# TRIALS IN PROGRESS: A RANDOMIZED, PLACEBO CONTROLLED, MULTICENTER PHASE 2 ADJUVANT TRIAL OF THE EFFICACY, IMMUNOGENICITY, AND SAFETY OF GI-4000 PLUS GEMCITABINE VERSUS GEMCITABINE ALONE IN PATIENTS WITH RESECTED PANCREAS CANCER WITH ACTIVATING RAS MUTATIONS

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## Summary

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.

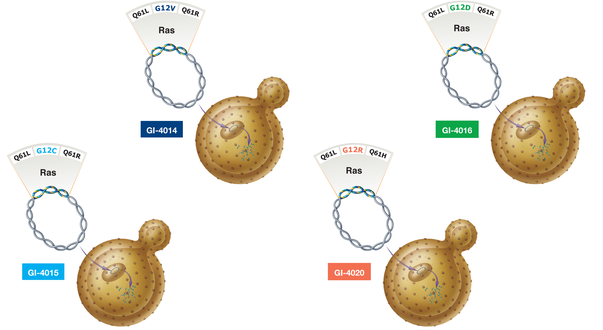
The spectrum of tumor types found to harbor Ras mutations is broad, reflecting the pivotal role Ras plays in regulating cell division. For some cancers such as NSCLC and colorectal cancer, the presence of a Ras mutation in the tumor has been associated with a significantly poorer prognosis.

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 one reason 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. Subjects' tumors are sequenced to identify 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 in 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.




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]). Patient tumors are sequenced to identify the specific Ras mutation contained in their tumor, and only the specific yeast with the matching mutation is administered to the patient.

## Clinical strategy in Ras mutation positive cancers

Annual incidence of mutated Ras-related cancers in the US

| | US Incidence | % mutated Ras | Mutated-Ras Related Cancers |
|---|---|---|---|
| Pancreas | 42,470 | 90% | 38,223 |
| NSCLC | 190,913 | 20% | 38,183 |
| Colorectal | 146,970 | 35% | 51,440 |
| Ovarian | 21,500 | 20% | 4,310 |
| Melanoma | 68,720 | 30% | 20,616 |
| Endometrial | 53,430 | 22% | 11,755 |
| Multiple myeloma | 20,580 | 25% | 5,145 |
| | 544,633 | | 169,671 |

There are ~170,000 new cases of Ras mutation positive cancers in the US annually. Strategically, GI-4000 will be explored in settings where it is possible to integrate GI-4000 with standard of care in settings of surgically or chemically reduced tumor burden and in patients having an estimated potential survival long enough for a therapeutic vaccine to make a difference. GI-4000 will be explored in a preliminary indication for each tumor type and then expanded to additional indications within a tumor type based on clinical results.

Resected pancreas cancer was selected as the initial target for GI-4000. Patients with resected pancreas cancer treated with standard of care gemcitabine have a median recurrence free survival of 14 months, median overall survival of 22 months, and a survival rate of 20% at 5 years. The post-resection patient population represents a compelling model of minimal residual disease for which targeted and active immunotherapy may decrease cancer recurrence and improve survival through elimination of microscopic disease.

## GI-4000-01 Phase 1 study

**Methods:** **Phase 1 monotherapy trial**

- Patient tumors were screened by surgical, biopsy or FNA tumor samples, laser capture microdissection, PCR amplifi cation and genomic DNA sequencing for product-associated K-, H- and N-ras gene mutations at codon 12 or 61.
- Patients were administered the specific GI-4000 series product matching their identified mutation.

**Open-label, dose escalation study (0.1, 0.3, 1, 10, 20 & 40 YU)**

- Subjects received 5 weekly subcutaneous doses
- 1 YU (Yeast Unit) = $10^7$ yeast cells

**33 refractory CRC (18) and pancreas cancer (15) subjects were enrolled**

- 90% Stage IV
- Average prior lines of therapy: 3

**Dosing regimen:**

| Day | Screen | 1 | 8 | 15 | 22 | 29 | 57 | 84 |
|---|---|---|---|---|---|---|---|---|
| Treatment | | X | X | X | X | X | | |
| Immunology performed | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |

**Results:** **Favorable safety profile**

- No drug-related SAEs
- No drug-related discontinuation due to AEs
- No dose-limiting toxicities (DLTs)
- Non-serious AEs limited to mild constitutional symptoms & injection site reactions

**Immune responses in late stage subjects**

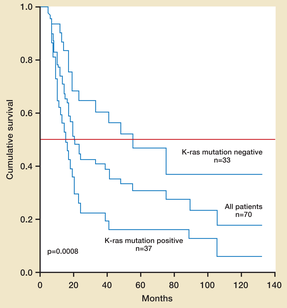
- > 90% of subjects had antigen-specific T cell immune responses
- > 57% of subjects had treatment emergent, antigen-specific T cell responses

**Five subjects survived beyond 48 week follow up**

- One pancreas cancer (790 days)
- Four CRC (1028, 883, 623 & 365 days)

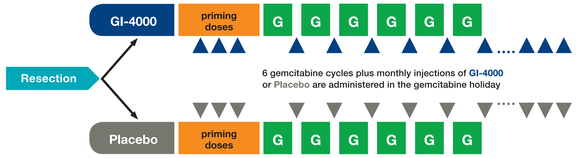
## Clinical rationale for Phase 2b study

A paper published in Gut in 2006 by Kim *et al* demonstrated the importance of mutated Ras as a predictor of outcome, even after successful surgery and adjuvant chemotherapy. This analysis was performed on resected pancreas cancer tissue samples from 70 patients evaluated with histologically clean margins / (R0) resections. This is similar to the population being evaluated in the ongoing phase 2 study with GI-4000 (GI-4000-02). The margins of 53% of subject tumors in the Kim *et al* study were positive for K-ras mutations by PCR; these patients had significantly worse survival. There was a 40 month difference in median overall survival (55 months vs. 15.5 months; p=0.0008) in samples with/without K-ras mutations in the margins by PCR. We believe that these findings suggest that a therapy targeting mutated Ras such as GI-4000 may improve outcomes in those patients with successful surgery and who are receiving adjuvant chemotherapy.




*Adapted from Kim et al. Gut. 2006; 55: 1598-1605*

## 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-4000-02 eligibility criteria

**Inclusion criteria:**

- Subjects must have resectable pancreas cancer, ductal adenocarcinoma type, with post-resection confirmation of non-metastatic disease.
- Confirmed product related mutation in Ras from tumor sample.
- ECOG performance status (PS) of ≤2 prior to randomization.
- Post-operative confirmed R0 or R1 resection status.
- Negative scratch test (immediate hypersensitivity, IgE mediated) to *S. cerevisiae*.

**Exclusion Criteria:**

- Non-resectable pancreatic cancer, or histologic types other than ductal adenocarcinoma, tumors classified as T4, or history of splenectomy.
- Prior chemotherapy, radiation therapy, targeted therapy, or immunotherapy for pancreatic cancer.
- History of another cancer within the last 5 years with the exception of localized basal or squamous cell carcinoma of the skin, stage 1A cervical cancer, or melanoma in situ.
- History of Crohn's disease or ulcerative colitis.
- History of major organ transplantation.
- Concurrent and chronic therapy with corticosteroids or any other immunosuppressive drugs.

## Demographics

| Variable | Resection Status | | Total |
|---|---|---|---|
| | R0 (n=77%) | R1 (n=23%) | |
| **Sex** | | | |
| Female | 42.6% | 37.5% | 41.5% |
| Male | 57.4% | 62.5% | 58.5% |
| **Race** | | | |
| White | 80.1% | 82.5% | 80.7% |
| Black | 7.4% | 10.0% | 8.0% |
| Asian | 5.1% | 2.5% | 4.5% |
| Hispanic | 6.6% | 5.0% | 6.3% |
| Other | 0.7% | 0.0% | 0.6% |
| **Age (Median age: 61 years)** | | | |
| 25 to <45 years | 6.6% | 0.0% | 5.1% |
| 45 to <65 | 54.4% | 62.5% | 56.3% |
| 65 to <75 | 29.4% | 25.0% | 28.4% |
| ≥75 years | 9.6% | 12.5% | 10.2% |

*The median age is 61 years; 59% of the study population is male, and resection status is R0 77% or R1 23%.*

# OTHER GI-4000 TRIALS

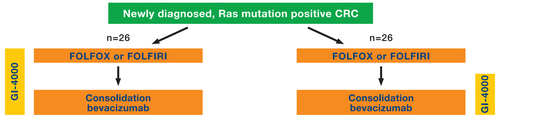
## GI-4000-03 NSCLC (n=24)


*Single center pilot study at Memorial Sloan Kettering Cancer Center*

Single arm, phase 2a consolidation therapy evaluating GI-4000 in subjects with Ras mutation positive NSCLC

- Patients must be NED at their first post-treatment re-staging assessment
- Patient tumor must contain a Ras mutation by direct sequencing to be eligible for the trial
- Dosing: GI-4000 for 3 weekly doses, followed by 6 monthly doses, followed by vaccinations every 3 months for up to 3 years
- Administration of study drug will continue according to this schedule until study withdrawal, disease recurrence, or death
- Endpoints will included safety, immunology, and matched case controls for efficacy

## GI-4000-05 CRC (n=52)




*Single center pilot study at the Lombardi Cancer Center at Georgetown University*

GI-4000-05 is a pilot open label, multi-arm, single center, therapeutic trial in patients with newly diagnosed Ras mutant positive metastatic colorectal cancer (Group A), or patients with Ras mutant positive colorectal cancer who have just completed first line-therapy with an oxaliplatin or irinotecan plus 5-FU and bevacizumab containing regimen (Group B). 52 subjects in two groups of 26 subjects each will be enrolled into the study.

## Conclusions

- Mutated Ras is an important cause of numerous cancers
- GI-4000 is the only novel, late-stage approach directly targeting cancers with mutated Ras

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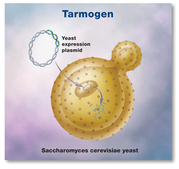
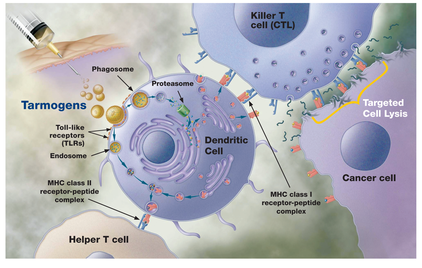
## Active immunotherapy with yeast-based Tarmogens

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.

## Trials in Progress: A Randomized, Placebo Controlled, Multicenter Phase 2 Adjuvant Trial of the Efficacy, Immunogenicity, and Safety of GI-4000 Plus Gemcitabine versus Gemcitabine alone in Patients with Resected Pancreas Cancer with Activating Ras Mutations

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### Background

Patients with resected pancreas cancer treated with standard of care gemcitabine (Gem) have a median recurrence free survival of 14 months, median overall survival of 22 months, and a survival rate of 20% at 5 years. The post-resection patient population represents a compelling model of minimal residual disease for which targeted and active immunotherapy may decrease cancer recurrence and improve survival through elimination of microscopic disease.

Activating mutations in ras occur early in the development of pancreas cancer and are subsequently maintained, being found in > 90% of pancreas cancer cases. This trial is designed to evaluate the efficacy, immunogenicity, and safety of GI-4000 plus Gem vs. placebo plus Gem in patients with resected pancreas cancer and an activating ras mutation.

GI-4000 is a proprietary immunotherapy designed to target cells with activating ras mutations using whole, heat-killed recombinant Saccharomyces cerevisiae yeast (called Tarmogens = Targeted Molecular Immunogens). Tarmogens have demonstrated selective killing of target cells expressing a number of cancer antigens including mutated ras in vivo by activating an antigen-specific T cell mediated response.

### Methods

The study population consists of subjects with pancreas cancer who have an activating mutation in ras and an R0 or R1 resection by the Whipple procedure.

Subjects are randomized to either GI-4000 plus Gem or placebo plus Gem; 3 weekly injections of GI-4000 40YU (or placebo) are followed by 6 cycles of Gem 1000 mg/m2 iv infusion (day 1, 8, 15 every 28 days). Monthly doses of GI-4000 or placebo are administered on the Gem off-weeks and continue monthly for up to 5 years or until subjects experience intolerance, disease recurrence, or death .

This trial uses a Bayesian statistical approach to analyze efficacy on a quarterly basis using time to recurrence as the primary efficacy endpoint and time to mortality as a key secondary efficacy endpoint. Enrollment is ongoing and may continue up to 200 subjects based on pre-specified treatment effects observed for RFS and OS.