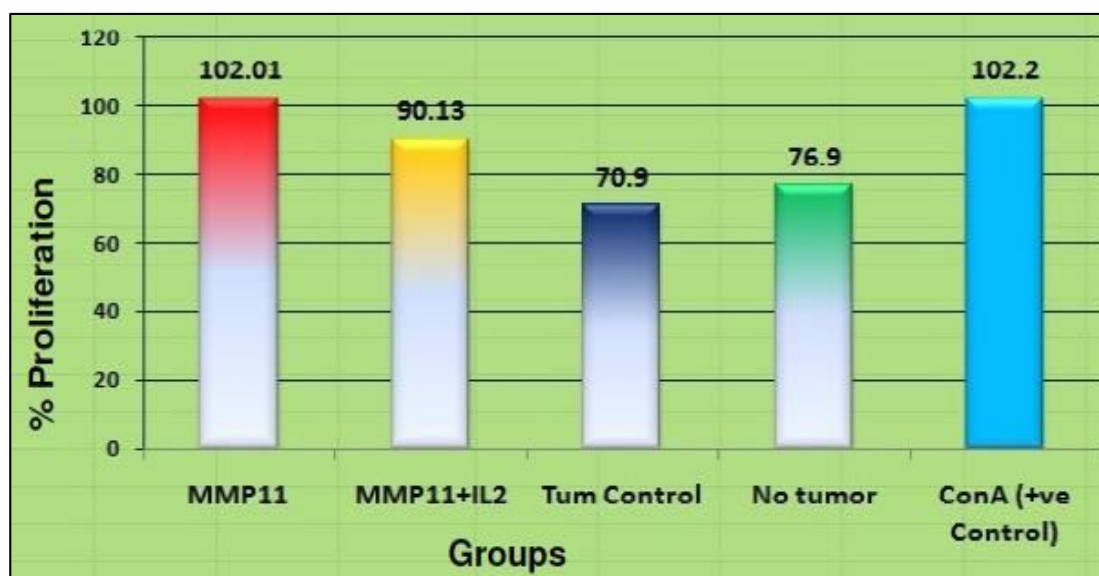


Fig 1: LTT-Percentage proliferation of lymphocytes after MMP-11 treatment

### Vaccine Efficacy and Tumor Progression

While the immune response to the vaccine was evident, the therapeutic effect on tumor regression was not as anticipated. Contrary to our expectations, the chimeric MMP-11 vaccine did not reduce tumor growth in the LA7 rat mammary cancer model. In fact, the vaccine may have inadvertently aggravated tumor progression, possibly due to interference with anti-tumor immune mechanisms. These findings underscore the complexity of cancer immunotherapy and the need for further optimization of vaccine strategies to enhance tumor targeting and immune response efficacy.

### Discussion

The results of the lymphocyte transformation test (LTT) indicate that the chimeric DNA vaccine targeting MMP-11 successfully elicited a T cell response in the LA7 rat mammary cancer model. The increase in T cell proliferation, especially in the pVAX1.MMP-11 group, supports the hypothesis that the vaccine can stimulate an immune response against MMP-11 expressing tumor cells. However, the failure of the vaccine to reduce tumor growth suggests that there may be additional factors at play, such as immune evasion mechanisms by the tumor or the need for more potent immune stimulators. Moreover, as MMP-11

promotes cancer growth (Zhang *et al.* 2016) [5], it is evident that the vaccine expressed MMP-11 which caused tumor growth.

The role of MMP-11 in cancer progression, including its involvement in extracellular matrix remodeling and tumor invasion, makes it an attractive target for immunotherapy. However, as seen in this study, the therapeutic application of MMP-11-based vaccines requires further refinement. Factors such as the timing of vaccine administration, the use of adjuvants, and the route of administration may all influence the success of such therapies.

### Conclusion

The therapeutic chimeric DNA vaccine targeting MMP-11 successfully induced a T cell-mediated immune response in the LA7 rat mammary cancer model, as demonstrated by the lymphocyte transformation test. Despite this immune activation, the vaccine did not result in the expected tumor regression, highlighting the need for additional research to optimize DNA vaccine strategies in cancer immunotherapy. Future studies will focus on enhancing the immunogenicity of the vaccine, exploring combination therapies, and further understanding the complex interplay between tumor microenvironment and immune responses.

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### References

1. Gherman LM, Chiroi P, Nuțu A, Bica C, Berindan-Neagoe I. Profiling canine mammary tumors: A potential model for studying human breast cancer. *The Veterinary Journal*. 2024;303:106055. doi: 10.1016/j.tvjl.2023.106055.
2. Kim GJ, Kim DH, Min KW, Chae SW, Kim SH, Son BK, Moon KM, Kim YH. Expression of UTX indicates poor prognosis in patients with luminal breast cancer and is associated with MMP-11 expression. *Applied Immunohistochemistry & Molecular Morphology*. 2020;28(7):544-550. doi: 10.1097/PAI.0000000000000795.
3. Klein R, Schwenk M, Heinrich R, Templeton DM. Diagnostic relevance of the lymphocyte transformation test for sensitization to beryllium and other metals. *Pure and Applied Chemistry*. 2004;76(6):1269-1281.
4. Ma B, Ran R, Liao HY, Zhang HH. The paradoxical role of matrix metalloproteinase-11 in cancer. *Biomedicine & Pharmacotherapy*. 2021;141:111899. doi: 10.1016/j.biopha.2021.111899.
5. Zhang X, Huang S, Guo J, Zhou L, You L, Zhang T, Zhao Y. Insights into the distinct roles of MMP-11 in tumor biology and future therapeutics (Review). *International Journal of Oncology*. 2016;48:1783-1793. doi: 10.3892/ijo.2016.3400.
6. Hamid R, Rotshteyn Y, Rabadi L, Parikh R, Bullock P. Comparison of alamar blue and MTT assays for high throughput screening. *Toxicology in vitro*. 2004;18(5):703-710. doi: 10.1016/j.tiv.2004.03.012. PMID: 15251189.