LB-191

Combination checkpoint inhibitor therapy: Anti-PD1 and Beta-alethine lead to complete responses of melanoma in a syngeneic mouse model

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ABSTRACT

Previously we reported that the combination of anti-PD1 and β-alethine completely stopped cancer growth in a syngeneic mouse melanoma model even under conditions when neither of the drugs had statistically significant effects as a single agent. In order to further examine this dramatic result, we extended the studies, evaluated immune parameters and tested if long-term immunity to re-challenge occurred.

Surprisingly, three low doses of anti-PD1, spread over 9 days, were sufficient to allow concurrent and continuing weekly dosing of β-alethine to not only halt tumor growth, but to reverse it. The established melanoma regressed over the subsequent 4-6 weeks. Even more striking was that the short course of anti-PD1, which failed to reduce cancer growth as a single therapy, altered the immune system such that subsequent treatment with β-alethine was effective. β-alethine therapy, beginning eight days after the conclusion of anti-PD1 therapy, when tumors averaged almost 50 mm², was sufficient to cause at least stabilization of cancer in all animals and complete response (no palpable tumor on repeated measurements) in the majority of animals. Statistical comparisons of all animals receiving combination therapy (either concurrently or sequentially) with controls resulted in significant differences using ANOVA for tumor size ($p = 0.005$) or chi-squared tests for tumor presence ($p < 0.0001$).

No toxicity was noted in treated animals. This is consistent with previous animal and GLP toxicity studies and the completed human Phase I/II trial. The human trial showed that β-alethine, as a single agent, caused no drug-related adverse events and lead to shrinkage or stabilization in all patients with lymphoma who were not anergic to recall antigens pre-trial.

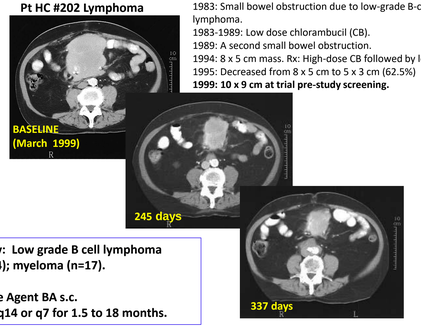
The final result of re-challenging the mice with the original Cloudman melanoma at a higher dose than the initial cancer inoculation level will be presented.

First BA patient, the pet cat "Mr. C"

- A ~13 y.o. male cat with advanced lymphosarcoma who had lost half his weight, was not eating, was hiding in the closet, scheduled for euthanasia. Owner obtained permission for compassionate use administration of beta-alethine.
- Single agent BA, 30 ng/kg *s.c.* q14, 5 doses
- Tumor decreased by 25% every two weeks; not palpable at 8 weeks, severe GI symptoms resolved; fur and skin returned to normal condition, all symptoms abated cat appeared normal and playful. Treatment was discontinued (AMA).
- Tumor recurred 9 weeks after therapy discontinued
- Treated with 60 ng/kg; tumors stopped growing. Lived for a total of 2 years following initial treatment; death appeared to be congestive heart failure & age (estimated at 15 years)
- Appeared on Fox News as part of a story about beta-alethine https://www.youtube.com/watch?v=xzOBvMMMntY

Introduction: HUMAN PHASE I/II

Pt HC #202 Lymphoma

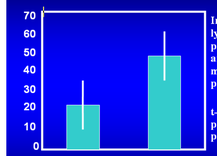


Pt. history
1983: Small bowel obstruction due to low-grade B-cell lymphoma.
1983-1989: Low dose chlorambucil (CB).
1989: A second small bowel obstruction.
1994: 8 x 5 cm mass. Rx: High-dose CB followed by low dose CB.
1995: Decreased from 8 x 5 cm to 5 x 3 cm (62.5%)
1999: 10 x 9 cm at trial pre-study screening.

Study: Low grade B cell lymphoma (n=14); myeloma (n=17).

Single Agent BA s.c.
2 µg q14 or q7 for 1.5 to 18 months.

Percent of Lymphocytes Positive for Surface TNF alpha *in Vivo*



Includes 8 lymphoma patients and 6 myeloma patients.

t-test for pre-post, p=0.006

By the end of the study, all patients had at least 25% of their cells positive for TNF, while only half had more than 4% positive initially.

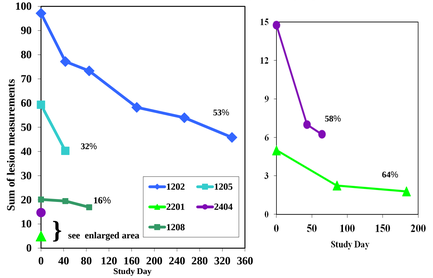
DAY 337: Two tumor lesions disappeared. One remained 1 cm². The fourth decreased by 60%. Overall tumors decrease 53% by bi-dimensional, 75% by volume.

Animal studies indicated a therapeutic index of over 1 million.

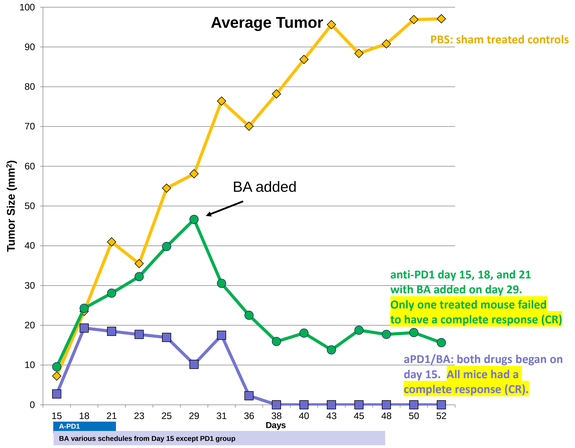
In people, no local or systemic drug related adverse events were observed, although transient neutropenia not clearly related to BA, and likely related to the underlying diseases, occurred in 3 patients.

Individual Human Patients Single Agent Rx

Decreased Tumor in 5 of 9 Immunocompetent Lymphoma Patients



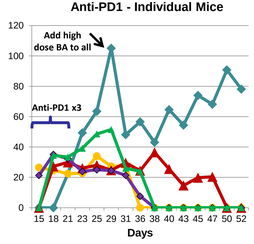
RESULTS

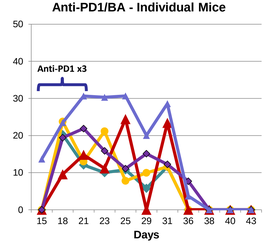


DBA mice were given the syngeneic Cloudman melanoma and treated with either vehicle, β-alethine (BA) weekly or q14 s.c, starting on day 15 and/or anti-PD1 i.p. on days 15, 18, and 21. BA alone was at 30 ng/kg or 30 mg/kg (not shown) and 30 mg/kg weekly with anti-PD1; anti-PD1 was 50 µg/mouse. Weekly BA (30 mg/kg) was added to the aPD1-only group starting on day 29. Both groups of mice receiving aPD1 (with either late or early BA) were rechallenged with Cloudman cells on day 50, two days after the last BA dose.

As single agent therapies, β-alethine (not shown) and anti-PD1 appeared to potentially slow the growth of the melanoma, but the combination was highly synergistic for both tumor elimination and resistance to re-challenge in almost all mice. **The combination differed significantly from PBS starting on day 21, and from aPD1 prior to the introduction of BA ($p \leq .05$, oneway ANOVA).** The aPD1 group similarly had smaller tumors than the PBS group, although not all differences were significant. Groups treated only with BA, at various doses and schedules, had significantly smaller tumors than controls on days 25 and 29 ($p < .05$), but the differences were not significant throughout the study. Overall, the group differences over time was significant (repeated measures ANOVA, $p = 0.007$)

All but one mouse receiving both BA and aPD1 had complete responses, including animals not given BA until day 29 when tumors averaged about 47mm² (chi-squared $p < 0.0001$ for CRs vs Controls).



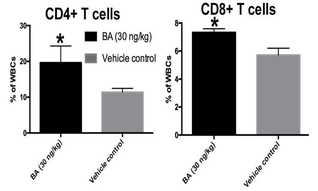


BA ONLY -- Biomarker Studies

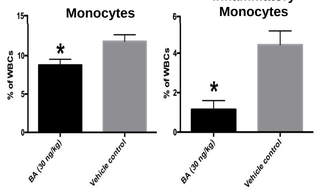
BA Only: % Change in Exhausted T-Cells

	CD4 Cells	CD8 Cells
PD1	-48	- 81
Lag 3	-32	- 94
Tim 3	- 34	- 95

Significant changes in checkpoint molecule expression in T cells 7 days after BA treatment in Cloudman tumor-bearing mice. All differences were at $p = 0.05$ or lower.



Percentages of circulating CD4+ and CD8+ T-cells monocytes in Cloudman tumor bearing mice that were BA treated vs vehicle control treated, 7 days after receiving the first dose of BA.



Percentages of circulating monocytes and inflammatory monocytes in Cloudman tumor bearing mice that were BA treated vs vehicle control treated, 7 days after receiving the first dose of BA.

Re-Challenge Studies

All mice with CR were injected with 3 times as many cells as originally required to establish tumors in 15-20 days. At 60 days post challenge only a single mouse had cancer. The one who did not resist re-challenge was the one who took longest to attain CR.

88% of CRs resisted re-challenge.

CONCLUSIONS

β-alethine (BA) is a small molecule drug that is:
- non-toxic,
- Modulates the immune system
- Examples single agent anti-lymphoma effects in people
- pan checkpoint inhibitor

synergizes with anti-PD1

Complete response (CR) is the typical (90%) response to combination therapy.

Results in Cancer Immunity

FUTURE DIRECTIONS PARTNERSHIPS

Clinical sites and corporate partners are sought for the next stages. Preclinical collaborations are also solicited. FET@FindCure.org

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Increased T cell cytotoxicity by Betathine™-induced upregulation of TNFα

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Abstract

Betathine (BT) is a low molecular weight disulfide that has previously been shown to exhibit in vivo antitumor activity in murine myeloma and melanoma models. We have shown that BT treatment of both human T cells and monocytes is associated with an increase in surface tumor necrosis alpha (TNFalpha) expression. Further, in T cells and monocytes that have been stimulated with PMA and ionomycin, the addition of BT results in a dose and time dependent increase in the percentage of high TNFalpha-expressing cells. Unlike TNFalpha upregulation produced by the commonly used thiol antioxidant N-acetyl-L-cysteine (NAC), the BT-induced increase in TNFalpha is observed consistently in different donors. This increase in surface TNFalpha is associated with elevated levels of TNFalpha mRNA. In addition, expression of TNFalpha receptor I is also significantly enhanced by BT treatment. The upregulation of surface TNFalpha by BT has functional consequences, in that, BT-treated T cells exhibit enhanced cytotoxic activity. Thus, increased TNFalpha expression may be one mechanism responsible for the antineoplastic activity of BT.

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Beta-Alethine Phase I/II Data: Immune Stimulation in Patients with Follicular Lymphoma and Myeloma with

Evidence of Tumor Response and No Significant Toxicity

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