Oncology 2025
The Cancer Control Challenge:
Better Understanding It to Better Face It

TRANSIENT PLANT EXPRESSION PLATFORM FOR THE PRODUCTION OF A RECOMBINANT GASTRIC CANCER ANTIGEN

Andrei Stéfano Queiroz Gonçalves[1], Debora Cristina Viana Pereira[1], Marcela Alves de Oliveira[1], Beatriz Modesta Moreira[1], Dayanne Wysllate Araujo Ribeiro[1], Jade Dias Valente[1], Myrth Soares Do Nascimento Remígio[1], Paulo Pimentel De Assumpção[1], Maria Izabel Florindo Guedes[2], Lívia Érika Carlos Marques[1]

[1]Oncology Research Center (NPO), Federal University of Pará (UFPA), Brazil
[2]Laboratory of Biotechnology and Molecular Biology (LBBM), State University of Ceará (UECE), Brazil

Introduction: Cancer is a complex and heterogeneous disease and is ranked as the second leading cause of death worldwide, according to data from the World Health Organization (WHO). Conventional treatments such as surgical resection, radiotherapy, and chemotherapy have limitations and can cause severe physical discomfort due to adverse effects. In this context, molecular studies and the discovery of biomarkers have been essential for the development of monoclonal antibodies (mAbs) applicable to immunotherapies and targeted therapies, offering a more personalized and less aggressive approach. However, the investment required for the development of such biologics is extremely high, and patents may last over 20 years, directly impacting treatment costs and limiting access to these medications. Therefore, it is necessary to implement more accessible platforms for the production of biologics to support research and the development of personalized therapeutic solutions. The transient plant expression platform using Nicotiana benthamiana leaves infiltrated with Agrobacterium tumefaciens represents an efficient alternative for the production of complex proteins and recombinant antigens, with cost-effective implementation, lower risk of contamination, scalable production, and rapid yield of the product of interest. **Objectives:** To establish the initial steps of a transient expression platform in Nicotiana benthamiana for the production of a recombinant gastric cancer antigen. **Methods:** The plant platform was implemented under controlled conditions (24 °C; 18 h photoperiod), and Nicotiana benthamiana growth was optimized through adjustments in humidity control and nutritional regimen, starting from the initial NPK 20-20-20 fertilizer formulation. The antigen sequence was retrieved from UniProt, and a search was conducted for distinct regions corresponding to the target isoform of interest in gastric cancer. SnapGene software was used to design the DNA construct optimized for cloning, and the sequence of interest was synthesized by the company GenScript. The recombinant antigen sequence was cloned into the binary vector pCaMGate-ER-ELP via LR Clonase reaction and transformed into *E. coli* DH10B. Recombinant clones were selected based on growth in LB medium containing 50 µg/mL kanamycin and confirmed

by PCR and agarose gel electrophoresis. Plasmid DNA was purified using a miniprep protocol. **Results:** The platform supports the growth of up to 20 plants, which reach optimal leaf size and appearance for infiltration by the 5th or 6th week after germination. Selection of recombinant vector-containing clones was achieved through growth on selective medium. Additionally, clone confirmation was based on the expected 1100 bp amplicon of the expression cassette amplified by PCR. **Conclusion:** A methodological framework was established for binary vector construction and plant growth optimization, which comprise the initial stages of the plant expression platform. In the next steps, Agrobacterium tumefaciens will be transformed, followed by leaf agroinfiltration for antigen expression and purification. The production of a recombinant antigen is highly relevant to oncology research and may be applied to monoclonal antibody generation, phage display-based antibody fragment selection, molecular characterizations, and immunoassays.

Keywords: Molecular farming; Nicotiana benthamiana; Tumor antigen; Recombinant proteins; Oncology