Poster No. 7999

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: α-Lactalbumin (aLA) is expressed in lactating breasts and 70% of triple-negative breast cancer (TNBC) but not at other times or in other tissues.[1] Based on the "retired protein hypothesis"[2] 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.[3]

Methods: We are performing a Phase I trial of recombinant human aLA with GMP-grade zymosan adjuvant in Montanide ISA 51 VG vehicle in 3 cohorts of subjects: Ia) patients with high-risk TNBC who have completed all standard treatment; Ic) patients with TNBC who have neoadjuvant chemotherapy and are receiving post-operative treatment with pembrolizumab +/- capecitabine; and Ib) patients with BRCA1, BRCA2, or PALB2 mutations who are undergoing risk-reducing mastectomies. Three vaccinations are given once every 2 weeks. Events of Common Terminology Criteria for Adverse Events (CTCAE) Grade ≥ 2 are considered dose-limiting toxicities (DLTs).

Results: We have vaccinated 21 patients in Group Ia, 3 in Group Ic, and 2 in Group Ib (NCT04674306). CTCAE toxicity by dose level (DL) is summarized in Results (Table 1) by grade for each study cohort. All DLTs were injection site reactions, with ulceration and need for incisional drainage representing the Grade 3 events. 19 of 26 patients assayed to date across all cohorts met protocol specified definitions of an immune response based on ELISPOT assays (Figure 2) to determine frequencies of T cells producing IFNγ and/or IL-17 in response to recombinant aLA, including 4 of 6 subjects at DL1.

Conclusions: DL1 is the maximum tolerated dose (MTD) to date and produces an immune response in most patients. The aLA vaccine given at DL1 has been tolerable to date when given alone or concurrently with pembrolizumab in patients treated for TNBC, and when given to healthy patients undergoing elective prophylactic mastectomy. Immune data in these new cohorts are reported here. Data from this trial will be insufficient to assess clinical efficacy and will be explored in Phase II.

References:

1. Tuohy, VK, et al., Cancers (Basel). 2016 Jun 16;8(6):56. doi: 10.3390/cancers8060056
2. Tuohy, VK, et al., Semin Immunol. 2020 Feb;47:101392. doi: 10.1016/j.smim.2020.101392
3. Jaini, R, et al., Nat Med. 2010 Jul;16(7):799-803. doi: 10.1038/nm.2161

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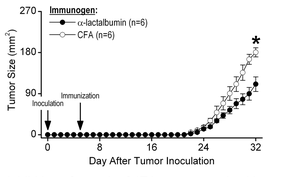
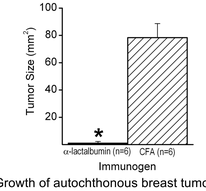
Background

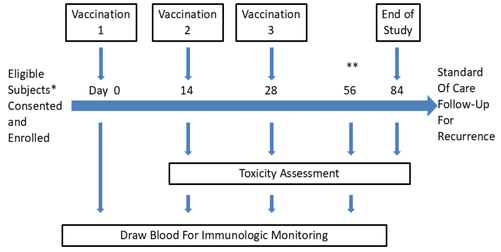


Inhibition of growth of 4T1 tumor growth with α-lactalbumin immunization 5 days after tumor inoculation (*P < 0.01).
Nat Med PMID: 20512124



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



*Stage IIA-IIIC or residual disease following neo-adjuvant chemotherapy in the TNBC cohort

**Subjects in the Prevention Cohort will have prophylactic mastectomy between days 57 and 84

Key Eligibility Criteria

TNBC Cohort (Ia)
- Pathologic Stage IIA-IIIC or residual disease following neo-adjuvant chemotherapy
- Completed all standard therapy
- Within 3 years of initial therapy for TNBC
- 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

Prevention Cohort (Ib)
- Have a high risk for developing TNBC, defined as: carrying a deleterious mutation in BRCA1, PALB2, or BRCA2
- Have scheduled risk reducing mastectomy at Cleveland Clinic Main Campus
- Normal serum prolactin and no prolactin-raising medications
- Adequate major organ function
- No current need for immunosuppression or systemic corticosteroid therapy
- No history of any invasive malignancy within the last 5 years

Pembrolizumab Cohort (Ic)
- Histologically proven invasive TNBC
- ≥ 1 months since last active therapy with chemotherapy (excluding Xeloda/capecitabine), radiation therapy, or surgery and at least 6 weeks of pembrolizumab therapy planned after the first dose of alpha-lactalbumin vaccine
- Normal serum prolactin and no prolactin-raising medications
- Adequate major organ function

Results

Figure 1. Subject Demographics by Study Phase

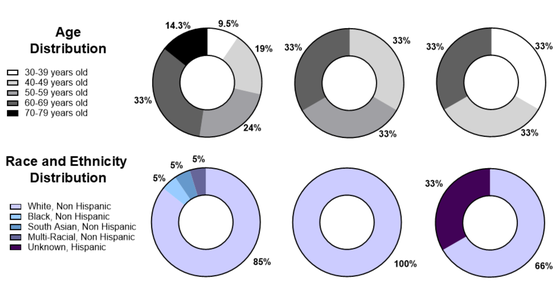


Table 1. Prevalence of Injection Site Reaction by Study Phase, Dose Level and CTCAE v5 Grade.

Group	Dose Level	aLA Dose (mcg)	Zymosan Dose (mcg)	Total Enrolled Subjects	Number of Subjects				
					G1	G2	G3	G4	G5
Ia	1	10	10	6	6				
	1b	50	10	5	3	1	1		
	2	100	10	6	5		1		
	3	500	10	3	1		2		
	2 (old)	100	100	1			1		
Ib	1	10	10	2	2				
Ic	1	10	10	3	3				

Immunologic Assessment Results

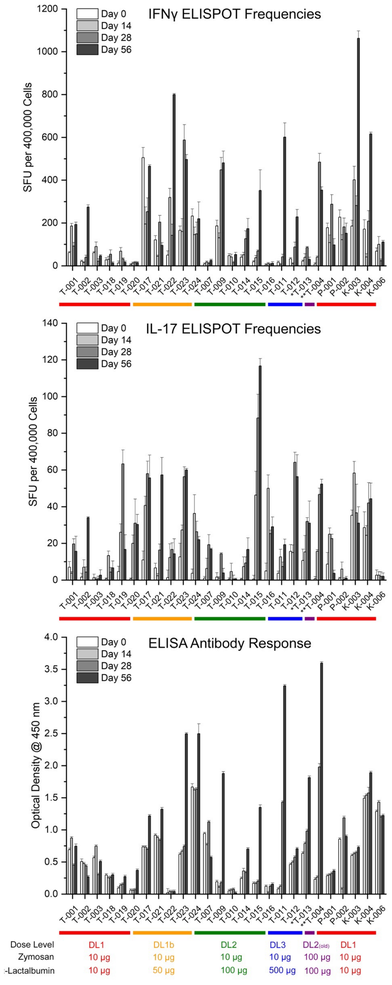


Figure 2. Immunologic responses from trial subjects tested to date (n=26). In the figure to the left, ELISPOT frequencies are presented as spot forming units (SFU) per 400,000 PBMCs in culture minus background. ELISA antibody response is presented as the optical density at 450 nm of alpha-lactalbumin specific IgG wells minus background at a plasma dilution of 1:400. In all cases, background wells contained all components except antigen. All data are from individuals coded by subject ID. Subject ID prefix T = Phase Ia (TNBC); P = Phase Ib (prevention); K = Phase Ic (Keytruda/pembrolizumab). All error bars represent ±SD.

*Third dose reduced to DL2 due to DLT in cohort
**No third dose administered due to DLT in subject.

Table 2. Statistical analysis of the ELISPOT and ELISA responses from trial subjects tested to date (n=26). The table the the right shows the results of the statistical analysis of the ELISPOT data for all 26 subjects tested. A significant increase over baseline (Day 0) was observed by Day 56 in the IFNγ ELISPOT (P = 0.04) and by Day 14 in the IL-17 ELISPOT (P < 0.0001). The IgG response by ELISA in plasma at 1:400 dilution was also significant by Day 56 (P = 0.03). No significance was observed by dose in any assay. The analysis includes data from all subjects across all three cohorts (Ia, Ib, and Ic) by dose. The linear mixed model used 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. Dose Level 2 (old) is excluded from analysis due to only one subject treated. P-values were calculated by likelihood ratio test. Red font indicates significance.

Statistical Analysis
ELISPOT and ELISA Results by Visit and Dose

Assay	Factor	Level	Estimated Mean	95% LCL	95% UCL	P-value
IFNγ ELISPOT	Visit	Day 0	55.17	8.22	143.71	0.04
		Day 14	55.79	8.31	145.36	
		Day 28	91.01	24.45	199.82	
		Day 56	131.15	46.95	257.67	
	Dose	1	74.71	15.51	178.18	0.97
		1b	77.97	7.45	222.90	
		2	93.76	17.95	228.87	
		3	76.26	2.47	252.61	
IL-17 ELISPOT	Visit	Day 0	4.85	0.83	12.21	<0.0001
		Day 14	15.74	7.15	27.67	
		Day 28	19.89	10.02	33.09	
		Day 56	20.16	10.22	33.44	
	Dose	1	8.57	2.32	18.78	0.27
		1b	18.58	5.22	40.16	
		2	9.42	1.73	23.26	
		3	23.13	5.67	52.39	
ELISA	Visit	Day 0	0.384	0.172	0.681	0.03
		Day 14	0.377	0.167	0.671	
		Day 28	0.452	0.218	0.770	
		Day 56	0.642	0.356	1.013	
	Dose	1	0.361	0.118	0.737	0.06
		1b	0.826	0.279	1.664	
		2	0.080	0.004	0.397	
		3	0.839	0.180	1.979	

Discussion, Conclusions, and Plans

- Among the doses studied, Dose Level 1 (DL1) is the maximum tolerated dose (MTD)
- IFNγ and/or IL-17 ELISPOT cellular immune responses were seen at all dose levels, including Dose Level (DL1)[1]
- IFNγ and/or IL-17 ELISPOT cellular immune responses were seen in the majority (73%) of patients[1]
- A statistically significant increase over baseline with time was observed in ELISPOT assays for both cytokines IFNγ and IL-17 as well as for antibody response in ELISA
- No statistically significant dose response was observed in any assay
- ELISA results may be underrepresented due to assay sensitivity limit with plasma diluted ≥ 1:400; plans to 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 DL1b will be examined

[1]The clinical protocol defines an antigen-specific immune response as the post-treatment development of ≥ 1/30,000 IFNγ-secreting (Type 1) or IL-17 secreting (Type 17) T cells in the peripheral blood monocytes in response to α-lactalbumin. If this level of response is present prior to therapy, a 3-fold increase of IFNγ-secreting (Type 1) or IL-17 secreting (Type 17) T cells in the peripheral blood monocytes will be an immunologic response.

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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.