Abstract # 2571

# TREATMENT OF RAS MUTATION-BEARING SOLID TUMORS USING WHOLE RECOMBINANT *S. CEREVISIAE* YEAST EXPRESSING MUTATED RAS: PRELIMINARY SAFETY AND IMMUNOGENICITY RESULTS FROM A PHASE 1 TRIAL

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## Abstract

**Background:** A number of tumors express activating, transforming mutations at codons 12 and 61 in the *ras* oncogene. We have previously shown that whole heat-inactivated recombinant *S. cerevisiae* expressing mutated Ras proteins induce protective cellular immunity as well as complete remission of established, carcinogen-induced, *ras* mutation-bearing lung tumors in mice *(Lu et al. 2004 Can Res 64, 5084)*.

**Methods:** GI-4014, GI-4015 and GI-4016 are recombinant yeast, each expressing a truncated and modified human Ras protein containing one of the three most common mutations at codon 12 (G12V, G12C, or G12D, respectively) and both of the most common mutations at codon 61 (Q61R and Q61L). In a four center Phase 1 trial, patients with advanced colorectal, pancreatic or non-small cell lung cancer, who have failed at least first line chemotherapy, have tumor samples subjected to genomic sequencing of the *K-*, *H-* and *N-ras* genes. If the tumor contains one of the target mutations, the subject receives 5 weekly subcutaneous doses of the corresponding product and is followed for an additional 56 days for safety, immunogenicity and tumor response.

**Results:** 67 patients have been consented, of whom 17 had product-related *ras* mutations in their tumors. Twelve patients have been treated. Of the 5 patients that completed dosing in the 0.1 YU cohort, all have shown mutation-specific treatment related T cell responses by proliferation and cytokine assays. No treatment-related serious adverse events have occurred and possibly treatment-related adverse events have been mild and infrequent.

**Conclusions:** Recombinant yeast represent a novel therapeutic approach for generating antigen-specific immune responses. Early data in humans suggest that, even at the lowest dose, Tarmogens generate mutation-specific cellular responses with an acceptable safety profile.

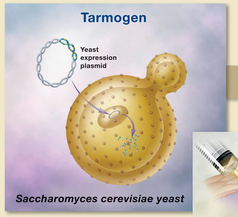

## Introduction

Tarmogens (targeted molecular immunogens) are whole, heat-killed recombinant *Saccharomyces cerevisiae* yeast engineered to express one or more target protein antigens to stimulate the immune system against diseased cells. They activate both an innate immune response via Toll-Like Receptors (TLRs), as well as an adaptive, antigen-specific immune response. Yeast are innately taken up by antigen presenting cells that stimulate cytotoxic T lymphocytes (a.k.a. "killer T cells"; CTLs) against the desired target. The use of these products provide a number of advantages over other immunotherapeutic approaches: they elicit potent CTL immune responses against cells expressing target antigens, are not neutralized by the host immune system upon repeated administration, do not require a patient-specific custom vaccine and are simple to manufacture.

A variety of solid tumors have key mutations in genes that govern the production of proteins involved in cell division. A limited number of mutations in the *ras* oncogene are expressed in many solid tumors including colorectal, pancreatic, ovarian and non-small cell lung cancers as well as malignant melanoma. These mutations result in constitutive Ras activation, leading to unregulated cellular proliferation.

GI-4000 consists of three different Tarmogens targeting the three common mutations at codon 12 as well as the two most common mutations at codon 61 in the *ras* gene. In previous animal studies, therapeutic dosing with GI-4000 induced mutation-specific, complete remission of tumors bearing the target Ras mutations *(Lu et al. 2004 Can Res 64, 5084)*.

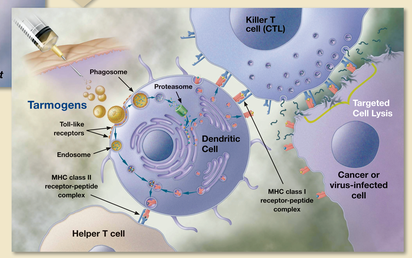
## Recombinant Yeast Elicit Both Innate and Adaptive Immune Responses




Heat-killed, recombinant *S. cerevisiae* transfected with genes encoding *ras* mutations at codons 12 and 61

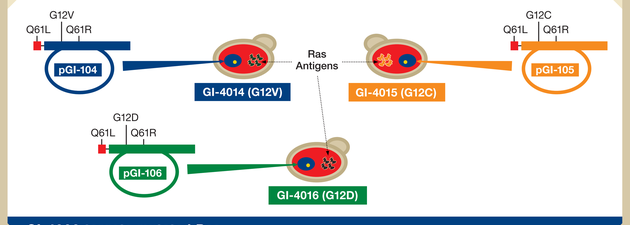
**Uptake of Tarmogens results in:**

- Activation of dendritic cells
- Presentation of antigens through both MHC class I and II
- Production of antigen specific T helper ($CD4^+$) and CTL ($CD8^+$) cells




## Products

- The GI-4000 series is comprised of GI-4014, GI-4015 and GI-4016 which expresses five activating Ras mutations.
- Each product contains the Ras Q61L and Q61R mutations but contain a different codon 12 mutation; G12V (in GI-4014), G12C (in GI-4015) and G12D (in GI-4016).
- All three products are manufactured and vialed separately, filed under a single IND.




**GI-4000 targets mutated Ras**

## Safety

- No treatment-related serious adverse events (SAEs) to date.
- No Grade 3/4 toxicities to date.
- A total of 9 adverse events were seen in the first two dose cohorts; all were mild in nature.
- Fatigue was the most common possibly-related adverse event in the first two cohorts.

## Methods

- Open-label, dose escalation study (0.1, 0.3, 1, 10 YU)
  1 YU (Yeast Unit) = $10^7$ yeast cells
- Previously treated advanced stage pancreatic, NSCLC or colorectal patients are eligible.
- Patient tumors are screened by surgical, biopsy or FNA tumor samples, laser capture microdissection, PCR amplification and genomic DNA sequencing for product-associated *K-*, *H-* and *N-ras* gene mutations at codon 12 or 61.
- Patients are administered GI-4014, GI-4015 or GI-4016 depending on the identified mutation.
- Treatment calendar:

| Day | Screen | 1 | 8 | 15 | 22 | 29 | 57 | 84 |
|---|---|---|---|---|---|---|---|---|
| Treatment received | | x | x | x | x | x | | |
| Immunology Performed | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |

- Study endpoints are safety, *in vitro* cellular immunogenicity and disease progression.
- The development of antigen-specific immune responses in patients is measured by *in vitro* LPA and ICCS assays.
  - LPA (Lymphocyte Proliferation Assay): PBMCs cultured with antigen for 6d and pulsed with $^3$H-thymidine to show antigen-specific T lymphocyte proliferation.
  - ICCS (Intracellular Cytokine Staining): PBMCs were cultured with antigen for 6d. Three color flow cytometry was used to determine antigen-specific IFN-gamma producing T cells (CD4 and CD8).

## Enrollment

- 67 patients consented (36 colorectal, 15 NSCLC, 16 pancreatic)
- 17 patients enrolled in the treatment phase after positive mutation screening
  - 1st two dose groups complete
  - 5 ongoing in 3rd dose group (1 YU); 2 awaiting start (either 1 or 10 YU)
  - Average age: 61
  - 7 male, 10 female

| Dose Level (YU) | Patient | Age | Sex | Tumor Type | Mutation Present | Cancer Treatment |
|---|---|---|---|---|---|---|
| 0.1 | 1001 | 75 | M | Colorectal | Q61L | Failed 4 lines |
| 0.1 | 1005 | 47 | F | Colorectal | G12D | Failed 4 lines |
| 0.1 | 2001 | 54 | F | Colorectal | G12D | Failed 4 lines |
| 0.1 | 2009 | 62 | M | Pancreatic | G12D | Failed 3 lines |
| 0.1 | 1015 | 76 | F | Colorectal | G12V | Failed 3 lines |
| 0.1 | 3002 | 60 | F | Colorectal | G12V | Failed 4 lines |
| 0.3 | 1018 | 45 | M | Colorectal | G12D | Failed 4 lines |
| 0.3 | 3005 | 64 | F | Pancreatic | G12D | Failed 2 lines |
| 0.3 | 2012 | 51 | M | Colorectal | G12V | Failed 4 lines |
| 0.3 | 1021 | 65 | M | Colorectal | G12V | Failed 3 lines |
| 1.0 | 1025 | 42 | M | Pancreatic | G12D | Failed 2 lines |
| 1.0 | 1026 | 59 | F | Colorectal | G12V | Failed 3 lines |
| 1.0 | 4001 | 74 | F | Pancreatic | G12V | Failed 3 lines |
| 1.0 | 3011 | 81 | F | Pancreatic | G12D | Failed 2 lines |
| 1.0 | 4007 | 53 | F | Pancreatic | Q61R | Failed 1 line |
| Awaiting start | 1030 | 73 | M | Pancreatic | G12V | Failed 1 line |
| Awaiting start | 2022 | 52 | F | NSCLC | G12C | Failed 3 lines |

## Immunology Results

**0.1 YU Dose Group**

- In the lowest dose group, all patients (6 of 6) exhibited treatment-related, antigen specific T cell immune responses.
  - 80% (4 of 5) evaluable patients developed treatment-related immune responses to the mutant epitope that was distinct from their tumor-associated mutation.
  - 100% (2 of 2) patients that survived 1-2 months post dosing had memory responses.

**0.3 YU Dose Group**

- In the 0.3 YU dose group, 50% (2 of 4) patients exhibited treatment-related antigen specific T cell immune responses.

**General**

- 90% (9 of 10) patients in the first two cohorts had pre-existing T cells capable of reacting to the mutant Ras epitopes expressed by the tumors.

**Responses**
- Responder
- Non-Responder
- Data not collected / insufficient blood

**General**
- Discontinuation due to patient death or disease progression
- Time point not yet reached

| Dose | Tumor type | Product | Patient number | Assay Type | Day 1 | Day 8 | Day 15 | Day 22 | Day 29 | Day 57 | Day 85 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.1 | Colorectal | 4016 | 1001 | LPA | | | | | | | |
| | | | | ICCS | | | | | | | |
| 0.1 | Colorectal | 4016 | 1005 | LPA | | | | | | | |
| | | | | ICCS | | | | | | | |
| 0.1 | Colorectal | 4016 | 2001 | LPA | | | | | | | |
| | | | | ICCS | | | | | | | |
| 0.1 | Pancreatic | 4016 | 2009 | LPA | | | | | | | |
| | | | | ICCS | | | | | | | |
| 0.1 | Colorectal | 4014 | 1015 | LPA | | | | | | | |
| | | | | ICCS | | | | | | | |
| 0.1 | Colorectal | 4015 | 3002 | LPA | | | | | | | |
| | | | | ICCS | | | | | | | |
| 0.3 | Colorectal | 4016 | 1018 | LPA | | | | | | | |
| | | | | ICCS | | | | | | | |
| 0.3 | Pancreatic | 4014 | 3005 | LPA | | | | | | | |
| | | | | ICCS | | | | | | | |
| 0.3 | Colorectal | 4014 | 2012 | LPA | | | | | | | |
| | | | | ICCS | | | | | | | |
| 0.3 | Colorectal | 4014 | 1021 | LPA | | | | | | | |
| | | | | ICCS | | | | | | | |
| 1.0 | Pancreatic | 4016 | 1025 | LPA | | | | | | | |
| | | | | ICCS | | | | | | | |
| 1.0 | Colorectal | 4014 | 1026 | LPA | | | | | | | |
| | | | | ICCS | | | | | | | |
| 1.0 | Pancreatic | 4014 | 4001 | LPA | | | | | | | |
| | | | | ICCS | | | | | | | |
| 1.0 | Pancreatic | 4016 | 3011 | LPA | | | | | | | |
| | | | | ICCS | | | | | | | |
| 1.0 | Pancreatic | 4014 | 4007 | LPA | | | | | | | |
| | | | | ICCS | | | | | | | |
| Awaiting Start | Pancreatic | 4014 | 1030 | LPA | | | | | | | |
| | | | | ICCS | | | | | | | |
| Awaiting Start | NSCLC | 4015 | 2022 | LPA | | | | | | | |
| | | | | ICCS | | | | | | | |

## Summary

- Tarmogens elicit antigen-specific immune responses in patients through activation of both innate and adaptive immunity.
- The majority of patients (75%) tested to date, developed product-mediated, antigen-specific T cell responses against the mutated Ras oncoprotein.
- No significant safety concerns to date.
- Phase 2 studies in NSCLC, pancreatic and colorectal cancers are being planned.