GI-5005 THERAPEUTIC VACCINE IMPROVES DEFICIT IN CELLULAR IMMUNITY IN *IL28B* GENOTYPE T/T, TREATMENT-NAÏVE PATIENTS WITH CHRONIC HEPATITIS C GENOTYPE 1 WHEN ADDED TO STANDARD OF CARE (SOC) PEG-IFN-ALFA-2A/RIBAVIRIN

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J.M. Vierling[1,2], J.G. McHutchison[3], I.M. Jacobson[4], T.D. Boyer[5], E.R. Schiff[6], W.M. Lee[7], M.L. Shiffman[8], N.C. Tsai[9], E.J. Lawitz[10], C. Coeshott[11], D. Bellgrau[11], J. Boyer[12], D.S. Schullery[12], C. Krebs[12], B. Armstrong[13], T.C. Rodell[11], D. Apelian[11]

[1]Department of Medicine and Surgery, Baylor College of Medicine, Houston, TX, [2]St. Luke's Episcopal Hospital, Houston, TX, [3]Duke Clinical Research Institute, Duke University Medical Center, Durham, NC, [4]Center for the Study of Hepatitis C, Weill Cornell Medical College, New York, NY, [5]Department of Medicine, University of Arizona College of Medicine, Tucson, AZ, [6]Center for Liver Diseases, University of Miami School of Medicine, Miami, FL, [7]University of Texas Southwestern Medical Center at Dallas, Dallas, TX, [8]Liver Institute of Virginia, Bon Secours Health System, Newport News, VA, [9]University of Hawaii, Honolulu, HI, [10]Alamo Medical Research, San Antonio, TX, [11]GlobeImmune, Inc., Louisville, CO, [12]University of Pennsylvania, Philadelphia, PA, [13]QST Consultations, Allendale, MI

Introduction

The majority of patients exposed to HCV develop chronic infection. However, approximately 20% are able to clear their infection during the acute phase without medical intervention. A strong HCV-specific T cell response has been associated with spontaneous resolution of infection. (1) Achievement of sustained virologic response (SVR) depends on the patient's ability to clear infected cells from the liver and requires long periods of antiviral suppression by standard of care (SOC) to allow a weak host immune response sufficient time to completely eliminate HCV infected cells. Substantial gains in the treatment of HCV could be achieved through a combination approach that inhibits viral replication (SOC or small molecule antivirals) and enhances HCV-specific cellular immune responses (GI-5005).

The GI-5005 Tarmogen® product consists of recombinant *S. cerevisiae* yeast expressing large conserved regions from HCV NS3 and Core proteins. The GI-5005-02 phase 2 study, described herein, is the first clinical study evaluating GI-5005 in combination with SOC versus SOC alone. We have previously shown in GI-5005-02 that GI-5005 plus SOC improved virologic, biochemical, and histologic responses, with particular virologic improvement in *IL28B* T/T patients. Presented here are the results of immunology testing in naïve patients grouped by *IL28B* genotype.

GI-5005-02 study design

Study Design; Arm 1- (n naïve=50, n T/T=6) 5 weekly followed by 2 monthly SC injections of 40YU GI-5005 (1YU=10^7 yeast), followed by 40YU GI-5005 monthly + SOC for 48 weeks or; Arm 2- (n naïve=46, n T/T=5) SOC alone. Subjects were tested for virologic, biochemical, histologic, and immunologic response as well as *IL28B* genotype.

Immunology background

Tarmogens are whole, heat-killed recombinant *S. cerevisiae* yeast modified to express one or more protein targets that are disease specific: conserved viral proteins, mutated cancer antigens, or overexpressed cancer antigens. Tarmogens stimulate both the innate and antigen-specific arms of the immune system to produce a highly specific and potent T cell response against the diseased cell, with little or no impact on healthy cells. (2) Tarmogens are administered to patients subcutaneously.

Tarmogens activate innate immunity via 'danger' signals at the site of injection:

- taken up by antigen presenting cells (APCs), such as dendritic cells (DCs)
- mimic microbial infection and are recognized via pattern recognition receptors
  - Toll-like receptors (3)
  - phagocytic receptors, such as dectin-1 and mannose receptors (4, 5)
  - activate APCs; migrate to draining lymph nodes/secrete cytokines (6, 7)

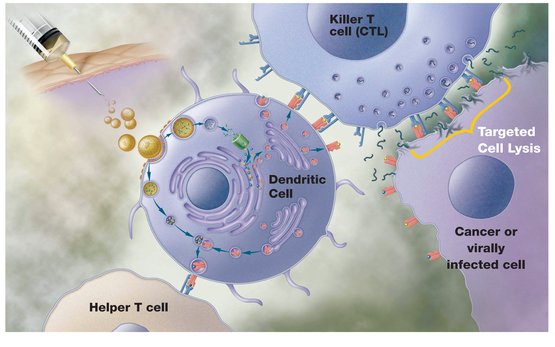
Tarmogens activate adaptive immunity systemically via antigen processing:

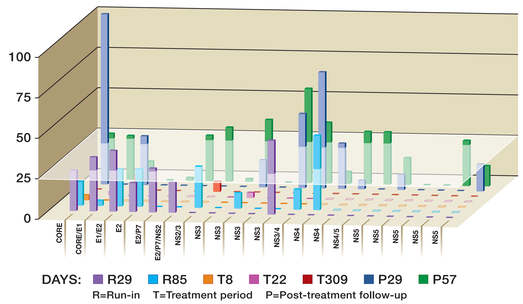
- target antigens are degraded into short peptides inside APCs
  - presented on APC surface to killer T cells (CD8) via MHC class I
  - presented on APC surface to helper T cells (CD4) via MHC class II
- two opposing $CD4^+$ T cell pathways are activated by fungi
  - helper (Th1) T cells, driven by IL12 and assist $CD8^+$ T cell activation
  - Th17 T cells (8, 9, 10), driven by IL6

Activation of Th17 improves effector/suppressor T cell ratios

- activation of Th17 reduces production of suppressive regulatory T cells (Tregs)
  - Tregs have been implicated in down-regulation of protective immune responses in chronic HCV (11)
- Th17 cells produce IL21, a cytokine that enhances the longevity of $CD8^+$ T cells

Tarmogens are unique immunotherapeutics that activate effector T cells and reduce the number and functionality of immuno-suppressive regulatory T cells. (12, 13)





The graph shows a representative example of the response to the overlapping (non-optimized) peptide pools for one T/T subject (169) in the Triple therapy arm. The increase from baseline at multiple timepoints in IFNγ producing cells per million PBMCs (y axis) is plotted against the peptide pools that were used for stimulation (x axis).

ELISpot methods and response algorithm

ELISpot immune analysis

- peripheral blood mononuclear cells (PBMCs) cryopreserved until assay
- all timepoints from a subject assayed on same day (longitudinal analysis)
- PBMCs thawed and incubated with HCV peptides *ex vivo*
  - panel of 407 overlapping peptides (15 to 20 mers) spanning all expressed HCV proteins
  - second panel of 93 peptides (8 to 12 mers) identified as cognate peptides for human $CD8^+$ T cell responses

T cell response analyzed by IFNγ production (hallmark of T cell activation)

- phorbol myristate acetate (PMA) plus ionomycin added as a positive control
- medium alone added to six replicate wells to generate control background values
- enumeration of cells (or "spots") per million PBMCs that produce IFNγ
- scores adjusted by subtracting the average background value from each peptide pool and also by correcting the peptide pool score at each timepoint by subtraction of the baseline value for that given peptide pool

Definition of categorical immune response

An algorithm was pre-specified to evaluate the IFNγ T cell response in terms of breadth, duration and magnitude since it has been previously shown that acutely infected HCV subjects generate T cell responses that are robust in magnitude and show broad HCV epitope recognition (1, 14, 15).

ELISpot Response Algorithm

For a subject to be deemed a responder, the following stringent criteria had to be met.

Overlapping (non-optimized) peptide ELISpot:

- 15 or more pools ≥ 25 spots at one visit
- Or at least 10 pools ≥ 25 spots at one visit with at least 2 pools positive (≥ 25) on more than one on-treatment measurement
- Or at least 5 pools ≥ 25 spots at one visit with at least one pool ≥ 150 spots

Non-overlapping, discrete (optimized) peptide ELISpot:

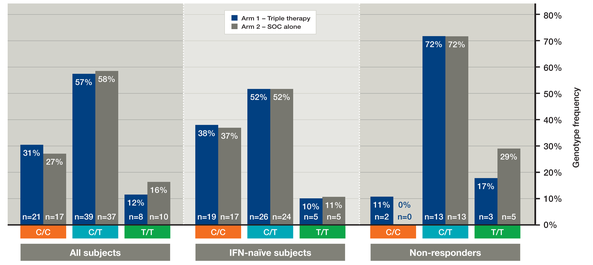
- 4 or more pools ≥ 75 spots at one visit
- Or at least 2 pools ≥ 75 spots with at least 1 pool positive (≥ 75) for more than one on-treatment measurement
- Or at least 2 pools ≥ 75 spots with at least 1 pool ≥ 150 spots

The IFNγ responses were evaluated by different treatment periods through Run-in (GI-5005 treated subjects only), triple therapy or SOC and the post treatment follow-up period.

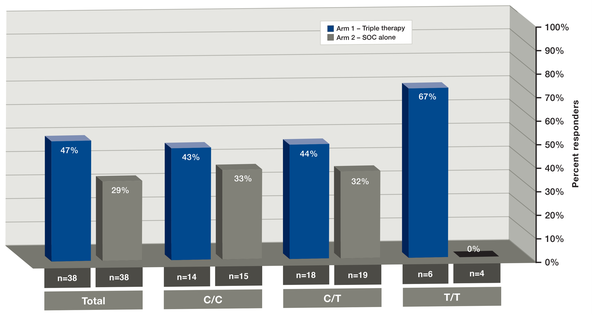
IL28B genotype and response to therapy

IL28B genotypes predict spontaneous clearance of HCV (16), and response to pegIFN/ribavirin therapy (17). The role of *IL28B* in acute clearance of HCV strongly suggests that it is a marker of cellular immunity. *IL28B* testing in GI-5005-02 showed excellent balance between the GI-5005 triple therapy and SOC groups. Important differences were noted for the different *IL28B* genotypes related to the timing and magnitude of HCV specific cellular immunity as measured by IFNγ ELISpot assay. GI-5005 triple therapy improved HCV specific cellular immunity as measured by IFNγ ELISpot assay in all *IL28B* subgroups (C/C; 43% vs 33%, C/T; 44% vs 32%, T/T; 67% vs 0%) as well as end of treatment viral clearance (C/C; 84% vs 76%, C/T; 69% vs 54%, T/T; 60% vs 20%) and SVR in C/C (74% vs 65%) and T/T groups (60% vs 0%). The greatest favorable treatment effect for GI-5005 was observed in the T/T group (ETR +40% and SVR +60%). The low levels of HCV specific cellular immune responses measured in the SOC *IL28B* T/T group suggest that poor cellular immunity may be the most significant deficit in these patients, and point to new models of pathogenesis and response to antiviral therapy.

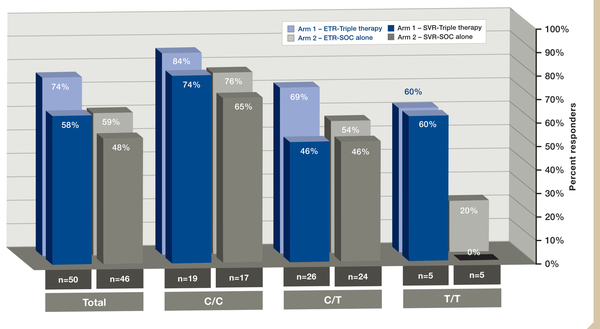
IL28B genotype is well balanced in GI-5005-02



Categorical cellular immune response *IL28B* subgroups (naïve)



Virologic response *IL28B* subgroups (naïve)

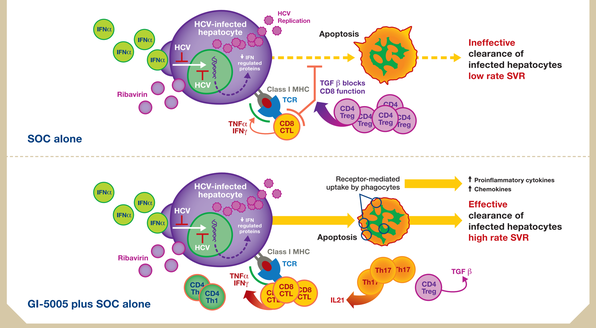


Hypothetical model of Tarmogen effects on immune mediated hepatic clearance

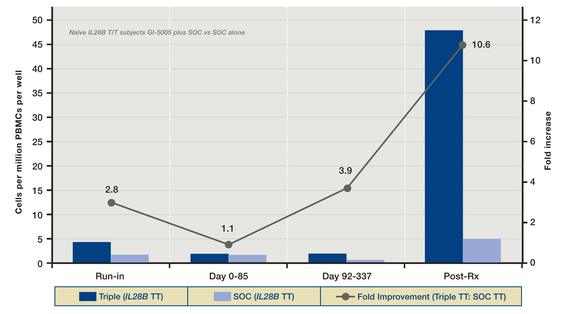
1) SOC alone: SOC inhibits viral replication, but hepatic clearance is poor due to low numbers of HCV specific $CD4^+$ and $CD8^+$ T cells in the liver and suppression of these effector T cells by suppressive regulatory T cells (Tregs).

2) GI-5005 plus SOC: activates the number and functionality of effector $CD4^+$ and $CD8^+$ T cells in the liver. Also reduces the number and functionality of Tregs via the Th17 pathway, further unbridling the favorable CD4/CD8 effects. Th17 cells also produce IL21, which increases the longevity of $CD8^+$ T cells.

Immune-mediated clearance of HCV

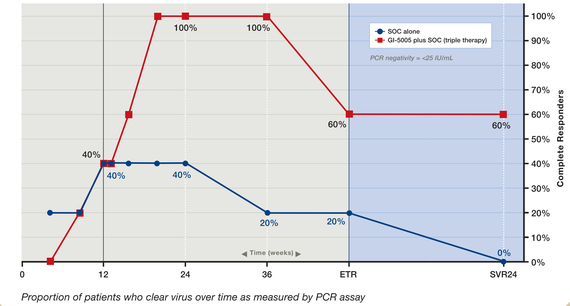


ELISpot assay/change from baseline



**SOC Day0-85 as comparator for both GI-5005 run-in, and GI-5005+SOC Day0-85 periods*

GI-5005-02 *IL28B* T/T complete response (IFN-naïve, ITT)



Proportion of patients who clear virus over time as measured by PCR assay

Conclusions

1) GI-5005 triple therapy subjects with *IL28B* T/T genotype had the greatest advantage in SVR as well as IFNγ ELISpot assay, indicating that GI-5005 is compensating for a deficit in T cell immunity in these subjects.

2) SOC *IL28B* T/T subjects had notably poorer virologic and IFNγ ELISpot responses than SOC C/C and C/T patients, indicating that the fundamental deficit in these patients is one of cellular immune response.

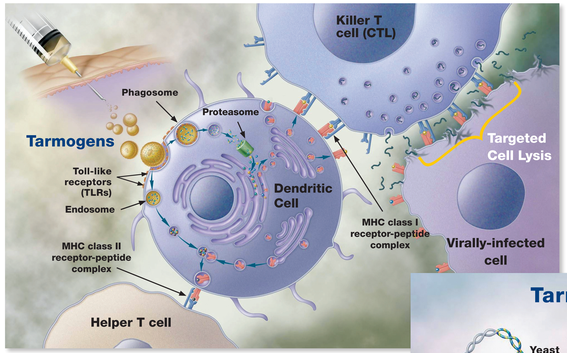
3) The relationship of GI-5005 immune and virologic response to *IL28B* status suggests an alternate model of pathogenesis for chronic HCV where differences in HCV specific cellular immunity play a major role in driving sustained response to antiviral therapy.

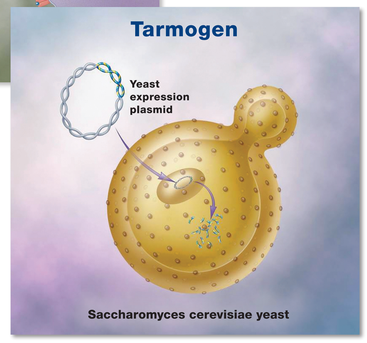
The consistency of the clinical immunology and virologic data provides further confidence in the SVR advantage observed in this phase 2 clinical trial.

GlobeImmune, Inc
1450 Infinite Drive
Louisville, Colorado 80027
tel 303-625-2700
fax 303-625-2710
www.globeimmune.com

Active immunotherapy with yeast-based Tarmogens

Tarmogens are whole, heat-killed recombinant *Saccharomyces cerevisiae* yeast modified to express one or more protein targets that stimulate the immune system against diseased cells. The target antigens are markers of diseased cells and can be conserved viral proteins, mutated proteins unique to cancer cells, or proteins over-expressed in cancer. To create a new Tarmogen, DNA encoding target protein antigens is engineered into a yeast expression plasmid. The heat-inactivated yeast, with the target protein inside, is administered as the Tarmogen product. Tarmogens stimulate the innate and antigen-specific immune system to produce a highly specific and potent T cell response against the diseased cell, with little or no impact on healthy cells.[1]





Tarmogens are administered subcutaneously and are avidly taken up by antigen presenting cells (APCs), such as dendritic cells and macrophages in a process mediated by Toll-like receptors (TLRs) found on the cell surface. Uptake of Tarmogens activates the APCs and results in their migration to lymph nodes and their production of immune-stimulating cytokines.[2,3]

Tarmogens are degraded inside APCs within hours and the target antigens are presented by MHC class I and II receptors on the APC surface. Tarmogens are initially digested in phagosomes, whereupon the antigens are delivered to the cytosol, and these proteins are cleaved by proteasomes into small peptides. These small peptides are loaded into newly folded MHC class I receptors in the secretory pathway of the APC. The peptide-MHC I receptor complex is shuttled to the surface of the APC, where the antigenic peptides are presented to CD8+ killer T cells (causing activation of these cells). Tarmogens are also digested in endosomes, and the product-associated peptides are loaded into MHC class II receptors for antigen presentation to CD4+ helper T cells (causing activation of these cells).[2,3]

Therapeutic benefit from the Tarmogen is driven by the targeted activation of the immune system. Tarmogens activate killer T cells capable of locating and destroying the target cancer or virally-infected cells. Repeated dosing with Tarmogens further increases the number of T cells available to eliminate diseased cells. In summary, Tarmogens couple the innate immune response to yeast with potent activation of antigen-specific cellular immune responses against cancer cells or virally infected cells.[3-4]

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Abstract

GI-5005 THERAPEUTIC VACCINE IMPROVES DEFICIT IN CELLULAR IMMUNITY IN *IL28B* GENOTYPE T/T, TREATMENT-NAÏVE PATIENTS WITH CHRONIC HEPATITIS C GENOTYPE 1 WHEN ADDED TO STANDARD OF CARE (SOC) PEG-IFN-ALFA-2A/RIBAVIRIN

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[1]Department of Medicine and Surgery, Baylor College of Medicine, Houston, TX, [2]St. Luke's Episcopal Hospital, Houston, TX, [3]Duke Clinical Research Institute, Duke University Medical Center, Durham, NC, [4]Center for the Study of Hepatitis C, Weill Cornell Medical College, New York, NY, [5]Department of Medicine, University of Arizona College of Medicine, Tucson, AZ, [6]Center for Liver Diseases, University of Miami School of Medicine, Miami, FL, [7]University of Texas Southwestern Medical Center at Dallas, Dallas, TX, [8]Liver Institute of Virginia, Bon Secours Health System, Newport News, VA, [9]University of Hawaii, Honolulu, HI, [10]Alamo Medical Research, San Antonio, TX, [11]GlobeImmune, Inc., Louisville, CO, [12]University of Pennsylvania, Philadelphia, PA, [13]QST Consultations, Allendale, MI

Background and aims: GI-5005 is a therapeutic vaccine that elicits HCV-specific T-cell responses. An earlier report from this study (J Hepatol. 2010; 52: S45) showed an improved rate of sustained virological response (SVR) in *IL28B* T/T subjects receiving GI-5005 + SOC compared to SOC alone. This abstract describes the impact of GI-5005 on HCV-specific T-cell responses in subjects with the *IL28B* T/T genotype.

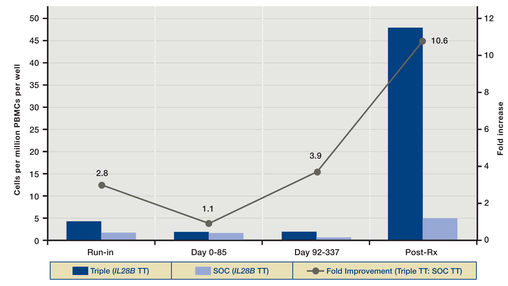
Methods: Arm 1- (n=50, $n_{T/T}$=6) 5 weekly followed by 2 monthly SC injections of 40YU GI-5005 (1YU=10^7 yeast), followed by 40YU GI-5005 monthly + SOC for 48 weeks or; Arm 2- (n=46, $n_{T/T}$=5) SOC alone. Subjects were tested for *IL28B* genotype. HCV-specific T-cell activation was analyzed by interferon-γ ELISpot assay of peripheral blood mononuclear cells (PBMCs) stimulated *ex vivo* with HCV peptide antigens.

Results: 1) In SOC subjects, HCV-specific cellular immune responses were up to 17-fold lower in *IL28B* T/T subjects compared to other *IL28B* subgroups (0.4 vs 6.6 T-cells/10^6 PBMCs/well).

2) In GI-5005 + SOC subjects, HCV-specific cellular immune responses were up to 5-fold higher in *IL28B* T/T subjects compared to other *IL28B* subgroups (47.5 vs 8.9 T-cells/10^6 PBMCs/well).

3) HCV-specific T-cell responses were increased by up to 10-fold in *IL28B* T/T subjects receiving GI-5005 + SOC compared to SOC alone (47.5 vs 4.5 T-cells/10^6 PBMCs/well; see Figure).

Change from baseline of HCV specific T-cells by ELISpot assay (*IL28B* T/T subjects; GI-5005+SOC compared to SOC alone)



*SOC Day0-85 as comparator for both GI-5005 run-in, and GI-5005+SOC Day0-85 periods

4) Improved HCV-specific immunity in GI-5005 +SOC treated *IL28B* T/T subjects correlated with the previously reported improvement in SVR (60% vs. 0%) compared to *IL28B* T/T subjects treated with SOC alone.

Conclusions: HCV specific T-cell responses are depressed in *IL28B* T/T subjects receiving SOC. HCV-specific T-cell responses were increased by 10-fold in *IL28B* T/T subjects receiving GI-5005 + SOC compared to SOC alone. GI-5005 + SOC can overcome and improve the blunted HCV-specific T cell responses in subjects with the *IL28B* T/T genotype and could redefine therapy for this subgroup of patients who are refractory to SOC.

Poster references

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