BriaVax Regressions in Metastatic Breast Cancer: Preliminary Results and Description of Pending Clinical Trial

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Abstract

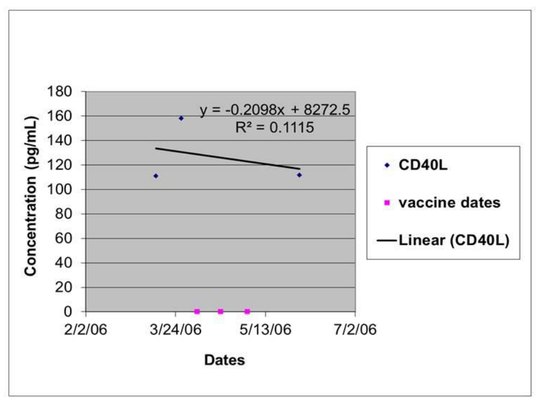
BriaVax (formerly SV-BR-GM-1) is a GM-CSF transfected human breast cancer cell line established in our laboratory. The cell line is ER/PR negative, HER2/neu very strong. We treated 4 patients under FDA protocol BB-IND 10312. Patients received 20,000 viable cells intra-dermally at 4 sites 48-72 hours after cyclophosphamide 300 mg/m^2. Interferon-α 5,000 U was injected into each inoculation site after 48 and 96 hrs. Inoculations occurred q2weeks x 3, then monthly x3. One patient progressed after 5 inoculations, one had a remarkable PR as below; median OS 35 mo. Patient A002 enjoyed a prompt and near-complete tumor regression of breast and lung metastases after only 3 inoculations, but relapsed widely 3 months after finishing the 6th and final injection per protocol. After FDA permission was obtained, vaccinations resumed; all sites of metastases, including CNS, again showed prompt regression. Cytokine assays on serial bleeds evaluated possible changes of IL-2, IL-2r, IL-5, IL-6, IL-12, CD40L and total IgG. Pt A002 was found to have a consistent increase in CD40L while receiving the 1st series of inoculations, R2=0.7623, and similar increase, R2=0.5619, for the 2nd. No other patients showed such changes in CD40L, and no other cytokines provided similar correlation. Also, immune monitoring using DTH responses, antibody assays, and screening for *in vitro* cell mediated immune responses were unrevealing. The protocol, on hold for logistic reasons, is now being reactivated.

Demographics

ID #	Age	ETHNICITY	PARITY	ECOG	MENOPAUSE	TNM Stage	Her2/neu	ER/PR	Sites of Disease
A001	73	Hispanic	G3P1AB2	1	Yes	T1N0M0	+	Neg/Neg	Chest wall, Axilla
A002	58	Caucasian	G4P3AB1	1	Yes	T2N3M1	-	ER+/PR+	Breast, Lung
A003*	74	Caucasian	G2P2	1	Yes	T4	-	NA.	Pelvis
B001	61	Caucasian	NA	2	Yes	T2NXM1	-	ER+/PR+	Rib cage, Humerus, Hip, Femur

*Ovarian Cancer Patient

Patient # B001 (Non-Responder): CD40L Levels in Serum at Various Time Points of Vaccine



Rationale

A pilot study of a genetically-engineered allogeneic whole-cell vaccine (BriaVax, previously known as SV-Br1-GM) demonstrated striking regressions in a metastatic breast cancer patient.

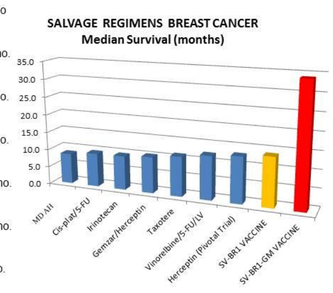
The responding patient had to cease therapy after 6 vaccines according to Phase I protocol, but then was again treated when she relapsed 90 days later, a unique experimental opportunity to demonstrate an impact of vaccine therapy.

Analysis of immunological surrogates identified a strong inverse correlation between tumor size and serum levels of CD40L.

This program is being reactivated, awaiting FDA approval.

Median OS of Prototype and Initial Genetic-Modified Vaccine Compared with Other Phase I Studies

AUTHOR	REGIMEN	N	Pre-RX CRITERIA	MED OS
Wheeler (2009)	MD Anderson Phase I	92	"Phase I Trial"	6.7 mo
O'Shaugnessy (2002)	Gemzar/Herceptin	38	3 Regimens	10.2 mo.
Perez (2004)	Irinotecan	51	Anthracycline &/or Taxane	9.6 mo.
Kucek (1999)	Cis-plat/5-FU	36	One or more previous Rx	9.5 mo.
Razis (2004)	Vinorelbine/5-FU/LV	35	Anthracycline and Taxane	12.3 mo.
Brodowicz (2000)	Docetaxol	19	One or more Anthracycline	11.2 mo.
Baselga (2001)	Herceptin (Pivotal Trial)	222	One or Two previous Rx	13 mo.
Wiseman (2009)	Cell Line Vaccine/GMCSF	14	Anthracycline &/or Taxane	12.1mo
	Genetically Engineered Vaccine	4	Anthracycline &/or Taxane	35 mo



Summary of Findings

- **Patient A002 had ~90% regression of tumors in the first series of vaccinations. CD40L but not other cytokines rose consistently.**
- **At relapse, CD40L levels had returned to pretreatment levels**
- **Revaccination was associated with regression of tumors and consistent rise in CD40L levels.**
- **Correlation coefficients in the two vaccination series were high in each case, R^2 = 0.76, 0.56.**

What is BriaVax? (BB-IND 10312)

- A therapeutic breast cancer whole-cell vaccine gene-transfected to produce GM-CSF
- Previous clinical trial with SV-BR1 unmodified cell-line show 12.1 month Median Survival in 14 Stage IV refractory patients
- Pre-vaccine treatment with low-dose cyclophosphamide
- Post-vaccine treatment with Interferon-α to boost dendritic cell activity

BriaVax (GEV) *in vitro* Studies

- **Circulating Antibody-** Modest (one titer) increase in responder
- **Cell Mediated Immunity-**
  - Delayed type hypersensitivity- Modest (2 mm) induration in responder
  - CD4+ mitogenic response (FACS) to tumor vaccine cells- **Not seen**
- **Circulating Serum Markers-**
  - Biological correlate of response

- **Potential serum markers studied**
  - IL-2
  - IL-2r
  - IL-5
  - IL-6
  - IL-10
  - IL-12
  - CD40L
  - Total IgG

Selected patients also studied for IFNγ, IL-1β, IL-4, IL-13, TNFα

Serum assays performed on serial specimens on **three breast cancer** patients before and at various intervals during vaccine trial

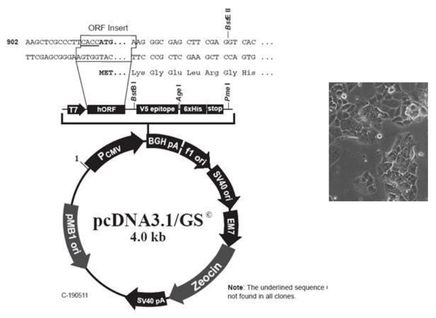
FINDINGS:

- CD40L levels increased consistently with vaccine injection, but only in the responding patient;
- No significant changes for other patients or other serum factors

Significance of CD40L

- **CD40L is transiently expressed by activated T cells, B cells and platelets, and variably induced on monocytic cells, NK cells, mast cells and basophils**
- **CD40L is one of the strongest natural stimulants for immune response, enhancing dendritic cell maturation, CD4+, CD8+, and NK cell activity**
- **CD40/CD40L pathway is critical for the development of protective anti-tumor immunity, provides a critical initial step in the development of humoral (B cells) and cellular immunity (T cells)**
- **CD40L down-regulates suppressor cells**
- **CD40L binding on cancer cells may directly mediate cytotoxic effects**
- **CD40L serum level is elevated in many cancers**
- **CD40L is released with platelet clumping, vascular disease, autoimmune and other inflammatory conditions**
- **CD40L is immunosuppressive in some models; the CD40/CD40L synapse permits tumors to manipulate both T cell and APC compartments, and thus having effects on supporting an immunosuppressive tumor microenvironment**

Characteristics of Vaccine



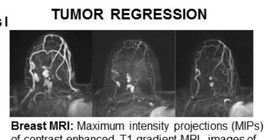
- ER/PR Negative
- Her2/NEU ++++
- Keratin +
- β-actin (PCR) +
- GM-CSF Secretes
- QA/QC

Plasmid Used for GM-CSF Gene Construct

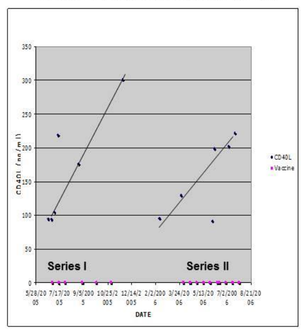
Induction and Re-induction Tumor Response in PT #A002 (Clinical Responder)

Series I

TUMOR REGRESSION

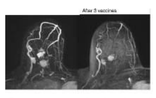


SERUM CONCENTRATION, CD40L



Breast MRI: Maximum intensity projections (MIPs) of contrast-enhanced T1 gradient MRI images of the right breast. **Left Image:** Baseline pre-vaccine. **Middle Image:** After 3rd vaccination (2 months); Note the regression of multiple lesions and reduced vascularity; **Right Image:** After 6th vaccination (5 months); Note the consistent decrease in number and size of masses.

Series II

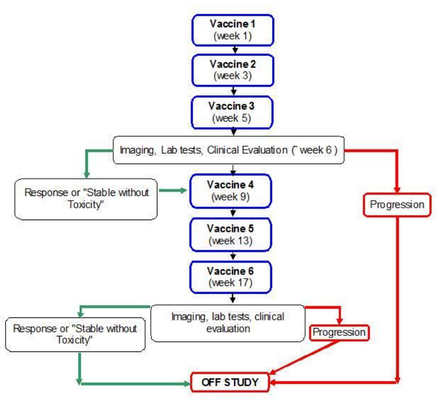


Left Image: Breast pre-vaccination baseline study showing relapse. **Right Image:** After 3rdvaccination (2 months). Note the diminution in size and number of lesions.

Conclusions

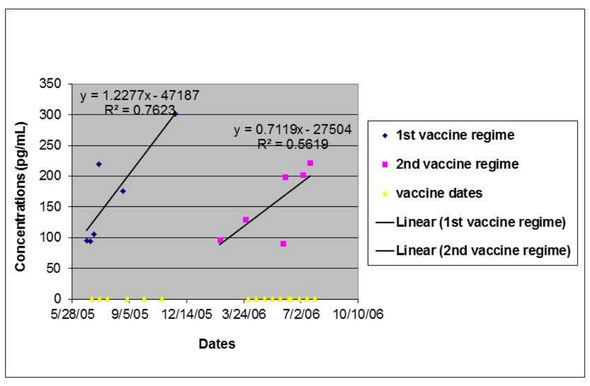
- **BriaVax vaccinations were associated with repeated regression of advanced metastatic breast cancer.** These findings are inconsistent with the notion that vaccines are inactive except in cases with very low, subclinical tumor burden. Confirmation studies must be a high priority.
- **Serum CD40L may be a potential surrogate marker of vaccine effect.** CD40L may be important in protective immune responses. Mackey's study (Mackey et al. Cancer Res. 1997) showed that inhibiting CD40L produced lowered protective and therapeutic effects in an experimental vaccine.
- **While closely correlated, in the present study, it is not clear if the increase of serum CD40L was involved in the mechanism of tumor regression**
- **We hypothesize that the action of BriaVax may be unique.** Unlike most other reported clinical trials, BriaVax showed efficacy in advanced disease, a very rapid response (2 months after starting), a rapid relapse (3 months after stopping), and a paucity of finding other humoral or cellular immune changes, the CD40L changes notwithstanding.

Clinical Trial Schema



Note: Grade III hypersensitivity or Grade IV toxicity at ANY step will warrant special review

Patient # A002 (Responder): CD40L Levels in Serum at Various Time Points of Vaccine (Detail)



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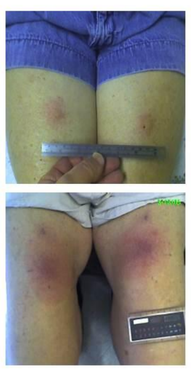
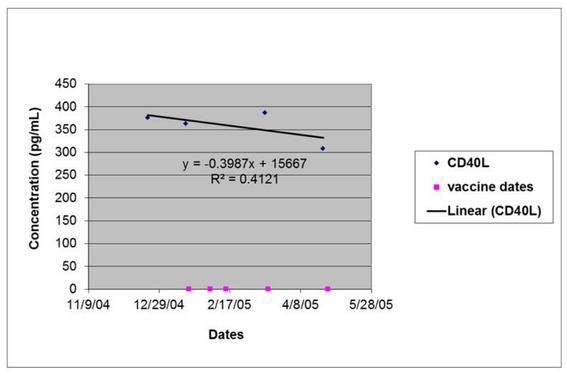
Vaccine Injection

- Intradermal injection into thighs and lower-abdomen
- Observe 20 min
- Document initial wheal and subsequent erythema and wheal
- Cutaneous reaction NOT considered hypersensitivity
- Local inflammation normalized <2 weeks even in the severe cases



Patient # A001 (Non-Responder): CD40L Levels in Serum at Various Time Points of Vaccine



BRIACELL
Immunotherapy approaches to breast cancer management