A NOVEL YEAST-BASED IMMUNOTHERAPEUTIC PRODUCT FOR CHRONIC HEPATITIS C VIRUS INFECTION

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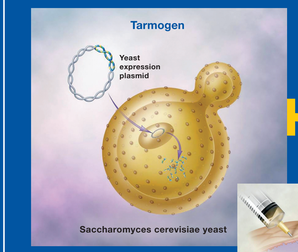
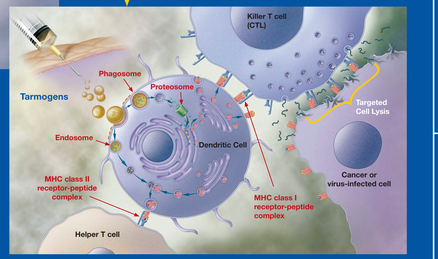
GlobeImmune Tarmogen Technology



Heat inactivated, recombinant *S. cerevisiae* transfected with the NS3-Core fusion gene (GI-5005)



Uptake of Tarmogens by DCs results in the activation of killer T cells against the HCV expressing cells

Abstract

Evidence suggests that control of hepatitis C (HCV) infection in humans requires an effective T cell response. Previous studies have shown that whole, heat inactivated, recombinant *Saccharomyces cerevisiae* yeast (Tarmogens™) are avidly phagocytosed by and directly activate dendritic cells which present disease-associated protein antigens to CD4 and CD8 T cells mediating antigen-specific protective and therapeutic anti-tumor immunity (Stubbs et al. Nature Med. 5:625, 2001; Lu et al., Cancer Res. 64:5084, 2004).

Tarmogens (**tar**geted **mo**lecular immuno**gens**) express reproducible amounts of target protein, induce cellular immune responses, activate antigen presenting cells, are not neutralized upon repeated administration, are non-toxic in pre-clinical studies, can be used for multiple antigen delivery and are straightforward to manufacture.

In this study, GI-5005, a Tarmogen containing an HCV NS3-Core fusion protein, was evaluated for its ability to induce protective and therapeutic cellular immunity in mice. GI-5005 immunogenicity was evaluated using cytotoxic T lymphocyte killing assays, lymphocyte proliferation assays, cytokine secretion profiles and *in vivo* tumor challenge models.

The results show that GI-5005 administration elicits potent HCV antigen-specific T cell responses. A Phase 1 study of GI-5005 in patients with chronic HCV infection is being initiated.

T cells from GI-5005 immunized mice kill virus-infected cells expressing HCV NS3 or Core

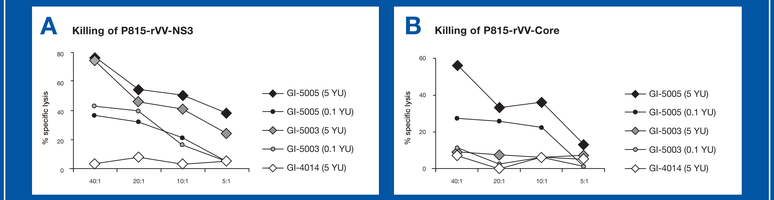


Fig. 1. A ^{51}Cr release assay was performed to demonstrate antigen-specific killing of P815 tumor cells infected with vaccinia virus expressing NS3 (A) or Core (B) proteins using T cells from BALB/c mice immunized with GI-5005, GI-5003 (produces mutant NS3); GI-4014 which is a control Tarmogen.

Dose-dependent induction of CTL with GI-5005

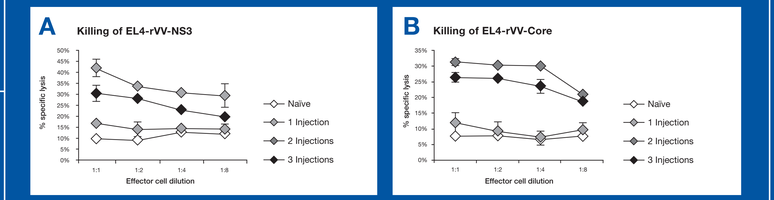
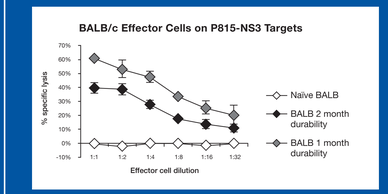


Fig. 2. C57BL/6 mice received one, two or three weekly subcutaneous doses of GI-5005. The isolated T cells were stimulated for five days with GI-5005. Then a ^{51}Cr release assay was carried out to evaluate CTL activity using EL4 tumor cells infected with vaccinia viruses expressing NS3 (A) or Core (B) proteins as target cells. A naïve control group was included.



Durability of CTL responses with GI-5005

Fig. 3. BALB/c mice received three weekly doses of GI-5005 or none, and were then sacrificed one and two months after the final dose. The T cells were stimulated with GI-5005 for 5 days *in vitro*, and a CTL assay was performed using P815 expressing NS3 as targets.

Mice immunized with GI-5005 reject tumors expressing HCV antigens

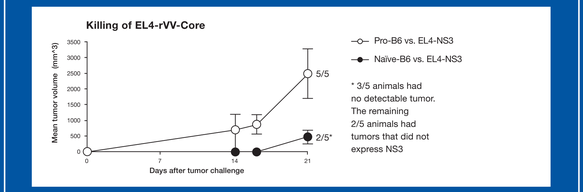


Fig. 4. C57BL/6 mice (5/group) challenged one week after immunization.

Antigen-specific T_H cells in protected mice

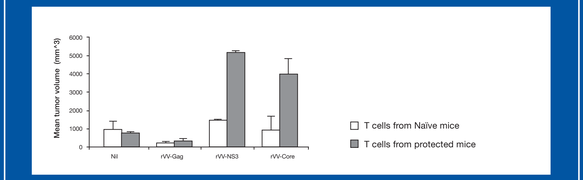


Fig. 5. BALB/c splenocytes isolated from animals that were protected from tumor challenge were evaluated in a standard lymphocyte proliferation assay after *in vitro* stimulation with vaccinia viruses expressing NS3, Core, HIV-Gag or nothing.

GI-5005 overcomes inadequate immunity

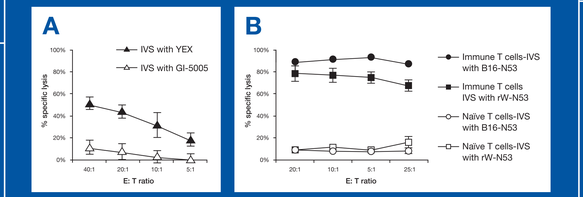


Fig. 6. (A) T cells derived from non-immunized, tumor challenged mice (B) T cells from GI-5005 immunized, tumor challenged mice. EL4-NS3 were used as target cells in a standard ^{51}Cr release assay. Naïve animals were included as control. YEX, yeast vector alone.

GI-5005 eliminates established tumors

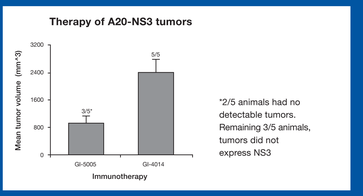


Fig. 7. BALB/c mice were injected with A20-NS3 tumor cells. One week later, the mice were treated with three weekly doses of GI-5005.

Cytokine profiles from immunized mice

Treatment	IVS	IL-2	IL-12	GMCSF	IFN-_	TNF-_
PBS (Naïve)	Con A	+	-	+	++	+
	LPS	-	+	-	-	++
	GI-5005	-	+	-	-	++
	rVV-NS3	-	-	-	-	-
GI-5005	Con A	+	-	+	++	+
	LPS	-	+	+	-	+++
	GI-5005	+	+	+++	+++	++++
	rVV-NS3	-	-	++	++	-

Table 1. BALB/c splenocyte culture supernatants were harvested after *in vitro* stimulation and analyzed for cytokine production

Conclusions

- GI-5005 induces dose-dependent NS3 and Core-specific CTL and T_H immune responses in mice
- GI-5005 boosts pre-existing immune responses
- HCV specific memory responses can be detected at least two months after administration of GI-5005
- GI-5005 induces therapeutic and protective immunity against HCV antigen-expressing tumors
- A Phase 1 study of GI-5005 in patients with chronic HCV infection is being initiated

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