

Interim Results of a Phase I/II Clinical Trial of a DNA Plasmid Vaccine (BHT-3021) for Type 1 Diabetes

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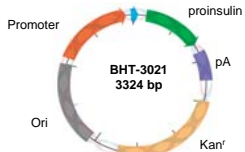
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Abstract

BHT-3021 is a tolerizing DNA vaccine for type 1 diabetes (T1D), encoding full-length human proinsulin. We present here interim results from a randomized, blinded, placebo-controlled phase I/II trial in T1D. Four doses of BHT-3021 are being tested (0.3 mg, 1 mg, 3 mg and 6 mg) and given as once weekly intramuscular injections for 12 weeks, after which the patients are followed in a blinded manner for 40 weeks. Data from the first cohort tested (1 mg) are presented here. The 1 mg dose was safe, well-tolerated and was associated with evidence of β cell preservation as well as tolerance to pancreatic autoantigens. β cell preservation was demonstrated by a mean 17% increase in C-peptide levels with BHT-3021 by week 15 after enrollment, whereas placebo patients experienced a mean 42% decrease in C-peptide. Immune tolerance was demonstrated by a mean 17% reduction in anti-insulin antibodies and 25% reduction in anti-GAD65 antibodies with BHT-3021 by week 15 after enrollment. In conclusion, in T1D patients preliminary data indicate that treatment with BHT-3021 may cause β cell preservation and induction of immune tolerance to pancreatic autoantigens.

Design & Methods

Plasmid: The structure of the BHT-3021 human proinsulin encoding DNA plasmid vaccine used in the trial is diagrammed below.



Subjects: Inclusion criteria include an age of 18 to 40 years at the time of T1D diagnosis, ≤ 5 years since the time of diagnosis, and the presence of antibodies to at least one diabetes-related antigen (insulin, GAD65, or IA2). Both C-peptide + and - patients were allowed in the first (1 mg) cohort, but subsequent cohorts only enrolled C-peptide + patients.

Design: A total of up to 72 T1D patients will be randomized to BHT-3021 or placebo (saline) in a 2:1 ratio. BHT-3021 or placebo is injected intramuscularly weekly for 12 weeks, after which patients are followed in a blinded manner for 40 weeks. Patients initially randomized to placebo may cross-over and receive 12 weeks of open label treatment with BHT-3021. Four doses of BHT-3021 are being tested: 0.3 mg, 1 mg, 3 mg and 6 mg.

Endpoints: The primary outcome measures are safety and changes in antigen-specific immune responses.

Immune response assays: Antibody levels to insulin, GAD65, and IA2 are being tested by radioimmune assay in a blinded and batched manner at the Barbara Davis Center. Autoreactive T cell responses are being tested by ELISpot and by tetramer analysis.

Results

Baseline patient characteristics: Both C-peptide positive and negative patients are included in Table 1 and Table 2 (adverse events), but only the C-peptide positive patients are included in the other analyses (Figs 1-5).

Table 1. Baseline patient characteristics (cohort 1)

	Placebo C-pep +	Placebo C-pep -	BHT-3021 C-pep +	BHT-3021 C-pep -
Number	2	3	7	3
Gender:				
Female	0	0	2	2
Male	2	3	5	1
Age:				
Mean	32.5	38	27.1	46.2
Range	32-33	20-56	19-48	31-54
Time from diagnosis (yrs):				
Mean	5.0	17	2.6	19.5
Range	1-9	1-44	0.4-11	17-21
Antibody positive (number of patients):				
Anti-insulin	2	3	5	3
Anti-GAD65	1	2	3	3
Anti-IA2	1	2	4	2

Adverse Events (AE): All treatment emergent AEs are listed in Table 2. All AEs were considered mild/moderate. No severe or worse adverse events were observed. Note that there were no substantial imbalances of AEs comparing placebo to BHT-3021.

Table 2. Incidence of all treatment-emergent AEs (cohort 1)

	Placebo n=5	BHT-3021 n=11*
Eye		
Conjunctivitis	0	1
Eye pain	1	0
Visual disturbance	0	1
Gastrointestinal		
Abdominal discomfort	0	1
Dyspepsia	0	2
Nausea	0	1
Diarrhea	1	0
Vomiting	0	1
General & administration site		
Application site bruising	0	1
Immune system		
Hypersensitivity	1	0
Infections and Infestations		
Nasopharyngitis	0	2
Upper respiratory tract infection	1	3
Viral infection	0	1
Musculoskeletal and connective tissue		
Back pain	0	1
Nervous system		
Headache	1	3
Vasovagal syncope	1	0
Skin and subcutaneous tissue		
Dermatitis	1	1
Ingrown nail	1	0
Pruritis	1	0
Eczema	0	1

*Includes one patient who crossed-over from placebo to BHT-3021

Results (cont.)

C-peptide (cohort 1)

C-peptide levels in patients were measured in a blinded fashion at a CLIA certified lab at week 0, week 5, week 15, month 6, month 9, and month 12 using a two hour mixed meal tolerance test (with the exception of the first three patients who were tested using glucagon stimulation). The AUC_{mean} (area under the curve per minute) values were calculated for each patient, and the absolute values at the week 15 and month 6 time points are graphed.

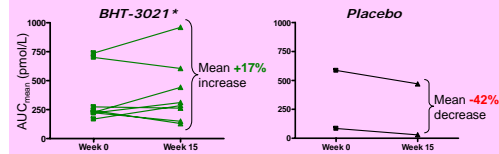


Figure 1. C-peptide preservation at week 15. Individual AUC_{mean} values are graphed and the mean change from baseline of each group is indicated. *Includes one patient who crossed-over from placebo to BHT-3021.

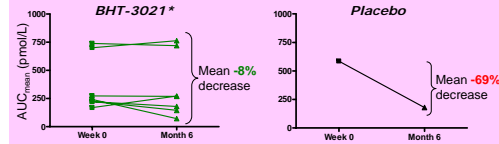


Figure 2. C-peptide preservation at month 6. Individual AUC_{mean} values are graphed and the mean change from baseline of each group is indicated. *Includes one patient who crossed-over from placebo to BHT-3021. The other placebo patient has yet to have the month 6 visit. The results in Figs 1 and 2 suggest that BHT-3021 can maintain C-peptide levels in T1D patients, whereas the placebo treated patients have a decline in C-peptide levels as expected with disease progression and ensuing β -cell destruction.

Autoantibodies (cohort 1)

Anti-insulin and anti-GAD65 Ab levels in patients were measured at weeks 0, 2, 4, 6, 8, and 15 in a blinded fashion at the University of Colorado using a radioimmunoassay in a CLIA certified lab. Samples were stored frozen until analyzed and all samples were tested in parallel to eliminate intra-assay variability.

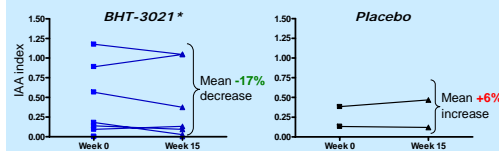


Figure 3. Anti-insulin Ab levels in C-peptide positive patients. Individual anti-insulin Ab values are graphed and the mean change from baseline of each group is indicated. *Includes one patient who crossed-over from placebo to BHT-3021.

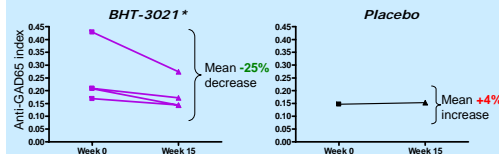


Figure 4. Anti-GAD65 Ab levels in C-peptide positive patients. Individual anti-GAD65 Ab values are graphed and the mean change from baseline of each group is indicated. *Includes one patient who crossed-over from placebo to BHT-3021. The results in Figs 3 and 4 demonstrate that BHT-3021 causes tolerance to pancreatic autoantigens as demonstrated by a decline in the autoantibody titers with treatment.

Results (cont.)

Crossover patient

The C-peptide levels and anti-insulin Ab levels of a single patient treated with placebo for 9 months and then crossed over to 1 mg of BHT-3021 were measured as in Figs 1-3. The relative change from week 0 was calculated as a percentage change. After crossover at month 9, the relative change in both C-peptide and anti-insulin Ab from month 9 is shown.

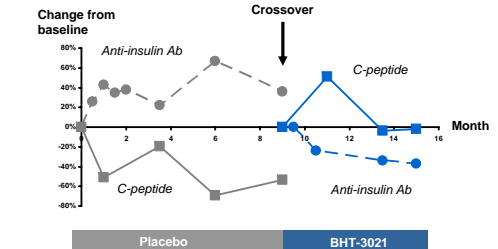


Figure 5. C-peptide and anti-insulin Ab change correlation in a single patient. This result shows that while on placebo, the C-peptide levels decline over 9 months in this patient and the anti-insulin Ab levels increase, but after crossover to BHT-3021 the C-peptide levels are maintained and the anti-insulin Ab levels decline in this same patient. The results imply that BHT-3021 may cause antigen-specific tolerance and halt pancreatic β -cell destruction. In other words, these data provide strong evidence of correlation of both tolerance and efficacy in this patient.

Summary & Conclusions

- Preliminary results demonstrate that 1 mg of BHT-3021: is safe and well-tolerated, appears to preserve pancreatic function, causes tolerance to pancreatic autoantigens.
- The 3 mg BHT-3021 cohort is fully enrolled.
- Currently enrolling the 0.3 mg and 6 mg BHT-3021 cohorts.

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