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For the degree of: Doctor of Philosophy
Department: Biological Sciences

Title: Experimental Therapy of Human Triple Negative Breast Cancer Using Oncolytic Tanapoxvirus Recombinants Expressing Interleukin-2 and Monocyte Chemoattractant Protein-1

Committee: Dr. Karim Essani, Chair
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Time/Place: Thursday, July 6, 2017
2:30 to 4:30 p.m.
1710 Wood Hall

Triple negative breast cancer (TNBC) is one of the most challenging breast cancer subtypes owing to the non-expression of therapeutic targets like estrogen, progesterone and human epidermal growth factor receptors by cancer cells, and the aggressive nature of this cancer subtype. Standard therapeutics such as chemo and radiotherapies have a limited role in the treatment of TNBC due to the associated severe toxicity and development of therapeutic resistance by cancer cells. Oncolytic viruses (OVs) are an emerging and promising treatment option for breast cancer with several viruses, including poxviruses under investigation in pre-clinical and clinical studies. Tanapoxvirus (TPV), a member of the genus Yatapoxvirus within the
family *poxviridae*, naturally infects humans and has a large (144 kilo base pairs) genome that can be easily maneuvered to engineer armed oncolytic TPVs, stably expressing transgenes. A distinct antigenicity among poxviruses with no cross-immunity with vaccinia virus and no reported direct human to human viral transmission are some of the desirable characteristics of TPV which make this virus a suitable OV candidate. Moreover, TPV causes a self-limiting, mild febrile illness in humans and the safety of virus can be further enhanced by ablating virulence genes from the viral genome. This study describes the oncolytic potential of a panel of TPV recombinants, some of which express immune modulatory proteins in human TNBC, both *in vitro* and *in vivo*.

Most TPV recombinants showed comparable replicability and ability to induce cell death in human TNBC cells, in culture, TPV recombinants expressing mouse interleukin-2 [TPV/Δ66R/mIL-2] and monocyte chemoattractant protein-1 (also known as CCL2) [TPV/Δ66R/mCCL2] regressed the MDA-MB-231 tumor xenografts in athymic nude mice significantly. Histological analyses of tumors showed that predominantly mononuclear immune cell response was induced against tumors by TPV recombinants, where the most striking effect was observed in tumors injected with TPV/Δ66R/mIL-2 in the form of deep infiltration of mononuclear cells into the tumor capsule. In this study, a computational tool, “Quick Codon Count,” which not only allows analyses of large genome data sets for codon usage, but also enables analyses of codon usage for each amino acid at the level of an open reading frame (ORF), has also been described. Development of the “Quick Codon Count” program is important as no currently available computational tools allows codon usage analyses for each amino acid at the level of ORF. Codon usage regulates the viral gene expression, hence understanding codon usage by pox and other viruses can guide the development of OVs.