WHOLE RECOMBINANT YEAST THERAPEUTIC VACCINE GENERATES HBV X, S, AND CORE ANTIGEN-SPECIFIC RESPONSES IN MURINE AND HUMAN T CELLS

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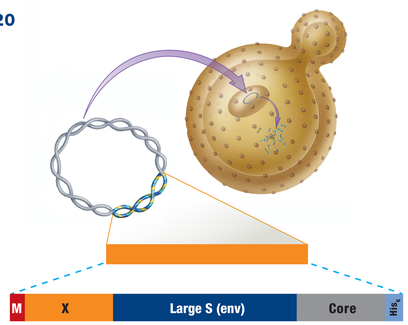
Introduction

Hepatitis B virus (HBV) is the leading cause of chronic liver infection. Worldwide 350 million people are chronically infected with HBV, of whom more than 620,000 die from liver-related disease annually. In the United States approximately 1.4 million Americans have chronic HBV. This disease is characterized by suboptimal T cell responses against HBV antigens. A therapeutic vaccine capable of generating an HBV-specific T cell immune response that is administered concomitantly in patients whose disease is under virologic control with a direct acting antiviral agent could be an attractive clinical option. Tarmogens® (targeted molecular immunogens) are recombinant, intact *Saccharomyces cerevisiae* yeast engineered to express high quantities of disease-related proteins inside the yeast cell. Tarmogens generate robust $CD4^+$ and $CD8^+$ T cell responses directed against these disease antigens and have been well tolerated to date in >300 trial subjects in GlobeImmune's oncology and hepatitis clinical trials. Eighteen HBV Tarmogens expressing variations of HBV X, S, Pol and Core antigens were engineered. One product expressing an X-S-Core fusion protein emerged as the lead candidate on the basis of favorable antigen expression and immunogenicity. Here we present the results of further testing of this candidate in murine and human cellular immunology assays.

GI-13020/GS-4774 X-SCore Tarmogen

The GI-13020 Tarmogen was created by transfecting *S. cerevisiae* yeast with an expression plasmid containing a nucleotide sequence encoding an HBV X-S-Core fusion protein. The Tarmogen expresses high intracellular levels of X-S-Core antigens.

GI-13020



M, proprietary metabolic stability signal; His6, hexahistidine tag for antigen quantification

Table 1: GI-13020 features broad HBV genotypic coverage

The X, S, and Core antigen sequences were chosen to incorporate many known epitopes (not shown) while preserving a high level of conservation across the major HBV genotypes (88 to 99%). These features should maximize the utility of GI-13020 for treatment of all of the commonly observed HBV genotypes.

HBV Antigen (No. residues)	% identity, GI-13020 (Gen. D) vs. Genotype:		
	A	B	C
X (60)	92	88	95
S (399)	95	95	96
Core (182)	97	96	99

Figure 1A/B: GI-13020 vaccination elicits HBsAg-specific $CD8^+$ T-cell responses in mice

To determine if HBsAg-specific immune responses were elicited in GI-13020-vaccinated mice, spleen cells from these mice were stimulated *in vitro* with peptide VWLSVIWM, a known murine class I MHC-restricted HBsAg epitope, and then stained with antibodies to CD8, TNFα, IL-2 and IFNγ. The results showed that GI-13020 vaccination elicits HBsAg-specific $CD8^+$ T cells displaying a multi-functional phenotype on the basis of the cytokines produced.

Figure 1A: GI-13020 vaccination elicits HBsAg-specific, IFNγ-producing $CD8^+$ T cells

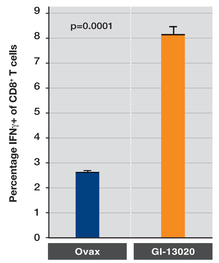
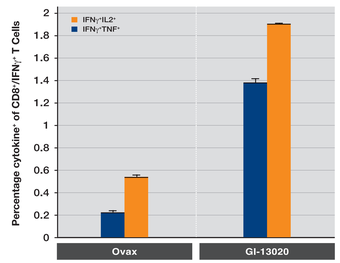


Figure 1B: Production of IL-2 and TNFα by $CD8^+IFN\gamma+$ T cells

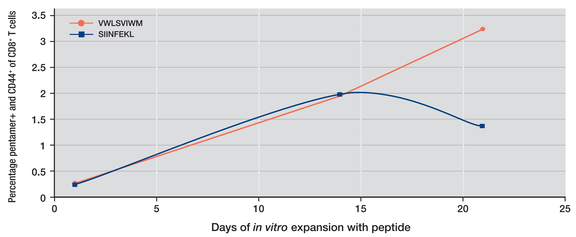


Vaccination with either Ovax or GI-13020

- *GI-13020 elicits* HBsAg-*specific $CD8^+$ T cell responses*
- *TNFα and IL-2 production were observed by $CD8^+IFN\gamma^+$ T cells*
- *Ovax: Ovalbumin-expressing Tarmogen (neg. ctrl)*
- *p value is for GI-13020 vs. Ovax (ANOVA) and applies to all cytokine results*

Figure 2: A HBsAg-specific T cell line was expanded from GI-13020-vaccinated mice

To further demonstrate the presence of HBsAg-specific T cells, an HBsAg-specific T cell line was created from immune cells of Tarmogen vaccinated mice. C57BL/6 mice were vaccinated once per week for three weeks with GI-13020 and spleen cells were then prepared and incubated with the HBsAg peptide VWLSVIWM or the irrelevant ovalbumin peptide SIINFEKL. Cells were stained with a VWLSVIWM-specific pentamer, and the percentage of activated ($CD44^+$) $CD8^+$ T cells that were HBsAg pentamer$^+$ was determined by flow cytometry. The results showed that by day 21, the T cell population stimulated with the irrelevant peptide began to contract whereas that stimulated with HBsAg peptide was expanding. Thus, GI-13020 vaccination elicits HBsAg specific T cells that can be expanded into a T cell line of known epitope specificity.



- *VWLSVIWM, an HBsAg-specific peptide*
- *SIINFEKL, an irrelevant peptide from chicken ovalbumin*

Figure 3A/B: GI-13020 elicits HBxAg-specific T cell responses

ELISpot and lymphocyte proliferation assays (LPAs) conducted in BALB/c mice demonstrated HBxAg-specific T cell responses arising from GI-13020 vaccination. Spleen cells from Yvec (yeast control) mice were stimulated *in vitro* for 4 days with 76 individual peptides spanning HBxAg, or recombinant full length X antigen. T cell activation was detected by IL-2 ELISpot (panel A) or ^{3}H Thymidine incorporation (panel B). The results indicate that GI-13020 elicits HBxAg-specific T cell responses that are detected upon stimulation with class I and II MHC peptides representing ~8% of the panel. The LPA response to full length recombinant HBxAg antigen showed a 32-fold GI-13020/Yvec response ratio, consistent with T cell responses that are highly specific for this antigen.

Figure 3A

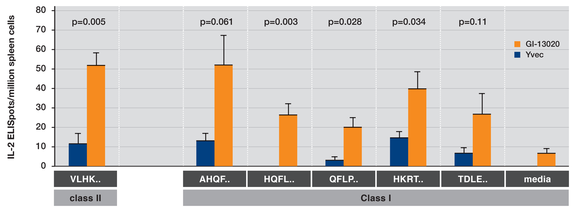
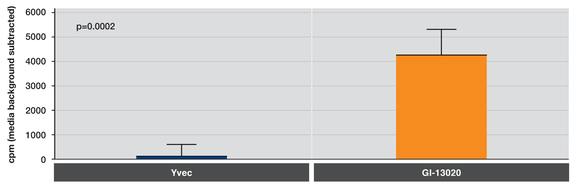


Figure 3B



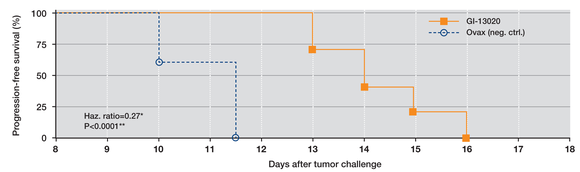
BALB/c mice vaccinated with Yvec or GI-13020.

- *Yvec: S. cerevisiae yeast without exogenous antigen*
- *p values shown are for GI-13020 vs. Yvec (ANOVA)*

Figure 4: GI-13020 protects mice from challenge with HBxAg-expressing tumors

The ability of a vaccine to elicit protective responses *in vivo* provides direct measures of its potency and relevance. To evaluate the protective capacity of GI-13020 vaccination against HBxAg-expressing targets, mice were thrice vaccinated with GI-13020 or Ovax (irrelevant control) Tarmogens and then challenged subcutaneously with an aggressive EL4 tumor cell line expressing HBxAg (EL4/X). Tumor growth rate was then caliper-measured for 18 days. The results show that the HBxAg component of GI-13020 generates a functional killing response that significantly slows the growth of the EL4/X tumor.

GI-13020 significantly slows growth of EL4-X tumors

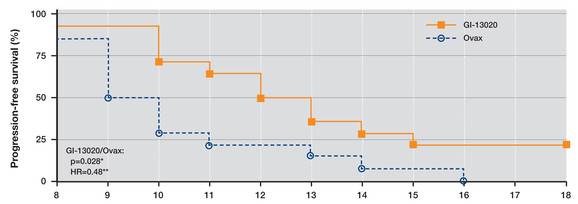


90,000 EL4/X tumor cells used for challenge n=10 mice/group
** DG Altman "Practical statistics for medical research" 1991 ** Log rank test from Kaplan-Meier analysis*

Figure 5: GI-13020 protects mice from challenge with HBV Core-expressing tumors

To demonstrate a relevant *in vivo* killing response targeting HBV Core antigen (HBcAg), mice were thrice vaccinated with GI-13020 or Ovax and then challenged subcutaneously with an EL4 tumor cell line expressing HBcAg (EL4/Core). Tumor growth rate was then caliper-measured for 18 days. The results show that the HBcAg component of GI-13020 generates a functional killing response that significantly slows the growth of HBcAg expressing tumors.

GI-13020 significantly slows growth of EL4/Core tumors



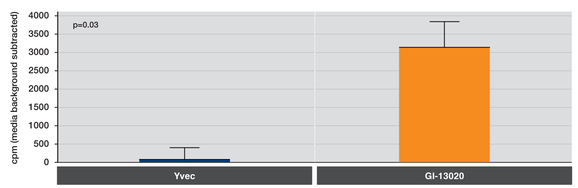
30,000 EL4/Core tumor cells used for challenge, n=14 mice/group
* Log rank test from Kaplan-Meier analysis ** DG Altman "Practical statistics for medical research" 1991

- *Ovax: Ovalbumin-expressing Tarmogen (negative ctrl)*

Figure 6: GI-13020 pulsed DCs stimulate HBc18-27 specific T cells *in vitro*

To evaluate the potential of GI-13020 to elicit T cell responses that are relevant to HBV clearance, Tarmogen-fed dendritic cells (DCs) were used to stimulate human T cells transduced with a T cell receptor recognizing an epitope associated with acute HBV clearance (HBc18-27). DCs were incubated with GI-13020 to achieve DC maturation/HBV antigen presentation. The mature DCs were then incubated with c18-27-TCR-transduced T cells in an IFNγ ELISpot assay to measure T cell activation. The results showed that HBc18-27 was presented by the pulsed DCs to the cognate T cells (cross-priming). The effect was antigen-specific as the ELISpot response was 23-fold higher for GI-13020 than for Yvec (yeast control)-pulsed DCs.

Cross-priming of HBc18-27 specific T cells by GI-13020- or Yvec-pulsed DCs



- *Yvec: S. cerevisiae yeast without exogenous antigen*

Conclusions

- GI-13020 is a whole yeast-based vaccine candidate engineered to elicit cellular immune responses targeting HBV-infected cells.
- X, S, and Core- targeted immune responses were generated by GI-13020 vaccination in mouse models
- Human DCs pulsed with GI-13020 activate Core - specific T cells, demonstrating cross-presentation of an epitope that is important in immunological control of HBV infection.

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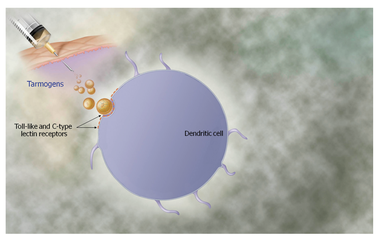
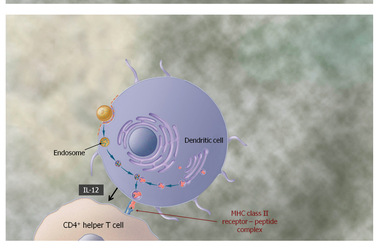
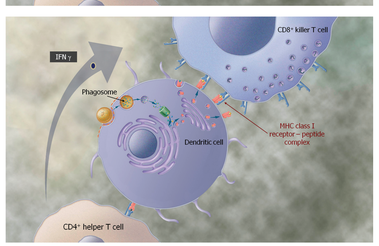
Active immunotherapy with yeast-based Tarmogens



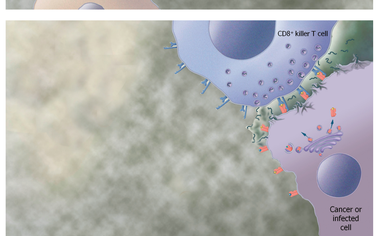
Administration of Tarmogens initially results in binding of the yeast to antigen-presenting cells, the most important of which are dendritic cells, near the injection site. The dendritic cells are activated as a result of the Tarmogens binding to Toll-like receptors and other receptor molecules on the surface of the dendritic cell, resulting in the activation of cytokine immune signaling molecules. The dendritic cell also engulfs the Tarmogen. Multiple Tarmogens may be taken up by the same dendritic cell.



The Tarmogen is processed by the dendritic cell in two ways. First, the Tarmogen is engulfed by endosomes and the protein inside the endosome is cut into shorter peptides fragments. These peptides are presented by Class II MHC molecules on the surface of the dendritic cell. In combination with IL-12, a cytokine that is produced by the dendritic cell, these MHC-peptide complexes on the surface of the dendritic cell are recognized by and activate cells involved in viral immunity called $CD4^+$ helper T cells.



Dendritic cells also process Tarmogens by engulfing them with phagosomes. This results in presentation of peptides, including the antigen from inside the Tarmogen, to $CD8^+$ killer T cells, via Class I MHC molecules on the surface of the dendritic cell, resulting in proliferation of identical antigen specific $CD8^+$ T cells. $CD4^+$ helper T cells are so named because one of their roles is to “help” activate killer T cells by expressing interferon gamma (IFNγ).



The newly activated $CD8^+$ killer T cells move throughout the body and identify any other cell that expresses the same disease protein as the one recognized by the $CD8^+$ killer T cells. Once the $CD8^+$ killer T cell finds another cell in the body containing the target protein, it can kill the cell using multiple mechanisms.

Whole recombinant yeast therapeutic vaccine generates HBV X, S, and Core antigen-specific responses in murine and human T cells

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Background and aims: Chronic hepatitis B is characterized by sub-optimal T cell responses to viral antigens. A therapeutic vaccine capable of restoring immune responses to HBV could potentially improve HBV S antigen seroconversion rates in the setting of specific antiviral therapy. A yeast-based immunotherapy platform (Tarmogens) was used to develop a clinical vaccine candidate (GI-13020) expressing HBV X (HBxAg), S (HBsAg), and core antigen (HBcAg) conserved across all HBV genotypes. The aim of our current study was to assess whether epitopes associated with acute HBV clearance are efficiently presented to T cells by GI-13020-pulsed dendritic cells (DCs).

Methods: Mice were subcutaneously immunized with 3 weekly doses of GI-13020 or empty vector yeast (control). One week later, T cell responses were evaluated by lymphocyte proliferation assay (LPA), interferonγ (IFNγ)/IL-2 ELISpot, intracellular cytokine staining (ICCS), and tumor challenge assays. Human T cells specific for HBc18-27 and HBs183-91 were incubated with GI-13020-pulsed DCs and IFNγ production was measured to evaluate presentation of relevant HBV epitopes to the T cells.

Results: Mice immunized with GI-13020 compared to controls showed induction of T cell responses specific for HBxAg by tumor protection (haz. ratio (hr)=0.27), LPA (32-fold increase vs. control), and IL-2 ELISpot (6-fold increase). Immune responses to HBcAg were also observed in tumor protection (hr=0.48), and responses to HBsAg were shown by the ability to expand HBs190-197 specific T cells ex vivo (13-fold increase vs. baseline by pentamer staining) and by IFNγ production by HBsAg-specific $CD8^+$ T cells (3.6 fold increase vs. control). HBsAg- or HBcAg-specific human T cells produced IFNγ following incubation with GI-13020-pulsed DCs (4.2 to 23-fold increase vs. control).

Conclusions: The GI-13020 vaccine candidate elicits HBxAg-, HBsAg- and HBcAg-specific T cell responses in murine models. HBsAg- and HBcAg-specific human epitopes associated with clearance of acute HBV infection are efficiently presented to virus-specific T cells by GI-13020-pulsed DCs. These data suggest that GI-13020 could be used to improve outcomes in chronic HBV patients, through the induction of HBV-specific T cell responses targeting infected hepatocytes.