YEAST-BASED TARMOGEN IMMUNOTHERAPY AGAINST BRAIN TUMORS EXPRESSING SELF ANTIGENS

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TARGETED MOLECULAR IMMUNOTHERAPY

Abstract

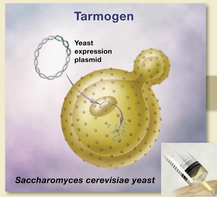
Tarmogens™ are whole, heat-inactivated *Saccharomyces cerevisiae* yeast containing defined quantities of target antigens to elicit T cell-mediated immune responses that recognize and ablate antigen-bearing tumors. Yeast are avidly taken up through a mechanism involving Toll-like receptors (TLRs) and pattern recognition receptors for glucan and mannan on antigen-presenting dendritic cells (DCs), initiating an innate immune response. This innate immune response drives DC maturation and secretion of pro-inflammatory cytokines. The innate immune response is followed by MHC-restricted presentation of yeast-expressed tumor antigens to initiate adaptive immune responses.

Tarmogens may be administered repeatedly without the generation of neutralizing host anti-yeast antibodies, thus boosting tumor antigen-specific immune responses against over-expressed self or mutated tumor antigens. We have previously reported that administration of Tarmogens expressing mutated Ras proteins caused complete therapeutic elimination of carcinogen-induced lung tumors containing the same mutated protein in mice (Cancer Res 64: 5084). In a Phase 1 trial treating colorectal and pancreas cancer patients with tumors expressing mutant Ras protein antigen, the Tarmogens were well-tolerated and demonstrated immunogenicity in the patients treated.

In an immunotherapeutic Fischer rat study, yeast encoding human or rat EGFR as the tumor antigen (GI-3000 Tarmogens) were tested against an intracranial glioma challenge. Subcutaneous and intranasal administration of GI-3000 expressing either rat or human EGFR proteins resulted in antigen-specific protection against an intracranial challenge with 9L glioma cells transfected with non-mutated rat or human EGFR.

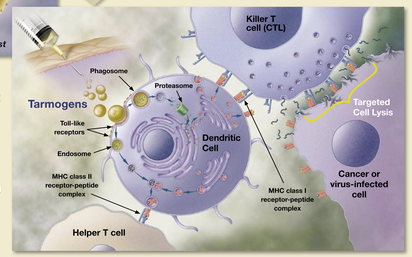
The immune-mediated tumor ablation was dependent on EGFR overexpression, as demonstrated by flow cytometry of tumor cells isolated and analyzed from responding and non-responding animals. The profile of cell surface EGFR density revealed an antigen threshold below which tumor cells were not eliminated. The absence of grossly observable off-target effects in tissues with normal levels of EGFR expression may be explained by the apparent requirement for EGFR overexpression to elicit immune recognition. These preclinical data indicate that Tarmogen administration is capable of breaking tolerance to overexpressed self antigens, supporting clinical evaluation in relevant cancer indications such as EGFR-overexpressing glioblastomas.

Recombinant yeast elicit both innate and adaptive immune responses



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



GI-3000 product series; Heat-killed, recombinant *S. cerevisiae* expressing human EGFR (GI-3001, -3006 and -3010) or rat EGFR (GI-3005)as a soluble truncated protein

1 YU = 10^7 yeast cells

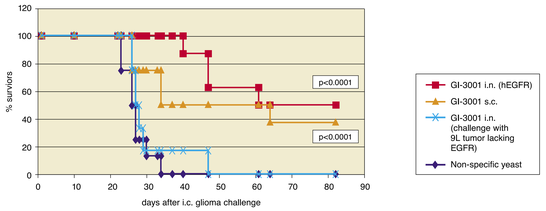


Figure 1: Tarmogen-mediated survival advantage in glioma challenge model

Fischer rats were immunized with either 1) non-specific yeast, 2) GI-3001 administered intranasally, or 3) GI-3001 administered subcutaneously. Rats were challenged with either 9L tumors lacking endogenous EGFR expression, or with 9L cells transfected to over express human EGFR. Initial doses administered at days -28, -21 and -7 with additional doses given on days 7 and 21 after implantation of 2,500 9L-hEGFR glioma cells. P values are versus non-specific yeast.

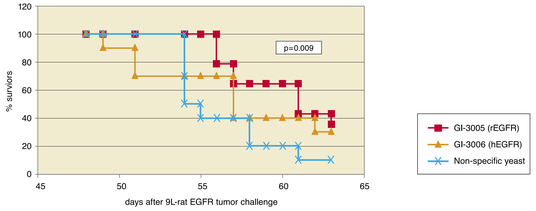


Figure 2: Rat EGFR vs. human EGFR as antigen against rat EGFR tumor challenge

Dosing of GI-1001 (non-specific yeast; 10 animals), GI-3005 (rat EGFR; 14 animals) or GI-3006 (human EGFR; 10 animals) by the i.n. route against intracranial challenge of 9L-rat EGFR glioma cells. Doses administered at days -35, -28, -14 and -7 with additional immunizations were given on days 7, 14, 21 and 56 after implantation of 5,000 9L glioma cells overexpressing rat EGFR protein. The P value is GI-3005 (rEGFR) vs. non specific yeast.

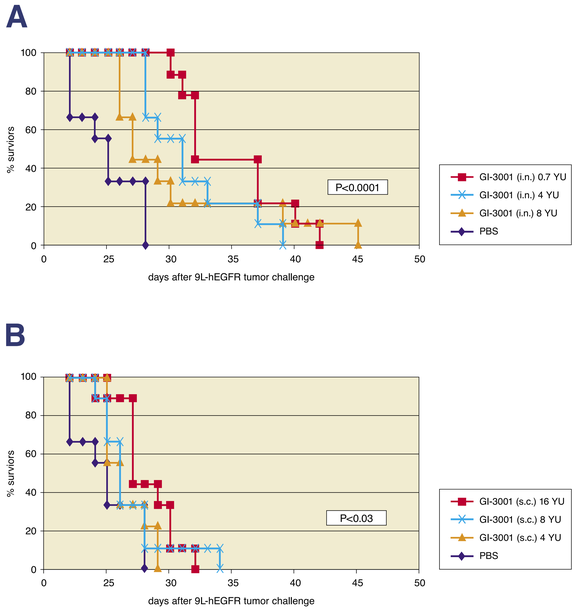


Figure 3: Intranasal and subcutaneous dosing of GI-3001

Kaplan-Meier survival graphs for intranasal (Panel A) or subcutaneous dosing (Panel B) of GI-3001 vs. placebo (PBS) on survival to intracranial 9L-hEGFR tumor challenge in rats; nine animals per group. Doses were administered on days -28, -21 and 7 prior to tumor challenge, then additional immunizations were given on days 7, 14, 28 and 42 after implantation of 2,500 9L-hEGFR glioma cells. In Panel A, the P value is for 0.7YU versus PBS. In Panel B, the P value is for 16YU versus PBS. See chart for P value matrix for all treatments.

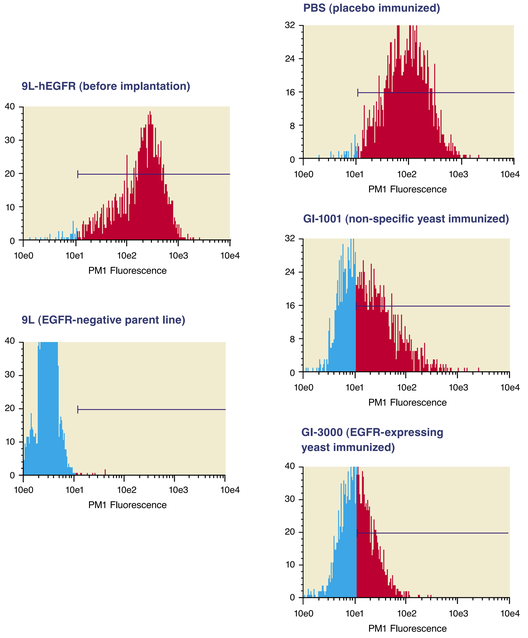


Figure 4: GI-3000 induces eradication of tumors over-expressing EGFR

Non-transfected 9L glioma cells (9L EGFR-negative) do not exhibit surface EGFR protein expression, as shown by EGF-ligand binding and immunoblot analyses. Transfection results in high-level hEGFR expression (9L-hEGFR before implantation). Animals immunized by the intranasal route with PBS, non-specific yeast, or EGFR-expressing yeast (GI-3000) were challenged with the cloned 9L-hEGFR expressing glioma cells. Intracranial tumors were analyzed by flow cytometry for EGFR expression. Levels of EGFR expression below 10e1 (in blue) correspond to 9L cells expressing little to no surface EGFR.

Log-rank statistical P values for survival benefit against intracranial tumor challenge with GI-3000 treatment

INTRANASAL VS. INTRANASAL		INTRANASAL Placebo	INTRANASAL Non-specific yeast
Intranasal GI-3000	0.7 YU	<0.0001	<0.0001
	4 YU	0.0005	0.1
	8 YU	0.02	0.4
Non-specific yeast	4 YU	0.07	n/a

SUBCUTANEOUS VS. SUBCUTANEOUS		SUBCUTANEOUS PLACEBO
Subcutaneous GI-3000	4 YU	0.2
	8 YU	0.3
	16 YU	0.03

INTRANASAL VS. SUBCUTANEOUS		SUBCUTANEOUS GI-3000 4 YU	SUBCUTANEOUS GI-3000 8 YU	SUBCUTANEOUS GI-3000 16 YU
Intranasal GI-3000	0.7 YU	<0.0001	0.0007	0.0006
	4 YU	0.002	0.01	0.03
	8 YU	0.06	0.1	0.6

This chart summarizes P values for the data presented in Figure 3. All P values refer to the statistical analysis comparing the treatment regimen described in the row to the regimen defined by the column heading. GI-3000 demonstrates a statistically significant survival advantage with both intranasal and subcutaneous administration, with intranasal administration demonstrating superiority. The most effective dose in rats is intranasal 0.7YU. Non-specific yeast was dosed at 4YU intranasally. Placebo is PBS buffer administered either subcutaneously or intranasally.

Conclusions

- GI-3000 demonstrated a survival benefit for rats in an intracranial glioma challenge model
- Tolerance to immune responses against *self*-antigen is overcome by Tarmogen administration
- Intranasal administration of GI-3000 is superior to subcutaneous administration in this model
- GI-3000 appears to preferentially target cells over-expressing EGFR, potentially limiting off target effects