GI-4000 VACCINE AS ADJUVANT CONSOLIDATION THERAPY IS IMMUNOGENIC FOLLOWING DEFINITIVE TREATMENT IN PATIENTS WITH STAGE I-III ADENOCARCINOMA OF THE LUNG WITH KRAS MUTATIONS

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Background

The GI-4000 Tarmogen is designed to target cancers caused by a mutation in the KRAS protein. Mutated KRAS proteins permanently remain in an activated state, resulting in unregulated cell division and tumorigenesis. Mutations in KRAS 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.

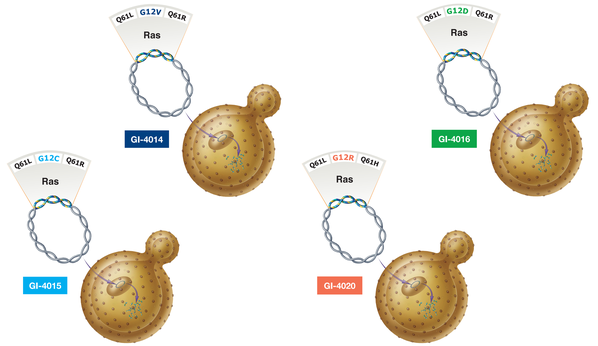
The spectrum of tumor types found to harbor KRAS mutations is broad, reflecting the pivotal role KRAS plays in regulating cell division. Approximately 90% of all pancreas cancer is caused by KRAS mutations, which may be one reason why pancreas cancer has such a dismal prognosis. For some cancers such as non-small cell lung cancer / NSCLC (~20%) and colorectal cancer / CRC (~35%), the presence of a KRAS mutation in the tumor has been associated with a significantly poorer prognosis.

Studies have shown that NSCLC tumors with KRAS 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 KRAS mutations. Additionally in colorectal cancer, subjects with tumors harboring KRAS mutations do not benefit from anti-EGFR antibodies such as cetuximab or panitumumab.

At Memorial Sloan Kettering (MSKCC), routine diagnostic molecular testing has been performed on all lung adenocarcinomas since 2006. This has allowed patients to be enrolled in mutation specific adjuvant clinical trials and has informed selection of chemotherapy at time of recurrence. Research regarding the independent prognostic significance of EGFR/KRAS mutations is on-going at MSKCC and elsewhere.

GI-4000 is a series of four recombinant yeast expressing the seven most common KRAS mutations. Subjects' tumors are sequenced to identify the specific KRAS 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 KRAS mutation matching the KRAS 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.

This study is a consolidation therapy trial evaluating GI-4000 in subjects with NSCLC treated with curative intent who are disease free at their first post-treatment re-staging assessment (1-4 months after completing therapy).



GI-4000 for mutated-KRAS cancers

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 KRAS mutations seen in human cancers. Each of the four yeast expresses a fusion protein of three different KRAS 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 KRAS mutation contained in their tumor, and only the specific yeast with the matching mutation is administered to the patient.

GI-4000-03 NSCLC (n=24)

*1 yeast unit (YU) = 10^7 yeast

Single center pilot study at Memorial Sloan Kettering Cancer Center

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

- 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 included safety, immunology, and matched case controls for efficacy

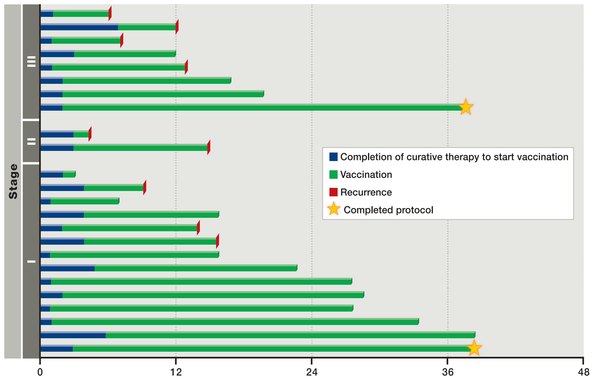
Inclusion criteria

- Pathologically proven stage I-III lung adenocarcinoma
- Patients must be NED (no evidence of disease) at their first post-treatment re-staging assessment
- Vaccine related KRAS mutation

Exclusion criteria

- Active immunosuppressive therapy
- History of Crohn's disease or ulcerative colitis
- History of allergy or positive skin test to *S. cerevisiae*

Patient disposition



Patient disposition is shown in the figure above, with key landmarks denoted for completion of first line therapy, use of consolidation vaccine therapy, recurrence, death (no mortality observed yet), and completion of 3 year vaccine regimen. Study follow up for at least 6 months has been completed in all subjects (1 subject withdrew from the study early) and the primary analysis milestone for safety and immunology has been achieved.

Patient demographics

Age (years)	Total (N=33)
Median	66
Minimum to Maximum	46 to 79
18 to < 45 years	0 (0%)
45 to < 65 years	15 (46%)
65 to < 75 years	13 (39%)
≥ 75 years	5 (15%)
Gender	
Male	10 (30%)
Female	23 (68%)
Race	
White	32 (97%)
Black/African American	1 (3%)
Asian	0 (0%)
Hispanic	0 (0%)
Other	0 (0%)

ECOG performance status

	n=	ECOG 0	ECOG 1
Stage I	14	9 (64%)	5 (36%)
Stage II	2	1 (50%)	1 (50%)
Stage III	8	3 (38%)	5 (63%)
	24	13 (54%)	11 (46%)

Subject disposition

Study disposition	Total (N=33)
Completed protocol requirements	2
Continuing the study	11
Discontinued the study early	20
Progressive disease	8
Patient withdrawal of consent	3
Relapse	0
Screen failures	9

ELISpot methods and response criteria

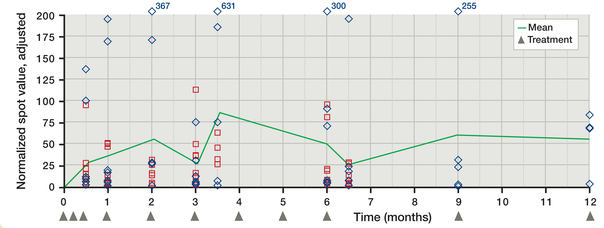
- Peripheral blood mononuclear cells (PBMCs) cryopreserved until assay
- All time points from a subject assayed on same day (longitudinal analysis)
- PBMCs thawed and incubated with KRAS peptides *ex vivo*
  – Positive control: the human T cell mitogen, phytohemagglutinin (PHA)
  – Negative controls: assay medium or a set of mismatched Ras peptides
  – Addition of KRAS peptides at 12.5 to 50 µg/ml
- T cell response analyzed by ELISpot for IFN-γ production (hallmark of T cell activation)
  – Enumeration of cells (or "spots") per million PBMCs that produce IFN-γ
- Two testing approaches were used. Algorithms were pre-specified to evaluate the IFN-γ T cell response. For a subject to be deemed a responder, the following criteria had to be met:
  – **Round 1: <u>10 subjects</u> with up to six months of immune sampling available**
  Response algorithm used: For baseline negative subjects*: at least one KRAS peptide pool with an increase from baseline of ≥ 25 IFN- γ + cells/10^6 PBMCs after subtraction of background (control), with a response that was at least 2x the assay background for that time point. For baseline positive subjects*: an on treatment response ≥ 25 IFN- γ + cells/10^6 PBMCs after subtraction of the background (control) with a response that was at least 2x the assay background, PLUS a second product related peptide response of ≥25 IFN- γ + cells/10^6 PBMCs after subtraction of the background (control) that was at least 2x the assay background.
  **Baseline response defined as: ≥ 25 IFN-γ+ cells/10^6 PBMCs with a response that was at least 2x the assay background.*
  – **Round 2: <u>7 subjects</u> with up to twelve months of immune sampling available**
  Response algorithm used: For baseline negative subjects†: at least one KRAS peptide pool with an increase from baseline of ≥ 25 IFN- γ + cells/10^6 PBMCs after subtraction of mismatched peptide response (control). For baseline positive subjects†: an on treatment response ≥ 25 IFN- γ + cells/10^6 PBMCs after subtraction of mismatched peptide response, PLUS a second product related peptide response of ≥ 25 IFN- γ + cells/10^6 PBMCs. In addition, potential positive responses for one subject were rejected because of very poor replicates plus high assay backgrounds.
  †Baseline response defined as: ≥ 25 IFN- γ + cells/10^6 PBMCs

ELISpot: categorical immune response to KRAS

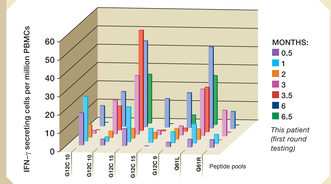
KRAS mutation specific immune response	8/17 (47%)
Treatment emergent immune response	5/9 (55%)
Improved immune response over baseline values using pre-specified criteria	3/8 (37%)

17/24 patients had adequate immune sampling to be analyzed by ELISpot assay, G12V patients (n=3) are still undergoing testing. 8/17 (47%) of the patients developed an immune response to mutant KRAS, 5/9 (55%) developed a treatment emergent response and 3/8 (37%) developed an improvement in a pre-existing baseline response based on pre-specified immunologic criteria.

ELISpot immune response to G12 mutation – all subjects



IFN-γ ELISpot responses are shown for 17 subjects over 12 months of treatment. **Green** line: the mean increase from baseline of the IFN-γ^+ cells for the sum of all G12 peptide pools. **Red** squares: values for ten subjects tested over the first six months of treatment. **Blue** diamonds: values for seven subjects tested over twelve months of treatment. Responses exceeding 200 spots (n=4) are shown as values of 200 to avoid excessive compacting of the y axis scale. Actual values are shown above.



Patient 013 KRAS peptide pools

This patient with a G12C mutation (first round testing) was scored as an immune responder to one G12C pool (G12C 15: a pool of four 15 mers encompassing the KRAS G12C mutation) due to ≥25 IFN-γ^+ cells/10^6 PBMCs, with a 5-fold increase in response over assay background (subtracted) – see immune response criteria in column 3. This patient also responds to one peptide pool (Q61L), a pool of 15 mers spanning the Q61L mutation present in Tarmogen (GI-4015) but not the patient's tumor.

	Grade 1	Grade 2	Grade 3
Dry skin	1		
Edema	2	1	
Fatigue	9	2	
Fever	1		
Injection site reaction	21		
Insomnia	1		
Muscle weakness		1	
Pain	2		1
Pruritus	2		
Rash		1	

Safety

GI-4000 was well tolerated and resulted in no SAEs considered related to therapy by the investigator, and only one subject discontinued therapy secondary G3 pain at the local injection site. Most patients experienced G1 injection site reactions and about one half experienced G1 fatigue. There have been no deaths.

Conclusions

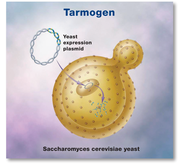
MSKCC's program of reflex testing of lung adenocarcinoma resection specimens permits the identification of patients for KRAS specific therapy. GI-4000 is immunogenic in targeting mutated KRAS as an adjuvant "consolidation" therapy in patients with stage I-III lung adenocarcinomas harboring KRAS mutations.

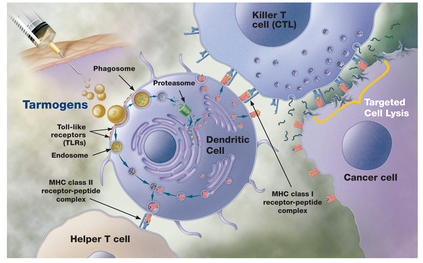
- GI-4000 was well tolerated
- 47% of the patients who were immune tested experienced a treatment emergent KRAS mutant specific cellular immune response
- These data warrant further study of GI-4000 in KRAS mutant lung adenocarcinomas and other cancers with these specific mutations

Memorial Sloan-Kettering Cancer Center

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.

GI-4000 vaccine as adjuvant consolidation therapy is immunogenic following definitive treatment in patients with stage I-III adenocarcinoma of the lung with KRAS mutations

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Background: All patients at MSKCC with lung adenocarcinoma undergo reflex testing for EGFR and KRAS mutations at the time of surgical resection. KRAS mutations occurred in 19% of resected lung adenocarcinomas at MSKCC from 2006- 4/2009. GI-4000 is a recombinant, yeast-based vaccine (*S. cerevisiae*) engineered to express one of 4 mutated KRAS oncoproteins.

Methods: GI-4000 was administered as adjuvant therapy to patients with stage I-III lung adenocarcinomas and G12C, G12D, or G12V KRAS mutations after completion of curative therapies. All patients were disease free at their first post-treatment assessment. GI-4000 was given for 3 weekly doses, then 6 monthly doses, then every 3 months for up to 3 years. The primary endpoint was vaccine-induced T cell responses documented by interferon-γ ELISpot assay in peripheral blood mononuclear cells (PBMCs) stimulated *ex vivo* with KRAS peptide pools from the specific mutation present in their tumor.

Results: 24 subjects were enrolled. Women=17, Stage IA=10, IB=4, II=2, III=8, median age 67 (range 50-80), G12C=15, G12V=3, G12D=6, median # of doses per subject =9 (range 1-16). To date, there have been no serious adverse events related to GI-4000. 17/24 patients had adequate immune sampling to be analyzed by ELISpot assay, G12V patients (n=3) are still undergoing testing. 8/17 (47%) of the patients developed an immune response to mutant KRAS, 5/9 (55%) developed a treatment emergent response and 3/8 (37%) developed an improvement in a pre-existing baseline response based on pre-specified immunologic criteria.

Conclusions: MSKCC's program of reflex testing of lung adenocarcinoma resection specimens permits the identification of patients for KRAS specific therapy. GI-4000 is immunogenic in targeting mutated KRAS as an adjuvant "consolidation" therapy in patients with stage I-III lung adenocarcinomas harboring KRAS mutations. These data warrant further study of GI-4000 in KRAS mutant lung adenocarcinomas and other cancers with these specific mutations.