LIST ALL AUTHORS and AFFILIATIONS – *underline presenting author*

Rodrigo Mora\(^1\), Yuk Pheel Park\(^1\), Natalie Atyeo\(^3\), Kristianna M. Fredenburg\(^2\), Dunrui Wang\(^2\), Edward K. L. Chan\(^1\)

\(^1\)Department of Oral Biology, University of Florida, Gainesville, FL 32608, USA; \(^2\)Laboratory of Cellular Oncology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA; \(^3\)Department of Pathology, Immunology, and Laboratory Medicine, University of Florida, Gainesville, FL 32610, USA

**TITLE**

Identification and Evaluation of Human Neurotrimin Protein to Develop Novel CAR-T Therapy for Head and Neck Cancer

**HYPOTHESIS:**

We hypothesize that neurotrimin can serve as a potential candidate target antigen for CAR-T immunotherapy.

**BACKGROUND/AIMS:**

Over 526,000 people worldwide are diagnosed with head and neck cancer annually. This year in the US alone, it is estimated that there will be 49,570 new cases and 9,700 deaths from oral cancer. Chimeric Antigen Receptor-T (CAR-T) immunotherapy has revolutionized cancer immunotherapy. Recently, CD19-CAR-T cells were approved to treat patients with leukemia. CAR-T cell immunotherapy involves modifying receptors on a patient’s T cells so that they bind to tumor cells via a specific antigen on the tumor cell surface. CAR-T therapy involves introducing a gene of interest into a human T cell so that it can express a tumor antigen binding receptor that is specific for the protein that is highly expressed on the tumor cell surface. These CAR-T cells can then be administered back to the same cancer patient from which the T cells were isolated, where they can target tumors that overexpress the protein that was used to develop our CAR construct. To design novel CAR-T cells, our immediate goal is to express the recombinant human neurotrimin (NTM) protein in Lemo21 bacteria strain and produce monoclonal antibodies to be able to detect NTM antigen.

**METHODS:**

To identify potential CAR-T target antigens, data from the Cancer Genomic Atlas (TCGA) were analyzed, comparing gene expression in head and neck cancer patients versus normal. To validate tumor antigen expression, three oral cancer cell lines (OQ01, HN, CAL27), an oral epithelial cell line (HOK) and 12 tumor specimens were analyzed by immunofluorescence. Oral tumor biopsies were stained with NTM antibody following antigen retrieval.

To express recombinant human NTM, we cloned human NTM cDNA in His6 tagged-pET28 plasmid. Using nickel column affinity chromatography, His-tagged recombinant NTM was purified from Lemo21 strain. Purification yield and purity were evaluated by coomassie brilliant blue staining and western blot analysis.

**RESULTS & CONCLUSIONS**

NTM was identified as a novel target antigen due to an over 6.4-fold increase of mRNA in 303 head and neck cancer patients compared to normal controls from TCGA data analysis. Immunofluorescent staining demonstrates that NTM was localized mainly to the cytoplasm and cell surface, with heterogeneous patterns of expression between cell lines. 11 out of 12 oral tumor biopsies were positively stained with commercial anti-NTM antibody and one specimen showed clear expression of NTM on the cell surface of the tumor. Moreover, we expressed and purified recombinant human NTM protein from Lemo21 strain to design of novel CAR construct. NTM is a glycosylphosphatidylinositol (GPI)-anchored adhesion molecule forming surface complexes that promote neurite growth. GPI-anchored proteins have been described as biomarkers for several cancers, including overexpression in epithelial ovarian tumors. Further work is ongoing to produce monoclonal antibody to detect purified recombinant human NTM protein.