PS4-06-19

Final results of a Phase I trial of an alpha-lactalbumin (aLA) vaccine for breast cancer

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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. Based on the "retired protein hypothesis" 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. Methods: We completed accrual to 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; Ib) patients with BRCA1, BRCA2, or PALB2 mutations who are undergoing risk-reducing mastectomies; and Ic) patients with TNBC who have residual cancer after primary chemo-immunotherapy and are receiving post-operative treatment with pembrolizumab +/- capecitabine. Three vaccinations were given once every 2 weeks. Events of Common Terminology Criteria for Adverse Events (CTCAE) Grade ≥ 2 are considered dose-limiting toxicities (DLTs). Blood was drawn prior to therapy as well as 14, 28, and 56 days after the first vaccination to assess cellular response using enzyme-linked immunosorbent spot (ELISPOT) assays of interferon-gamma and interleukin-17 production in response to aLA and for humoral response by enzyme-linked immunosorbent assay (ELISA). The breast tissue of participants in the Phase Ib cohort were or will be examined for occult lactational foci and inflammatory changes. Results: We vaccinated 26 patients in Cohort Ia, 4 in Cohort Ib, and 5 in Cohort Ic. CTCAE toxicity by dose level (DL) is summarized in Table 2 by grade for each study cohort. All adverse events (AEs) were injection site reactions, with ulceration and need for incisional drainage representing the Grade 3 DLT events. 26 of 35 patients (74%) across all cohorts met protocol specified definitions of an immune response based on ELISPOT assays that quantify frequencies of T cells producing IFNγ or IL-17 in response to recombinant aLA; these data included 4 of 6 subjects in Cohort Ia at DL1 and 10 of 15 subjects (67%) in all cohorts treated at DL1. Conclusions: DL1 is the maximum tolerated dose (MTD) by protocol definition, produced at worst Grade 1 toxicity in a total of 13 of 15 subjects, and produced an immune response in most patients, based on the criteria prospectively defined in the study protocol. DL1e was tolerable in 5 of 5 subjects but was not determined the MTD per our 3+3 trial design. Immune data for all subjects in the trial, including new data across all cohorts, are reported here and will inform the design of subsequent Phase II trials. Funding Source: Department of Defense (W81XWH-17-1-0592 and W81XWH-17-1-0593).

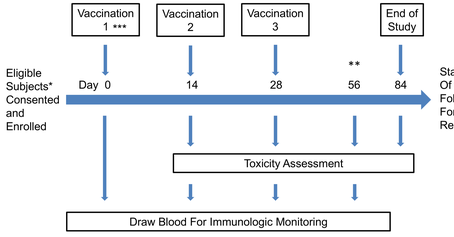
Key Eligibility Criteria And Study Design

All Cohorts
- Normal serum prolactin and no prolactin-raising medications
- No lactation within 6 months of study start
- Adequate major organ function
- Performance Status 0-1
- No other invasive cancer for five years
- No immunosuppressive therapy or steroids
- ≥ 4 weeks since prior chemotherapy or radiation (excluding capecitabine in cohort Ic)

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 and not diagnosed while pregnant
- No evidence of recurrence or metastasis on restaging scans

Prevention Cohort (Ib)
- Have a high risk for developing TNBC, defined as: carrying a deleterious mutation in BRCA1, BRCA2, or PALB2
- Have scheduled risk reducing mastectomy at Cleveland Clinic Main Campus
- 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 and not diagnosed while pregnant
- ≥ 1 month since last active therapy with chemotherapy (except capecitabine), radiation therapy, or surgery and at least 6 weeks of pembrolizumab therapy planned after the first dose of alpha-lactalbumin vaccine
- Residual invasive cancer after neoadjuvant chemotherapy + pembrolizumab



*Stage IIA-IIIC (cohort Ia) or residual disease following neo-adjuvant chemo- or chemo-immunotherapy (cohorts Ia and Ic)

**Subjects in the Ib cohort had prophylactic mastectomy between days 57 and 84

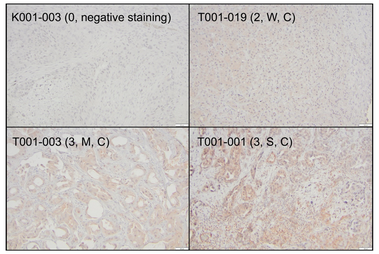
***Vaccination given during post-op pembrolizumab (cohort Ic)

Results

	TNBC Cohort Ia						Prevention Cohort Ib	Pembrolizumab Cohort Ic
	Number of Subjects (% of Total Subjects per Dose Level)							
	Dose Levels (mcg aLA/zymosan)							
	1 (10/10)	1b (50/10)	1e (10/20)	2 (old) (100/100)	2 (100/10)	3 (500/10)	1 (10/10)	1 10/10)
Age								
30-39	-	1 (20)	1 (20)	-	1 (16.7)	-	2 (50)	1 (20)
40-49	1 (16.7)	2 (40)	3 (60)	-	-	1 (33.3)	1 (25)	1 (20)
50-59	1 (16.7)	-	-	1 (100)	2 (33.3)	1 (33.3)	1 (25)	1 (20)
60-69	4 (66.7)	1 (20)	-	-	2 (33.3)	-	-	2 (40)
70-79	-	1 (20)	1 (20)	-	1 (16.7)	1 (33.3)	-	-
Ethnicity								
Hispanic	-	-	-	-	-	-	-	1 (20)
Non-Hispanic	6 (100)	5 (100)	5 (100)	1 (100)	6 (100)	3 (100)	4 (100)	4 (80)
Race								
White	6 (100)	4 (80)	5 (100)	1 (100)	4 (66.7)	3 (100)	4 (100)	4 (80)
Black	-	-	-	-	1 (16.7)	-	-	-
South Asian	-	-	-	-	1 (16.7)	-	-	-
Multi Racial	-	1 (20)	-	-	-	-	-	-
Declined/ Unk.	-	-	-	-	-	-	-	1 (20)

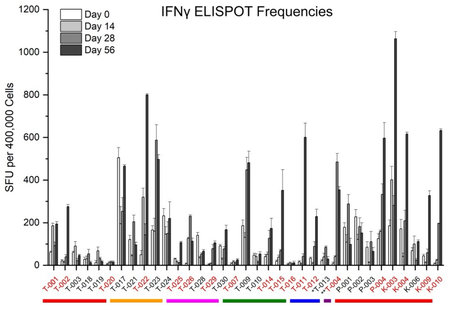
Table 1. Subject demographic summary. Numbers and percentages for all cohorts and dose levels are shown above for all 35 trial subjects.

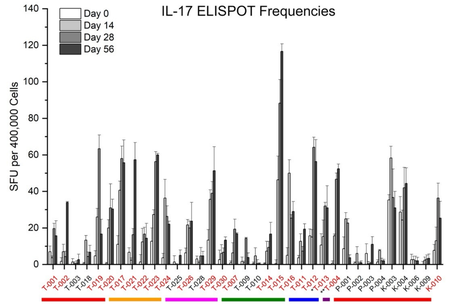
Figure 1. Immunohistochemistry (IHC) of primary tumors of study subjects detects a range of alpha-lactalbumin protein expression. Sections from subjects' primary tumors prior to vaccination were stained for the presence of alpha-lactalbumin protein and were scored by a clinical breast pathologist in a blinded manner according to the following criteria: **% Positivity** as 0 = <1% staining, 1 = 1-33% staining, 2 = 34-66% staining, 3 = 67-100% staining; **Average Intensity** as weak (W), moderate (M), or strong (S); **Localization** as cytoplasmic (C) or nuclear (N). Representative core biopsy sections exhibiting various aLa expression levels are shown to the right with subject codes and scores. Magnification is 200X; scale bars indicate 100 μm. IHC analysis and correlation with immune response and clinical outcomes is ongoing.

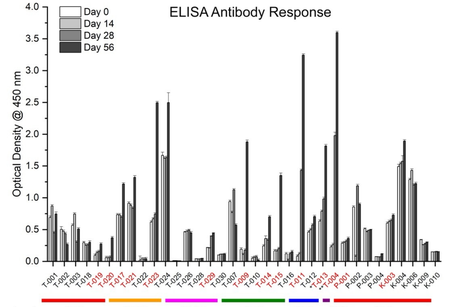


	TNBC Cohort Ia						Prevention Cohort Ib	Pembrolizumab Cohort Ic
	Dose Levels (mcg aLA/zymosan)							
	1 (10/10)	1b (50/10)	1e (10/20)	2 (old) (100/100)	2 (100/10)	3 (500/10)	1 (10/10)	1 10/10)
Total Subjects Enrolled	6	5	5	1	6	3	4	5
Injection Site Reactions, Number of Subjects (%)								
Grade 1	6 (100)	3 (60)	5 (100)	-	5 (83)	-	4 (100)	3 (60)
Grade 2	-	1 (20)	-	-	-	1 (33)	-	-
Grade 3	-	1 (20)	-	1 (100)	1 (17)	2 (66)	-	2 (40)
Grade 4	-	-	-	-	-	-	-	-
Grade 5	-	-	-	-	-	-	-	-

Table 2. Prevalence of injection site reactions by study cohort, dose level, and CTCAE v5 grade. Toxicity consisted predominantly of injection site reactions characterized by erythema, swelling, lump formation, pruritus, and in severe cases ulceration with delayed healing. Injection site reactions accounted for all dose-limiting toxicity (DLT). No other DLTs were observed.







Dose Level	DL1	DL1b	DL1e	DL2	DL3	DL2$_{new}$	DL1
Zymosan	10 μg	10 μg	20 μg	10 μg	10 μg	100 μg	10 μg
α-Lactalbumin	10 μg	50 μg	10 μg	100 μg	500 μg	100 μg	10 μg

Figure 2. Individual immunologic data from all trial subjects (n=35) demonstrates a 74% response rate to the vaccine. 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. Subjects in red font developed prospectively defined immune response which is defined for ELISPOT as ≥ 1 in 30,000 SFU or triple baseline (Day 0) at Day 56. Across the study, 74% of subjects demonstrated a positive response in the IFNγ and/or IL-17 ELISPOT assays. The ELISA is a correlative objective and subjects were deemed positive based on a substantial increase in signal over baseline (Day 0) by Day 56 concurrent with increasing antigen-specific IgG titers over time. *Third dose reduced to DL2 due to DLT in cohort. **No third dose administered due to DLT in subject.

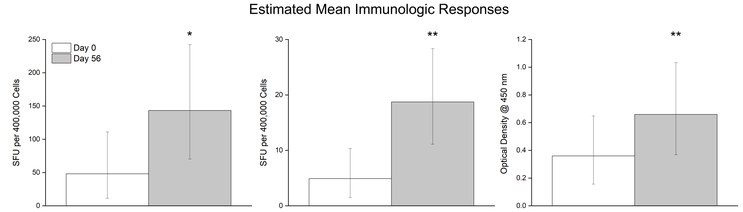


Figure 3. Estimated ELISPOT and ELISA means and statistical analysis of all trial subjects (n=35) demonstrate a significant immunologic response to the vaccine. The figure above shows the results of the statistical analysis of the immunologic data for all 35 subjects across all cohorts and dose levels. A significant increase over baseline (Day 0) was observed by Day 56 in the IFNγ ELISPOT (*P = 0.0001) 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 < 0.0001). No significance was observed by dose level in any assay. The analysis includes data from all subjects across all three cohorts (Ia, Ib, and Ic) and all doses. 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. Batch correction was used in all calculations. Error bars represent 95% confidence intervals. *P = 0.0003 and **P < 0.0001 with Dunnett's adjustment applied.

Discussion, Conclusions, and Plans

- **Among the doses studied, Dose Level 1 (DL1) is the maximum tolerated dose (MTD).**
  - DL1 produced no greater than Grade 1 AEs in all subjects tested except 2 of the 5 in the pembrolizumab cohort.
  - Dose Level 1e (DL1e) was tolerated in 5 of 5 subjects tested and may be further explored for Phase II.
  - All dose-limiting toxicities (DLTs) were injection site reactions; no other DLTs were observed.
- **DL1 is a usable optimal immunologic dose based on toxicity and IFNγ and IL-17 ELISPOT responses in subjects not concurrently treated with pembrolizumab. Toxicity at DL1 with concurrent pembrolizumab might be acceptable for high risk cancer patients.**
- **IFNγ and/or IL-17 ELISPOT cellular immune responses were seen in the majority (74%) of patients.[1]**
  - IFNγ and/or IL-17 ELISPOT cellular immune responses were seen at all dose levels, including DL1.[1]
  - Statistically significant increases over baseline with time were observed in ELISPOT assays for IFNγ and IL-17, and in ELISA.
  - No statistically significant dose response was observed in any assay.
- Immunohistochemistry (IHC) of subjects' primary tumors for alpha-lactalbumin protein revealed a range of expression from absent to strong. Analysis and correlation to immune response and clinical outcomes is ongoing.
- Consenting subjects will be followed for five years after completing the study.
- **All primary study endpoints were met and these results and follow-up data will inform the design of Phase II studies.**

[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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