PRECLINICAL DEVELOPMENT OF YEAST-BASED IMMUNOTHERAPY FOR CHRONIC HEPATITIS C VIRUS INFECTION

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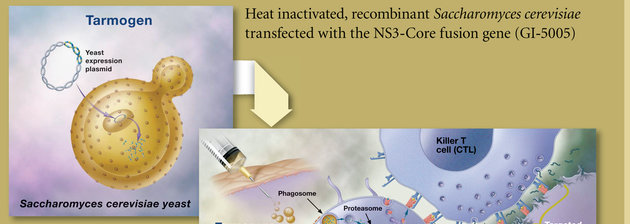
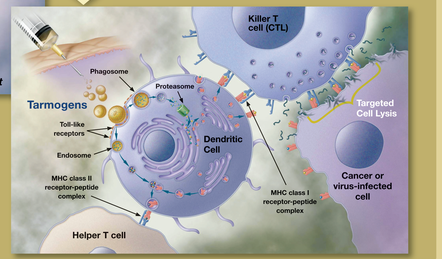
Tarmogen Technology



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



Uptake of Tarmogens by dendritic cells results in the activation of killer T cells against HCV infected cells

Abstract

Background: Evidence suggests that control of hepatitis C infection in humans requires effective T cell-mediated immunity. Previous studies have demonstrated that recombinant, heat-inactivated *Saccharomyces cerevisiae* yeast (Tarmogens™) are phagocytosed by and directly activate dendritic cells that present disease-associated proteins contained within the Tarmogen to CD4⁺ and CD8⁺ T cells. Tarmogens are capable of mediating both therapeutic as well as prophylactic antigen-specific anti-tumor effects (*Lu et al. 2004 Can Res 64, 5084*). In this study, a Tarmogen that produces an HCV NS3-Core fusion protein (GI-5005) was evaluated for its ability to induce protective and therapeutic immunity in mice.

Methods: C57BL/6 and BALB/c mice were injected subcutaneously with GI-5005. Immunogenicity was determined using assays that measure antigen-specific lymphocyte proliferation, cytokine secretion, and cytotoxicity. A surrogate model for hepatitis C infection employing HCV antigen-expressing syngeneic tumor cells implanted in mice was used to assess both preventative immunity and therapeutic efficacy.

Results: Immunization with GI-5005 induced dose-dependent NS3 and Core antigen-specific cytotoxic T cell and helper T cell activity associated with secretion of IL-2, IFN-γ, GM-CSF and TNF-α. Protective immunity was demonstrated in mice that were immunized prior to tumor challenge with NS3-expressing tumor cells. Therapeutic efficacy was demonstrated in mice that were immunized seven days after implantation of NS3-expressing tumor cells. No significant systemic adverse effects have been observed upon repeated administration of Tarmogens in mice, rats, rabbit and macaques.

Conclusion: GI-5005 was found to elicit both protective and therapeutic cytotoxic T cell and helper T cell mediated responses specific for HCV antigens. A Phase 1 study is being initiated to test GI-5005 in humans chronically infected with hepatitis C virus.

Introduction

Tarmogens (targeted molecular immunogens) are whole, heat-killed recombinant *Saccharomyces cerevisiae* yeast engineered to express one or more target protein antigens, and activate both an innate immune response via Toll-Like Receptors (TLRs), as well as an adaptive, antigen-specific immune response. GI-5005 was engineered to express a hepatitis C virus (HCV) fusion protein comprised of large segments of NS3 protease and Core protein sequences. These proteins were chosen as targets for immunotherapy because they are essential for virus replication, contain multiple epitopes that are recognized by both CD4⁺ and CD8⁺ T cells in acute and chronic infections, and are highly conserved among the different HCV genotypes. GI-5005, by expressing multiple antigens, was designed to induce a broad cellular immune response, which is thought to be necessary to achieve a sustained viral response and HCV clearance in patients.

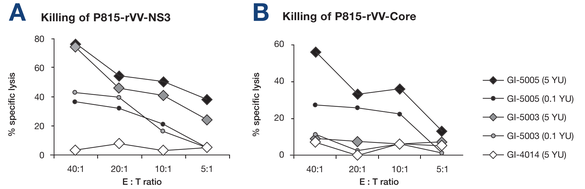


Figure 1. T cells from GI-5005 immunized mice kill virus-infected cells expressing HCV NS3 or Core

^{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 Tarmogen expressing a non-HCV protein.

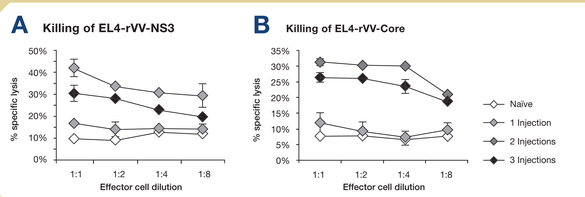


Figure 2. Dose-dependent induction of CTLs with GI-5005

C57BL/6 mice received one, two or three weekly subcutaneous doses of GI-5005. Isolated T cells were stimulated for five days with GI-5005. 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.

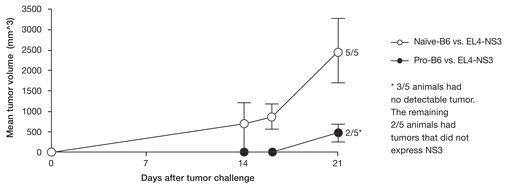


Figure 3. Mice immunized with GI-5005 reject tumors expressing HCV antigens

C57BL/6 mice (5/group) challenged with EL 4-NS3 cells one week after immunization with GI-5005.

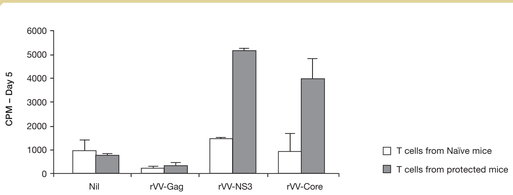


Figure 4. Antigen-specific T_H cells in protected mice

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.

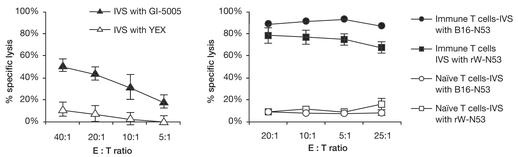


Figure 5. GI-5005 overcomes antigen ignorance

(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.

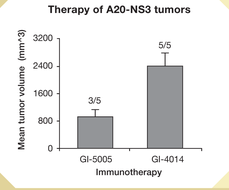


Figure 6. GI-5005 eliminates established tumors

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

Treatment	IVS	IL-2	IL-12	GM-CSF	IFN-γ	TNF-α
PBS (Naïve)	Con A	+	-	+	++	+
	LPS	-	+	-	-	++
	GI-5005	-	+	-	-	++
	rVV-NS3	-	-	-	-	-
GI-5005	Con A	+	-	+	++	+
	LPS	-	+	+	-	+++
	GI-5005	+	+	+++	+++	++++
	rVV-NS3	-	-	++	++	-

Table 1. Cytokine profiles from immunized mice

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

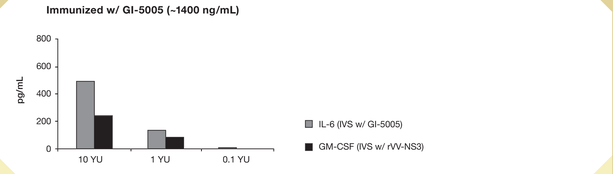


Figure 7. Dose-dependent cytokine production in response to GI-5005

Pro-inflammatory cytokine secreting cells induced with different doses of GI-5005. Spleen cells (10^6 spleen cells/well) from BALB/c mice that received three weekly injections of 0.1, 1 or 10 YU of GI-5005 were stimulated *in vitro* with GI-5005 (2 x 10^6 yeast cells/well) or rVV-NS3 (100 x 10^6 pfu/well). Cell-free supernatants for cytokine analysis were collected at 72 h (IVS w/ GI-5005) or 120 h (IVS w/ rVV-N53) after IVS.

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
- GI-5005 induces proinflammatory cytokines in mice
- 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