PREVALENCE OF K-RAS MUTATIONS IN PANCREAS CANCER AND DEVELOPMENT OF RAS-TARGETED IMMUNOTHERAPY

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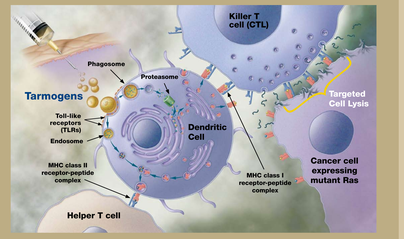
Targeting mutated Ras for cancer immunotherapy

Aberrant constitutive signaling by Ras oncoproteins with mutations at codons 12, 13 and 61 drive uncontrolled cell proliferation and tumorigenesis. GI-4000 is a targeted immunotherapy that specifically eliminates tumor cells expressing mutated Ras. Patient tumors are genotyped to identify specific *ras* point mutations. Here we describe the preclinical and clinical data for triggering mutated Ras-specific T cell immunity, and the development of new GI-4000 products to meet the demand for a more comprehensive attack on mutant Ras-expressing cancers.



GI-4000 Tarmogen® products are whole, heat-killed recombinant *Saccharomyces cerevisiae* yeast that express mutated Ras proteins. Tarmogens couple the activation of the innate and adaptive immune systems. The yeast-expressed mutated Ras protein is digested into peptides for both (MHC class I and II) pathways of antigen presentation to produce a highly specific and potent T cell response. The GI-4000 Tarmogen-activated CD8 killer T cells provide systemic surveillance and eliminate the mutated Ras-expressing tumor cells, with little or no impact on healthy cells.

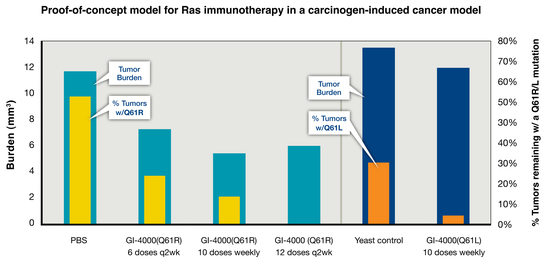


Fig. 1. GI-4000 triggered Ras mutation-specific tumor ablation

A/J mice received a single injection of urethane and 2 weeks later initiated dosing with Tarmogens or mock (PBS or yeast alone) control. At 14 weeks post-urethane exposure, tumors were excised, and the total tumor burden was calculated (dark bars). Ras genotyping (light bars) was performed on the remaining tumors (10-30) from several mice per group. GI-4000(Q61R) demonstrated dose-dependent complete elimination of all tumors driven by Q61R-mutated K-Ras (yellow). Likewise, when mice were administered GI-4000(Q61L) Tarmogens, the tumors bearing Q61L-mutated K-Ras were also selectively eliminated (orange).

BEDSIDE

Fig. 2. Original GI-4000 Tarmogen yeast products for clinical use

GI-4000-01: Phase 1 clinical study

Design:
- Open-label, dose escalation, monotherapy safety study (0.1, 0.3, 1, 10, 20 and 40YU per dose)
- Previously treated Ras mutant-positive pancreas, NSCLC or colorectal patients were eligible.
- 33 patients were administered GI-4014, GI-4015 or GI-4016 based on their genotyped *ras* mutation.

Day	Screen	1	8	15	22	29	57	84
Treatment received		X	X	X	X	X		
Immunology Performed		*	*	*	*	*	*	*

Results:
- No-product related SAEs, DLTs or discontinuations.
- Mutant Ras-specific T cell immune responses observed in 90% of treated subjects.
- Five long-term survivors, all with Stage IV disease at enrollment.

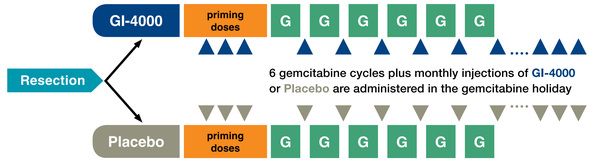


Fig. 3. GI-4000-02: Phase 2 study design

GI-4000-02 is a randomized, double-blind trial evaluating GI-4000 vs. placebo in combination with 6 cycles of adjuvant gemcitabine in patients with successfully resected pancreas cancer (R0 or R1). This study will enroll 100 patients at 40 US centers and 15 international centers. 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.

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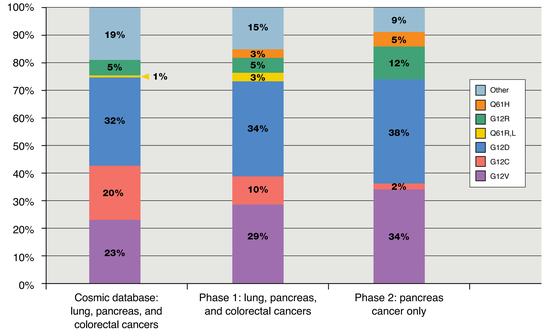

Fig. 4. Frequency of K-Ras mutations: expected vs. observed

Data obtained from the COSMIC database (http://www.sanger.ac.uk/genetics/CGP/cosmic/) shows the most frequent mutations in these cancers to be G12D, G12V and G12C. The K-Ras mutations included in the original series of GI-4000 Tarmogens were selected based on their prevalence in pancreas, NSCLC and colorectal cancers. After genotyping patient tumors in the Phase 1 clinical study, significant percentages of the patients were found to have G12R and Q61H mutations in K-Ras which was translated into the development of GI-4020 (Fig. 6).

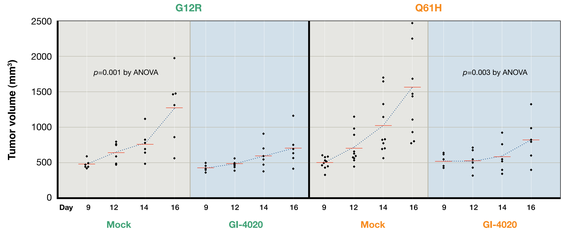

Fig. 5. GI-4020 specifically activated cytotoxic T cells against RasG12R- or RasQ61H-expressing EL4 tumor cells

C57BL/6 mice were immunized with 6 doses (q2wk) of GI-4020 (See Fig. 6). Isolated CD8 T cells mixed with target EL4 cells expressing RasG12R (left) or RasQ61H (right) mutants were implanted into naïve recipient mice and tumor size was monitored.

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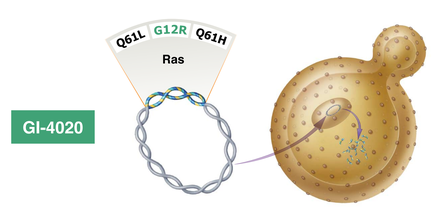

Fig. 6. Design of a new clinical GI-4000 product

Based on the higher than anticipated frequency of Ras G12R (12%) and Q61H (5%) mutations in pancreas cancer, GlobeImmune engineered a new Tarmogen to broaden the scope of treatable patients. The new product, GI-4020, contains Q61L, G12R and Q61H in the same format as the other GI-4000 products.

Fig. 7. GI-4020 timeline: product design through patient dosing

The major phases leading to the clinical introduction of GI-4020 from initial design took 10 months. This GI-4020 Tarmogen increases by 28% the number of genotyped patients eligible for pancreas cancer immunotherapy with GI-4000 products.

Conclusions

- Administration of GI-4000 yeast products targeting mutated Ras leads to T cell-mediated selective ablation of tumor cells.
- Tumor genotyping revealed an under-reported incidence of Ras G12R and Ras Q61H in pancreas cancer.
- The development of GI-4020 took only 10 months from design to clinical usage, demonstrating the speed at which a novel Tarmogen product can be developed for clinical use.
- Combined with tumor genotyping, the GI-4000 product series, including GI-4020, would be used for personalized cancer immunotherapy to treat 93% of pancreas cancer driven by mutated Ras.

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Prevalence of G12R or Q61H K-Ras mutations in pancreas cancer and development of Ras-targeted immunotherapy

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Background: Aberrant constitutive signaling by Ras oncoproteins with mutations at codons 12, 13 and 61 drive uncontrolled cell proliferation and tumorigenesis. We have developed targeted immunotherapy to specifically eliminate tumor cells expressing mutated Ras and are genotyping pancreas cancer for the incidence of specific *ras* point mutations.

GI-4000 tarmogens for immunotherapy with whole, heat-killed yeast expressing mutated Ras proteins were originally a three product series targeting tumors expressing *ras* codon 12 mutations encoding G12V (GI-4014), G12C (GI-4015) or G12D (GI-4016). Each of the original products also targeted Q61R and Q61L mutations. In preclinical studies, the original GI-4000 tarmogens stimulated mutation-specific ablation of tumor cells when the Ras antigen mutation expressed in yeast matched that found in the tumor. Ras mutation-specific cellular immune responses were shown in 90% of subjects from a Phase 1 study of GI-4000 in colorectal and pancreas cancer.

Experimental procedures: Tissue was obtained by surgical resection of tumors from 124 US and India subjects with Stage I and II pancreas cancer screened for enrollment in a placebo-controlled Phase 2 trial of adjuvant gemcitabine chemotherapy plus immunotherapy with GI-4000 tarmogens. Tumors were genotyped for K-, N- and H-*ras* DNA sequences by nested PCR amplification including peptide-nucleic acid oligomer clamping, followed by DNA sequencing. The GI-4020 tarmogen was engineered to express G12R- and Q61H- mutated Ras protein. CD8 T cells from mice immunized with GI-4020 or control yeast were mixed with tumors expressing G12R- or Q61H-mutated Ras, then implanted and monitored for control of tumor growth.

Results: Tumor genotyping for the Phase 2 study revealed that 83% of pancreas cancers had K-Ras mutations, predominantly G12V or G12D. However, 17% of pancreas tumors harbor G12R or Q61H mutations, where as 5-6% of tumors harbor the Q61H mutation, which was at much greater frequency than previously documented. Preclinical studies demonstrated that administering GI-4020, which targets these mutations, specifically activated T cells against tumors with G12R- or Q61H-mutated Ras.

Conclusions: G12R- or Q61H-mutated Ras is found in pancreas cancer with much higher incidence than previously reported. GI-4020 immunotherapy targets these 2 mutations and is being tested in the ongoing Phase 2 trial. The current four GI-4000 products now cover 93% of mutated Ras-bearing pancreas cancers.