PROTEOMIC SIGNATURE PREDICTS RESPONSE TO A THERAPEUTIC VACCINE IN PANCREAS CANCER, ANALYSIS OF GI-4000-02

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Introduction

GI-4000 is a product series designed to elicit an immune response against cells with activating *ras* mutations using heat-killed *Saccharomyces cerevisiae yeast* (named Tarmogens: Targeted Molecular Immunogens) genetically engineered to express Ras G12 and Q61 mutations.

Tarmogens activate antigen-specific T cell-mediated immune responses that have been shown to kill target cells expressing a number of cancer antigens including mutated Ras. In addition to increasing effector T cells, Tarmogens specific for other oncology targets have been shown to cause a reciprocal decrease the number and function of human regulatory T cells (Tregs) *in vitro*. The ability of Tarmogens to suppress Treg cells could be an important attribute for an immunotherapeutic in the treatment of cancer.

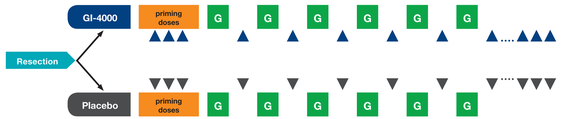
Therapeutic protection was previously demonstrated in a murine model of lung cancer. Activating mutations in ras occur in >90% of pancreas cancer cases and ~25% cases of NSCLC. GI-4000 has been evaluated in phase 2 trials in these indications and has demonstrated:

- In a multicenter, placebo-controlled phase 2b pancreas cancer study, a 3-month improvement in median overall survival, or OS (p=ns) in a pre-specified subgroup was observed, without however a corresponding improvement in survival in the overall study population.
  - Generation of interferon-γ (IFNγ) T cell responses in subjects receiving GI-4000: 7/15 (46.7%) GI-4000 subjects vs. 1/12 (8.3%) placebo subjects (p=0.043) had an IFNγ response to their G12 mutation.
- In a phase 2 NSCLC trial:
  - An indication of improved OS in subjects treated with GI-4000 compared to case-matched controls: HR=0.567, p=0.240.

Here we present the results of a retrospective analysis of 90 pre-treatment blood samples from the 4000-02 study. The goal of the analysis was to identify a pre-treatment companion diagnostic test that could predict which subjects are likely to respond to treatment with GI-4000 to assist in subject selection for future clinical trials. BDX-001, the resulting potential proteomic companion diagnostic test, appeared to predict whether a subject treated with GI-4000 and the chemotherapy drug gemcitabine in this trial would have improved recurrence free and overall survival compared to gemcitabine alone.

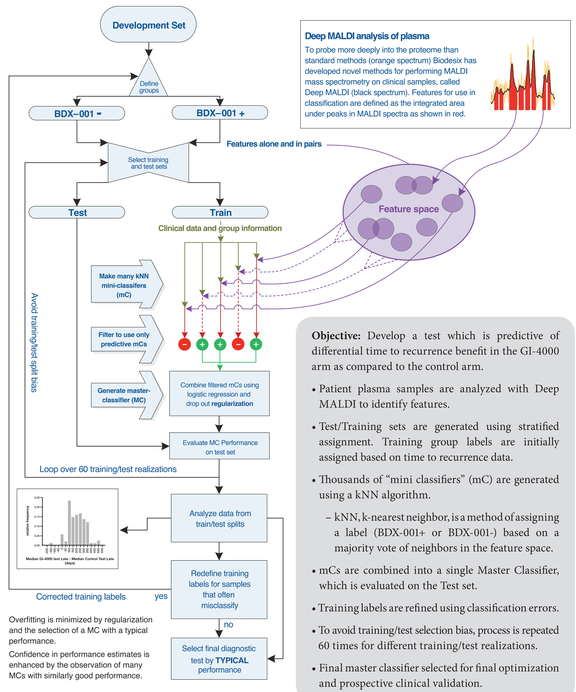
GI-4000 consists of four different heat-inactivated *S. cerevisiae* yeast GI-4014, GI-4015, GI-4016 and GI-4020 expressing seven common *ras* mutations in human cancers. Each of the four yeast expresses a fusion protein of three different *ras* mutations. Each protein product expressed in the yeast contains 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 *ras* mutation contained in their tumor, and only the specific yeast with the matching mutation is administered to the patient.

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 subjects with resected pancreas cancer (R0 or R1). This study enrolled 176 subjects at 31 US centers and 8 international centers. Subjects received 3 priming doses of study drug or placebo prior to initiation of adjuvant gemcitabine therapy, followed by monthly doses of study drug or placebo in the 2 week holiday between cycles of gemcitabine. Study therapy is administered until disease recurrence or withdrawal, with recurrence-free survival (primary), OS and immune response as endpoints for the trial.

BDX-001 Development Process

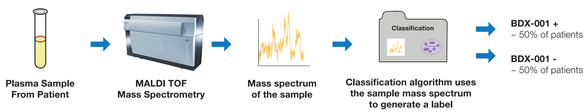


Objective: Develop a test which is predictive of differential time to recurrence benefit in the GI-4000 arm as compared to the control arm.

- Patient plasma samples are analyzed with Deep MALDI to identify features.
- Test/Training sets are generated using stratified assignment. Training group labels are initially assigned based on time to recurrence data.
- Thousands of "mini classifiers" (mC) are generated using a kNN algorithm.
  - kNN, k-nearest neighbor, is a method of assigning a label (BDX-001+ or BDX-001-) based on a majority vote of neighbors in the feature space.
- mCs are combined into a single Master Classifier, which is evaluated on the Test set.
- Training labels are refined using classification errors.
- To avoid training/test selection bias, process is repeated 60 times for different training/test realizations.
- Final master classifier selected for final optimization and prospective clinical validation.

BDX-001 Companion Diagnostic - Work Flow

After test is selected and all parameters locked, a patient sample is analyzed as follows:



Baseline plasma samples used for BDX-001 development were balanced between arms and representative of overall clinical trial population

Demographics (age, gender and race) and baseline characteristics predicted to be independently associated with outcome for the 90 sample dataset used to create BDX-001 were compared to the overall trial population.

Demographics and baseline characteristics were well balanced between GI-4000 and placebo arms within the BDX-001 dataset.

The BDX-001 data set appears to be generally representative of the overall study with two possibly meaningful imbalances, one favoring the non-BDX group and one favoring the BDX group in terms of predicted outcome. Resection status, age and gender were well balanced between the BDX-001 dataset and the non-BDX-001 dataset.

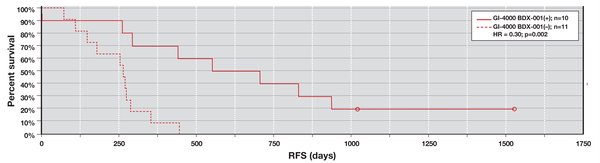
There was an imbalance in ECOG performance status between the two datasets with 92.3% of the BDX-001 group having performance status of 0 or 1 compared with 76.8% in the non-BDX dataset. However, in the non-BDX group 14% were not reported vs. 0% in the BDX-001 group. This imbalance is therefore probably not meaningful, as most subjects in both groups had PS 0-1. The unreported group would most likely have been PS 0-1 if reported.

There was an imbalance in lymph node involvement with 72.2% of the BDX-001 group having more than one node involved vs. 46.5% in the non-BDX group and 15.6% having no positive nodes in the BDX group vs. 34.9% in the non-BDX group so from a nodal status perspective, the BDX group had more extensive disease at baseline than the overall study population.

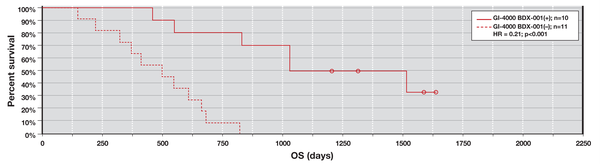
BDX-001 – a predictive test for response to GI-4000 treatment

The goal of this work was to identify a pre-treatment companion diagnostic test that could predict which subjects are likely to respond to treatment with GI-4000+gemcitabine, but didn't predict a difference in RFS or OS in the placebo+gemcitabine arm.

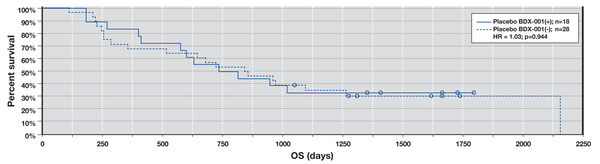
BDX-001 identifies a 12.2 month difference in mRFS in GI-4000+gemcitabine treatment group



BDX-001 predicts a 25.8 month improvement in mOS in GI-4000+gemcitabine treatment group



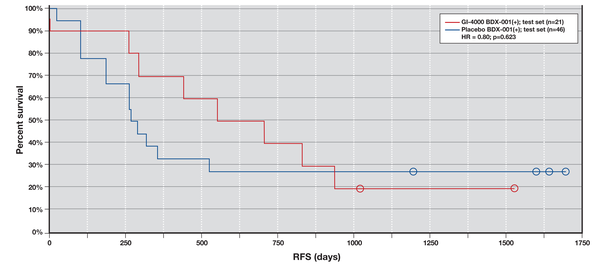
BDX-001 – no difference in OS seen in placebo+gemcitabine group



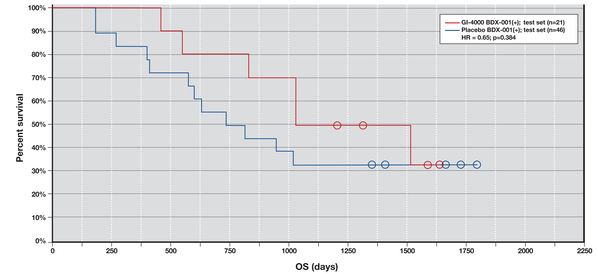
BDX-001 companion diagnostic predicts improved RFS and OS in exploratory analysis

When BDX-001 was applied to the patient samples not used in its training, BDX-001 appears to select patients that have better recurrence free survival and overall survival. In BDX-001(+) subjects treated with GI-4000 and gemcitabine, there was an 11.7 month improvement in median recurrence free survival, and a 16.6 month improvement in median overall survival, compared with BDX-001(+) subjects treated with placebo and gemcitabine. Overall, approximately one-half of subjects tested are BDX-001(+).

11.7 month improvement in median recurrence free survival (mRFS)



16.6 month improvement in median overall survival (mOS)



Conclusions

- The 90 patient samples used in this analysis were balanced between the GI-4000 and placebo arms and were representative of the overall 176 subject population
- Using BDX-001 to select subjects, the GI-4000+gemcitabine treated group demonstrated an 11.7 month improvement in mRFS vs. placebo+gemcitabine treated group
  - 21 months mRFS (GI-4000+gemcitabine) vs. 9 months (placebo+gemcitabine)
- Using BDX-001 to prospectively select subjects, the GI-4000+gemcitabine treated group demonstrated an 16.6 month improvement in mOS
  - 42 months mOS (GI-4000+gemcitabine) vs. 25 months (placebo+gemcitabine)
- The BDX-001 companion diagnostic should be prospectively validated in resected pancreas cancer

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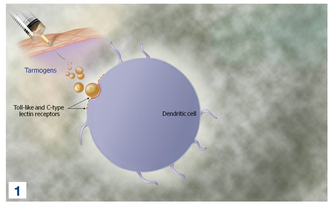
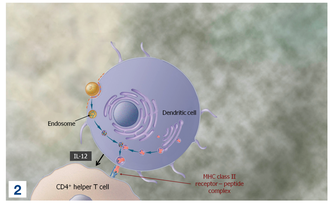
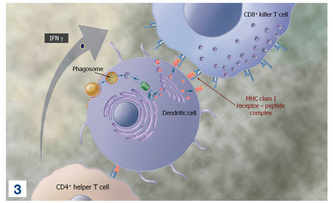
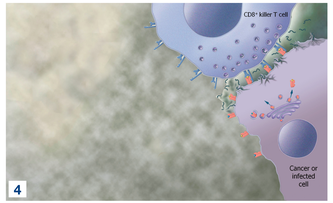
Active immunotherapy with yeast-based Tarmogens









A Tarmogen consists of intact, heat-inactivated yeast containing a target protein. Immunization with a Tarmogen results in antigen-specific cellular immune responses against the target protein and reduction in the number of abnormal cells containing the same target antigen. Tarmogens also reduce the number and function of regulatory T cells, thus further enabling the antigen-specific cellular immune response. Tarmogens target the molecular profile that distinguishes a diseased cell from a normal cell but are not required to be custom manufactured for each individual patient. Tarmogens are manufactured by a process that yields a stable, off-the-shelf product candidate that is disease- or antigen-specific. While some antibody may be generated against the yeast, the antibody does not block the activity of the yeast, allowing for repeated administration and boosting of the immune response with additional administrations. The following graphics and corresponding text describe the mechanism by which we believe Tarmogens work.

As shown in **graphic 1**, administration of Tarmogens initially results in binding of the yeast to white blood cells called antigen-presenting cells, the most important of which are known as dendritic cells, near the injection site. The dendritic cells are activated as a result of the Tarmogens binding to molecules called Toll-like receptors and other receptor molecules on the surface of the dendritic cell, resulting in the activation of immune signaling molecules called cytokines. The dendritic cell then engulfs the Tarmogen. Multiple Tarmogens may be taken up by the same dendritic cell.

The Tarmogen is processed by the dendritic cell in two ways, as shown in **graphic 2**. First, the Tarmogen is engulfed by subcellular bodies known as endosomes and the protein inside the endosome is cut into shorter fragments called peptides. These peptides are presented by Class II MHC molecules on the surface of the dendritic cell. In combination with IL-12, a cytokine that is produced by the dendritic cell, these MHC-peptide complexes on the surface of the dendritic cell are recognized by and activate cells involved in viral immunity called CD4+ helper T cells.

Dendritic cells also process Tarmogens by engulfing them with different subcellular bodies called phagosomes, as shown in **graphic 3**. This results in presentation of peptides, including the antigen from inside the Tarmogen, to cells, known as CD8+ killer T cells, via Class I MHC molecules on the surface of the dendritic cell, resulting in proliferation of identical antigen specific CD8+ T cells. CD4+ helper T cells are so named because one of their roles is to “help” activate killer T cells by expressing a cytokine called interferon gamma, IFNγ.

The newly activated CD8+ killer T cells move throughout the body and identify any other cell that expresses the same disease protein as the one recognized by the CD8+ killer T cells. Once the CD8+ killer T cell finds another cell in the body containing the target protein, it can kill the cell using multiple mechanisms as demonstrated in **graphic 4**. In addition to generating these antigen-specific T cell immune responses, Tarmogens also reduce the number and function of regulatory T cells, the component of the immune system that suppresses immune responses of other cells. Regulatory T cells represent an important mechanism built into the immune system to prevent excessive reactions. We believe that suppression of regulatory T cells could further enhance the ability of antigen-specific T cells to eliminate diseased cells.

Proteomic signature predicts response to a therapeutic vaccine in pancreas cancer, analysis of GI-4000-02

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Background

We have previously reported that adjuvant treatment with a therapeutic vaccine targeting the mutated Ras oncogene product generated mutation-specific T cell responses associated with a trend toward improved survival in patients with post-operative residual disease (R1 resections) but no improvement in the overall population1. Initial analysis of 90 pretreatment plasma samples using matrix assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry (MS) showed the potential to predict improved RFS and OS for treatment with GI-4000/gemcitabine, but not placebo/gemcitabine.

Methods

We have developed a novel technique, combining methods used in recent advances in learning theory (‘deep learning’) with newly-refined MS techniques that allow exploration deeper into the proteome to create diagnostic tests. Using 500,000 laser shot Deep MALDI spectra2 more than 700 mass spectral features were identified. A subset of these was used to create many multivariate classifiers that were filtered for performance and combined using dropout regularization. This method allows the use of smaller training sets and so left a test set with which performance of the signature could be independently assessed. This new methodology was used to create a test (BDX-001) to identify patients likely to benefit from the addition of GI-4000 to gemcitabine.

Results

Using BDX-001 for stratification, subjects who are BDX-001(+) demonstrated a 499 day advantage in median OS when treated with GI-4000/gemcitabine vs. placebo/gemcitabine. Additionally, these subjects demonstrated a 351 day improvement in median RFS. BDX-001 did not predict response for placebo/gemcitabine treated subjects. These results were obtained using only test set data, and although the small sample size prohibited statistical significance, it should give an unbiased test performance estimate to be validated independently.

Conclusions

BDX-001 is a test developed using novel proteomic and learning theory methods that appears to predict treatment response to GI-4000 in resected pancreas cancer patients, potentially identifying patients with improved RFS and OS in the GI-4000/gemcitabine arm. We plan to prospectively validate BDX-001 as a companion diagnostic in a future study of GI-4000 in pancreas cancer..

References

1. Richards et al, ESMO GI. Annals of Oncology, June 2012 23 (suppl 4)

2. Duncan et al, ASMS 2013, http://asms.inmerge.com/Proceedings/2013Proceedings.aspx.