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IDENTIFICATION OF ANTIBODY RESPONSES INDUCED IN PATIENTS WITH METASTATIC HORMONE-REFRACTORY PROSTATE CANCER (MHRPC) TREATED WITH GVAX IMMUNOTHERAPY FOR PROSTATE CANCER

T. Harding[1], M. Nguyen[1], K. Koprivnikar[1], G. Huan-Tu[1], N. Sacks[1], E. J. Small[2], K. Jooss[1]

[1]Cell Genesys, Inc., South San Francisco, CA 94080; [2]University of California, San Francisco, Comprehensive Cancer Center, San Francisco, CA 94143

ABSTRACT

Introduction: GVAX immunotherapy for prostate cancer is comprised of 2 allogeneic prostate carcinoma cell lines (PC-3 and LNCaP) that have been modified to secrete GM-CSF. It has been tested in patients (pts) with mHRPC in 2 multicenter Phase II trials, G-9803 and G-0010. The subset of pts in these 2 trials who received doses comparable to the dose used in ongoing Phase 3 trials showed median survival of 34.9 months (m) and 35.0 m, respectively.
Methods: Immunotherapy-induced antibody (Ab) responses were evaluated in 14 pts from the G-0010 trial whose actual survival exceeded that predicted by the Halabi nomogram using 3 methods: i) serological analysis of gene expression (SEREX), ii) protein chip analysis, iii) screening pre-defined prostate cancer antigens (Ags). Responses observed in at least 2 of the 14 pts were then further examined in all evaluable G-0010 pts (n=65). Ab response was evaluated for potential association with survival using the Cox regression model, adjusted for prognostic factors and dose group.
Results: Analysis of Ab responses in 14 pts yielded 411 candidate Ags. 318 of these Ab-responses were found in only 1/14 pts screened, with 93 responses seen in ≥ 2 pts. Preliminary data from all G-0010 pts suggest that titers of Ab to the PC-3-derived HLA-A24 or FLJ14668 proteins may be associated with survival. Among HLA-A24 haplotype negative pts, the HLA-A24 Ab-positive pts (n=30) had a median survival of 43 m vs. 18 m in Ab-negative pts (n=28), HR=0.53, p=0.05. Pts with Ab to FLJ14668 protein (n=34) had a median survival of 43 m vs. 21 m in Ab negative pts (n=31), HR=0.34, p=0.002.
Conclusions: GVAX immunotherapy for prostate cancer induces a IgG Ab response to a broad panel of immunotherapy-derived antigens. The majority of proteins recognized targeted are pt-specific; however, a smaller group of higher frequency Ab targets were identified. Apparent associations of Ab titers of HLA-A24 and FLJ14668-specific IgG with actual survival were observed. Phase II immunomonitoring studies are designed to identify Ab candidates that will be evaluated further in 2 on-going 600 pt phase 3 trials of GVAX immunotherapy for prostate cancer with the goal of identifying potential biomarkers of response and new diagnostic assays for the disease.

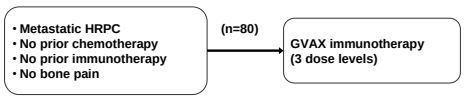
INTRODUCTION

The investigational immunotherapy, GVAX immunotherapy for prostate cancer, is comprised of 2 allogeneic prostate carcinoma cell lines (PC-3 and LNCaP) that have been modified to secrete GM-CSF. The rationale for employing a GM-CSF-transduced immunotherapy is to use whole tumor cells as the antigen source while the secreted GM-CSF, a potent immunostimulatory cytokine, induces dendritic-cell growth, activation and recruitment. Murine GM-CSF-secreting murine tumor cell immunotherapies (mGVAX) induce potent anti-tumor immunity in numerous murine models. Clinical trials of human GVAX immunotherapies in multiple cancers have demonstrated clinical and immunologic activity. GVAX immunotherapy for prostate cancer has been investigated in a multicenter phase 2 trial G-0010 (Figure 1), demonstrating safety and feasibility, as well as preliminary evidence of immunologic and clinical activity.

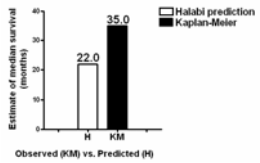
OBJECTIVE

The objective of these research studies was to retrospectively investigate antibody induction in pts treated with GVAX immunotherapy for prostate cancer in the phase 2 G-0010 study utilizing 3 different techniques: SEREX, protein microarrays and screening of defined PCa tumor-associated antigens to identify potential biomarker(s) of clinical response or proteins that could be used for new diagnostic assays for the disease.

Figure 1: Phase 2 Trial of GVAX Immunotherapy in HRPC (G-0010): Design, dosing and overall survival



- **Low-dose group -** **Level 1: 100 × 10⁶ cells q28d × 6 (n=3)**
  Level 2: 200 × 10⁶ cells q28d × 6 (n=30)
- **Mid-dose group -** **Level 3: 200 × 10⁶ cells q14d × 12 (n=25)**
- **High-dose group -** **Level 4: 300 × 10⁶ cells q14d × 12 (n=3)**
  Level 5: prime with 500 × 10⁶ cells then 300 × 10⁶ cells q14d × 11 (n=19)



G-0010‡

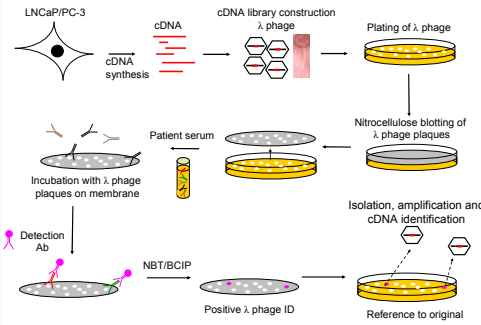
‡Includes 22 patients from the high-dose groups

METHODS

PATIENT SELECTION: G-0010 patients were selected for SEREX analysis (n=14), ProtoArray analysis (n=12) or defined PCa tumor-associated antigen screening (n=5) when based upon their actual survival exceeded that predicted by the Halabi nomogram.

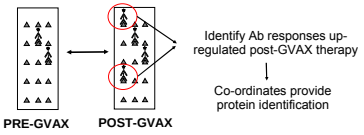
SEREX ANALYSIS: Patient sera post-GVAX immunotherapy for prostate cancer were immuno-screened at a 1:200 dilution against λgt11 phage display libraries expressing proteins derived from PC-3 and LNCaP cells genetically modified to express GM-CSF (Figure 2) cellular RNA. Immunoreactive clones were sequenced to identify encoded transgenes and then screened against pre-treatment serum to assess antibody induction over the course of dosing.

Figure 2: Overview of SEREX analysis



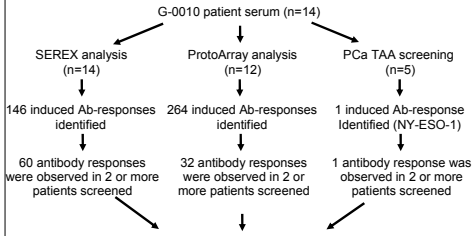
PROTOARRAY ANALYSIS: Patient serum pre- and post-GVAX immunotherapy for prostate cancer were separately screened at a 1:500 dilution against v4.0 ProtoArray® Human Protein Microarray's (Figure 3), which contain 8,000 full length proteins arrayed in duplicate on a microscope slide. Antibody reactivity to each protein on the array was compared for pre- and post-treatment across the whole group of patients using M-statistics. P-value of <0.05 considered significant.

Figure 3: Overview of protein micorarray analysis



PRE-DEFINED PROSTATE CANCER ANTIGEN SCREENING: Patient serum pre- and post-GVAX immunotherapy for prostate cancer were screened separately using western blots and ELISA at a 1:200-500 dilution range against a panel of 20 selected purified prostate cancer tumor-associated antigens (TAAs) including: prostate-specific antigen (PSA), prostate-specific membrane antigen (PSMA), NY-ESO-1, prostatic acid phosphatase (PAP), prostase, prostate stem cell antigen (PSCA), p53, carcinoembryonic antigen (CEA), Her2/Neu, survivin, telomerase, Cyclin-B1, α-methylacyl-CoA-racemase (AMACR) and glucose-regulated protein 78 (GRP78).

ANTIGEN SCREENING

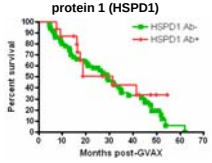


- Screening of 411 proteins indicates that the majority (318; 77.3 %) of induced Ab responses are unique or occur at low frequency (n=1/14) in G-0010 patients
- A group of 93 proteins (22.6%) are found where the induction of an antibody response is observed in more than 1 patient (n≥2/14)
- 20 proteins selected as targets for further development (WB, ELISA, Luminex)
- Screen all available serum samples (G00-10: n=65)
- Analyses: Correlate presence/absence of antibody responses with overall survival using the Cox regression model, adjusted for prognostic factors and dose group.

SURVIVAL ANALYSIS

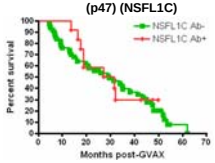
Figure 4: G-0010 survival analysis for HSPD1 and NSFL1C antibody responders

Heat shock 60kDa protein 1 (HSPD1)



HSPD1 Ab +ve MST 31m (n=15)
HSPD1 Ab –ve MST 29m (n=50)
p=0.687

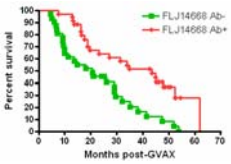
NSFL1 (p97) cofactor (p47) (NSFL1C)



NSFL1C Ab +ve MST 29m (n=12)
NSFL1C Ab –ve MST 29m (n=53)
p=0.489

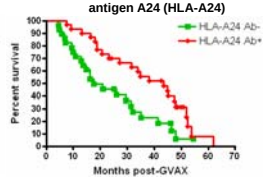
Figure 5: G-0010 survival analysis for FLJ14668 and HLA-A24 Ab responders

FLJ14668 Protein



FLJ14668 Ab+ve MST 43m n=34
FLJ14668 Ab-ve MST 21m n=31
HR= 0.34
p=0.002

Human Leukocyte antigen A24 (HLA-A24)



HLA-A24 Ab+ve MST 43m n=30
HLA-A24 Ab-ve MST 18m n=28
HR=0.53
p=0.05

Figure 6: G-0010 FLJ14668 antibody survival analysis by dose level

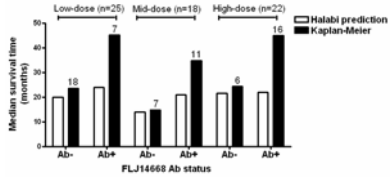
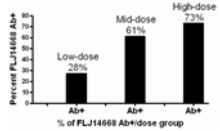


Figure 7: G-0010 FLJ14668 antibody dose-response



SUMMARY

- GVAX immunotherapy for prostate cancer has been investigated in a multicenter phase 2 trial, G-0010, demonstrating a median survival of 35.0 m at the dose comparable to that being used in the ongoing Phase 3 studies
- Three different antigen discovery techniques have been used to investigate patient immune response following GVAX immunotherapy for prostate cancer: SEREX, protein microarrays and defined PCa antigen screening
- GVAX immunotherapy for prostate cancer induced antibody responses recognize a broad panel of antigens and are predominantly patient-specific (unique) or induced at a low frequency
- A select group of GVAX prostate-induced antibody responses are found at higher frequency in treated patients
- An association was observed between survival benefit and a subset of higher frequency antibody responses, such as HLA-A24 and FLJ14668
- The survival advantage observed when comparing Halabi predicted and actual survival was limited to patients with an antibody response to FLJ14668 or HLA-A24 and was not observed in antibody negative population.
- The impact of HLA-A24 and FLJ14668 antibody responses on survival are independent of dose and number of treatments received (multivariate analyses)
- GVAX immunotherapy for prostate cancer is being investigated in two ongoing international Phase 3 trials (VITAL-1 and VITAL-2)
- Selected, candidate antibody biomarkers will be examined in VITAL-1 and -2 studies for further verification
- Candidate antibody biomarkers will also be examined for their role in PCa progression as potential tumor-associated antigens

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