Abstract Presentation Number: 3035

Phase I trial of alpha-lactalbumin vaccine in high-risk operable triple-negative breast cancer

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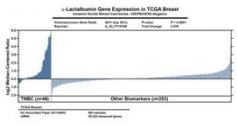
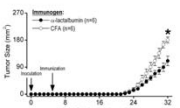
Abstract

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 inhibited growth of established breast tumors and provided protection from development of autochthonous tumors in transgenic murine models of breast cancer and against 4T1 transplantable breast cancer 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. ELISpot assays to determine frequencies of T cells producing IFNγ and IL-17 in response to recombinant aLA and ELISA assays of antibody response to aLA will be available in December 2022.
Conclusion: Dose level 2 appears to be the maximum tolerated dose. Accrual to dose levels 1 and 2 will be expanded to further define toxicity and immunologic effects. Accrual of patients with BRCA1 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.

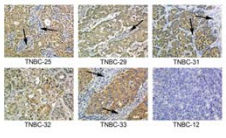
Introduction



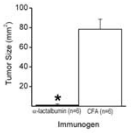
- α-lactalbumin normally expressed only in lactating breast tissue
- Oncomine database search of TCGA shows overexpression of α-lactalbumin gene in TNBC *vs.* other breast cancers



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). Arrows show no immunostaining in tumor stroma.
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

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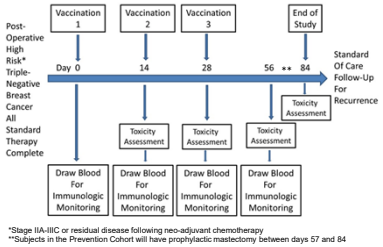
Conflict of Interest Statement
The vaccine technology discussed in the abstract and poster has been licensed to Anixa Biosciences, Inc. (San Jose, CA). VKT and JMJ are the inventors on issued and pending patents related to the vaccine technology discussed in this manuscript and may earn royalties for such if the vaccine becomes commercially successful. In addition, VKT and 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.

Methods and Materials



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

Endpoints:

- Maximum Tolerated Dose (MTD)
- Lowest Immunologic Dose:
  The lowest dose producing significant increases in IFNγ and IL-17 in response to α-lactalbumin by ELISpot assay
- Optimal Immunologic Dose (Recommended for Phase II):
  The lowest tolerated dose producing a maximal immunologic response

Dose Levels

Dose Level	α-Lactalbumin	Zymosan	Notes
1	10 μg	10 μg	
2	100 μg	10 μg	
3	500 μg	10 μg	DLT experienced, dose will not be used
Original 2	100 μg	100 μg	DLT experienced, dose will not be used
2b	100 μg	30 μg	
2c	100 μg	60 μg	
2d	200 μg	10 μg	If dose level 2b proves too toxic
2e	200 μg	30 μg	If dose level 2c proves too toxic

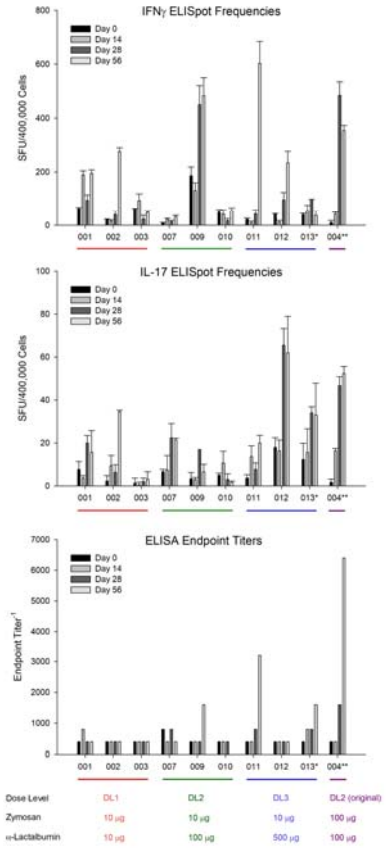
Results

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
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
* Immunologic studies pending

Results



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.

Results

Worst Toxicity by Dose Level

Dose Level	α-Lac dose (mcg)	Zymosan dose (mcg)	Cumulative Number				
			Patients	Grade 0	Grade 1	Grade 2	Grade 3
1	10	10	3		3		
2	100	10	6		6		
3	500	10	3		2		1
Original 2	100	100	1				1

Toxicity has consisted predominantly of injection site reactions characterized by erythema, swelling, lump formation, pruritis, and in severe cases ulceration with delayed healing.

Discussion, Conclusions, and Plans

- Varying degrees of antigen-specific T cell responses were observed at all dose levels
- Per protocol, dose levels 1 and 2 are being expanded to 6 subjects each
- Based on current data, dose level 2 appears to be the maximum tolerated dose
- Additional dose levels will be explored
- Dose expansion cohorts in BRCA1/PALB2 carriers planning to undergo prophylactic bilateral mastectomy have opened
- Dose expansion cohort with concurrent pembrolizumab in the adjuvant setting is planned

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