Phase I Trial of alpha-lactalbumin vaccine in high risk operable triple negative breast cancer (TNBC) and patients at high genetic risk for TNBC

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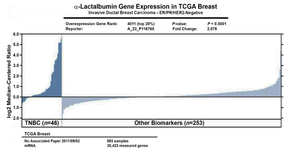
Abstract

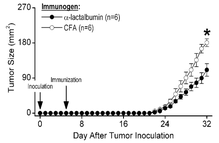
Background: Triple-negative breast cancer (TNBC) has a poor prognosis and may be associated with germline mutations. α-Lactalbumin (aLA) is expressed in lactating breasts but not at other times or in other tissues. Expression of aLA is found in 70% of TNBC (PMID: 27322324) so could be an immunologic target for TNBC based on the "retired protein hypothesis" (PMID: 31926646). In pre-clinical studies, vaccination with aLA provided protection from development of autochthonous tumors in transgenic murine models of breast cancer and inhibited growth of established 4T1 transplantable breast tumors in BALB/c mice (PMID: 20512124). **Methods:** To determine the safety and immunogenicity of aLA, patients with early stage TNBC are being entered in a Phase I trial of aLA with GMP-grade zymosan adjuvant in Montanide ISA 51 VG vehicle. Subjects receive 3 vaccinations given once every 2 weeks. Events of Common Terminology Criteria for Adverse Events (CTCAE) Grade ≥ 2 are considered dose-limiting toxicities (DLTs). **Results:** CTCAE toxicity by dose level is summarized below. All DLTs were injection site reactions, with ulceration and need for incisional drainage representing the Grade 3 events. 12 of 16 patients assayed to date have met protocol specified definitions of an immune response based on ELISpot assays to determine frequencies of T cells producing IFNγ or IL-17 in response to recombinant aLA. **Conclusion:** Dose level 1 appears to be the maximum tolerated dose. Based on immune response, additional intermediate dose levels will be studied. An additional cohort of patients receiving concurrent anti-PD1 treatment is being accrued. Accrual of patients with BRCA1/2 or PALB2 mutations planning to undergo prophylactic mastectomy is beginning in order to define the toxicity and immunologic effects in this group and to determine whether inflammatory changes from occult lactational foci will be produced.

Funding Source: Department of Defense (W81XWH-17-1-0592 and W81XWH-17-1-0593)

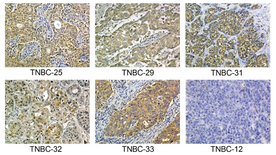
Background



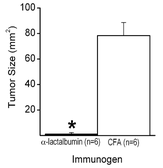
- α-lactalbumin normally expressed only in lactating breast tissue
- Oncomine database search of TCGA shows overexpression of α-lactalbumin gene in TNBC *vs.* other breast cancers



Inhibition of growth of 4T1 tumor growth with α-lactalbumin immunization 5 days after tumor inoculation (*P < 0.01).
Nat Med PMID: 20512124



Immunohistochemical detection of α-lactalbumin protein in parenchyma of human TNBC tumors. 5/6 (83%) showed reactivity ranging from weak (TNBC-32) to moderate (TNBC-33).
Cancers PMID: 27322324



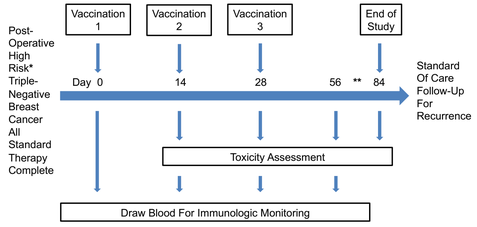
Growth of autochthonous breast tumors in 10-month-old MMTV-*neu* mice immunized with α-lactalbumin at 8 weeks of age (*P = 0.0004).
Nat Med PMID: 20512124

Study Design

Subject Eligibility

- Pathologic Stage IIA-IIIC or residual disease following neo-adjuvant chemotherapy
- Completed all standard therapy
- Within 3 years of initial therapy for triple-negative breast cancer
- No evidence of recurrence or metastasis on restaging scans
- Normal serum prolactin and no prolactin-raising medications
- Adequate major organ function
- Performance Status 0-1

Trial Schema



*Stage IIA-IIIC or residual disease following neo-adjuvant chemotherapy
**Subjects in the Prevention Cohort will have prophylactic mastectomy between days 57 and 84

Results

Patient Characteristics

Subject ID	Age	Race	Reproductive History	Breast Fed?	TNM	Stage	Treatment Regimen	Prior Treatment -> Vaccine (d)
001	44	White	G3 P3 A0 L3	No	T1 N0 M0	IIA	AC-T	862
002	66	White	G3 P2 A1 L2	No	T2 N2 M0	IIIC	AC-TCb	735
003	65	White	G2 P2 A0 L2	No	T2 N0 M0	IIA	AC	478
018	57	White	G1 P1 A0 L1	No	T1 N1 M0	III	AC-T Abraxane Xeloda	646
019	62	White	G4 P4 A0 L4	Yes	T3 N3 M0	IIIC	TC-AC Pembro Xeloda	305
020	61	White	G3 P2 A0 L2	No	T1 N0 M0	IB	TC-AC Xeloda	171
007	71	White	G5 P5 A1 L4	Yes	T1 N1 M0	IIB	AC-T Xeloda	348
009	57	Asian	G0 P0 A0 L0	N/A	T2 N0 M0	IIB	AC-T	515
010	70	White	G1 P1 A0 L1	No	T2 N0 M0	IIA	AC-T	467
014	33	White	G0 P0 A0 L0	N/A	T2 N0 M0	IIIA	AC-T Xeloda	280
015	53	Afr. Am.	G2 P2 A0 L2	Yes	T1 N1 M0	IIIA	AC-T Xeloda	30
016	61	White	G2 P2 A0 L2	No	T1 N0 MX	IIIA	AC-TCb Xeloda	671
011	71	White	G2 P1 A1 L1	No	T3 N0 M0	IIB	AC-TCb Xeloda	753
012	45	White	G2 P2 A0 L2	Yes	T2 N1 M0	IIB	TC-AC Pembro	41
013	59	White	G1 P1 A0 L1	No	T2 N1 M0	IIB	AC-T Xeloda	172
004	50	White	G5 P3 A2 L3	Yes	T2 N2 M0	IIIB	AC-T Xeloda	43

Dose Levels of α-lactalbumin/zymosan (mcg) are color-coded: 10/10; 100/10; 500/10; 100/100
Treatment: A, adriamycin; C, cyclophosphamide; T, taxol/taxotere; cb, carboplatin

Safety: Worst Toxicity by Dose Level

Dose Level	Alpha-Lac dose (mcg)	Zymosan dose (mcg)	n Patients	n Grade 1	n Grade 2	n Grade 3
1	10	10	6	6		
2	100	10	6	5		1*
3	500	10	3	1**		2
Original 2	100	100	1			1***

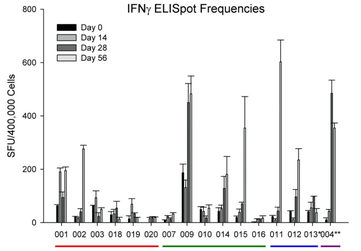
*Grade 3 after recurrence and pembrolizumab (reviewed with DSMC)

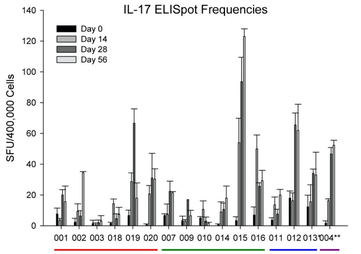
**3rd dose reduced in one subject after a Grade 3 event in a different subject at that dose level

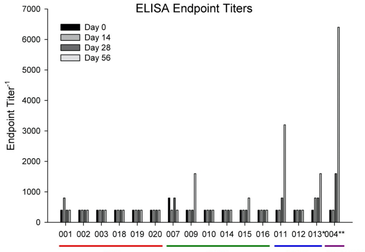
***3rd dose held

Results

Immunologic Assessment







Dose Level	DL1	DL2	DL3	DL2 (old)
Zymosan	10 μg	10 μg	10 μg	100 μg
α-Lactalbumin	10 μg	100 μg	500 μg	100 μg

Immunologic responses from trial subjects tested to date. ELISpot frequencies are presented as spot forming units (SFU) per 400,000 PBMCs in culture minus background. ELISA endpoint titers are presented as the greatest dilution at which signal (mean optical density of sample minus background) was reliably detected. In all cases, background wells contained all components except antigen. All data are from individuals coded by subject ID. All error bars represent ±SD.
*Third dose reduced to DL2 due to DLT in cohort; **No third dose administered due to DLT in subject.

Statistical Analysis

Statistical Analysis of IFNγ and IL-17 ELISpot Results by Visit and Dose[1]

Cytokine	Factor	Level	Estimated Mean	95% LCL	95% UCL	*P*-value
IFNγ	Visit	Day 0	29.58	7.03	67.68	0.03
		Day 14	39.93	12.47	82.94	
		Day 28	58.77	23.80	109.29	
		Day 56	102.99	54.17	167.34	
	Dose	1	36.53	7.59	87.09	0.65
		2	60.71	20.28	122.77	
		3	69.61	13.74	168.45	
IL-17	Visit	Day 0	2.43	0.18	7.26	0.0001
		Day 14	12.31	5.62	21.59	
		Day 28	17.03	8.93	27.71	
		Day 56	17.13	9.00	27.84	
	Dose	1	8.20	2.22	17.93	0.57
		2	9.22	2.77	19.42	
		3	16.81	4.69	36.42	

[1]The linear mixed model can account for correlations between measurements over time within the same patient. Due to right skewness, raw data was transformed to square root scale and estimated were back-transformed to raw scale for reporting. *P*-values were calculated by likelihood ratio test. Red font indicates significance

Statistical analysis of the ELISpot responses from trial subjects tested to date. The table to the left shows the results of the statistical analysis of the ELISpot data for all 16 subjects tested. A significant increase over baseline (Day 0) was observed by Day 56 in the IFNγ ELISpot (*P* = 0.03) and by Day 14 in the IL-17 ELISpot (*P* = 0.0001). Although the estimated mean spot frequencies for both cytokines increased with dose, this effect was not significant. No significance was observed across visit or dose in the ELISA data (not shown). The violin plots above illustrate the table data with visits represented by the x-axis and dose levels color coded.

Discussion, Conclusions, and Plans

- Among the doses studied, Dose Level 1 is the maximum tolerated dose (MTD)
- IFNγ and IL-17 ELISpot responses were seen at all dose levels
- IFNγ and IL-17 ELISpot responses were seen in the majority of patients
- A statistically significant increase over baseline with time was observed in both ELISpot assays
- No statistically significant dose response was observed in either ELISpot assay
- Humoral responses by ELISA were detected only at high dose levels
- ELISA results may be due to assay sensitivity limit with plasma diluted ≥ 1:400; will re-test in the 1:50 – 1:400 range
- Dose Level 1 is a usable optimal immunologic dose based on toxicity and IFNγ and IL-17 ELISpot responses, but additional dose levels between DL1 and DL2 will be examined:

Dose Level	Alpha-Lac dose (mcg)	Zymosan dose (mcg)	n Patients
1b	50	10	3-6
1c	50	50	3-6
1d*	50	30	3-6
*If dose level 1c is too toxic			

- 2 new cohorts are being studied:
  1. Patients receiving post-operative pembrolizumab after having been found to have residual disease following neoadjuvant chemo-immunotherapy
  2. BRCA1, BRCA2, and PALB2 carriers planning prophylactic risk-reducing mastectomies.

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Conflict of Interest Statement
The vaccine technology discussed in the abstract and poster has been licensed to Anixa Biosciences, Inc. (San Jose, CA). JMJ is an inventor on issued and pending patents related to the vaccine technology presented here and may earn royalties for such if the vaccine becomes commercially successful. In addition, JMJ have received equity in Anixa Biosciences, Inc. in the form of stock options. The abstract and poster were prepared without any input or coercion whatsoever from the licensee.

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