A RANDOMIZED PHASE 2 ADJUVANT TRIAL OF RESECTED SUBJECTS WITH RAS MUTATION BEARING PANCREAS CANCER TREATED WITH GI-4000 AND GEMCITABINE OR GEMCITABINE ALONE: A SAFETY ANALYSIS OF THE FIRST 100 TREATED SUBJECTS

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Introduction

The GI-4000 Tarmogen is designed to target cancers caused by a mutation in the Ras protein. Mutated Ras proteins permanently remain in an activated state, resulting in unregulated cell division and tumorigenesis. Mutations in Ras are found in approximately 30% of all human tumors and represent the underlying cause of approximately 170,000 new cases of cancer in the US annually.

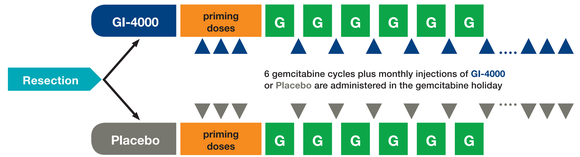
The spectrum of tumor types found to harbor Ras mutations is broad, reflecting the pivotal role Ras plays in regulating cell division. For some cancers such as NSCLC and colorectal cancer, the presence of a Ras mutation in the tumor has been associated with a significantly poorer prognosis.

Studies have shown that NSCLC tumors with Ras mutations are associated with a lack of response to tyrosine kinase inhibitors such as erlotinib and gefitinib. Further, in some studies, chemotherapy has also shown poorer clinical outcomes for NSCLC subjects with Ras mutations. Additionally in colorectal cancer, subjects with tumors harboring Ras mutations do not benefit from anti-EGFR antibodies such as cetuximab or panitumumab.

Approximately 90% of all pancreas cancer is caused by Ras mutations, which may be why pancreas cancer has such a dismal prognosis. Conventional cancer therapies to date have had a limited impact on disease outcome in pancreas cancer. Pancreas cancer is rarely curable with a median survival of 9-12 months and an overall 5-year survival rate of 5% for all stages. Among subjects whose disease is considered to be surgically resectable, 50% will die from recurrent disease within two years. Because of the central role for mutated-Ras activation of tumor proliferation, T cell immune mediated elimination of cells harboring mutant Ras proteins could result in activity in a broad range of human cancers.

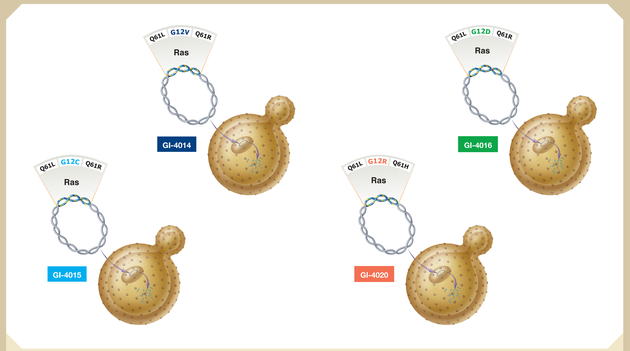
GI-4000 is a series of 4 yeast that express the 7 most common Ras mutations. Subject tumors are sequenced to elucidate the specific Ras mutation contained in their tumor, and the corresponding Tarmogen with the same mutated protein is administered. GI-4000 is subject specific, as the subject only receives the Tarmogen with the Ras mutation matching the Ras mutation causing their tumor. However, GI-4000 is not a custom manufactured product; each Tarmogen in the GI-4000 series is manufactured and vialed separately, and is available off-the-shelf.

GI-4000-02 is a double-blind, randomized, active-control adjuvant phase 2b trial in resected pancreas cancer. The study population includes subjects with resected pancreas cancer who have a product-related Ras mutation and an R0 or R1 resection by Whipple procedure. The data presented herein represent blinded safety data from the first 100 subjects enrolled in this study.



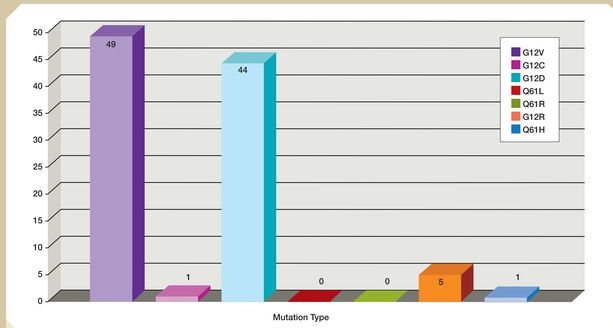
GI-4000-02: Phase 2 study design

GI-4000-02 is a randomized, double-blind trial evaluating GI-4000 vs. placebo in combination with 6 cycles of adjuvant gemcitabine in subjects with successfully resected pancreas cancer (R0 or R1). This study will enroll at least 100 subjects at 40 US centers and 15 international centers. A Bayesian statistical approach will be used to evaluate efficacy through multiple sequential analyses. Study enrollment may be expanded up to a total of 200 subjects based on the results of these analyses. Subjects receive 3 priming doses of study drug or placebo prior to initiation of gemcitabine therapy, followed by monthly doses of study drug or placebo, continuing until disease recurrence. Recurrence-free survival (RFS) is the primary endpoint.



GI-4000 targets mutated Ras

GI-4000 consists of four different heat-inactivated *S. cerevisiae* yeast GI-4014, GI-4015, GI-4016 and GI-4020 expressing the seven most common Ras mutation seen in human cancers. Each of the four yeast expresses a fusion protein of three different Ras mutations. Each protein product expressed in the yeast contain two mutations at codon 61 (glutamine to arginine [Q61R] or glutamine to histidine [Q61H], and glutamine to leucine [Q61L], plus one of four different mutations at codon 12 (either glycine to valine [G12V], glycine to cysteine [G12C], glycine to aspartate [G12D], or glycine to arginine [G12R]).



Ras mutations identified in the first 100 subjects

Approximately 90% of pancreas tumors harbor a mutation in the Ras oncogene. In the first 100 subjects of the GI-4000-02 study, the most common mutations were at codon 12 (glycine to valine [G12V] and glycine to aspartate [G12D]). The less common mutations that were identified were at codon 12 and 61 (glycine to cysteine [G12C], glycine to arginine [G12R], and glutamine to histidine [Q61H]).

Demographics

Variable	Resection Status		Total (n=100)
	R0 (N=77)	R1 (N=23)	
Sex			
Female	32 (41.6%)	8 (34.8%)	40 (40.0%)
Male	45 (58.4%)	15 (65.2%)	60 (60.0%)
Race			
White	60 (77.9%)	18 (78.3%)	78 (78.0%)
Black	7 (9.1%)	3 (13.0%)	10 (10.0%)
Asian	6 (7.8%)	1 (4.3%)	7 (7.0%)
Hispanic	4 (5.2%)	1 (4.3%)	5 (5.0%)
Other	0 (0.0%)	0 (0.0%)	0 (0.0%)
Age			
25 to <45 years	6 (7.8%)	0 (0.0%)	6 (6.0%)
45 to <65	43 (55.8%)	13 (56.5%)	56 (56.0%)
65 to <75	20 (26.0%)	5 (21.7%)	25 (25.0%)
≥75 years	8 (10.4%)	5 (21.7%)	13 (13.0%)

The median age is 61 years; 60% of the study population is male, and resection status is R0 77% or R1 23%.

Adverse events summary

Variable	Resection Status		Total (N=100)
	R0 (N=77)	R1 (N=23)	
All adverse events			
Number of adverse events	1865	546	2411
Number of subjects who had an adverse event	74 (96.1%)	22 (95.7%)	96 (96.0%)
Number of adverse events resulting in study drug discontinuation	10	6	16
Number of subjects with an adverse event resulting in study drug discontinuation	8 (10.4%)	5 (21.7%)	13 (13.0%)
Treatment emergent serious adverse events			
Number of treatment emergent serious adverse events	37	22	59
Number of subjects who had a treatment emergent serious adverse event	23 (29.9%)	10 (43.5%)	33 (33%)

33% of subjects had a total of 59 treatment emergent SAEs. All grade 3-4 AEs occurring in > 5% of the population: neutropenia, abdominal pain, anaemia, and fatigue. There have been an equivalent number of discontinuations, serious adverse events, adverse events, and deaths compared to published data.

Exposure to study drug

Doses of Study Drug Administered	Resection Status		Total (N=100)
	R0 (N=77)	R1 (N=23)	
Median (doses)	13.0	10.0	13.0

The median exposure to gemcitabine is 6 cycles. The median exposure to study drug (GI-4000 or placebo) is 13 doses/42.2 weeks.

Grade 3-4 adverse events

Grade 3 and 4 adverse events occurring ≥ 3 times		
Event term	**Total # of events on GI-4000-02 (n=100)**	**# Of subjects experiencing each event (N=100)**
Neutropenia	54	28
Neutrophil count decreased	16	5
Anaemia	12	10
Fatigue	10	9
Abdominal pain	10	8
Thrombocytopenia	6	3
Dehydration	6	5
Hypertension	5	5
Bile duct obstruction	5	5
Gastrointestinal haemorrhage	5	3
Abdominal abscess	4	4
Hyperglycaemia	4	3
Nausea	4	3
Small intestinal obstruction	4	3
Hypokalaemia	3	3
Dyspnoea	3	3
Granulocytopenia	3	3
Hyperbilirubinaemia	3	3
Hypoglycaemia	3	3
Back pain	3	2
Hyponatraemia	3	2
Malnutrition	3	2

Subject disposition

Variable	Resection status		Total (N=100)
	R0 (n=77)	R1 (n=23)	
Study disposition			
Continuing study	50 (64.9%)	7 (30.4%)	57 (57.0%)
Discontinued study	27 (35.1%)	16 (69.6%)	43 (43.0%)
Study drug disposition			
Continuing treatment	30 (39.0%)	2 (8.7%)	32 (32.0%)
Discontinued treatment	47 (61.0%)	21 (91.3%)	68 (68.0%)

Conclusions

- Safety profile of the first 100 subjects (in a blinded pooled analysis) appears to be similar to the safety profile of adjuvant gemcitabine alone post resection in pancreas cancer subjects.
- The most common grade 3-4 adverse events (occurring in at least 10% of the subjects) seen in the first 100 subjects were neutropenia, neutrophil count decreased, and anaemia.
- Based on quarterly Bayesian analyses, enrollment may be expanded to up to 200 subjects.
- Unblinded safety and efficacy data results will be available upon completion of the trial.

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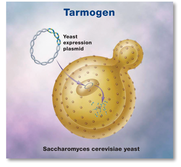
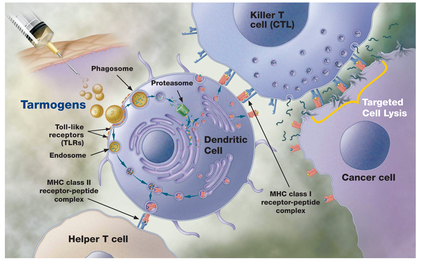
Active immunotherapy with yeast-based Tarmogens

Tarmogen® products 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 final 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]

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]

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]

For more information, visit www.globeimmune.com.

[1] Munson et al. "Coupling Innate and Adaptive Immunity with Yeast-Based Cancer Immunotherapy" Chapter 9; Cancer Vaccines and Tumor Immunity. January 2008
[2] Bernstein et al. "Recombinant Saccharomyces cerevisiae (yeast-CEA) as a potent activator of murine dendritic cells." Vaccine (2008) 26, 509-521.
[3] Wansley et al. "Vaccination with a Recombinant Saccharomyces cerevisiae Expressing a Tumor Antigen Breaks Immune Tolerance and Elicits Therapeutic Antitumor Responses" Clinical Cancer Research. July 2008.
[4] Haller et al. "Whole recombinant yeast-based immunotherapy induces potent T cell responses targeting HCV NS3 and Core proteins" Vaccine (2007) 25, 1452-1463.

A randomized phase 2 adjuvant trial of resected subjects with ras mutation bearing pancreas cancer treated with GI-4000 and gemcitabine or gemcitabine alone: a safety analysis of the first 100 treated subjects.

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Background: Subjects with resected pancreas cancer have a high relapse rate despite standard adjuvant treatment with gemcitabine. Approximately 90% of pancreas tumors harbor a mutation in the Ras oncogene. GI-4000 is a series of 4 whole, heat-inactivated recombinant *S. cerevisiae* yeast, each engineered to express a different mutated Ras oncoprotein. GI-4000 exhibited an excellent safety profile when administered as a single agent in a phase 1 clinical trial.

Methods: The study population includes subjects with resected pancreas cancer who have a product-related Ras mutation and an R0 or R1 resection by Whipple procedure. Subjects were randomized 1:1 to receive GI-4000 or placebo weekly for 3 weeks starting 21-35 days post surgery, monthly during 6 months of gemcitabine therapy, and monthly as a monotherapy thereafter until recurrence, death, or discontinuation. Gemcitabine was administered at 1000mg/m^2 d1,8,15 of a 28 day cycle for 6 cycles.

Results: The median age is 61 years; 60% of the study population is male, and resection status is R0 77% or R1 23%. The median exposure to gemcitabine is 6 cycles. The median exposure to study drug (GI-4000 or placebo) is 11 doses/35 weeks. 33% of subjects had a total of 57 treatment emergent SAE (5% [thought to be] related to GI-4000, 7% [thought to be] related to gemcitabine). 35 deaths and 2 discontinuations due to AE have been reported. Treatment emergent SAEs occurring in > 2% of the population include: small intestinal obstruction and abdominal abscess. Treatment emergent grade 3-4 AEs occurring in > 5% of the population: anemia, neutropenia, abdominal pain, and fatigue. There have been an equivalent number of discontinuations, serious adverse events, adverse events, and deaths compared to published data.

Conclusions: The blinded pooled safety data for the first 100 treated subjects in adjuvant combination (gemcitabine plus GI-4000 and gemcitabine alone) compares well to published data for gemcitabine alone. Based on quarterly Bayesian analyses, enrollment may be expanded to up to 200 subjects. Unblinded safety and efficacy data results will be available upon completion of the trial.