Phase II Study of the GI-4000 *KRAS* Vaccine After Curative Therapy in Patients With Stage I-III Lung Adenocarcinoma Harboring a *KRAS* G12C, G12D, or G12V Mutation

Jamie E. Chaft,[1] Anya Litvak,[1] Maria E. Arcila,[2] Payal Patel,[1] Sandra P. D'Angelo,[1] Lee M. Krug,[1] Valerie Rusch,[3] Alicia Mattson,[4] Claire Coeshott,[4] Bernard Park,[3] David M. Apelian,[4] Mark G. Kris,[1] Christopher G. Azzoli[1]

Abstract

This phase II study evaluated the feasibility, immunogenicity, and safety of GI-4000, a yeast derived vaccine expressing mutant KRAS (Kirsten rat sarcoma viral oncogene homolog) proteins, in patients with early stage KRAS mutant lung cancers who completed curative therapy. Twenty-four patients received the genotype matched GI-4000 vaccine for ≤ 3 years or until disease recurrence or intolerance. GI-4000 was found to be well tolerated and immunogenic when used as consolidation therapy in patients with stage I-III KRAS mutant lung cancers.

Introduction: Patients with early-stage lung cancer have a high risk of recurrence despite multimodality therapy. *KRAS*-mutant lung adenocarcinomas are the largest genetically defined subgroup, representing 25% of patients. GI-4000 is a heat-killed recombinant *Saccharomyces cerevisiae* yeast–derived vaccine expressing mutant KRAS proteins. The present phase II study assessed the feasibility, immunogenicity, and safety of the GI-4000 vaccine in patients with early-stage, *KRAS*-mutant lung cancer. **Materials and Methods:** Patients with stage I-III *KRAS*-mutant lung cancer who completed curative therapy were enrolled. The patients received the genotype matched GI-4000 vaccine for ≤ 3 years or until intolerance, disease recurrence, or death. The KRAS antigen T-cell response was assessed using the interferon-gamma enzyme-linked immunospot assay in peripheral blood mononuclear cells. The study was powered to detect an immune response in ≥ 25% of patients. **Results:** A total of 24 patients were enrolled over 28 months. No vaccine-related serious adverse events occurred. One patient withdrew consent because of pain at the injection site. The study met its primary endpoint, with 50% of patients developing an immune response to mutant *KRAS*. The median number of vaccinations received was 15 (range, 1-19). Ten patients experienced disease recurrence, and 6 died. Compared with the genotypically matched historical controls, the recurrence rates were equivalent but overall survival showed a favorable trend. **Conclusion:** GI-4000 was well tolerated and immunogenic when used as consolidation therapy in patients with stage I-III *KRAS*-mutant lung cancer. The patterns of recurrence and death observed in the present study can be used to design a randomized study of GI-4000 with overall survival as the primary endpoint.

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[1]Thoracic Oncology Service, Division of Solid Tumor Oncology, Department of Medicine, Memorial Sloan-Kettering Cancer Center and Weill Cornell Medical College, New York, NY
[2]Diagnostic Molecular Pathology Service, Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, NY
[3]Department of Surgery, Memorial Sloan-Kettering Cancer Center, New York, NY
[4]GlobeImmune, Inc, Louisville, CO

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Address for correspondence: Jamie E. Chaft, MD, Thoracic Oncology Service, Division of Solid Tumor Oncology, Department of Medicine, Memorial Sloan-Kettering Cancer Center, 300 East 66th Street, New York, NY 10065
E-mail contact: chaftj@mskcc.org

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Introduction

Patients with early-stage non–small cell lung cancer (NSCLC) treated with surgery alone have had relatively poor overall survival, with 5-year survival estimates ranging from 73% for stage IA to 9% for stage IIIB.[1] For patients with inoperable or unresectable stage I-III NSCLC, external beam radiation therapy can be used with curative intent. Stage for stage, patients with NSCLC treated with radiation therapy have had a slightly lower chance of cure than patients treated with complete surgical resection.[2,3] Cisplatin-based chemotherapy improves the 5-year overall survival for patients with completely resected stage II-III NSCLCs by approximately 4% to 15% compared with surgery alone.[4,5] Thus, better consolidation therapies, such as therapeutic vaccines or targeted therapies, are sorely needed.

One area of drug development currently under investigation is the manipulation of the immune system to target cells harboring mutant proteins through vaccine therapy. One of the largest immunotherapy trials for patients who received curative intent treatment of locally advanced lung cancer was recently reported[6]—1513 patients were randomized to receive tecemotide (L-BLP25), a MUC1 antigen-specific immunotherapy capable of inducing a T-cell response to MUC1, versus placebo after completing chemoradiotherapy for unresectable, stage III NSCLC. No significant difference was found in overall survival with the administration of tecemotide after chemoradiotherapy compared with placebo in this patient population. However, for the predefined subgroup of patients who had received concurrent chemoradiotherapy, consolidation therapy with tecemotide improved median overall survival by 10 months, and confirmatory studies are planned.

Vaccines that target KRAS proteins are another venue of active exploration. The proto-oncogene *KRAS* (Kirsten rat sarcoma viral oncogene homolog) is the most commonly mutated oncogene in lung cancers, with mutations detectable in approximately 25% of tumors, most of which occur in codon 12, 13, and 61.[7] Unlike patients with lung cancers harboring epidermal growth factor receptor (EGFR) mutations, patients with *KRAS*-mutant lung cancer do not have a targeted treatment option and may have a worse prognosis.[8-11] Because of the frequency and therapeutic implications of *KRAS* mutations for patients with lung cancer, the Molecular Diagnostic Laboratory at Memorial Sloan-Kettering Cancer Center has offered reflex testing of every lung adenocarcinoma specimen for the presence of a *KRAS* mutation since 2006.[12]

GI-4000 is a series of 4 heat-inactivated *Saccharomyces cerevisiae* yeast products, each expressing a unique combination of 3 RAS mutations and collectively targeting 7 RAS mutations commonly observed in human cancers. The RAS fusion proteins expressed in yeast contain 2 of 3 mutations at codon 61 (glutamine to arginine [Q61R] plus glutamine to leucine [Q61L] or glutamine to histidine [Q61H]), plus 1 of 4 different mutations at codon 12 (glycine to valine [G12V], glycine to cysteine [G12C], glycine to aspartate [G12D], or glycine to arginine [G12R]). Thus, GI-4000 is manufactured as 4 individual product configurations with the subnames of GI-4014, GI-4015, GI-4016, and GI-4020, depending on the mutated RAS oncoprotein the product is engineered to express.

In preclinical studies, GI-4000 has been administered to mice and rabbits in both live and heat-killed forms, and no significant toxicity has been observed.[13] When the present study was designed, a phase I study of GI-4000 in patients with solid tumors had been conducted, with complete data on file at GlobeImmune, Inc. Of 31 subjects treated, 18 (58%) had advanced colorectal cancer and 13 (42%) advanced pancreatic cancer. No patients with NSCLC were included in that study. Dose escalation proceeded throughout the entire dose-escalation scheme without dose-limiting toxicity or therapy-related serious adverse events. The highest dose tested in the phase I study (40 yeast units or 40 YU, with 1 YU = 10^7 yeast cells) was selected for use in subsequent phase II studies. Most subjects (> 72%) in the phase I study had measurable immune responses as assessed using the lymphocyte proliferation assay and/or intracellular cytokine staining for interferon-gamma (IFNγ) during treatment.

The primary objective of the present study was to evaluate the T-cell–mediated immune response to GI-4000 in patients with stage I-III NSCLC and GI-4000–related mutation in *KRAS* after completion of potentially curative therapy (surgery and/or radiation therapy and/or chemotherapy). The secondary objectives were to evaluate the tolerability of GI-4000 in this setting and to compare the recurrence rates and overall survival to those of matched controls.

Materials and Methods

Patient Selection

The Memorial Sloan-Kettering Cancer Center institutional review board approved the treatment protocol. To qualify for enrollment, the patients had to have stage I-III NSCLC and *KRAS* G12C, G12V, or G12D mutations by direct sequencing of exon 2. All patients were treated with curative intent and were disease free at their first post-treatment assessment based on history, physical examination findings, and computed tomography (CT) findings (1-4 months after completion of all therapy). Patients with stage III NSCLC were also required to have a magnetic resonance imaging or CT scan of the brain documenting no brain metastases within 6 months of study entry. All patients were required to have an ECOG performance status of ≤ 2.

The exclusion criteria included a history of splenectomy, a history of Crohn's disease or ulcerative colitis, concurrent and chronic therapy with corticosteroids or any other immunosuppressive drugs, pregnancy or nursing, a history of an allergy to *S. cerevisiae,* immediate hypersensitivity to a skin test for *S. cerevisiae* or delayed hypersensitivity to a skin test to *Candida albicans* or *Trichophyton,* or a history of another cancer within the past 5 years, with the exception of localized basal or squamous cell carcinoma of the skin, stage IA cervical cancer, or melanoma in situ.

Study Design and Intervention

This was an open-label, single-institution, phase II study that evaluated GI-4000 in patients with early-stage lung adenocarcinoma and *KRAS* mutations. The primary endpoint was the immune response to mutated KRAS proteins. The secondary endpoint was tolerability. The exploratory endpoints included a comparison of clinical outcomes to genotypically matched-controls. After enrollment and baseline history taking, physical examination, and laboratory studies, 24 patients were vaccinated with their individual genotype-matched vaccine from the GI-4000 series for 3 weekly doses and 6 monthly doses, and then every 3 months for ≤ 3 years (19 doses). The vaccinations were administered starting 1 to 4 months after completion of standard treatment. Vaccination

with study drug proceeded for ≤ 3 years or until study withdrawal, disease recurrence, or death. The specific GI-4000 product contained the RAS mutation in the subject's tumor (GI-4014 for G12V, GI-4015 for G12C, and GI-4016 for G12D). For patients with synchronous primary lung tumors, GI-4000 was directed at the higher stage tumor. The dose of GI-4000 used in the present study was 40 YU (4 × 10 YU subcutaneous injections, 1 in each extremity). Immunologic adjuvant agents to potentiate the response were not used. The safety evaluations included physical examinations, blood tests (including complete blood count, comprehensive metabolic panel, and coagulation studies), urinalysis, and electrocardiograms. Patients underwent surveillance CT scans of the chest every 6 months.

In Vitro T-Cell Responses

The primary objective of the present study was to determine the frequency of GI-4000–mediated KRAS antigen–specific T-cell responses documented using the IFNγ enzyme-linked immunospot (ELISpot) assay in peripheral blood mononuclear cells (PBMCs). ELISpot assays are well-described and highly sensitive methods for the measurement of low-frequency antigen-specific T-cell responses. Blood was collected during screening and on day 1, day 15, and months 1, 2, 3, and 6 and then every 3 months thereafter for isolation of PBMCs. All blood samples for each individual patient were thawed and tested on the same day so that longitudinal analysis was possible. For the ELISpot assay, the PBMCs were stimulated in vitro with KRAS peptide pools that contained the mutation present in the patient's tumor. In select patients, the PBMCs were also tested with a mismatched set of KRAS peptide pools, which were identical to the matched set except at position G12. All conditions were set up in triplicate. The numbers of cells (or "spots") that produced IFNγ as a result of activation by these peptides were enumerated, and the numbers were adjusted by subtraction of either the response to the assay medium or the response to the appropriate mis-mismatched pool. If a T cell recognizes 1 of the peptides in the ELISpot matrix, it can be stimulated to produce IFNγ, which can be detected by an enzyme-labeled anti-IFNγ antibody and a precipitable chromogenic substrate. A positive ELISpot result required ≥ 1 peptide pool with > 25 spots/million PBMCs over the assay background and baseline signal. For patients with a baseline response of 25 spots/million for the mutation in their tumor, a positive test required at least a twofold increase in the tumor-specific signal after treatment, plus the emergence of a second product-related response (ie, position 61 for a position 12 tumor mutation) of ≥ 25 spots/million PBMCs. A patient was considered to have an immune response if a response was detected at any assessment point after baseline. Treatment with GI-4000 would be considered sufficiently active to warrant additional evaluation in a large, comparative, multicenter study if ≥ 6 of the 24 patients (≥ 25%) achieved an immunologic response using the ELISpot assay. In addition, a cumulative response was assessed for each subject by summing the responses to all KRAS peptide pools (after adjustment for controls) at each postbaseline point.

Statistical Analysis

The survival time was recorded from the date of surgery to the date of death. For patients who did not undergo surgical resection, the last date of radiation therapy was substituted for the surgical date. The median survival time and 1-, 2-, and 3-year survival rates were calculated using the Kaplan-Meier method. A contemporaneous comparison group matched for year of diagnosis, age, sex, *KRAS* genotype, and 7th edition stage was collected in an approximate 3:1 control/study ratio, although the numbers were limited by the availability of contemporaneous patients with stage 2 disease. This group was used to compare recurrence and survival using the Kaplan-Meier method with a hazard ratio for survival adjusted for age, sex, and stage.

Results

Patient Characteristics

A total of 24 patients were enrolled into the study from February 2008 to July 2010. The characteristics of the 24 subjects are summarized in Table 1. Thirteen patients had single *KRAS* G12C mutations, 3 patients had single G12V mutations, and 6 patients had single G12D mutations. Two patients had synchronous primary lung tumors with 2 distinct *KRAS* mutations (1 with *KRAS* G12C and G12A and 1 with *KRAS* G12C and G12V). Twelve patients were treated with surgery alone, 8 patients with surgery plus chemotherapy, and 1 with surgery, radiation, and chemotherapy. Three patients were treated with definitive radiation plus chemotherapy.

Table 1 Patient Characteristics (n = 24)

Characteristic	Median (Range) or n (%)
Age (years)	65 (47-78)
Sex	
Women	17 (71)
Men	7 (29)
Stage	
I	12 (50)
II	5 (21)
III	7 (29)
Smoking status	
Never	0
Former	23 (96)
Current	1 (4)
KRAS G12C	13
KRAS G12V	3
KRAS G12D	6
KRAS G12C and G12V[a]	1
KRAS G12C and G12A[a]	1
Initial treatment	
Surgery	12 (50)
Surgery plus chemotherapy	8 (33)
Surgery, chemotherapy, plus radiation	1 (4)
Chemotherapy and radiation	3 (13)

[a]Patients with synchronous primary lung tumors and different *KRAS* (Kirsten rat sarcoma viral oncogene homolog) mutations; in both cases, the tumor with the *KRAS* G12C mutation was the higher stage tumor.

The observation time before the initiation of the vaccine for each patient, grouped by stage, is displayed graphically in Figure 1 and ranged from 2 to 10 months, with a median of 4 months. Patients with resected stage I disease had a shorter period of observation before initiation of the vaccine (range, 3-5 months; median, 3 months) compared with patients with stage II-III (range, 3-10; median, 5 months; $P = .0007$, Student's t test). The median period receiving the vaccine for the entire population was 25 months (range, 1 day to 36 months), with a median of 15 doses administered. Eleven patients completed the vaccine protocol, 10 patients stopped the vaccine because of disease recurrence, and 3 patients chose not to complete the vaccination program. The duration of receiving the vaccine for each patient, grouped by stage, at the time of this report is displayed in Figure 1.

KRAS *Antigen-Specific Immune Response*

Twenty patients had evaluable baseline samples. Specimens were not obtained in 4 patients. One patient was tested using the ELISpot assay, but the data were deemed not interpretable owing to the extremely high assay backgrounds. Nine of 13 patients with a negative test at baseline developed a treatment-emergent response, and 3 of 6 patients with a pre-existing baseline response had an increased response over baseline that met the prespecified immunologic criteria. Altogether, 50% (12 of 24) of all patients developed an antigen-specific immune response to mutant *KRAS* and thus met the study's primary endpoint.

The cumulative responses to the KRAS peptide pools at each point tested are shown in Figure 2. These responses increased from baseline during a 12-month period, with a peak mean response at 3.5 months (after 6 doses). The response plateaued after approximately 9 months of treatment.

Toxicity

The data from the patients who had received any GI-4000 vaccination were included in the safety analysis. The patients were followed up for adverse events during and after each vaccination. The common, clinically relevant toxicities are summarized in Table 2. No serious adverse events or grade 4 toxicities were recorded. One patient (4%, 95% CI 0.1%-21%) withdrew consent after the first vaccination because of grade 3 pain in the arms and thighs underlying the injection sites that lasted for 3 days. Twenty-one patients had grade 1 injection site reactions. Other possible vaccine-related side effects noted included dry skin, peripheral edema, fatigue, fever, rash, and pruritus. We did not observe any clinical signs of autoimmune disease or abnormal biochemical or hematologic parameters related to the vaccinations.

Recurrence Rate and Overall Survival

A graphic depiction of the duration receiving the vaccine and disease-free and overall survival for all 24 patients is presented in Figure 1. Patients receiving GI-4000 had a median observation time of 3.2 years. Ten patients experienced disease recurrence. The rates of disease recurrence were comparable to those of the genotypically matched control group. Of the 6 patients with disease recurrence who underwent repeat biopsy with *KRAS* testing, the original *KRAS* mutation was detected in 5 patients and no mutation was detected in 1 patient. Six deaths were observed, all secondary to disease progression. For all 24 GI-4000–treated patients, the 1-, 2-, and

Figure 1 Patient Outcomes and Observation Summary

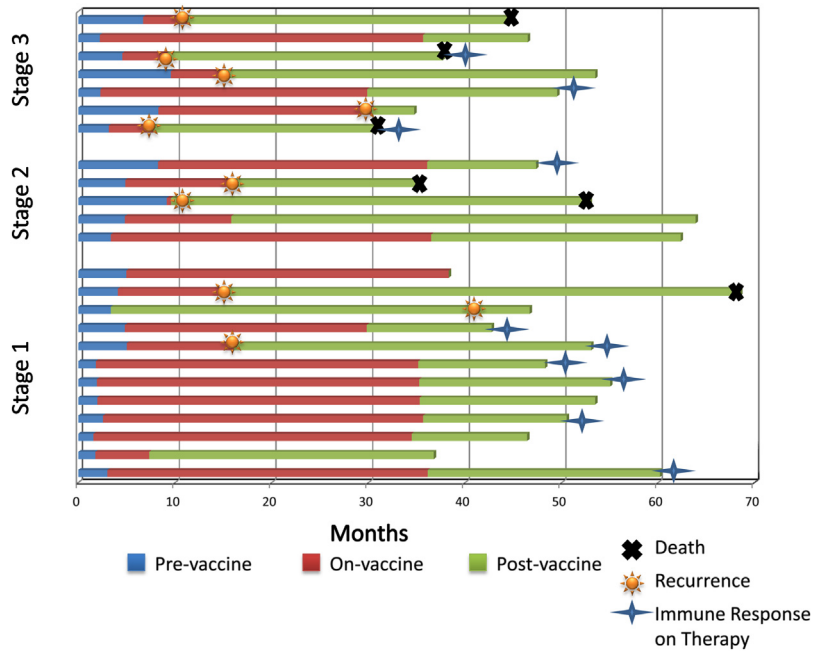
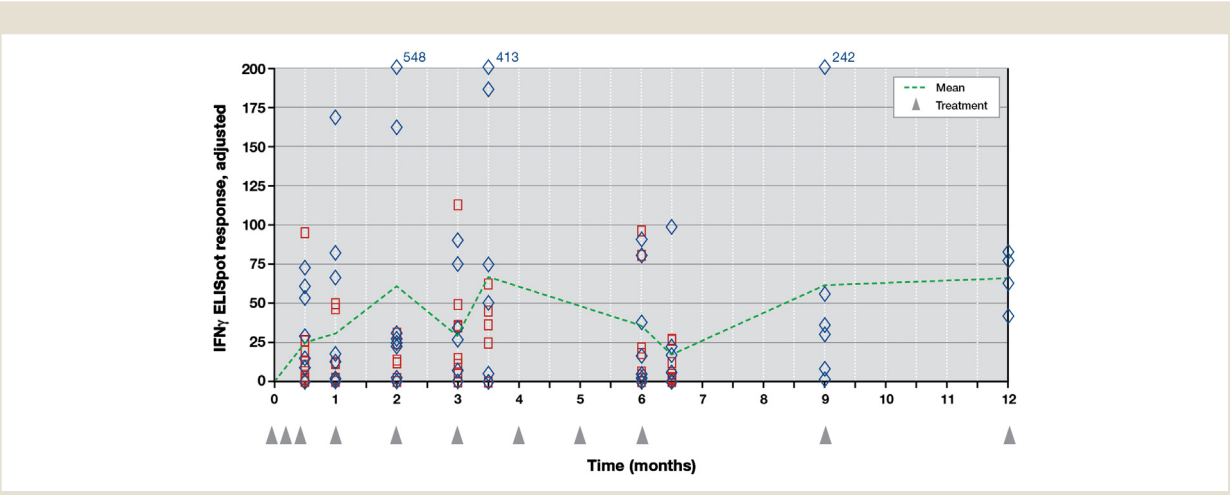


Figure 2 Cumulative Interferon-Gamma (IFNγ) Enzyme-Linked Immunospot (ELISpot) Response to KRAS G12 Mutations. IFNγ ELISpot Responses Shown for 19 Subjects During a 12-Month Period. Green Line, Mean Increase From Baseline of IFNγ-Producing Cells for Sum of All KRAS G12 Peptide Pools; Red Squares, Values for 10 Subjects Assayed Tested During First 6 Months of Treatment; and Blue Diamonds, Values for 9 Subjects Tested During 12 Months of Treatment. Responses > 200 Spots (n = 3) Graphed as Values of 200 to Avoid Excessive Compacting of Y Axis Scale. Actual Values Indicated Above. Gray Arrows, Timing of Immunizations



3-year survival was 100%, 100%, and 92% (22 of 24), respectively. The 1-, 2-, and 3-year survival in the genotypically matched, covariate-adjusted control group was 92%, 86%, and 80%, respectively (Table 3). The sample size was insufficient to specifically compare the clinical outcomes of the immune responders (n = 12) versus nonresponders (n = 7).

Discussion

Our study has provided the first data on the toxicity, feasibility, and immunogenicity of consolidation therapy with GI-4000 in patients with stage I-III *KRAS*-mutant lung cancer who had completed curative therapy. The most important finding we have reported is that 50% of patients (12 of 24) mounted an immune response to the vaccine. No serious adverse events or autoimmune disease were attributed to the vaccinations. Finally, the observed rates of death tended to be lower than those in the genotypically matched controls.

Point mutations at specific positions of the *KRAS* gene are the most common oncogenic driver mutations in a number of malignancies and have been found in 25% of patients with lung adenocarcinomas. Several preclinical models have shown that helper T cells and cytotoxic T cells can recognize such point mutations.[14-16] Furthermore, mutant *KRAS* is an attractive vaccine target in lung adenocarcinoma because of the specificity of mutant RAS to cancer cells and the near absolute lack of presence in normal cells. Therefore, patients with resected disease and *KRAS* mutations are a unique population of patients amenable to a personalized clinical trial.

The administration of GI-4000 as a consolidation therapy is a novel approach, with the ultimate goal of improving disease-free and overall survival in patients with *KRAS*-mutant malignancies. GI-4000 has previously been tested in several studies. One phase I study showed that GI-4000 was well tolerated and immunogenic (data on file, GlobeImmune, Inc). The preliminary results of a randomized, placebo-controlled, double-blind, multicenter, phase II adjuvant trial of GI-4000 combined with gemcitabine versus gemcitabine alone in 176 patients with resected pancreatic cancer with a vaccine-specific RAS mutation demonstrated a trend in improved overall survival in a preplanned subgroup of patients (39 of 176) with R1 resection (2.6-month advantage in median overall survival and 16% advantage in 1-year survival).[17] This trend was more pronounced in the GI-4000–treated immune responders. Currently, a study combining GI-4000 and bevacizumab as continuation therapy after completion of first-line cytotoxic chemotherapy with FOLFOX or FOLFIRI in patients with colorectal cancer (n = 52) is underway (ClinicalTrials.gov registration no. NCT01322815).[18]

In our study, GI-4000 was found to be well tolerated and immunogenic when used as consolidation therapy for patients with stage I-III *KRAS*-mutant lung cancers. Although this was a small single-arm study, these findings support the hypothesis that a

Table 2 Summary of Treatment Emergent Adverse Events

Toxicity	Grade (n)				
	0	1	2	3	4
Injection site reaction	4	21	0	0	0
Fatigue	13	10	1	0	0
Pain in injection site	21	2	0	1	0
Peripheral edema	21	2	1	0	0
Dry skin	23	1	0	0	0
Fever	23	1	0	0	0
Muscle weakness	23	0	1	0	0
Pruritus	23	1	0	0	0
Rash	23	0	1	0	0

Table 3 GI-4000 Versus MSKCC-Matched Controls

Variable	GI-4000 (n = 24)	Matched Controls (n = 64)
Median age at diagnosis (years)	63	66
Sex		
Male	7 (29)	21 (33)
Female	17 (71)	43 (67)
Stage		
1	12 (50)	42 (66)
2	5 (21)	2 (3)
3	7 (29)	20 (31)
KRAS mutation		
G12C	15 (63)	26 (41)
G12V	3 (13)	12 (19)
G12D	6 (25)	10 (16)
Other	0 (0)	16 (25)
Recurrence-free survival per year (%)		
1	88	82
2	71	71
3	67	67
Overall survival per year (%)		
1	100	92
2	100	86
3	92	80

Hazard ratio for survival, 0.58; $P = .32$.
Abbreviations: KRAS = Kirsten rat sarcoma viral oncogene homolog; MSKCC = Memorial Sloan-Kettering Cancer Center.

correlation exists between immune response and survival. In the future, data from our study can be used to design a prospective, randomized trial to detect the efficacy of GI-4000 as consolidation therapy in patients with stage I-III KRAS-mutant lung cancers after multimodality therapy, with overall survival as the primary endpoint.

Clinical Practice Points

- We report the safety and immunogenicity of the GI-4000 KRAS vaccine in patients with early-stage *KRAS* mutant lung cancers who have completed standard curative therapy.
- Our study met its primary endpoint, with 50% of patients developing an immune response to mutant *KRAS*. We also found that administration of GI-4000 *KRAS* vaccine genotypically matched to the individual patient's cancer was feasible and well tolerated. Compared with genotypically matched historical controls, the observed rates of death tended to be lower.
- Data from our phase II study can be used to design a prospective, randomized trial to detect the efficacy of GI-4000 as consolidation therapy for patients with stage I-III KRAS-mutant lung cancers after multimodality therapy, with overall survival as the primary endpoint.

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Disclosure

A.M. and C.C. are employees of GlobeImmune, Inc. At the time of study conduct, D.M.A. was an employee of GlobeImmune Inc and had stock in the company. All other authors declare that they have no competing interests.

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