Bacteriophage Virus-like Particles: A Novel Technology for Vaccine Development

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Introduction

Despite marked advancement in breast cancer treatment over the years, breast cancer relapse with distal metastases occurs in 20-30% of cases despite early detection. Breast cancer stem cells (BCSC) have unique biological properties that represent a cellular reservoir for the relapse, metastatic evolution and progression of the disease. Therefore, the development of novel therapeutics targeting BCSC and thereby inhibiting breast cancer metastasis are urgently needed.

The xCT (SLC7A11) protein is the light chain of the cysteine-glutamate antiporter system xc^-, which regulates detoxification of reactive oxygen species by controlling the production of the antioxidant glutathione (GSH). We showed that xCT is highly expressed in breast cancer and in BCSCs. Importantly, inhibiting xCT function in vitro and in vivo resulted in increased susceptibility to chemotherapeutic agents and decreased metastatic disease.

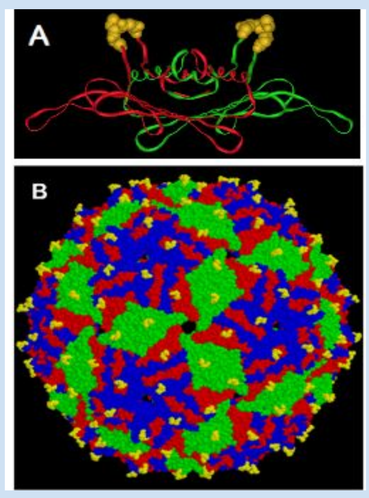
We used an immunotherapeutic approach to target xCT using Agilvax's innovative and proprietary bacteriophage MS2 virus-like particle (VLP) technology. This technology can be used as a platform to display heterologous target antigens in a multivalent format that virtually guarantees strong immunogenicity, and includes epitopes that may not be immunogenic in the context of natural infection and the display of self-antigens to break B-cell tolerance. Vaccines that link target antigens in a dense, repetitive array on the surface of VLPs and provide a source of T helper epitopes can induce particularly robust, high-titer antibody responses. Using our technology, we have developed vaccines against self-antigens (PCSK9), as well as select pathogens (*Staphylococcus aureus,* malaria and Nipah virus). With the knowledge gained from these successes, we are now working to identify and produce vaccines targeting cancer stem cells.

Agilvax's VLP technology

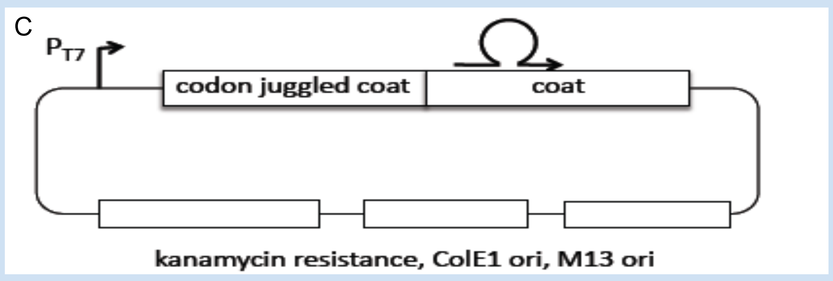
Advantages:

- Elicits strong immune responses.
- Generates antibody responses to self-antigens.
- High-level production and scalable expression from *E. coli.*
- VLPs are amenable to dry powder formulation, mitigating the need for cold chain.
- Agilvax's lead vaccine candidates are undergoing GMP product development studies with the assistance of the NIH NIAID DMID Product Development Program.

Bacteriophage VLPs



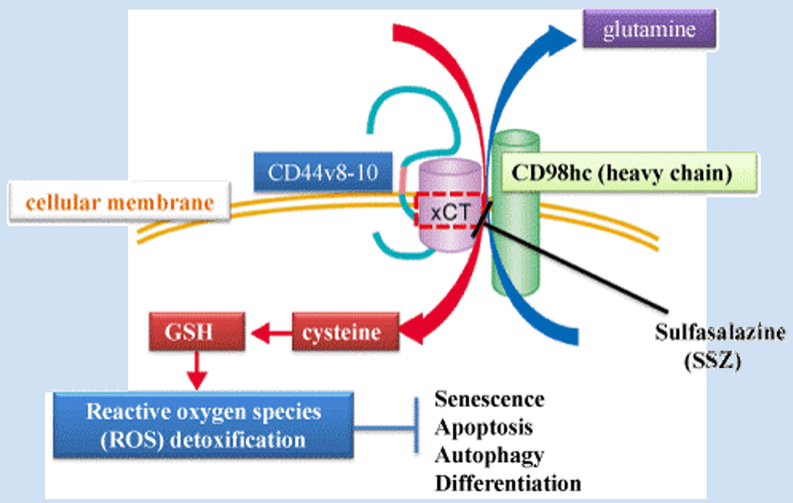
The structure of MS2. (A) The coat protein dimer with the two identical chains in red and green ribbons, and the AB-loops emphasized in yellow space fill. (B) The MS2 VLP with the AB-loops highlighted in yellow. Coat protein exists in three slightly different conformations, here shown in red, blue, and green. (C) The structure of pDSP62, the vector used for construction of MS2 VLPs.



Peabody *et al*. (2008). Immunogenic display of diverse peptides on virus-like particles of RNA phage MS2. *J Mol Biol*, 380(1), 252-263.

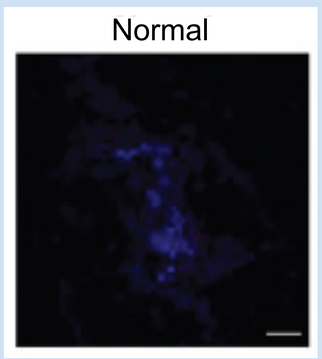
Chackerian *et al*. (2011). Peptide epitope identification by affinity selection on bacteriophage MS2 virus-like particles. *J Mol Biol*, 409(2), 225-237.

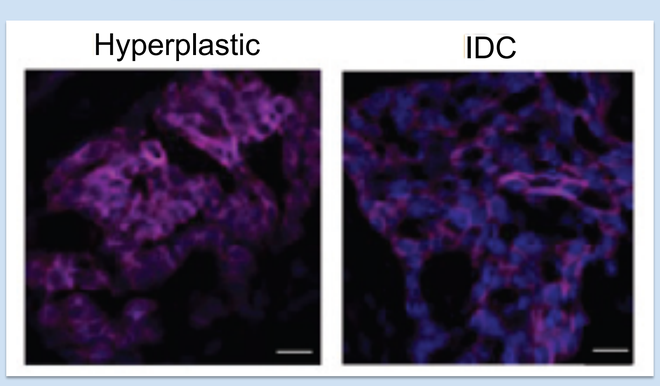
Normal role of xCT



The xCT protein is the light chain of the cysteine-glutamate antiporter system xc^-, which exports glutamate to the extracellular space and concomitantly imports cystine into the cytosol, where it is rapidly reduced to cysteine. The xCT pathway regulates detoxification of reactive oxygen species by controlling the production of the antioxidant glutathione (GSH). Reproduced from Yoshida and Saya (2014). *Clin Exp Pharmacol*, 4(2), 147.

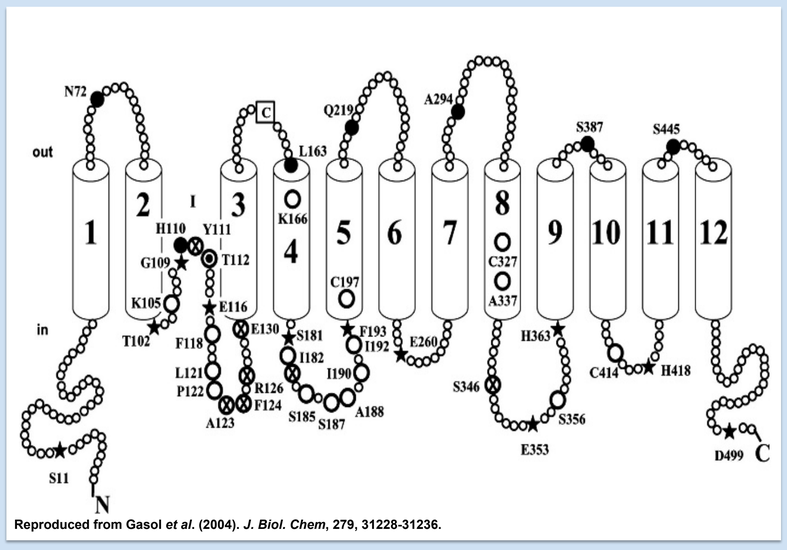
xCT is overexpressed in human breast cancer tissues





xCT expression in human normal and neoplastic breast tissues. Immunofluorescence of xCT (red) in normal, hyperplastic, and invasive ductal carcinoma (IDC). Nuclei are counterstained with DAPI (blue). Scale bar is 20 µm. Adapted from Lanzardo et al. (2016), *Cancer Res*, 76(1), 62-72.

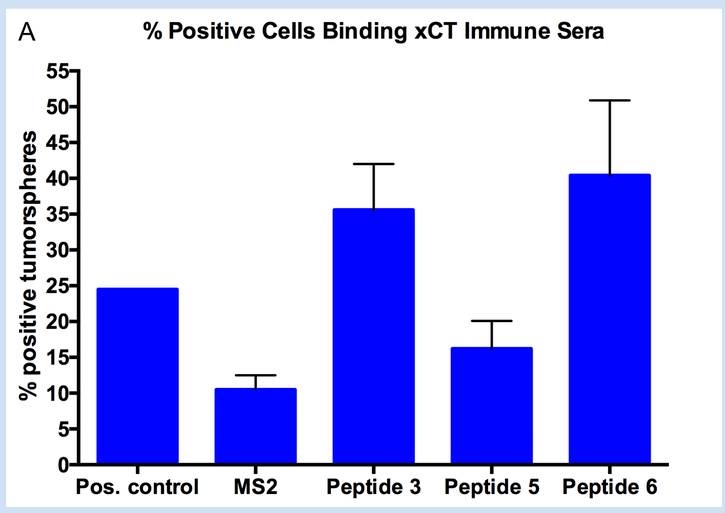
Structure of human xCT

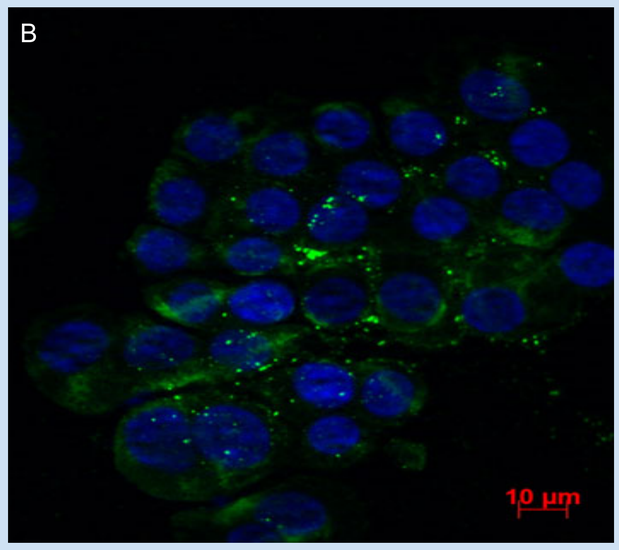


Reproduced from Gasol *et al.* (2004). *J. Biol. Chem*, 279, 31228-31236.

Topographical model of human xCT. VLP expression plasmids displaying selected peptides of human xCT were constructed. Amino acid sequences were codon optimized for expression in E. *coli* and were inserted into the AB-loop of MS2. Peptide 2 and peptide 6 are 100% conserved between human and rodents, while peptide 5 has a single, conservative amino acid substitution.

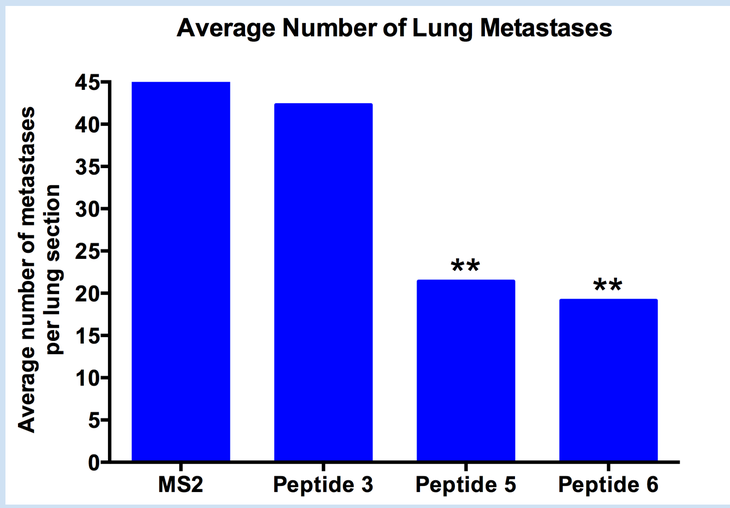
xCT VLPs elicit antibodies that bind to BCSCs





Immune sera (1:50) from xCT or control (MS2) VLP immunized mice were incubated with HCC-1806 derived tumorspheres. FITC conjugated anti-mouse IgG was used to detect antibodies bound to the cells. (A) FACS analysis of the percentage of tumorsphere cells that bound to the immune sera. As a positive control for xCT binding, we used a commercially available xCT polyclonal antibody (Santa Cruz). (B) Immunofluorescence analysis showing antibody binding (green) to a tumorsphere from a xCT Peptide 6 immunized mouse. Cell nuclei are stained with DAPI (blue).

Immunization with xCT VLPs inhibits lung metastases



Mice immunized with xCT or MS2 control VLPs were injected (i.v.) with $5x10^4$ mouse tubo-derived tumorspheres. After 20 days, animals were euthanized and lungs were removed, sectioned, and the number of metastatic foci were measured. The number of mice were MS2 (n=2), xCT Pep 3 (n=5), xCT Pep 5 (n=5) and xCT Pep 6 (n=4). Metastatic lesions were counted from two lung sections per animal. Data presented is the average number of lesions per slide. Two-tailed student *t*-test was used to determine significance. ** indicates a p-value < 0.01.

Conclusions

- xCT VLP immunization elicited antibodies that bound to xCT expressing tumorspheres.
- xCT VLP immunization significantly reduced the number of lung metastases.
- xCT immunotherapy has the potential to be an effective therapy for the treatment of aggressive forms of breast cancer.