A PHASE 2 ADJUVANT TRIAL OF GI-4000 PLUS GEM VS. GEM ALONE IN RAS MUTATION+ RESECTED PANCREAS CANCER: R1 SUBGROUP AND PROTEOMIC ANALYSIS

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Introduction

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 patients 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.

Additionally, 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 benefit 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. In colorectal cancer, subjects with tumors harboring Ras mutations do not benefit from anti-EGFR antibodies such as cetuximab or panitumumab.

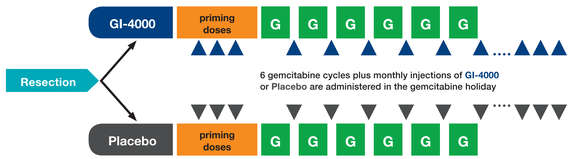
The GI-4000 Tarmogen is designed to target cancers caused by a mutation in the Ras protein. Mutated Ras proteins permanently remain in an activated state, resulting in unregulated cell division and tumorigenesis. Mutations in Ras are found in approximately 30% of all human tumors and may be associated with approximately 180,000 new cases of cancer in the US annually.

GI-4000 is a family of four different Tarmogens. Each Tarmogen product is a heat-inactivated *S. cerevisiae* yeast expressing a unique combination of three Ras mutations, collectively targeting the seven most common Ras mutations seen in human cancers. In these trials, a patient's tumor is sequenced to identify the specific Ras mutation contained in the tumor. Only the corresponding off-the-shelf Tarmogen containing the identified mutated protein is administered to the subject. Each Tarmogen in the GI-4000 series is manufactured and vialed separately.

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 seven common Ras mutations in human cancers. Each of the four yeast expresses a fusion protein of three different Ras mutations. Each protein product expressed in the yeast contains 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.

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 enrolled 176 subjects at 40 US centers and 15 international centers. A Bayesian statistical approach was used to evaluate efficacy through multiple sequential analyses. Subjects receive 3 priming doses of study drug or placebo prior to initiation of adjuvant gemcitabine therapy, followed by monthly doses of study drug or placebo in the 2 week holiday between cycles of gemcitabine. Study therapy is administered until disease recurrence, with recurrence-free survival as the primary endpoint for the trial.

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 pancreas 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 pancreas 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.

Patient-specific demographics (R1s)

Characteristic	GEM + GI-4000 n=19	GEM + Placebo n=20	Total n=39
Mean age (SD)	62.1 (10.2)	62.4 (9.8)	62.3 (9.9)
Female	6 (31.6%)	9 (45.0%)	15 (38.5%)
Male	13 (68.4%)	11 (55.0%)	24 (61.5%)
White	15 (78.9%)	17 (85.0%)	32 (82.1%)
Black	3 (15.8%)	1 (5.0%)	4 (10.3%)
Asian	0 (0.0%)	1 (5.0%)	1 (2.6%)
Hispanic	1 (5.3%)	1 (5.0%)	2 (5.1%)
Other	0 (0.0%)	0 (0.0%)	0 (0.0%)

Disease-specific demographics (R1s)

Characteristic		GEM + GI-4000 n=19	GEM + Placebo n=20	Total n=39
Primary Tumor	pT1 – Tumor Limited to Pancreas, 2 cm or Less in Greatest Dimension	0 (0.0%)	0 (0.0%)	0 (0.0%)
	pT2 – Tumor Limited to Pancreas, More Than 2 cm in Greatest Dimension	2 (10.5%)	1 (5.0%)	3 (7.7%)
	pT3 – Tumor Extends beyond Pancreas without Involvement of Celiac Axis	14 (73.7%)	18 (90.0%)	32 (82.1%)
	pT4 – Tumor Involves Celiac Axis or Super Mesenteric Artery	3 (15.8%)	0 (0.0%)	3 (7.7%)
	Not reported	0 (0.0%)	1 (5.0%)	1 (2.6%)
Regional Lymph Nodes	Node negative	4 (21.1%)	2 (10.0%)	6 (15.4%)
	Node positive	15 (78.9%)	17 (85.0%)	32 (82.1%)
	Not reported	0 (0.0%)	1 (5.0%)	1 (2.6%)
CA 19-9 (U/mL) Post-operative	N	18	18	36
	Mean (SD)	45.8 (64.0)	27.7 (35.5)	36.8 (51.8)
	Median	31.3	11.2	24.9
	Min to Max	1 to 282	1 to 120	1 to 282
	Normal	11 (57.9%)	13 (65.0%)	24 (61.5%)
	Abnormal	7 (36.8%)	5 (25.0%)	12 (30.8%)
	Not reported	1 (5.3%)	2 (10.0%)	3 (7.7%)

Study disposition

176 total subjects enrolled in GI-4000-02 – enrollment completed June 2010

- 39 R1 subjects
- 137 R0 subjects

R1 demographics

- Balanced for baseline demographics
  - Stage, gender, race, age
- Disease specific factors unfavorable against the GI-4000/gemcitabine arm vs. placebo/gemcitabine arm
  - 3/19 (16%) T4s in GI-4000 arm vs. 0 in control arm
  - Post-operative CA19-9 levels higher in GI-4000 arm
    - Mean 46U/mL vs. 28U/mL
    - Median 31U/mL vs. 11U/mL

Safety – treatment emergent adverse events (TEAE, R1s)

Any TEAE occurring in >20%	GEM + GI-4000 (n=19)	GEM + Placebo (n=20)
Anaemia	9 (47.4%)	7 (35.0%)
Nausea	9 (47.4%)	10 (50.0%)
Back Pain	7 (36.8%)	3 (15.0%)
Diarrhoea	7 (36.8%)	9 (45.0%)
Fatigue	7 (36.8%)	10 (50.0%)
Neutropoenia	7 (36.8%)	9 (45.0%)
Dyspnoea	6 (31.6%)	2 (10.0%)
Oedema Peripheral	6 (31.6%)	6 (30.0%)
Pyrexia	6 (31.6%)	6 (30.0%)
Abdominal Pain	5 (26.3%)	7 (35.0%)
Constipation	5 (26.3%)	8 (40.0%)
Dizziness	5 (26.3%)	3 (15.0%)
Injection Site Pain	5 (26.3%)	0 (0.0%)
Insomnia	5 (26.3%)	4 (20.0%)
Vomiting	4 (21.1%)	6 (30.0%)
Weight Decreased	4 (21.1%)	4 (20.0%)
Dyspepsia	3 (15.8%)	4 (20.0%)
Anorexia	3 (15.8%)	6 (30.0%)
Dysgeusia	3 (15.8%)	4 (20.0%)
Depression	1 (5.3%)	6 (30.0%)
Abdominal Distension	1 (5.3%)	5 (25.0%)
Cellulitis	1 (5.3%)	4 (20.0%)
Decreased Appetite	0 (0.0%)	4 (20.0%)

The frequencies of adverse events were comparable between treatment groups and consistent with events expected in the population being studied. The TEAEs occurring with notably higher rates in the GI-4000 treated arm included back pain, dyspnoea and injection site pain. The TEAEs occurring with notably higher rates in the control arm included anorexia, depression, abdominal distension, cellulitis and decreased appetite. The study was monitored by an independent data safety monitoring board at least twice a year for four years with no evidence of significant novel toxicities.

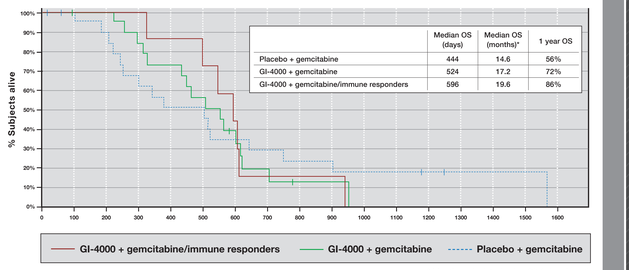
Ras-specific immune response by ELISpot (R1 subject only)

The production of interferon-gamma (IFNγ) by T cells in response to mutated Ras peptides is a hallmark of antigen-specific $CD4^+$ and $CD8^+$ T cell activation and is measured by ELISpot assay. PBMCs were collected pre-treatment and at various timepoints during treatment and were cryopreserved until assay so that longitudinal analysis was possible. Thawed PBMCs were incubated with Ras peptide pools that expressed the matched Ras mutation and with a control set of mismatched peptide pools. Data are expressed as numbers of $IFN\gamma^+$ cells ("spots") per million PBMC after subtraction of baseline score and correction by the appropriate control, mismatched peptide pool.

Categorical Immune Response:
A categorical immune response was defined by the following prespecified criteria: at least one peptide pool or single peptide with an increase from baseline of >= 25 $IFN\gamma^+$ cells/10^6 PBMCs after subtraction of the control (mismatched peptide) response. Additionally the raw score for the specific peptides before subtraction of mismatch control must increase on treatment (increase of >= 25 $IFN\gamma^+$ cells/10^6 PBMCs)

Results:
Twenty seven R1 subjects had sufficient sample for ELISpot testing. GI-4000 showed a greater rate of immune responses to mutated Ras by IFNγ ELISpot assay; GI-4000 7/15 (46.7%) vs. placebo 1/12 (8.3%) (p=0.043 by two-tailed Fisher's exact test).

Overall Survival (R1 only; ITT)

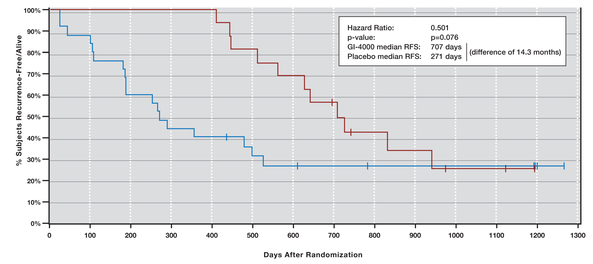


* Months calculated using 30.4 days/month

Exploratory proteomic signature is associated with improved RFS and OS

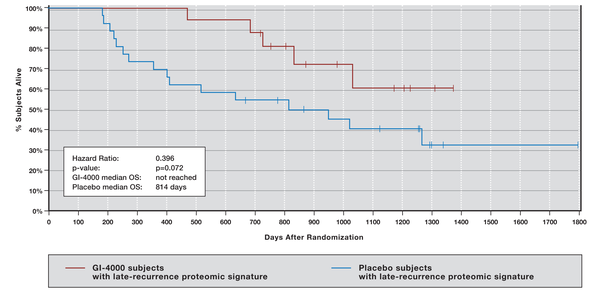
Exploratory MALDI ToF mass-spectrometry proteomics analysis was performed by Biodesix, Inc. on baseline plasma samples from 90 R1 and R0 subjects from this study. This analysis revealed a multi-peak proteomic signature that predicts late- versus early-recurrence in the GI-4000 group, regardless of resection status (R0 vs. R1). When used to evaluate overall survival, the proteomic signature also predicted better and worse overall survival for subjects in the GI-4000 group. Importantly, this multi-peak signature did not predict recurrence or survival in the placebo group and suggested that patients classified in the late-recurrence group may have better outcomes on GI-4000 than on placebo.

RFS by treatment group for subjects with the late-recurrence proteomic signature*



*The late-recurrence proteomic signature was observed at baseline in 16/44 of the GI-4000 subjects, 26/46 of the placebo subjects, and 42/90 subjects overall. Proportions of patients with the early versus late signature may vary in future studies.

OS by treatment group for subjects with the late-recurrence proteomic signature



Cross-validation analysis confirmed the ability of the signature to stratify patients according to RFS in the GI-4000 group, but not in the placebo group. However, these results are exploratory and validation in an independent sample set is essential.

Conclusions

GI-4000 in combination with adjuvant gemcitabine showed evidence of a clinically meaningful effect on survival in Ras mutation positive R1 pancreas cancer subjects, including:

- 2.6 month improvement in median OS (17.2 months compared to 14.6 months); an 18% relative improvement
- 5.0 month improvement in median OS for GI-4000 immune responders (19.6 months compared to 14.6 months); a 34% relative improvement
- 16% advantage in one-year survival (72% vs. 56%); a 30% relative improvement
- 1 month improvement in median RFS (9.6 months for GI-4000/gemcitabine vs. 8.5 months); a 13% relative advantage

GI-4000 was immunogenic and well tolerated in R1 subjects:

- 7/15 (47%) in the GI-4000/gem arm vs. 1/12 (8%) in the placebo/gem arm had Ras mutation specific T cell response
- GI-4000 has been well tolerated to date with no evidence of significant novel toxicities

A multi-peak proteomic signature was identified that was associated with improved clinical outcomes in the GI-4000 treated group but not the placebo group (both R0 and R1 subjects combined):

- The multi-peak proteomic classifier should be prospectively validated and, if positive, could be used to select patients for a randomized phase 3 adjuvant trial in resected pancreas cancer

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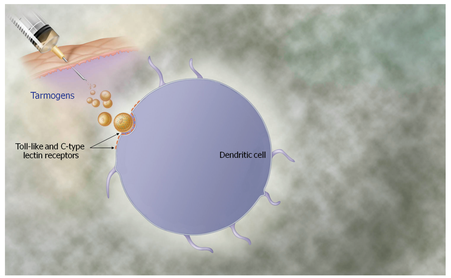
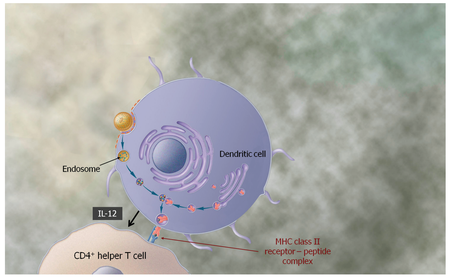
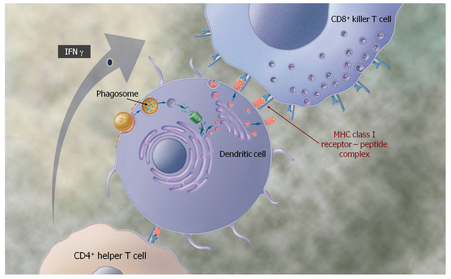
Active immunotherapy with yeast-based Tarmogens



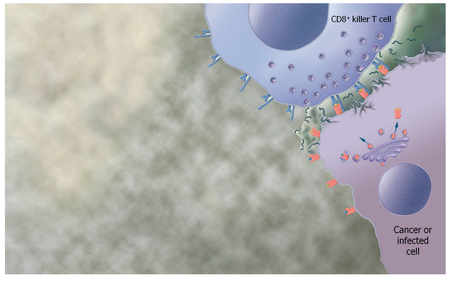
Administration of Tarmogens initially results in binding of the yeast to antigen-presenting cells, the most important of which are dendritic cells, near the injection site. The dendritic cells are activated as a result of the Tarmogens binding to Toll-like receptors and other receptor molecules on the surface of the dendritic cell, resulting in the activation of cytokine immune signaling molecules. The dendritic cell also engulfs the Tarmogen. Multiple Tarmogens may be taken up by the same dendritic cell.



The Tarmogen is processed by the dendritic cell in two ways. First, the Tarmogen is engulfed by endosomes and the protein inside the endosome is cut into shorter peptides fragments. These peptides are presented by Class II MHC molecules on the surface of the dendritic cell. In combination with IL-12, a cytokine that is produced by the dendritic cell, these MHC-peptide complexes on the surface of the dendritic cell are recognized by and activate cells involved in viral immunity called $CD4^+$ helper T cells.



Dendritic cells also process Tarmogens by engulfing them with phagosomes. This results in presentation of peptides, including the antigen from inside the Tarmogen, to $CD8^+$ killer T cells, via Class I MHC molecules on the surface of the dendritic cell, resulting in proliferation of identical antigen specific $CD8^+$ T cells. $CD4^+$ helper T cells are so named because one of their roles is to "help" activate killer T cells by expressing interferon gamma (IFN_γ).



The newly activated $CD8^+$ killer T cells move throughout the body and identify any other cell that expresses the same disease protein as the one recognized by the $CD8^+$ killer T cells. Once the $CD8^+$ killer T cell finds another cell in the body containing the target protein, it can kill the cell using multiple mechanisms.

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Background: Patients with resected pancreas cancer treated with standard of care gemcitabine have a median overall survival of 22 months (vs. 20 months with observation). Activating mutations in *ras* occur in > 90% of pancreas cancer cases. 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. This trial is designed to evaluate the efficacy, immunogenicity, and safety of GI-4000 plus gemcitabine in patients with Ras mutant positive resected pancreas cancer.

Methods: The study enrolled 176 subjects with Ras mutant positive adenocarcinoma of the pancreas post resection randomized 1:1 to GI-4000 plus gemcitabine or placebo plus gemcitabine (stratified by resection status; R0 or R1). Three weekly injections of GI-4000 or placebo were followed by 6 cycles of gemcitabine 1000 mg/m2 iv (day 1, 8, 15 every 28 days). Monthly GI-4000 or placebo were administered on the gemcitabine off-weeks and continued monthly until disease recurrence, intolerable toxicity, or death. The primary endpoint is recurrence-free survival. Data for the 39 R1 subjects (GI-4000 N=19, Placebo N=20) have been unblinded and analyzed.

Results: The GI-4000 group had an 11.4 week advantage in median overall survival (524 Days vs. 444 Days), 16% advantage in 1 year survival (72% vs. 56%), and a 4.6 week advantage in median RFS (287 Days vs. 255 days). The GI-4000 group showed a significantly higher rate of mutation specific T cell response to Ras by ELISpot assay; 7/15 (47%) vs. 1/12 (8%), p=0.043, with a more pronounced survival benefit in GI-4000 treated immune responders; 21.7 week advantage in median survival (596 Days vs. 444 Days) compared to placebo. No significant novel toxicities have been observed to date.

Conclusions: GI-4000 in combination with adjuvant gemcitabine showed a clinically meaningful point estimate for the treatment effect on survival in R1 subjects with Ras mutant positive adenocarcinoma of the pancreas. GI-4000 was immunogenic and well tolerated. Ras specific immune response was associated with a more pronounced benefit in median survival. These data warrant further study in a definitively powered clinical trial for GI-4000 in the adjuvant setting in R1 subjects. The results for the R0 cohort will be reported when the data are mature.