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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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Beta-alethine (BA) stimulates T and B-cell functions and has anti-melanoma, myeloma and breast cancer activity in mice. Human PBMCs in culture respond to BA with a coordinated increase in cytokine mRNAs including TNFα, TNFβ, IFNγ and IL-2; lymphocytes become cytotoxic and armed with surface TNFα. Design: In order to determine safety, efficacy and immune stimulation, 35 pts with low grade B cell lymphoma or myeloma (MM) were administered single agent BA s.c. (2ug) once every 7 or 14 days for up to 18 months at one of 5 sites in Canada or the USA. Pts were evaluated for continuation after 6 injections. Immune system response was evaluated by delayed type hypersensitivity (DTH) to recall antigens and the change in TNFα on the surface of lymphocytes. 15 lymphoma and 17 MM pts are evaluable for safety, 14 lymphoma and 17 MM pts for DTH and tumor responses, and 8 lymphoma and 6 MM pts for TNF effects. Results: No local or drug related systemic toxicity was observed, although transient neutropenia not clearly related to BA occurred in 3 pts. Surface TNFα was increased from baseline to end of study (p=0.006) among both lymphoma and MM pts. 5/10 pts who were anergic pre-study developed DTH after 3 to 5 doses of BA. 5 lymphoma pts had tumor reductions of 16-64%, assessed as sum of 2D cross products measured by CT scan and/or physical exam. Maximal tumor response included decreases of: 64% (after 6 mo. of BA), 58% (at d64), 53% (12 mo.), 32% (d43) and 16% (d85). All 5 responding pts were DTH+ pre-study. The other 4 DTH+ pts had stable disease after 6 injections. In contrast, 3/5 anergic pts had increased disease, and 2/5 were stable. This relationship of pre-study DTH status and tumor response was significant by a Fisher's Exact test (p=.02). Nine MM pts previously had an autologous stem cell transplant, and 5 others had chemotherapy. One MM pt had a 50% reduction in BJ protein; 3 others had stable disease or small decreases for periods of 9-12 months; 13 had increasing paraprotein. Discussion: Drug related toxicity was not seen with this low dose and 2 schedules; future studies may use higher doses. Immune stimulation was observed, including DTH response to infectious disease recall antigens and lymphocyte surface TNFα. Although numbers are small, effectiveness in lymphoma appears greater in pts who start the study with more functional immune systems as determined by pre-study DTH testing. All 9 DTH+ lymphoma pts ended the study with decreases in tumor or stable disease. In contrast, pts anergic pre-study had increased tumor (3/5) or stable disease. While other explanations are possible, these data are consistent with BA modulating the immune system of DTH positive pts in a manner allowing reduction of lymphoma. Similarly, the lack of response to BA in heavily pre-treated myeloma pts may reflect the immunosuppressive effects of high-dose chemotherapy. The observed stimulation of immune responses and anti-lymphoma activity seen in the more immunocompetent patients suggests future trials are warranted.

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Study Design

- Open label, non-randomized Phase I / II

- Eligible patients had:
  i) measurable multiple myeloma with quantifiable M-protein or measurable low grade follicular B-cell lymphoma
  ii) maximal response to one or more chemotherapy regimens, or with indolent disease not yet requiring therapy.
  iii) Performance Status #2

Study Treatment and Endpoints

- A 2 ug SC injection of β-alethine:
  - every 2 weeks (25 patients)
  - weekly (7 patients)

- Patients evaluated for treatment continuation following 6 doses

- Study Endpoints
  - safety
  - test for immune modulation
  - provide early evidence for efficacy

Patients enrolled

- Low Grade B Cell Lymphoma
  - 15 patients enrolled
  - median age = 51.00
  - previous regimens = 0-4

- Multiple Myeloma
  - 17 patients enrolled
  - median age = 56.50
    previous regimens = 0-10 (9 ABMT)

Safety

Preclinical

- MTD in rats ∃200mg/kg i.v. bolus
- MTD in rabbits ∃300mg/kg i.v. infusion
- Therapeutic Dose 30 nanograms/kg
- Therapeutic Index over 1 million (animal data)

Initial Clinical Studies

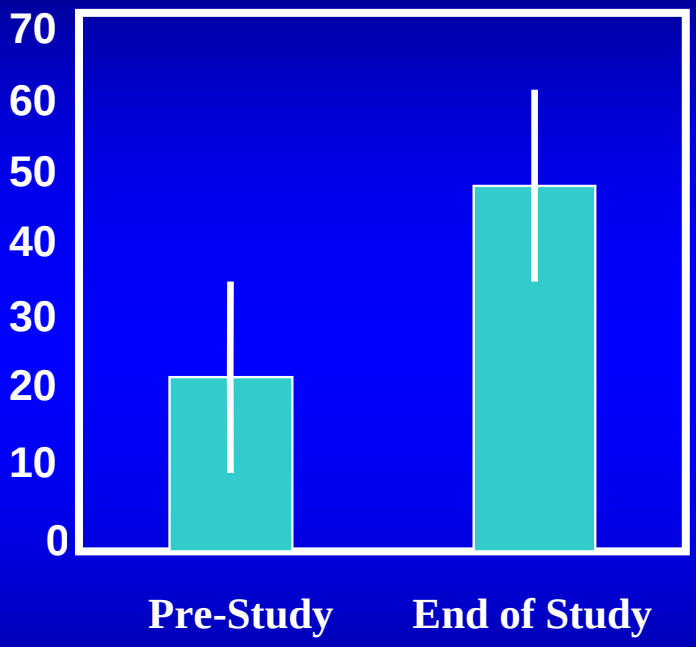
- 32 patients (9 post BMT) had 4 or more doses

- Observed intercurrent illnesses- each seen in 1 patient:
  - Gastrointestinal upset, Arm pain, Runny nose, Pneumonia, Inflammation of face and eyes

- Potentially drug related side effects:
  - Soft stools, Increased appetite each seen in 1 patient
  - Transient neutropenia in three patients, no clear relation to therapy

Immune Stimulation

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



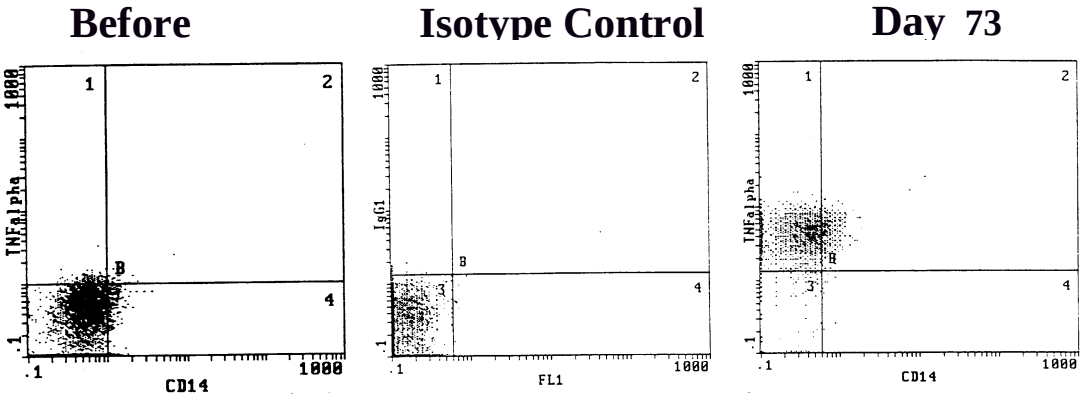
Includes 8 lymphoma patients and 6 myeloma patients.

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.

5 of the 10 patients who were anergic pre-study developed DTH after 3 to 5 doses.

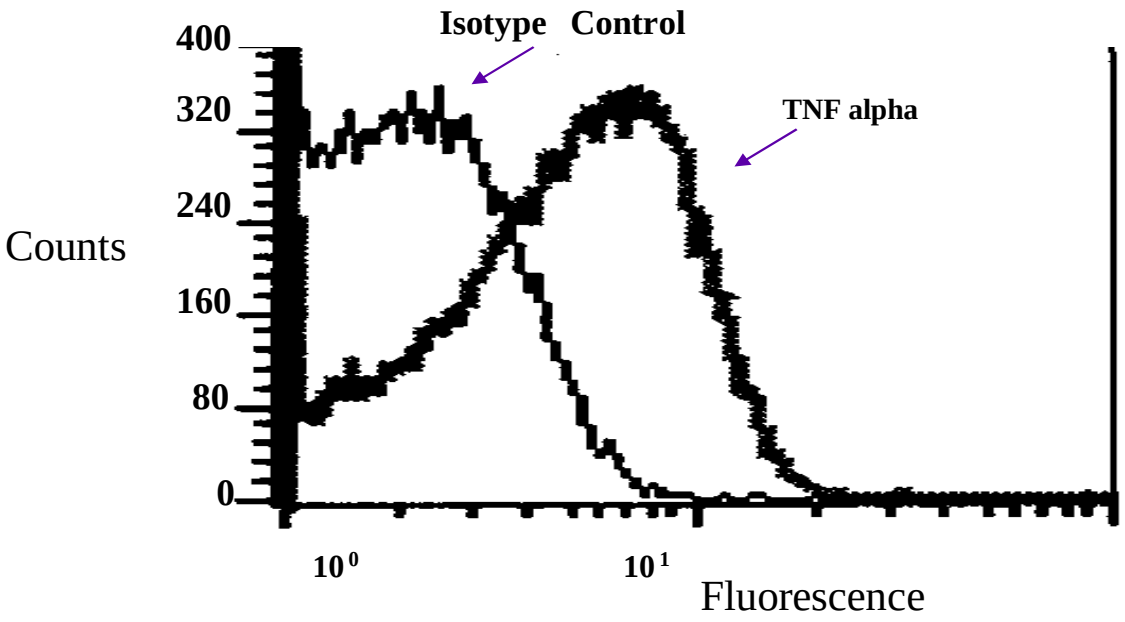
TNF-α on Lymphocytes in Patient #1-101



**(73 days from 1st injection
6 hours from last injection)**

Note: During this time, patient disease decreasing, as indicated by M-protein.

β-alethine Increases TNFα on Lymphocytes

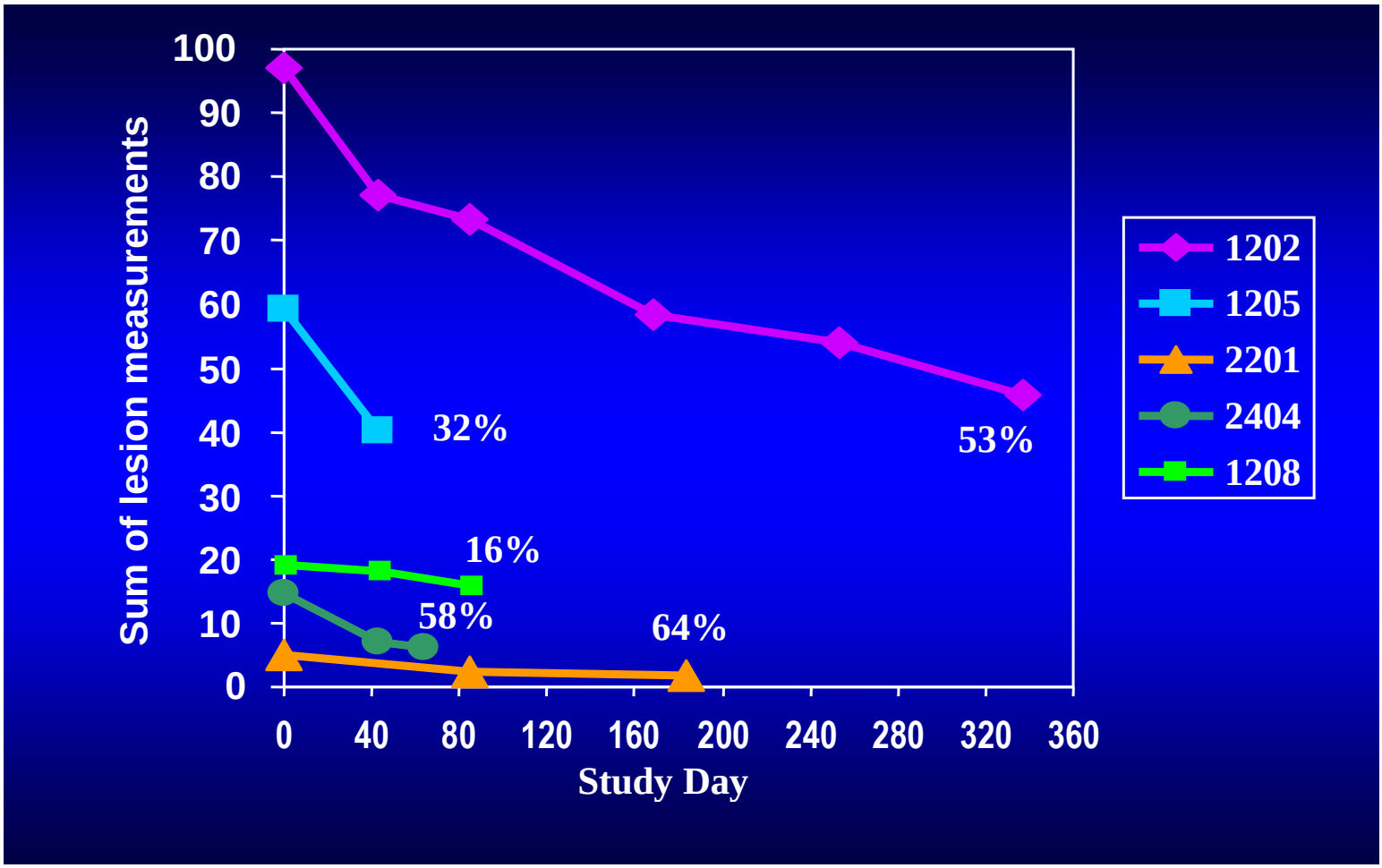


Overall Tumor Response

Response	Lymphoma	Multiple Myeloma
PR	3	1
MR	1	1
Stable	7	4
POD	3	9

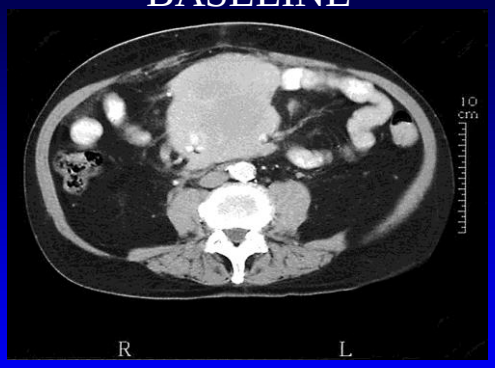
PR (>50% reduction in bidimensional disease) and MR (> 20% reduction) were defined by best response on therapy. Disease was evaluated as stable (steady state of disease documented to be present for at least 4 weeks from the start of therapy, no appearance of new lesions) or POD (>25% increase in bidimensional disease, may include appearance of new lesions) after 6 doses of β-alethine.

Responses in 5/14 Lymphoma Patients

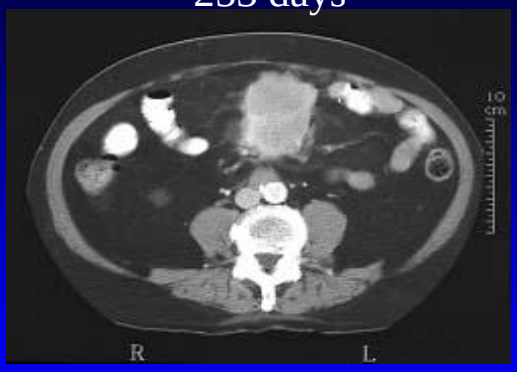


Tumor Response: Patient #202, H.C.

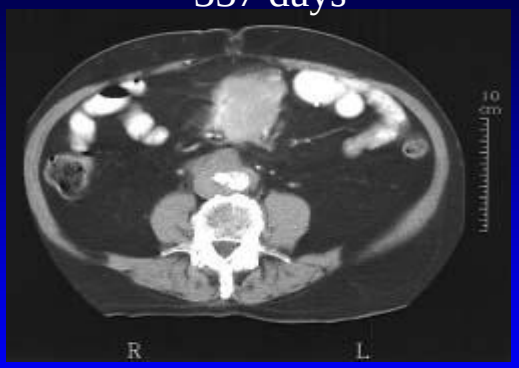
BASELINE



253 days



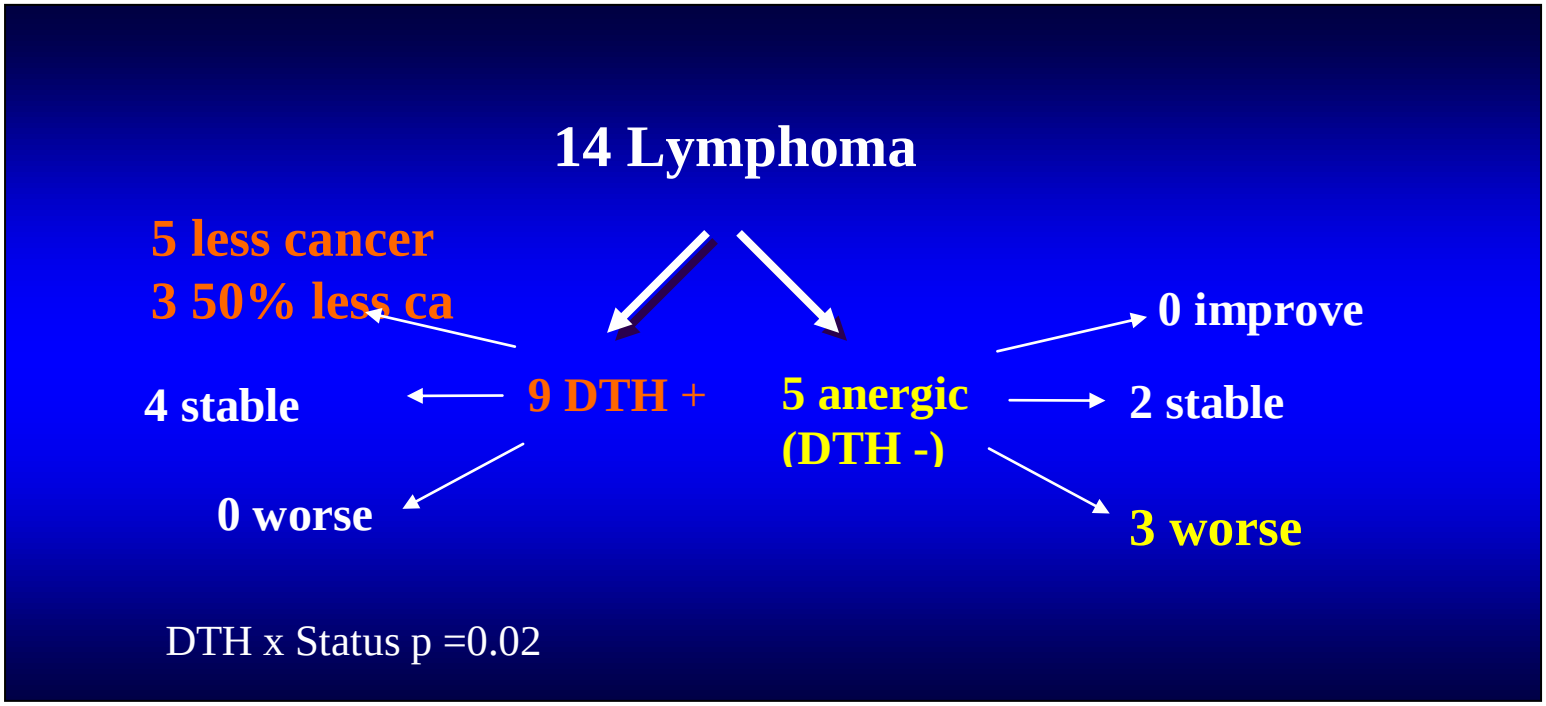
337 days



Prior 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% decrease)
1999: 10 x 9 cm at trial pre-study screening

Responses in Lymphoma Patients correlate with Baseline Immune Function



Conclusions

Data to Date Indicate β-alethine

- is Non-toxic
- Increases surface TNF
- Has anti-lymphoma effects, particularly in patients with better baseline immune function
- Warrants further evaluation:
  - Dose finding study with immunological endpoints
  - Additional tumor types
  - Combination with other therapy
  - Potential in minimal residual disease