Malignancy in breast tissue is most notorious and common life-threatening clinical conditions in women around the globe. Human breast is made up of lobules, duct, fatty tissue, connective tissue, blood vessels and lymph vessels and it is reported that most of the breast carcinomas activate in ductal cells/tissue. Different types of breast cancer are categorized in terms of cancer tissue localization and these are in situ carcinoma of ducts i.e. DCIS which is most happening mammary carcinoma (Non-invasive) on duct of milk, invasive carcinoma of ducts i.e. IDC that are invasive kind of carcinoma of mammary and reported in the duct of milk, infiltrating lobular carcinoma (ILC) or invasive lobular carcinoma which is characterized by the occurrence of carcinoma in lobules of mammary tissue of women and inflammatory breast cancer (IBC) which is most uncommon breast carcinoma. Mechanical diagnosis of carcinomas (cancer) in breast part, mammography remains the backbone platform for screening of breast cancer along with imaging by magnetic resonance procedure and ultrasound, but few protein markers also reported as promising tools.

MUC1 is a glycosylated protein consists of different domains include extra cellular region, spacing fragment of membrane and a cytosolic protein sequence. MUC1 is present in higher concentration in breast carcinoma condition but having less and aberrant glycosylation. Cancer antigen 15-3 is reported as secretory product of mucin 1 and reported as one of the widely used marker of diagnostic interest and prognostic interest for cancer in human breast. CA 15-3 is a glycoprotein that is predictable with a DF3 and 115D8 mAb. DF3 antibody detects DTRPAPGS; 8 amino acid sequence of tandem repeats (TR) in MUC1 protein and reported as antibody of detection in a sandwich assay while 115D8 monoclonal antibody intersect with glycosylated portion of 20 aa tandem repeats (TR) acts as the capture antibody in sandwich assay. Cancer antigen 15-3 is a MUC1 peptide fragment extensively used for the diagnosis of carcinoma in breast in women.

In this research work, to mimic CA15-3 antigen, a protein sequence have been designed, containing 10 tandem repeats and 20 tandem repeats with few approximate repeats out of MUC1 protein expressed in CHO K1 and AD293 mammalian cells to ensure post translational modifications. ‘Gluc’ protein (Gaussia luciferase) cDNA sequence was incorporated at N terminal along with gene of interest for secretion of protein outside the cells. Protein was characterized by western blot using cancer
antigen 15-3 antibody as well as confirmed for the presence of epitopes similar to the ones, as recognized by 115D8 mAb and DF3 mAb on Siemens CLIA based platform. It was found that AD293 expressing MUC1 product (MUC20TR) contains 20 tandem repeats, having highest yield than MUC20TR expressed in CHO K1 cells. It was also found that MUC1 proteins contain 10 tandem repeats (MUC1RS) was better expressed in secretory form but the activity was much lower that MUC20TR expressed in AD293. The purified recombinant MUC20TR from AD293 clone is used for the preparation of “Control”, so that it can be use for the development of immuno assay (CLIA, ELISA etc.).

Some time, high concentrations of CA 15-3 before operation are allied with wrong result of assay in patient. Therefore, an emergent need was still exists to findout secondary marker to boost specificity of tissue or serum based detection of breast cancer. In different studies, MUC1/Y protein is found as a part of MUC1 gene empty of tandem repeats array and its flanking amino acid sequences was found in different malignancy or carcinoma associated with breast. Several research studies suggested that MUC1/Y antigen would be a good target for therapy potential. Second step of this research study, recombinant human MUC1/Y was highly expressed (under T7 promoter control) and purified from Escherichia coli BL21-DE3 strain and developed a repertoire of monoclonal antibodies, which bound to recombinant MUC1/Y protein. However, several generated mAb shown immuno cross reactivity against His tag or against human serum form normal person. However, only 9A842G6 clone mAb specifically reacted to recombinant MUC1/Y protein. mAb generated by 9A842G6 clone shown Specific fluorescence signal with recognized tissue sections of breast cancer and did not bind to fibroid adenoma sections. Immuno blotting method was used to confirm the specific reactivity of mAb generated by 9A842G6 clone against MUC1/Y protein. Therefore, the observations suggest that MUC1/Y has good potential and could be established as secondary protein marker for diagnosis of carcinoma in breast along with CA15-3 or CA15-3 like antigen (MUC20TR).