YEAST-BASED TARMOGEN IMMUNOTHERAPY FOR CHRONIC HCV INFECTION

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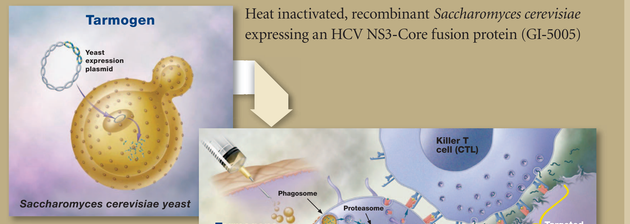
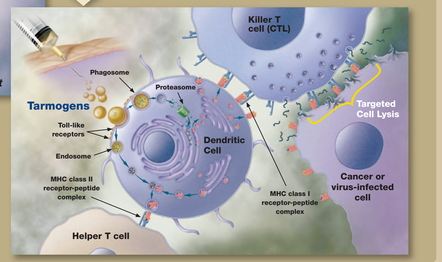
Tarmogen Technology



Heat inactivated, recombinant *Saccharomyces cerevisiae* expressing an HCV NS3-Core fusion protein (GI-5005)



Uptake of Tarmogens by dendritic cells results in the activation of HCV antigen-specific helper and cytotoxic T cells.

Abstract

Numerous reports suggest that individuals who successfully control primary infection with hepatitis C virus (HCV) have robust T cell-mediated immunity directed against a broad range of HCV antigens. In contrast, patients living with chronic hepatitis infection appear to have a much weaker cellular immune response which suggests that boosting T cell activity will have significant clinical value. Recombinant *Saccharomyces cerevisiae* yeast (Tarmogens™) expressing a variety of cancer antigens have been shown to elicit CD4⁺ and CD8⁺ T cells that are capable of mediating specific protective and therapeutic anti-tumor activity in animals (Nature Med. 5:625, 2001; Cancer Res. 64:5084, 2004) and are currently being investigated in a Phase 1b trial in humans. In this study, a Tarmogen that produces an HCV NS3-Core fusion protein (GI-5005) was evaluated for its ability to induce HCV-specific cellular immune responses including protective and therapeutic immunity in mice.

Subcutaneous injection of heat-inactivated GI-5005 in mice induced potent NS3 and Core antigen-specific helper and cytotoxic T cell activities as demonstrated by lymphocyte proliferation, cytotoxicity and cytokine secretion assays, the latter showing that Tarmogen immunization induced a predominantly Th1 response. HCV-specific T cell effector responses were observed for at least 10 weeks after immunization. Studies involving up to 12 weekly injections showed that the Tarmogens were well tolerated and demonstrated a lack of vector neutralization. A surrogate mouse model of HCV infection employing HCV antigen-expressing syngeneic tumor cells was used to assess preventative and therapeutic efficacy. Mice that were immunized either prior to or seven days after challenge with NS3-expressing tumor cells displayed reduced tumor burden or complete tumor regression.

In conclusion, GI-5005 has been shown to elicit durable, HCV-specific, T cell-mediated protective immunity and therapeutic efficacy in mice. GI-5005 is currently being tested in a Phase 1b study in patients chronically infected with HCV.

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.

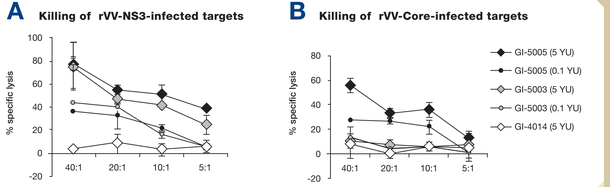


Figure 1. Dose-dependent induction of HCV antigen-specific CTL with GI-5005

Spleen cells from BALB/c mice that were immunized weekly for three weeks with either GI-5005, GI-5003 (producing HCV NS3 alone) or GI-4014 (expressing human mutant K-Ras) were tested for their ability to kill syngeneic P815 target cells infected with recombinant vaccinia virus (rVV) encoding HCV NS3(A) or Core (B) in a standard chromium-release assay. YU=yeast unit (1 YU = 10^7 heat-inactivated, whole yeast cells).

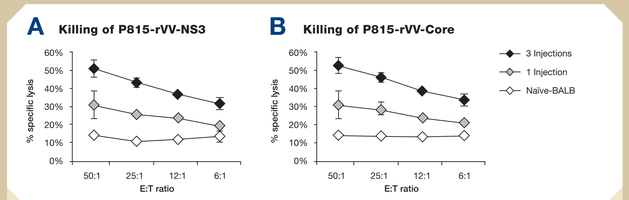


Figure 2. Dose frequency-dependent induction of HCV NS3- and Core-specific CTL with GI-5005

Spleen cells from BALB/c mice that received one or three weekly subcutaneous injections of 5 YU GI-5005 were stimulated *in vitro* for 5 days with GI-5005 before being assayed for cytotoxicity on ^{51}Cr-labeled P815 tumor cells infected with rVV-NS3 (A) or rVV-Core (B). Results are expressed as the mean +/- S.D. for triplicate samples.

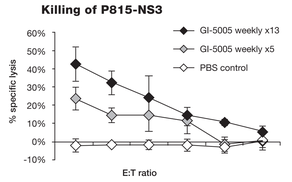


Figure 3. Lack of vector neutralization upon repeated weekly injection of GI-5005

BALB/c mice were immunized weekly for 5 or 13 consecutive weeks with PBS or 5 YU GI-5005 and sacrificed 14 days thereafter. Isolated spleen cells were stimulated in vitro for 5 days with GI-5005 and tested for killing of P815 target cells stably expressing plasmid DNA encoding HCV-NS3 protein.

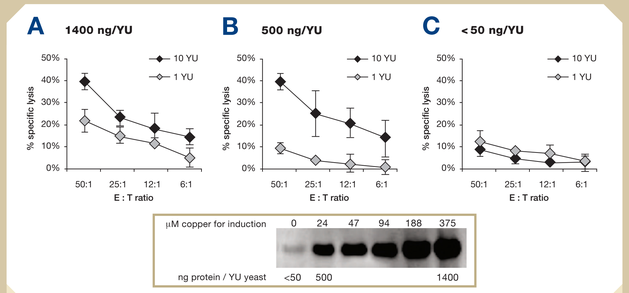


Figure 4. Antigen expression-dependent induction of NS3-specific CTL activity

BALB/c mice were immunized with 1 or 10 YU of GI-5005 manufactured to produce different amounts of the NS3-Core fusion protein. A ^{51}Cr release assay was used to evaluate CTL activity using P815-NS3 target cells.

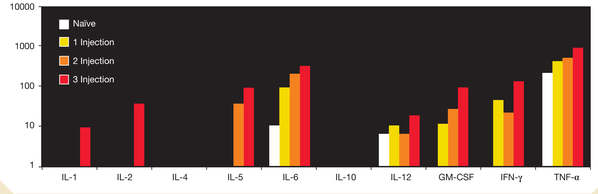
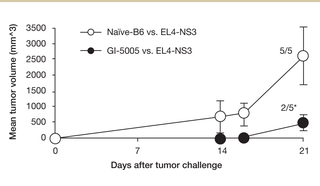


Figure 5. Dose frequency-dependent induction of Th1-type cytokine-producing T cells with GI-5005

Spleen cells from BALB/c mice that were immunized the indicated number of times with 5 YU GI-5005 were stimulated *in vitro* with GI-5005. After 24-hours, cell-free supernatants were isolated and cytokines detected using a Luminex assay.



* 3/5 had no detectable tumor. The remaining 2/5 had tumors that did not express NS3.

Figure 6. Mice immunized with GI-5005 reject tumors expressing HCV antigens

Mice immunized with GI-5005 reject tumors expressing HCV antigens. C57BL/6 mice (5/group) were challenged with EL4-NS3 lymphoma cells 7 days after immunization with GI-5005.

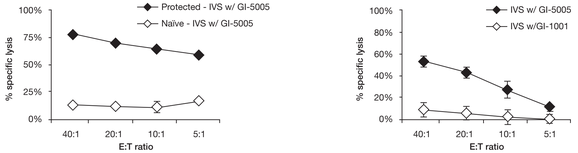


Figure 7. GI-5005 boosts pre-existing immune responses to NS3

T cells isolated from protected (tumor free) or non-protected mice were used in a ^{51}Cr release assay to evaluate NS3-specific CTL.

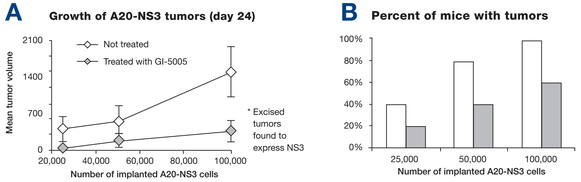


Figure 8. Tumor immunotherapy with GI-5005

BALB/c mice were injected subcutaneously with A20-NS3 tumor cells. One week later, the mice were treated with three weekly doses of 5 YU GI-5005.

Conclusions

- GI-5005 induces dose-dependent NS3 and Core-specific immune responses based upon yeast number, schedule, and antigen content
- GI-5005 induces Th1-type proinflammatory cytokines
- GI-5005 induces protective and therapeutic immunity against HCV antigen-expressing tumor cells
- A Phase 1b study of GI-5005 in patients with chronic HCV infection is currently ongoing