AX09: An Immunotherapy Candidate Targeting the Breast Cancer Stem Cell Protein xCT

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Abstract

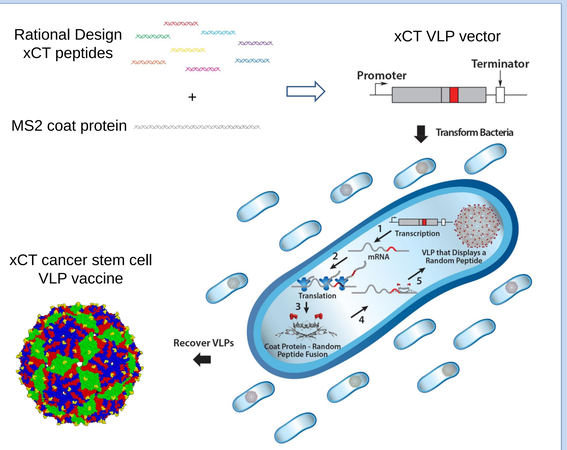
Breast cancer stem cells (BCSC) have unique biological properties that represent a key cellular reservoir for relapse, metastatic progression and therapeutic resistance. Aggressive forms of breast cancer, such as triple negative breast cancer (TNBC), are enriched in BCSCs and have limited therapeutic options.

AX09 is an immunotherapy candidate based on Agilvax's virus-like-particle technology that targets the cystine-glutamate antiporter system protein xCT (SLC7A11). xCT regulates detoxification of reactive oxygen species by controlling the production of the antioxidant glutathione, protecting cells against oxidative and chemical insults. The xCT protein is overexpressed in BCSC and forms a membrane complex with CD44 and MUC-1C. xCT protein levels are elevated in a high percentage of invasive mammary ductal tumors and its expression correlates with poor prognosis. xCT expression is upregulated by chemotherapy and inhibition of xCT function sensitizes BCSC and xCT expressing senescent tumor cells to a variety of chemotherapeutic agents *in vitro* and *in vivo*. In contrast, expression of xCT is limited to a few normal cell types and xCT knockout mice show no developmental or growth defects suggesting therapeutic inhibition of xCT activity would have limited effects on non-cancerous cells.

Immunization with AX09 elicited a strong antibody response and induced high levels of IgG2a antibodies. Immune sera from AX09 mice bound to $CD24^{-}CD44^{+}xCT^{+}$ BCSC analyzed by FACS and immunofluorescence. To assess if AX09 immunization would decrease metastases, purified BCSC were injected into the tail vein of vaccinated female BALB/c mice. In multiple independent experiments, immunization with AX09 conferred a significant reduction in the number of lung metastases compared to vaccination with control VLP alone. Using a therapeutic mouse model, immunization with AX09 after the formation of tumors also reduced lung metastases. Preliminary studies show that AX09 immunization results in an influx of NK and CD8 T-cells into the tumor site suggesting vaccination with AX09 alters the tumor microenvironment. Studies optimizing AX09 formulation and assessing the efficacy of AX09 in combination with traditional and emerging breast cancer therapies are ongoing.

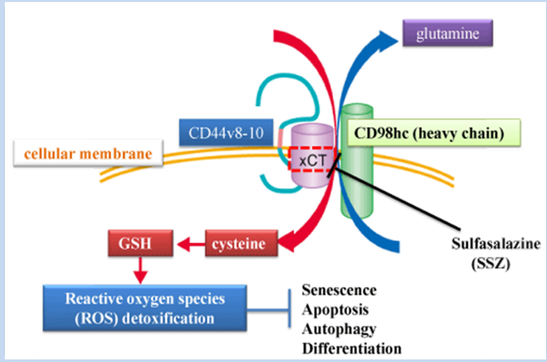
Agilvax's VLP Technology

- Highly immunogenic heterologous antigen display technology
- Antigen display by simple genetic insertion or chemical conjugation
- High level expression and scalable production in *E. coli*
- VLPs are readily purified from contaminating bacterial proteins and LPS
- Encapsulate endogenous TLR7 adjuvant (ssRNA), requiring no additional exogenous adjuvants
- Dry powder formulation eliminates the need for cold chain

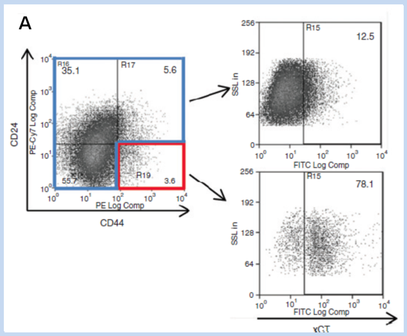


xCT Biology

The xCT protein is the light chain of the cystine/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). xCT protein forms a membrane embedded complex with the v8-10 splice variant of CD44 in many solid cancer stem cells. The small molecule SSZ inhibits xCT activity, but has vast off target effects. Reproduced from Yoshida and Saya (2014). *Clin Exp Pharmacol*, 4(2), 147.



xCT is Expressed in Breast Cancer Stem Cells



(A) TUBO cells were subjected to FACS analysis. Cells were stained with CD44 and CD24 and the breast cancer stem cell (CD24-CD44+; ~3.6%) and non-stem cell populations were evaluated for xCT expression. (B) Passage 3 tumorspheres derived from TUBO cells were evaluated for xCT expression by immunofluorescence. P3 tumorspheres are also positive for the cancer stem cell markers OCT4, Sca-1 and Thy1.1.

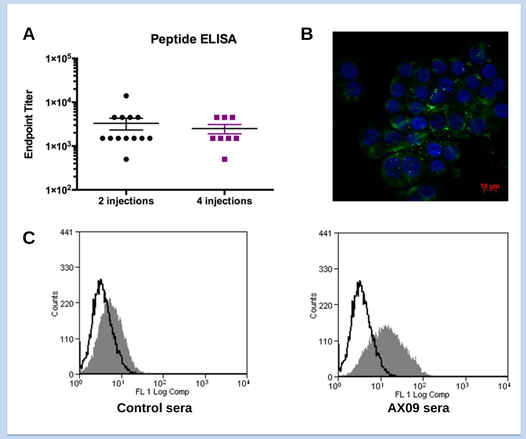
Immunization with AX09 Elicits Antibodies that Bind to BCSC

AX09 induced antibody responses were evaluated by ELISA, IF and FACS.

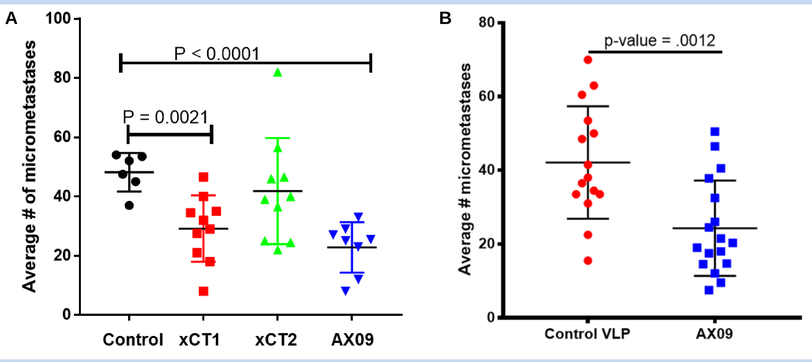
(A) Peptide ELISA. Immune sera from AX09 were serially diluted and incubated in xCT peptide coated wells. Endpoint titers were determined by the highest dilution that was 2-fold over background. Each dot represents an individual animal.

(B) Immunofluorescence. Antibodies (green) from AX09 immunized mice bound to HCC-1806 derived tumorspheres. Cell nuclei are stained with DAPI (blue).

(C) FACS. AX09 or control immune sera (1:50) were incubated with HCC-1806 derived tumorspheres. Single cell suspensions were generated and FITC conjugated anti-mouse IgG was used to detect antibodies bound to the cells.



AX09 Inhibits Lung Metastases: Preventative Model



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 enumerated. Metastatic lesions were counted from at least two H&E stained lung sections per animal. Each dot represents the average of an individual animal and is combined from two (A) or four independent experiments (B). Two-tailed student's *t*-test was used to determine significance. A p-value < 0.05 is considered significant.

AX09 Inhibits Lung Metastases: Therapeutic Model

(A) Passage 1 tumorspheres derived from the triple negative breast cancer cell line 4T1 were injected into the inguinal mammary fat pad. When tumors reached 1.5-2 mm, mice were immunized with AX09 (loop display) or Q-beta AX09 (linear display) and boosted 14 days later. 2 weeks after boost, animals were euthanized and lungs were removed, sectioned, and the number of metastatic foci were enumerated. Metastatic lesions were counted from at least two H&E stained lung sections per animal. Each dot represents the average of an individual animal. (B) An additional cohort of mice were also given a single i.v. administration of doxorubicin (10mg/Kg) at the time of the first immunization. Lung metastases were evaluated as in A.

Conclusions

A VLP based therapeutic candidate (AX09) represents the best approach to target xCT because:

- Antibodies have been shown to inhibit xCT activity
- AX09 induces high titer oligo antibody responses
- AX09 stimulates broad immune responses (e.g. T cells, NK)
- AX09 has anti-tumor activity *in vitro* and *in vivo*
- AX09 is based on a well established VLP platform/technology that has shown efficacy and safety in the clinic