**SUPPLEMENTARY MATERIALS**

**Humoral immune response against non-targeted TUMOR antigens after treatment with sipuleucel-T and its association with IMPROVED clinical outcome**

**Authors**: Debraj GuhaThakurta1, Nadeem A. Sheikh1, Li-Qun Fan1, Harini Kandadi1, T. Craig Meagher1, Simon J. Hall2, Philip W. Kantoff3, Celestia S. Higano4, Eric J. Small5, Thomas A. Gardner6, Kate Bailey1, Tuyen Vu1, Todd DeVries1, James B. Whitmore1, Mark W. Frohlich1,¶, James B. Trager1,\*, Charles G. Drake7,\*

**Affiliations:** 1Dendreon Corporation, Seattle, WA; 2Mount Sinai School of Medicine, New York, NY;  3Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA;  4University of Washington School of Medicine and Fred Hutchinson Cancer Research Center, Seattle, WA; 5University of California, San Francisco, CA; 6Indiana University Simon Cancer Center, Indianapolis, IN, 7Johns Hopkins University School of Medicine and the Brady Urological Institute, Baltimore, MD

\* **Corresponding authors**: JBT and CGD

Email: jtrager@dendreon.com; cdrake@jhmi.edu

¶ Current address: Juno Therapeutics, 307 Westlake Avenue North, Suite 300, Seattle, WA 98109, USA

# SUPPLEMENTARY METHODS

## Isolation and storage of serum samples

Blood samples were overnight shipped in ambient temperature.  Upon receipt, serum was immediately isolated, aliquoted into cryovials and stored long-term in monitored -80°C freezers ([1](#_ENREF_1)) until execution of assays.

## Quantification of serum IgGs using ProtoArray®

ProtoArray v5.0 (Life Technologies Corporation) ([2-6](#_ENREF_2)) used proteins expressed using a baculovirus/Sf9 expression from Invitrogen’s UltimateTM ORF (open reading frame) collection, or from Gateway® collection of kinase clones developed by Protometrix. All ProtoArray assays were performed by Life Technologies Corporation using the manufacturer’s recommended protocols. Microarray slides were blocked in blocking buffer (50 mM HEPES, 200 mM NaCl, 0.01% Triton X-100, 25% glycerol, 20 mM reduced glutathione, 1.0 mM DTT, 1X Synthetic Block) at 4 °C for 1 hour. After blocking, arrays were rinsed once with freshly prepared PBST buffer (1X PBS, 0.1% Tween 20, and 1 X Synthetic Block). Arrays were then probed with a 1:500 dilution of each serum sample diluted in 5 mL of PBST buffer. Arrays were incubated for 90 minutes at 4 °C in QuadriPERM 4-well trays (Greiner) with gentle agitation. After incubation, slides were washed five times (5 minutes per wash) in 5 ml PBST Buffer in 4-well trays. An Alexa Fluor®647-conjugated goat anti-human IgG antibody diluted in 5 ml PBST buffer to a 1.0 μg/ml final concentration was added to each array and allowed to incubate with gentle shaking at 4 °C for 90 minutes. After incubation, secondary antibody was removed and arrays were washed as described above. Arrays were dried by spinning in a table top centrifuge equipped with a plate rotor at 200x gravity for 2 minutes, then scanned using the fluorescent microarray Tecan PowerScanner.

GenePix 6.0 software was used to map human proteins in the array list file to each array image with a fixed feature size of 130 μm (diameter). After aligning the arrays using spots from the AlexaFluor-conjugated and murine antibodies printed in each subarray, the features were resized by the GenePix software to best fit the feature. Pixel intensities for each spot on the array were determined from the software and saved to a file. All quantified spot files were processed using the LifeTechnology ProtoArray Prospector software to determine which proteins interacted with the samples. The software performed background correction and Robust Linear Model normalization (RLM) ([7](#_ENREF_7)) using appropriate control spots on the microarray.

Prior to analyses, we filtered out signals on the microarray with low intensity across all samples and those from target antigens that did not have a known GenBank identifier (i.e., the target protein was poorly annotated or not annotated). This left IgG measurements to 7,221 protein isoforms on the ProtoArray, corresponding to 6,255 unique target antigens, with which all the subsequent analyses were performed.

## Assessment of serum IgG responses post-treatment using Luminex® xMAP®

Luminex xMAP ([8](#_ENREF_8)) uses multiplexed antigen-coupled, spectrally-distinguishable, fluorescent beads to quantify antibody levels in serum. GST-tagged proteins were conjugated to the beads using an anti-GST antibody bound to the beads, and proteins that were not GST-tagged were directly (covalently) conjugated to the beads. Serum samples were profiled at a 1:200 dilution. A protein signal assay and control assays (negative and positive) were run in parallel with the captured antigens and experimental samples. BSA captured directly to the beads, and GST captured on anti-GST-conjugated beads were used as negative controls. Across the samples evaluated, the median fluorescence intensity of IgGs against BSA was <100 and that against GST was <500, indicating low background signal. Positive controls included anti-human IgG (to indicate the presence of serum in the assayed sample) and human IgG (to indicate the presence of secondary antibody).

All signals from Luminex xMAP were log2-transformed prior to analyses. A subset of patients’ serum samples (n=120) was initially assayed in triplicate to evaluate the technical reproducibility of the platform. Within a batch, the median coefficient of variation (CV) for triplicate samples was low (<5%) for every evaluated antigen. Therefore, the remaining serum samples were assayed in single runs, with controls. To avoid batch effects, the pre- and post- treatment serum samples from patients were run with same lot of antigen-conjugated beads.

# SUPPLEMENTARY RESULTS

## Identification of treatment-induced serum IgGs to secondary antigens using protein microarrays

*Overlap of target antigens of the most highly induced IgGs with genes over-expressed in prostate tumors*

We examined if the antigens against which IgGs were induced at week 10 were enriched for genes reported as over-expressed in prostate tumors in the largest reported study of gene expression in prostate tumor and normal tissues ([9](#_ENREF_9), [10](#_ENREF_10)). We considered genes that were over-expressed in at least 33% of prostate tumors (primary and metastatic combined) relative to normal prostate tissues, which gave a list of 678 genes. Of these 678 genes, 152 were represented as protein products on the ProtoArray. We evaluated the overlap of these 152 proteins with the antigens against which serum IgG levels had increased from pre-treatment levels at week 10 in IMPACT. The targets of the 100 and 50 most highly induced IgGs overlapped significantly with these 152 products; 6 targets of the top 100 (p=0.012, hypergeometric test) and 4 targets of the top 50 (p=0.013) overlapped with the 152 products of genes over-expressed in prostate tumors.

## Confirmation of IgG responses to secondary antigens with Luminex xMAP

*Descriptions of the 10 candidate antigens evaluated using Luminex xMAP and their roles in cancer (prostate cancer) if known*

### LGALS3: Lectin, galactoside-binding, soluble, 3 (Galectin-3)

LGALS3, a multifunctional lectin with diverse expression ([11](#_ENREF_11), [12](#_ENREF_12)), is known to have roles in cell adhesion, migration ([13](#_ENREF_13)) and prostate cancer progression ([11](#_ENREF_11), [14](#_ENREF_14)). It is highly expressed in prostate tumors with expression decreasing in hormone-resistant tumors ([15](#_ENREF_15)). Alterations in the cytoplasmic/nuclear expression pattern of LGALS3 correlate with prostate carcinoma progression ([16](#_ENREF_16)). LGALS3 knock-down leads to reduced cell migration, invasion, cell proliferation, and tumor growth in the prostates of nude mice ([17](#_ENREF_17)). It is reported to be a pro-angiogenic molecule and a mediator of vascular endothelial growth factor (VEGF)- and basic fibroblast growth factor (bFGF)- mediated angiogenic responses ([18](#_ENREF_18)). LGALS3 is a binding partner of K-Ras and activates K‑Ras‑mediated signaling ([19](#_ENREF_19), [20](#_ENREF_20)). It is phosphorylated by c-Abl, a process that is modulated by PTEN ([21](#_ENREF_21)); the native but not the phosphorylated form of LGALS3 is cleaved by PSA ([21](#_ENREF_21)), potentially altering receptor-mediated signaling.

### ANPEP/CD13/GP150: Aminopeptidase N

ANPEP was originally identified as a myeloid cell surface peptidase that plays a role in antigen presentation ([22](#_ENREF_22)), is also selectively expressed in endothelial cells, and plays important roles in vascular endothelial morphogenesis during angiogenesis ([23](#_ENREF_23)). It has been proposed to induce angiogenesis through interaction with another pro-angiogenic protein, LGALS3 ([24](#_ENREF_24)) (see above). The cooperative expression of ANPEP in both cancer cells and nonmalignant stromal cells within the tumor microenvironment promotes angiogenesis, tumor growth, and metastasis ([25](#_ENREF_25)). In prostate tumors, expression of ANPEP is a prognostic marker and is associated with Gleason score and disease recurrence after radical prostatectomy ([26](#_ENREF_26), [27](#_ENREF_27)).

### CACNG1: Calcium channel, voltage-dependent, gamma subunit 1

L-type voltage dependent calcium channels are composed of five subunits ([28](#_ENREF_28)). The protein encoded by CACNG1 is the  subunit ([29](#_ENREF_29)) of the skeletal muscle 1,4-dihydropyridine-sensitive calcium channels. It is an integral membrane protein that plays a role in excitation-contraction coupling. No role for CACNG1 in cancer development has yet been described.

### FBXO6: F-box protein 6 (FBX6)

The F-box proteins constitute one of the four subunits of the ubiquitin protein ligase complexes called SCFs (SKP1-cullin-F-box), which function in phosphorylation-dependent ubiquitination ([30](#_ENREF_30)). These proteins are divided into 3 classes: Fbws contain WD-40 domains; Fbls contain leucine-rich repeats; and Fbxs, which contain either different protein-protein interaction modules or no recognizable motifs. FBXO6 belongs to the Fbxs class. FBOX6-dependent Chk1 degradation may contribute to S-phase checkpoint termination, and a defect in this mechanism may increase tumor cell resistance to certain anticancer drugs ([31](#_ENREF_31)).

### ECE1: Endothelin converting enzyme 1

ECE1 is involved in proteolytic processing of endothelin precursors to biologically active peptides. ECE1 generates endothelin-1 (or ET-1) from its inactive precursor, big-ET-1; ET-1 is a well-characterized driver for prostate tumor growth and metastasis ([32-35](#_ENREF_32)). Blockade of the ET-1 receptor (ETA) is being tested in the clinic for treatment of prostate cancer ([36-38](#_ENREF_36)).

### KRAS: v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog

K-Ras is a member of the mammalian Ras protein family. Oncogenic activating mutations in or aberrant expression of K-Ras is implicated in various malignancies, including prostate carcinomas. Among metastatic prostate tumors, 32% exhibit K-Ras mutation or over-expression ([9](#_ENREF_9)) and 90% exhibit activation of the Ras/Raf signaling pathway ([9](#_ENREF_9)).

### ERAS: Embryonic stem-cell expressed Ras

E-Ras is a member of the small GTPase Ras protein family. Initially found only in embryonic stem (ES) cells, E-Ras plays a crucial role in the transformation of transplanted ES cells to teratomas ([39](#_ENREF_39)). In gastric carcinomas, it is expressed (as determined by immunohistochemistry) in about 40% of the tumors; expression was found to be significantly associated with metastasis to the liver (*p*<0.0001) and lymph nodes (*p*<0.05) ([40](#_ENREF_40)). E-Ras is not yet characterized in the context of prostate cancer.

### KLK2/hK2: Kallikrien-related peptidase 2

KLK2 is primarily expressed in prostatic tissue ([41](#_ENREF_41)) and is responsible for cleaving pro-prostate-specific antigen (PSA) into its enzymatically active form ([42](#_ENREF_42)). It is highly expressed in prostate tumor cells and may be a marker for prostate cancer risk and detection ([43-47](#_ENREF_43)). Both PSA and KLK2 are produced by the same secretory epithelial cells in the prostate, and KLK2 is highly expressed in poorly differentiated cancer cells ([48](#_ENREF_48)).

### LGALS8 (Galectin-8): Lectin, galactoside-binding, soluble, 8 (Galectin-8, Prostate carcinoma tumor antigen 1 [PCTA-1])

LGALS8 was originally identified as a prostate carcinoma tumor antigen by surface epitope mapping and expression cloning ([49](#_ENREF_49)). It is widely expressed in tumor tissues, including all the TNM (tumor-node-metastasis) stages of prostate tumors ([15](#_ENREF_15)). Antibody responses to LGALS8 were observed in metastatic prostate cancer patients post- treatment with GVAX therapy (a whole cell prostate cancer vaccine comprised of two allogeneic prostate carcinoma cell lines, LNCaP and PC-3, modified to secrete GM-CSF) ([5](#_ENREF_5)).

### TSPAN13: Tetraspanin 13

TSPAN13 is a member of a diverse group of membrane-spanning proteins ([50](#_ENREF_50)). In multiple gene expression data-sets, TSPAN13 was overexpressed in prostate cancer tissue compared to normal prostate tissue ([51](#_ENREF_51)). In immunohistochemistry analyses of prostate cancer tissue microarrays, TSPAN13 was overexpressed in 80% of prostate cancer samples ([51](#_ENREF_51)). TSPAN13 expression inversely correlates with Gleason score (p=0.01) and with presence of prostatic intraepithelial neoplasia (PIN) in prostate tumor tissue (p=0.04) ([51](#_ENREF_51)).

# SUPPLEMENTARY TABLE LEGENDS

**Table ST1: Baseline clinical characteristics of patients in IMPACT (**[**52**](#_ENREF_52)**)**. The information for subgroups of patients from whom serum samples were analyzed in this study at different time points post-treatment is given, along with the information for the full set of patients enrolled in the IMPACT study.

**Table ST2: Baseline clinical characteristics of patients in ProACT**. The information for subgroups of patients in ProACT from whom serum samples were analyzed in this study at different time points post-treatment is given.

**Table ST3: Increase in levels of IgG against candidate antigens at weeks 2 and 22 in IMPACT as measured with ProtoArray (refer to Table 1 in the main text).** Increase in the serum levels of IgGs against 10 candidate antigens at weeks 2 and 22 after completion of sipuleucel-T treatment. Data for PAP (primary antigen) are shown for reference. The average fold-increase post-treatment, p-value from moderated paired t-test (limma), estimated false discovery rate (% FDR, Benjamini and Hochberg procedure), and ranks of antigens, sorted by average fold-increase in corresponding serum IgG levels, are given.

**Table ST4: Protein reagents used in Luminex xMAP assays.** Expression system used for production of proteins, purification tags (if any), related protein or nucleotide identifier, protein provider, product number from the provider (if known), estimated purity of the protein (as assessed by SDS-PAGE or size exclusion chromatography [SEC]), and method of conjugation of proteins to Luminex beads are given.

**Table ST5: Overlap of the number of patients who were IgG responders to different antigens at week 10 after treatment in the sipuleucel-T arm of IMPACT.** P-values (within parentheses) were computed using the hypergeometric test.

**Table ST6: Evaluation of IgG responses to candidate antigens at week 12 in ProACT using Luminex xMAP.** The antigen selection source, p-value for serum IgG level increase vs baseline, and the number (%) of patients with 2- or 5-fold increase in IgG level after treatment are given.

**Table ST7: Association of post-treatment changes in serum levels of IgG at week 10 with OS in the sipuleucel-T arm of IMPACT. (A)** Association of log2 of fold-change of serum IgG level with OS, **(B)** Association of IgG responses (≥2-fold increase in serum IgG level post-treatment) with OS. Univariate and multivariate Cox model (adjusted for baseline serum log-PSA and log-LDH) statistics are shown (refer to Table ST12 for similar data from weeks 2 and 22 of IMPACT).

**Table ST8: Comparison of OS in sipuleucel-T-treated IgG responders and IgG non-responders at week 10 with that in control patients in IMPACT.** IgG response was defined as ≥2-fold increase in serum IgG level at week 10. A multivariate Cox model was fit with the patient groups (control, IgG non-responder, and IgG responder) along with baseline serum PSA (log) and LDH (log). HRs for IgG responders and IgG non-responders relative to control patients (and the associated p-values, adjusted for baseline PSA and LDH) are given. A univariate Cox model gives the corresponding unadjusted p-values comparing control patients to IgG responders and IgG non-responders (refer to Table ST13 for similar data from weeks 2 and 22 of IMPACT).

**Table ST9: Evaluation of IgG responses against candidate antigens at weeks 2 and 22 in IMPACT using Luminex xMAP.** The antigen selection source, p-value of change in IgG level vs pre-treatment, average fold-increase in IgG level vs pre-treatment (across the patients evaluated), the number (%) of patients with ≥2- or ≥5-fold increase in IgG level after treatment are given. The rightmost column gives the p-value for the comparison of fold-change of IgG levels in the two arms using a one-sided Wilcoxon rank sum test (HA: fold-change in IgG levels post-treatment is higher in the sipuleucel-T treated group).

**Table ST10: Assessment of IgG responses against candidate antigens at weeks 4 and 20 in ProACT using Luminex xMAP.** The antigen selection source, p-value for IgG increase, and number (%) of patients with ≥2- or ≥5-fold increase after treatment are given.

**Table ST11: Overlap of the number of sipuleucel-T-treated patients who were IgG responders to antigens across the post-treatment time points in IMPACT**.The number of sipuleucel-T patients evaluated at weeks 2, 10, and 22 were 142, 93, and 60, respectively; data were available from 81 patients for both week 2 and week 10, and from 52 patients for both week 10 and week 22.IgG response was defined as ≥2-fold increase in serum IgG level post-treatment. The number of responses at each time point and the overlaps are given along with p-values (from hypergeometric test). **(A)** Overlap of IgG responses across theweek 2 and week 10 time points, **(B)** Overlap of IgG responses across theweek 10 and week 22 time points.

**Table ST12: Association of changes in serum IgG levels with OS at week 2 or 22 in the sipuleucel-T arm of IMPACT. (A)** Association of log2 of fold-change of serum IgG level with OS, **(B)** Association of IgG responses (≥2-fold increase in serum IgG level post-treatment) with OS. Univariate and multivariate model (adjusted for baseline serum log-PSA and log-LDH) statistics are shown (refer to Table ST7 for similar data from week 10 of IMPACT).

**Table ST13: Comparison of OS in sipuleucel-T-treated IgG responders and IgG non-responders with that in control patients at weeks 2 and 22 in IMPACT.** IgG response was defined as ≥2-fold increase in serum IgG level. A multivariate Cox model was fit with the patient groups (control, IgG non-responder, and IgG responder) along with baseline serum PSA (log) and LDH (log). HRs for IgG responders and IgG non-responders relative to control patients (and the associated p-values, adjusted for baseline PSA and LDH) are given. A univariate Cox model gives the corresponding unadjusted p-values comparing control patients to IgG responders and IgG non-responders (refer to Table ST8 for similar data from week 10 of IMPACT).

# SUPPLEMENTARY FIGURE LEGEND

**Figure SF1: Schematic of sipuleucel-T treatment doses (infusions) and serum collection time points in IMPACT and ProACT.** Other than pre-treatment, time points referred to in the text are relative to completion of treatment. Tx, treatment.

**Figure SF2:** **Shown in this figure are four Kaplan-Meier (KM) plots comparing the OS of patients in the sipuleucel-T arm of IMPACT with no IgG response at week 10 (IgG responses = 0) to patients with ≥1 IgG responses (A), ≥2 IgG responses (B), ≥3 IgG responses (C), or ≥4 IgG responses (D) at week 10.** IgGs to the six confirmed secondary antigens (namely, PSA, KLK2, K-Ras, E-Ras, LGALS8 and LGALS3) were considered for this analysis. Total numbers of patients in the analyses are given at the top right corner of each plot, and the numbers of patients in the two groups are given at the bottom left. The p-value estimate of the difference in OS between the two groups, and the hazard ratios [with 95% CI] of the IgG responder group relative to the non-responder group are given within each figure. **A**: KM plots of patients with no IgG response at week 10 (blue) and ≥1 IgG response to any of the six antigens (magenta; n=67; HR=0.57, 95% CI 0.29, 1.10; p=0.09; multivariate Cox model adjusted for baseline log(PSA) and log(LDH)). **B**: KM plots of patients with no IgG response at week 10 (blue; n=26) and ≥2 IgG responses to any of the six antigens (magenta; n=52; HR=0.51, CI 0.25, 1.03; p=0.06). In this plot, patients with 1 IgG response were not shown. **C**: KM plots of patients with no IgG response at week 10 (blue; n=26) and ≥3 IgG responses to any of the six antigens (magenta; n=34; HR=0.39, CI 0.17, 0.92; p=0.03). In this plot, patients with 1 or 2 IgG responses were not shown. **D**: KM plots of patients with no IgG response at week 10 (blue; n=26) and ≥4 IgG responses to any of the six antigens (magenta; n=26; HR=0.30, CI 0.11, 0.82; p=0.02). In this plot, patients with 1, 2, or 3 IgG responses were not shown. The number of patients with IgG responses ≥5 was small (n≤17), therefore the groups of patients with ≥5 IgG responses were not analyzed.

# SUPPLEMENTARY TABLES

|  | **Table ST1. Baseline clinical characteristics of patients in IMPACT.** |  |
| --- | --- | --- |
|  | **Characteristic** |  | **Full Data Set** |  | **Pre-Tx & Wk 2 Pairs** |  | **Pre-Tx & Wk 10 Pairs** |  | **Pre-Txt & Wk 22 Pairs** |  |
|  |  | **Control****(n=171)** | **Sip-T****(n=341)** |  | **Control****(n=62)** | **Sip-T****(n=142)** |  | **Control****(n=39)** | **Sip-T****(n=93)** |  | **Control****(n=16)** | **Sip-T****(n=60)** |  |
|  | Median age (range), y |  | 70(40-89) | 72(49-91) |  | 70(40-87) | 72(49-88) |  | 70(53-87) | 71(50-89) |  | 69(53-85) | 71(50-89) |  |
|  | Race, % of pts | White |  | 91.2 | 89.4 |  | 85.5 | 88 |  | 87.2 | 86 |  | 100 | 83.3 |  |
|  | Black |  | 4.1 | 6.7 |  | 8.1 | 6.3 |  | 5.1 | 8.6 |  | 0 | 10 |  |
|  | Other |  | 4.7 | 3.8 |  | 6.4 | 5.6 |  | 7.7 | 5.4 |  | 0 | 6.7 |  |
|  | Median time since diagnosis (range), y |  | 7.1(0.9-21.5) | 7.1(0.8-24.5) |  | 5.4(0.97-17.6) | 7.2(0.84-24.5) |  | 5.4 (1.5-16.6) | 7.5(0.8-19.8) |  | 5.4(2.1-17.6) | 7.4(0.8-19.8) |  |
|  | Median predicted survival, mo |  | 21.2 | 20.3 |  | 20.1 | 18.5 |  | 19.5 | 19.1 |  | 21.03 | 20.2 |  |
|  | ECOG performance status of 0, % of pts |  | 81.3 | 82.1 |  | 85.5 | 75.4 |  | 79.5 | 76.3 |  | 75 | 81.7 |  |
|  | Gleason score ≤7, % of pts) |  | 75.4 | 75.4 |  | 56.5 | 59.2 |  | 46.2 | 55.9 |  | 62.5 | 53.3 |  |
|  | Primary Gleason grade, % of pts | ≤3 |  | 41.5 | 42.2 |  | 32.3 | 32.4 |  | 23.1 | 29 |  | 31.2 | 30 |  |
|  | ≥4 |  | 58.5 | 57.8 |  | 67.7 | 67.6 |  | 76.9 | 71 |  | 68.8 | 70 |  |
|  | Disease location | Bone only |  | 43.3 | 50.7 |  | 45.2 | 51.4 |  | 41 | 57 |  | 50 | 55 |  |
|  | Soft tissue only |  | 8.2 | 7.0 |  | 11.3 | 6.3 |  | 10.3 | 6.4 |  | 0 | 13.3 |  |
|  | Bone and soft tissue |  | 48.5 | 41.9 |  | 43.5 | 42.3 |  | 48.7 | 36.6 |  | 50 | 31.7 |  |
|  | No. bone mets | 0-5 |  | 42.7 | 42.8 |  | 45.2 | 40 |  | 56.4 | 48.4 |  | 68.8 | 58.3 |  |
|  | 6-10 |  | 14.6 | 14.4 |  | 12.9 | 12.1 |  | 2.6 | 10.8 |  | 0 | 8.3 |  |
|  | >10 |  | 42.7 | 41.9 |  | 41.9 | 47.9 |  | 41 | 40.9 |  | 31.2 | 33.3 |  |
|  | Bisphosphonate use, % of pts |  | 48 | 48.1 |  | 51.6 | 52.1 |  | 48.7 | 50.5 |  | 62.5 | 46.7 |  |
|  | Previous prostate cancer therapy, % of pts | Androgen-deprivation therapy |  | 100 | 100 |  | 100 | 100 |  | 100 | 100 |  | 100 | 100 |  |
| Combined androgen blockade |  | 82.5 | 81.1 |  | 75.8 | 80.3 |  | 74.4 | 77.4 |  | 68.8 | 86.7 |  |
|  |
|  | Medical or surgical castration alone  |  | 17.5 | 18.2 |  | 24.2 | 19.7 |  | 25.6 | 22.6 |  | 31.2 | 13.3 |  |
|  | Orchiectomy |  | 7.6 | 9.4 |  | 3.2 | 9.9 |  | 5.1 | 7.5 |  | 6.3 | 3.33 |  |
|  | Chemotherapy |  | 15.2 | 19.6 |  | 17.7 | 25.7 |  | 12.8 | 19.4 |  | 18.8 | 21.7 |  |
|  | Docetaxel |  | 12.3 | 15.5 |  | 12.9 | 21.4 |  | 7.7 | 12.9 |  | 18.8 | 11.7 |  |
|  | Radical prostatectomy |  | 34.5 | 35.5 |  | 32.3 | 35.9 |  | 43.6 | 40.9 |  | 43.8 | 37.9 |  |
|  | Radiation to prostate or prostate bed |  | 53.2 | 54.3 |  | 62.9 | 47.9 |  | 56.4 | 50.5 |  | 56.2 | 48.3 |  |
|  | Baseline pain score, % of pts | 0 |  | 52.6 | 51.5 |  | 58.1 | 56.3 |  | 51.3 | 52.7 |  | 43.8 | 55 |  |
|  | > 0 |  | 47.4 | 48.5 |  | 41.9 | 43.7 |  | 48.7 | 47.3 |  | 56.2 | 43.3 |  |
|  | Median (range) laboratory values | Serum prostate specific antigen (ng/ml) |  | 47.2(6.2-3745.3) | 51.7(5.2-8005.6) |  | 52.7(6.5-1519.1) | 57.08(5.1-2056) |  | 36.8(6.5-384.2) | 38.5(5.2-1352.9) |  | 18.8(6.5-182.7) | 28.9(5.2-1017.6) |  |
| Serum prostatic acid phosphatase (U/L) |  | 3.2(0.6-147.2) | 2.7(0.6-466.1) |  | 3.3(0.59-109.0) | 2.4(0.6-128.4) |  | 2.4(0.6-93.0) | 1.5(0.6-129.6) |  | 1.4(0.6-80.6) | 1.3(0.6-129.6) |
| Alkaline phosphatase (U/L) |  | 109(43.0-2813.0) | 99(18.0-2396.0) |  | 97.0(43.0-2813.0) | 106.0(42.0-2396.0) |  | 80(43-607) | 90(45-799) |  | 80.5(48.0-269.0) | 89.5(42.0-799.0) |
| Hemoglobin (g/dL) |  | 12.7(9.0-15.4) | 12.9(8.4-17.9) |  | 12.7(9.0-15.4) | 12.6(9.3-15.8) |  | 12.3(9.4-15.4) | 12.7(9.3-15.8) |  | 12.6(10.9-15.4) | 13.0(9.4-14.9) |
| Lactate dehydrogenase (U/L) |  | 193(101.0-1662.0) | 194(84.0-637.0) |  | 191(101.0-654.0) | 195.5(115.0-598.0) |  | 193(133-407) | 196(115-369) |  | 210.5(152.0-303.0) | 190.5(129.0-369.0) |
| White-cell count (cells/mm3) |  | 6000(2830-13000) | 6200(3170-15610) |  | 5860(3080-11760) | 6100(3170-12240) |  | 6350(3080-11760) | 6020(3170-12240) |  | 6125.0(3600.0-10800.0) | 5850.0(3170.0-12240.0) |
| Total absolute neutrophil count (cells/mm3) |  | 4100(1550-8980) | 4000(1350-9960) |  | 3945(1870-8390) | 4010(1350.0-8700.0) |  | 4340(1890-8390) | 3950(1350-8700) |  | 4265.0(2240.0-6620.0) | 3790.0(1760.0-8700.0) |
|  | Pts, patients, Sip-T, sipuleucel-T; Tx, treatment. |  |

|  |  |  |
| --- | --- | --- |
|  | **Table ST2. Baseline clinical characteristics of patients in ProACT.** |  |
|  | **Characteristic** |  | **Sip-T: Pre-Tx & Wk 4 Pairs (n=33)** |  | **Sip-T: Pre-Tx & Wk 12 Pairs (n=26)** |  | **Sip-T: Pre-Tx & Wk 20 Pairs (n=19)** |  |
|  | Median age (range), y |  | 70 (56-88) |  | 69.5 (56-88) |  | 70 (62-88) |  |
|  | Race, % of pts |  |  |  |  |  |  |  |
|  | White |  | 94 |  | 96.2 |  | 94.7 |  |
|  | Black |  | 6 |  | 3.8 |  | 5.3 |  |
|  | Other |  | 0 |  | 0 |  | 0 |  |
|  | Median time since diagnosis (range), y |  | 8.4 (1.1-20.5) |  | 8.4 (1.1-20.5) |  | 9.3 (3.4-20.5) |  |
|  | ECOG performance status of 0, % of pts |  | 30.3 |  | 38.5 |  | 42.1 |  |
|  | Gleason score ≤7, % of pts  |  | 42.4 |  | 40 |  | 42.1 |  |
|  | Previous prostate cancer therapy, % of pts |  |  |  |  |  |  |  |
|  | Androgen-deprivation therapy |  | 100 |  | 100 |  | 100 |  |
|  | Combined androgen blockade |  | 96.9 |  | 96.2 |  | 100 |  |
|  | Medical or surgical castration alone |  | 3 |  | 3.8 |  | 0 |  |
|  | Orchiectomy |  | N/A |  | N/A |  | N/A |  |
|  | Chemotherapy |  | 24.2 |  | 19.2 |  | 26.3 |  |
|  | Docetaxel |  | 24.2 |  | 19.2 |  | 26.3 |  |
|  | Radical prostatectomy |  | 54.5 |  | 53.8 |  | 63.2 |  |
|  | Radiation to prostate or prostate bed |  | 36.4 |  | 34.6 |  | 26.3 |  |
|  | Median serum PSA (range), ng/ml |  | 20 (5.8-1299.2) |  | 19.1 (5.7-1299.2) |  | 11.9 (5.7-1299.2) |  |
|  | ECOG, Eastern Cooperative Oncology Group; PSA, prostate-specific antigen; Sip-T, sipuleucel-T; Tx, treatment; Wk, week(s). |  |

|  | **Table ST3. Increase in levels of IgG against candidate antigens at weeks 2 and 22 in IMPACT as measured with ProtoArray (refer to Table 1 in the main text).** |  |
| --- | --- | --- |
|  | **Antigen** |  | **Week 2** |  | **Week 22** |  |
|  |  | **Average Fold-Change** | **P-value** | **FDR (%)** | **Rank By Fold-Change** |  | **Average Fold-Change** | **P-value** | **FDR (%)** | **Rank By Fold-Change** |  |
|  | LGALS3 |  | 3.03 | 7.73E-08 | 0.019 | 3 |  | 4.16 | 2.65E-09 | 0.001 | 1 |  |
|  | CACNG1 |  | 3.30 | 2.07E-06 | 0.029 | 2 |  | 3.64 | 6.81E-06 | 0.589 | 2 |  |
|  | ANPEP |  | 3.31 | 4.04E-07 | 0.025 | 1 |  | 3.44 | 4.74E-06 | 0.489 | 3 |  |
|  | FBXO6 |  | 2.56 | 4.18E-07 | 0.025 | 7 |  | 3.24 | 1.75E-07 | 0.032 | 4 |  |
|  | ECE1 |  | 2.64 | 2.07E-05 | 0.037 | 6 |  | 2.59 | 4.23E-05 | 1.087 | 5 |  |
|  | ERAS |  | 2.07 | 1.51E-05 | 0.035 | 41 |  | 1.95 | 1.64E-04 | 1.424 | 34 |  |
|  | TSPAN13 |  | 1.86 | 4.77E-04 | 0.149 | 135 |  | 2.01 | 1.11E-04 | 1.317 | 22 |  |
|  | PAP |  | 2.11 | 4.99E-07 | 0.026 | 30 |  | 1.93 | 1.83E-05 | 0.778 | 37 |  |
|  | LGALS8 |  | 1.69 | 2.17E-04 | 0.091 | 374 |  | 1.78 | 6.86E-04 | 1.723 | 78 |  |
|  | KRAS |  | 1.90 | 6.29E-06 | 0.032 | 99 |  | 1.94 | 1.60E-06 | 0.192 | 35 |  |
|  | KLK2 |  | 2.20 | 1.68E-06 | 0.029 | 19 |  | 1.49 | 1.23E-03 | 2.006 | 487 |  |
|  | Fold-change, ratio of serum IgG level at time point and at pre-treatment; FDR, False discovery rate. |  |

|  |  |  |
| --- | --- | --- |
|  | **Table ST4. Protein reagents used in Luminex xMAP assays.** |  |
|  | **Antigen Name or Symbol** |  | **Expression System** | **Purification Tag (if any)** | **Protein or Nucleotide ID** | **Protein Provider** | **Product Number (if any)** | **Assessed Purity (Method)** | **Luminex Conjugation** |  |
|  | PAP |  | Mammalian (CHO) | HIS | P15309 | Dendreon |  | >95% by SEC | Direct |  |
|  | PA2024 |  | Insect (BV/Sf21) | HIS |  | Dendreon |  | >95% by SEC | Direct |  |
|  | Tetanus Toxoid |  | Inactived Tetanus Toxoid |  | Tetanus Toxoid from Clostridium tetani | List Biological Laboratories, INC | 191B |  | Direct |  |
|  | PSA (KLK3) |  | Mammalian (HEK293) | HIS | P07288 | Sino Biological | 10771-H08H | >95% by SDS-PAGE | Direct |  |
|  | PSMA |  | Mammalian (HEK293) | MYC/DDK | NM\_004476 | Origene Technologies | TP318310 | >80% by SDS-PAGE | Direct |  |
|  | LGALS3 |  | E. coli |  | P17931 | Sino Biological | 10289-HNAE | >97% by SDS-PAGE | Direct |  |
|  | CACNG1 |  | Mammalian (HEK293 derivative) | GST | NM\_000727.2 | LifeTechnologies | NA |  | GST- Ab |  |
|  | ANPEP |  | Mammalian (HEK293) | HIS | NP\_001141.2 | Sino Biological | 10051-H08H | >97% by SDS-PAGE | Direct |  |
|  | FBXO6 |  | Mammalian (HEK293 derivative) | GST | NM\_018418.2 | LifeTechnologies | NA |  | GST- Ab |  |
|  | ECE1 |  | Mammalian (HEK293) | MYC/DDK | NM\_001113349 | Origene Technologies | TP326153 | >80% by SDS-PAGE | Direct |  |
|  | ERAS |  | Mammalian (HEK293) | MYC/DDK | NM\_181532.2 | Origene Technologies | TP310965 | >80% by SDS-PAGE | Direct |  |
|  | TSPAN13 |  | Mammalian (HEK293 derivative) | GST | NM\_014399.2 | LifeTechnologies | NA |  | GST-Ab |  |
|  | LGALS8 |  | Mammalian (HEK293) | GST | AAF19370.1 | Sino Biological | 10301-H09E | >95% by SDS-PAGE | Direct |  |
|  | KRAS |  | E. coli | HIS | AAH13572.1 | Sino Biological | 12259-H07E | >90% by SDS-PAGE | Direct |  |
|  | KLK2 |  | Mammalian (HEK293) | MYC/DDK | NM\_005551.3 | Origine Technologies | TP302667 | >80% by SDS-PAGE | Direct |  |
|  |  |  |

|  |  |  |
| --- | --- | --- |
|  | **Table ST5. Overlap of the number of patients who were IgG responders to different antigens at week 10 in the sipuleucel-T arm of IMPACT.** |  |
|  |  |  | **n (p-value)** |  |
|  | **Antigen (n)** |  | **PAP (69)** |  | **PSA (36)** |  | **LGALS3 (26)** |  | **ERAS (39)** |  | **LGALS8 (23)** |  | **KRAS (37)** |  | **KLK2 (41)** |  |
| **Antigen (n)** | **PA2024 (86)** |  | 68 (0.001) |  | 36 (0.028) |  | 26 (0.092) |  | 37 (0.372) |  | 22 (0.445) |  | 37 (0.024) |  | 39 (0.327) |  |
| **PAP (69)** |  | - |  | 33 (0.002) |  | 25 (0.001) |  | 31 (0.227) |  | 20 (0.087) |  | 35 (1.4E-4) |  | 32 (0.305) |  |
| **PSA (36)** |  | - |  | - |  | 19 (3.26E-05) |  | 24 (1.0E-4) |  | 12 (0.101) |  | 23 (1.8E-4) |  | 25 (9.6E-05) |  |
| **LGALS3 (26)** |  | - |  | - |  | - |  | 22 (2.2E-07) |  | 13 (0.001) |  | 19 (5.94E-05) |  | 21 (9.8E-06) |  |
| **ERAS (39)** |  | - |  | - |  | - |  | - |  | 13 (0.083) |  | 21 (0.016) |  | 37 (5.5E-19) |  |
| **LGALS8 (23)** |  | - |  | - |  | - |  | - |  | - |  | 11 (0.253) |  | 13 (0.127) |  |
| **KRAS (37)** |  | - |  | - |  | - |  | - |  | - |  | - |  | 23 (0.004) |  |
|  |  |  |

|  |  |  |
| --- | --- | --- |
|  | **Table ST6. Evaluation of IgG responses to candidate antigens at week 12 in ProACT using Luminex xMAP.** |  |
|  | **Antigens Tested** |  | **Sipuleucel-T (n=26)** |  |
|  | **Selection Source** |  | **Antigen** |  | **P-value** |  | **≥2-fold up-reg, n (% of pts)** |  | **≥5-fold up-reg, n (% of pts)** |  |
|  | Controls |  | PAP |  | 4.47E-08 |  | 21 (80.8) |  | 16 (61.5) |  |
|  |  | PA2024 |  | 1.49E-08 |  | 25 (96.2) |  | 21 (80.8) |  |
|  |  | Tetanus toxoid  |  | 4.21E-02 |  | 1 (3.8) |  | 0 (0) |  |
|  | Known PCa antigen |  | PSA |  | 2.06E-04 |  | 5 (19.2) |  | 2 (7.7) |  |
|  | ProtoArray Candidates |  | LGALS3 |  | 4.32E-03 |  | 4 (15.4) |  | 0 (0) |  |
|  |  | ERAS |  | 1.09E-03 |  | 10 (38.5) |  | 4 (15.4) |  |
|  |  | LGALS8 |  | 1.04E-06 |  | 2 (7.7) |  | 2 (7.7) |  |
|  |  | KRAS |  | 1.09E-03 |  | 5 (19.2) |  | 2 (7.7) |  |
|  |  | KLK2 |  | 3.65E-03 |  | 8 (30.8) |  | 1 (3.8) |  |
|  | Pts, Patients; PCa, Prostate cancer; Upreg, Upregulation. |  |

**Table ST7: Association of post-treatment changes in serum levels of IgG at week 10 with OS in the sipuleucel-T arm of IMPACT.**

|  |  |  |
| --- | --- | --- |
|  | **Table ST7A. Association of log2 of fold-change of serum IgG level with OS.** |  |
|  |  |  | **Change in IgG Level** |  | **HR and P-value** |  |
| **≥ Median** |  | **< Median** | **Univariate****Cox Model** |  | **Multivariate****Cox Model** |
|  | **Antigen** |  | **n (% of total)** |  | **Deaths, n (%)** |  | **Median OS (mo)** |  | **n (% of total)** |  | **Deaths, n (%)** |  | **Median OS (mo)** |  | **HR (95% CI)** |  | **P-value** |  | **HR (95% CI)** |  | **P-value** |  |
|  | PA2024 |  | 47(50.54) |  | 21 (44.68) |  | 26.3 |  | 46 (49.46) |  | 18 (39.13) |  | 28.04 |  | 1.09(0.91-1.29) |  | 0.347 |  | 1.07(0.9-1.28) |  | 0.446 |  |
|  | PAP |  | 47(50.54) |  | 16 (34.04) |  | 26.3 |  | 46 (49.46) |  | 23 (50) |  | 27.12 |  | 0.94(0.81-1.09) |  | 0.413 |  | 0.94(0.81-1.1) |  | 0.442 |  |
|  | Tetanus toxoid |  | 47(50.54) |  | 21 (44.68) |  | 27.12 |  | 46 (49.46) |  | 18 (39.13) |  | 26.5 |  | 0.86(0.58-1.27) |  | 0.453 |  | 0.78(0.52-1.18) |  | 0.233 |  |
|  | PSA |  | 47(50.54) |  | 13 (27.66) |  | NA |  | 46 (49.46) |  | 26 (56.52) |  | 22.03 |  | 0.65(0.49-0.88) |  | 0.005 |  | 0.63(0.46-0.86) |  | 0.003 |  |
|  | LGALS3 |  | 47(50.54) |  | 15 (31.91) |  | 28.9 |  | 46 (49.46) |  | 24 (52.17) |  | 26.3 |  | 0.64(0.41-1) |  | 0.051 |  | 0.6(0.38-0.96) |  | 0.035 |  |
|  | ERAS |  | 47(50.54) |  | 17 (36.17) |  | 28.9 |  | 46 (49.46) |  | 22 (47.83) |  | 26.3 |  | 0.82(0.63-1.06) |  | 0.124 |  | 0.79(0.6-1.02) |  | 0.075 |  |
|  | LGALS8 |  | 47(50.54) |  | 19 (40.43) |  | 26.5 |  | 46 (49.46) |  | 20 (43.48) |  | 27.12 |  | 0.84(0.57-1.24) |  | 0.384 |  | 0.83(0.56-1.24) |  | 0.369 |  |
|  | KRAS |  | 47(50.54) |  | 16 (34.04) |  | 26.5 |  | 46 (49.46) |  | 23 (50) |  | 26.3 |  | 0.86(0.66-1.13) |  | 0.292 |  | 0.83(0.63-1.11) |  | 0.218 |  |
|  | KLK2 |  | 47(50.54) |  | 18 (38.3) |  | 28.9 |  | 46 (49.46) |  | 21 (45.65) |  | 26.5 |  | 0.79(0.6-1.04) |  | 0.096 |  | 0.75(0.57-1) |  | 0.051 |  |
|  |  |  |

**Table ST7: Association of post-treatment changes in serum levels of IgG at week 10 with OS in the sipuleucel-T arm of IMPACT.**

|  |  |  |
| --- | --- | --- |
|  | **Table ST7B. Association of IgG responses (≥2-fold increase in serum IgG level post-treatment) with OS.** |  |
|  |  |  | **Change in IgG Level** |  | **HR and P-value** |  |
| **IgG Responder** |  | **IgG Non-responder** | **Univariate****Cox Model** |  | **Multivariate****Cox Model** |  |
|  | **Antigen** |  | **n (% of total)** |  | **Deaths, n (%)** |  | **Median OS (mo)** |  | **n (% of total)** |  | **Deaths, n (%)** |  | **Median OS (mo)** |  | **HR (95% CI)** |  | **P-value** |  | **HR (95% CI)** |  | **P-value** |  |
|  | PA2024 |  | 86(92.47) |  | 35 (40.7) |  | 27.12 |  | 7(7.53) |  | 4 (57.14) |  | 28.9 |  | 1.06(0.37-3.04) |  | 0.907 |  | 1.03(0.36-2.98) |  | 0.952 |  |
|  | PAP |  | 69(74.19) |  | 25 (36.23) |  | 26.5 |  | 24(25.81) |  | 14 (58.33) |  | 27.12 |  | 0.78(0.4-1.52) |  | 0.459 |  | 0.77(0.39-1.52) |  | 0.454 |  |
|  | Tetanus toxoid |  | 10(10.75) |  | 4 (40) |  | 28.9 |  | 83(89.25) |  | 35 (42.17) |  | 26.5 |  | 0.78(0.28-2.21) |  | 0.646 |  | 0.75(0.26-2.12) |  | 0.582 |  |
|  | PSA |  | 36(38.71) |  | 10 (27.78) |  | NA |  | 57(61.29) |  | 29 (50.88) |  | 22.98 |  | 0.42(0.2-0.86) |  | 0.018 |  | 0.38(0.19-0.8) |  | 0.010 |  |
|  | LGALS3 |  | 26(27.96) |  | 4 (15.38) |  | NA |  | 67(72.04) |  | 35 (52.24) |  | 25.38 |  | 0.27(0.1-0.76) |  | 0.013 |  | 0.25(0.09-0.72) |  | 0.010 |  |
|  | ERAS |  | 39(41.94) |  | 14 (35.9) |  | 28.9 |  | 54(58.06) |  | 25 (46.3) |  | 26.3 |  | 0.63(0.32-1.21) |  | 0.161 |  | 0.55(0.28-1.08) |  | 0.085 |  |
|  | LGALS8 |  | 23(24.73) |  | 7 (30.43) |  | NA |  | 70(75.27) |  | 32 (45.71) |  | 26.5 |  | 0.74(0.32-1.68) |  | 0.469 |  | 0.76(0.33-1.73) |  | 0.510 |  |
|  | KRAS |  | 37(39.78) |  | 12 (32.43) |  | NA |  | 56(60.22) |  | 27 (48.21) |  | 26.5 |  | 0.77(0.38-1.53) |  | 0.452 |  | 0.77(0.39-1.55) |  | 0.466 |  |
|  | KLK2 |  | 41(44.09) |  | 17 (41.46) |  | 28.9 |  | 52(55.91) |  | 22 (42.31) |  | 26.5 |  | 0.81(0.43-1.53) |  | 0.520 |  | 0.73(0.38-1.4) |  | 0.348 |  |
|  |  |  |

|  |  |  |
| --- | --- | --- |
|  | **Table ST8. Comparison of OS in sipuleucel-T-treated IgG responders and IgG non-responders at week 10 with that in control patients in IMPACT.** |  |
|  |  |  | **Control** |  | **Change in IgG Level** |  | **HR and P-value** |  |
| **Univariate Cox Model** |  | **Multivariate Cox Model** |  |
|  | **Antigen** |  | **n (% of total)**  | **Deaths,****n (%)** | **Median OS (mo)** |  | **IgG Responder** |  | **IgG Non-responder** |  | **Control vs IgG Responder** | **Control vs IgG Non-responder** |  | **Control vs IgG Responder** | **Control vs IgG Non-responder** |  |
| **n (% of total** **Deaths)** | **n (%)** | **Median OS (mo)** |  | **n (% of total** **Deaths)** | **n (%)** | **Median OS (mo)** | **HR (95% CI)** | **P-value** | **HR** **(95% CI)** | **P-value** |  | **HR** **(95% CI)** | **P-value** | **HR** **(95% CI)** | **P-value** |  |
|  | PA2024 |  | 39 (29.55) | 23 (58.97) | 22.06 |  | 86 (65.15) | 35 (40.7) | 27.12 |  | 7 (5.3) | 4 (57.14) | 28.9 |  | 0.56(0.33-0.95) | 0.032 | 0.55(0.19-1.61) | 0.275 |  | 0.51(0.3-0.88) | 0.015 | 0.51(0.17-1.51) | 0.223 |  |
|  | PAP |  | 39 (29.55) | 23 (58.97) | 22.06 |  | 69 (52.27) | 25 (36.23) | 26.5 |  | 24 (18.18) | 14 (58.33) | 27.12 |  | 0.51(0.29-0.9) | 0.021 | 0.68(0.35-1.33) | 0.257 |  | 0.47(0.26-0.83) | 0.010 | 0.63(0.32-1.24) | 0.178 |  |
|  | PSA |  | 39 (29.55) | 23 (58.97) | 22.06 |  | 36 (27.27) | 10 (27.78) | NA |  | 57 (43.18) | 29 (50.88) | 22.98 |  | 0.31(0.15-0.66) | 0.002 | 0.76(0.44-1.31) | 0.324 |  | 0.27(0.12-0.58) | 7.41E-04 | 0.71(0.41-1.24) | 0.230 |  |
|  | LGALS3 |  | 39 (29.55) | 23 (58.97) | 22.06 |  | 26 (19.7) | 4 (15.38) | NA |  | 67 (50.76) | 35 (52.24) | 25.38 |  | 0.19(0.07-0.56) | 0.002 | 0.72(0.42-1.21) | 0.215 |  | 0.16(0.06-0.49) | 1.09E-03 | 0.66(0.38-1.12) | 0.123 |  |
|  |  |  |  |

|  |  |  |
| --- | --- | --- |
|  | **Table ST9. Evaluation of IgG responses against candidate antigens at weeks 2 and 22 in IMPACT using Luminex xMAP.** |  |
|  | **Antigen** |  | **Week 2** |  | **Week 22** |  |
|  |  | **Sip-T (n=142)** |  | **Control (n=62)** | **Sip-T vs Control** |  | **Sip-T (n=60)** |  | **Control (n=16)** |  | **Sip-T vs Control** |  |
|  | **Selection Source** | **Symbol or Name** |  | **P-value (pre vs post)** | **n (%) ≥2-fold up-reg** | **n (%) ≥5-fold up-reg** |  | **P-value (pre vs post)** | **n (%) ≥2-fold up-reg** | **n (%) ≥5-fold up-reg** | **P-value (fold-change, Sip-T vs Control)** |  | **P-value (pre vs post)** | **n (%) ≥2-fold up-reg** | **n (%) ≥5-fold up-reg** |  | **P-value (pre vs post)** | **n (%) ≥2-fold up-reg** | **n (%) ≥5-fold up-reg** |  | **P-value (fold-change, Sip-T vs Control)** |  |
|  | Controls | PAP |  | 3.59E-22 | 92 (64.8) | 65 (45.8) |  | 0.500 | 0 (0) | 0 (0) | 4.05E-19 |  | 4.19E-09 | 35 (58.3) | 23 (38.3) |  | 0.029 | 3 (18.8) | 0 (0) |  | 5.41E-04 |  |
|  | PA2024 |  | 4.82E-25 | 119 (83.8) | 103 (72.5) |  | 0.559 | 3 (4.8) | 1 (1.6) | 8.97E-25 |  | 1.18E-10 | 52 (86.7) | 43 (71.7) |  | 0.088 | 4 (25) | 1 (6.2) |  | 7.55E-07 |  |
|  | Tetanus Toxoid |  | 2.77E-15 | 23 (16.2) | 1 (0.7) |  | 0.287 | 1 (1.6) | 0 (0) | 6.80E-07 |  | 1.07E-02 | 11 (18.3) | 2 (3.3) |  | 0.281 | 2 (12.5) | 0 (0) |  | 2.56E-01 |  |
|  | Known PCa antigen | PSA |  | 6.58E-16 | 35 (24.6) | 21 (14.8) |  | 0.428 | 0 (0) | 0 (0) | 2.97E-09 |  | 3.56E-07 | 18 (30) | 8 (13.3) |  | 0.058 | 1 (6.2) | 0 (0) |  | 1.86E-02 |  |
|  | ProtoArray Candidates | LGALS3 |  | 1.71E-15 | 41 (28.9) | 13 (9.2) |  | 0.572 | 3 (4.8) | 0 (0) | 3.68E-09 |  | 1.06E-05 | 8 (13.3) | 1 (1.7) |  | 0.126 | 1 (6.2) | 0.00 |  | 5.08E-02 |  |
|  | ERAS |  | 1.39E-16 | 60 (42.3) | 25 (17.6) |  | 0.761 | 3 (4.8) | 0 (0) | 6.62E-11 |  | 2.46E-06 | 23 (38.3) | 6 (10) |  | 0.096 | 3 (18.8) | 0 (0) |  | 1.75E-02 |  |
|  | LGALS8 |  | 8.43E-17 | 36 (25.4) | 17 (12) |  | 0.002 | 2 (3.2) | 0 (0) | 1.29E-05 |  | 2.45E-05 | 13 (21.7) | 1 (1.7) |  | 0.029 | 0 (0) | 0 (0) |  | 1.35E-01 |  |
|  | KRAS |  | 1.20E-18 | 57 (40.1) | 22 (15.5) |  | 0.819 | 1 (1.6) | 0 (0) | 3.90E-13 |  | 9.71E-07 | 14 (23.3) | 4 (6.7) |  | 0.106 | 1 (6.2) | 0 (0) |  | 4.45E-02 |  |
|  | KLK2 |  | 6.37E-16 | 52 (36.6) | 18 (12.7) |  | 0.724 | 2 (3.2) | 1 (1.6) | 8.07E-10 |  | 2.46E-06 | 19 (31.7) | 2 (3.3) |  | 0.149 | 1 (6.2) | 1 (6.2) |  | 2.38E-02 |  |
|  | PCa, prostate cancer. |  |

|  |  |  |
| --- | --- | --- |
|  | **Table ST10. Assessment of IgG responses against candidate antigens at weeks 4 and 20 in ProACT using Luminex xMAP.** |  |
|  | **Antigens Tested** |  | **Week 4 (n=33)** |  | **Week 20 (n=19)** |  |
|  | **Selection Source** | **Antigen** |  | **P-value** | **≥2-fold up-reg, n (%)** | **≥5-fold up-reg, n (%)** |  | **P-value** | **≥2-fold up-reg, n (%)** | **≥5-fold up-reg, n (%)** |  |
|  | Controls | PAP |  | 3.49E-10 | 29 (87.9) | 26 (78.8) |  | 3.62E-05 | 13 (68.4) | 10 (52.6) |  |
|  | PA2024 |  | 2.33E-10 | 32 (97) | 32 (97) |  | 5.72E-06 | 17 (89.5) | 14 (73.7) |  |
|  | Tetanus toxoid  |  | 1.19E-03 | 2 (6.1) | 0 (0) |  | 4.30E-01 | 1 (5.3) | 0 (0) |  |
|  | Known PCa antigen | PSA |  | 2.02E-07 | 11 (33.3) | 2 (6.1) |  | 3.64E-02 | 2 (10.5) | 0 (0) |  |
|  | ProtoArray Candidates | LGALS3 |  | 1.47E-07 | 12 (36.4) | 2 (6.1) |  | 1.66E-01 | 2 (10.5) | 0 (0) |  |
|  | ERAS |  | 1.53E-05 | 13 (39.4) | 7 (21.2) |  | 6.68E-02 | 6 (31.6) | 3 (15.8) |  |
|  | LGALS8 |  | 1.16E-09 | 8 (24.2) | 2 (6.1) |  | 2.01E-02 | 1 (5.3) | 0 (0) |  |
|  | KRAS |  | 2.81E-06 | 14 (42.4) | 4 (12.1) |  | 5.21E-02 | 2 (10.5) | 2 (10.5) |  |
|  | KLK2 |  | 1.03E-04 | 11 (33.3) | 3 (9.1) |  | 9.09E-02 | 4 (21.1) | 1 (5.3) |  |
|  | PCa, prostate cancer. |  |

**Table ST11: Overlap of the number of sipuleucel-T-treated patients who were IgG responders to antigens across post-treatment time points in IMPACT.**

|  |  |  |
| --- | --- | --- |
|  | **Table ST11A. Overlap of IgG responses across the weeks 2 and 10 post-treatment time points.** |  |
|  | **Antigens** |  | **Wk 2, n** |  | **Wk 10, n** |  | **Overlap****(Wk2 - Wk10)** |  | **P-value** |  |
|  | PA2024 |  | 67 |  | 74 |  | 63 |  | 9.54E-02 |  |
|  | PAP |  | 53 |  | 60 |  | 46 |  | 5.23E-04 |  |
|  | PSA |  | 21 |  | 30 |  | 17 |  | 2.33E-06 |  |
|  | LGALS3 |  | 25 |  | 23 |  | 13 |  | 2.35E-03 |  |
|  | ERAS |  | 34 |  | 35 |  | 25 |  | 2.92E-06 |  |
|  | LGALS8 |  | 19 |  | 17 |  | 13 |  | 1.21E-07 |  |
|  | KRAS |  | 32 |  | 31 |  | 19 |  | 1.72E-03 |  |
|  | KLK2 |  | 31 |  | 36 |  | 25 |  | 2.25E-07 |  |
|  |  |  |

|  |  |  |
| --- | --- | --- |
|  | **Table ST11B. Overlap of IgG responses across the weeks 10 and 22 time points.** |  |
|  | **Antigens** |  | **Wk 10, n** |  | **Wk 22, n** |  | **Overlap****(Wk10 – Wk22)** |  | **P-value** |  |
|  | PA2024 |  | 47 |  | 46 |  | 45 |  | 2.68E-04 |  |
|  | PAP |  | 37 |  | 31 |  | 29 |  | 2.19E-05 |  |
|  | PSA |  | 24 |  | 16 |  | 15 |  | 3.60E-06 |  |
|  | LGALS3 |  | 17 |  | 7 |  | 5 |  | 3.09E-02 |  |
|  | ERAS |  | 26 |  | 20 |  | 17 |  | 6.86E-05 |  |
|  | LGALS8 |  | 13 |  | 9 |  | 9 |  | 1.94E-07 |  |
|  | KRAS |  | 20 |  | 13 |  | 11 |  | 1.38E-04 |  |
|  | KLK2 |  | 26 |  | 17 |  | 14 |  | 1.27E-03 |  |
|  |  |  |

**Table ST12: Association of changes in serum IgG levels at week 2 or 22 with OS in the sipuleucel-T arm of IMPACT.**

|  |  |  |
| --- | --- | --- |
|  | **Table ST12A. Association of log2 of fold-change of serum IgG level with OS.** |  |
|  | **Time point** | **Antigen** |  | **Change in IgG Level** |  | **HR and P-value** |  |
| **≥ Median** |  | **< Median** | **Univariate Cox Model** |  | **Multivariate Cox Model** |  |
|  |  | **n (% of total)** | **Deaths, n (%)** | **Median OS (mo)** |  | **n (% of total)** | **Deaths, n (%)** | **Median OS(mo)** |  | **HR (95% CI)** | **P-value** |  | **HR (95% CI)** | **P-value** |  |
|  | **Week 2** | PA2024 |  | 71 (50) | 34 (47.89) | 23.44 |  | 71 (50) | 35 (49.3) | 25.38 |  | 0.95(0.85-1.06) | 0.347 |  | 0.94(0.84-1.05) | 0.294 |  |
|  | PAP |  | 71 (50) | 30 (42.25) | 25.38 |  | 71 (50) | 39 (54.93) | 22.98 |  | 0.9(0.8-1) | 0.049 |  | 0.9(0.8-1.02) | 0.094 |  |
|  | PSA |  | 71 (50) | 31 (43.66) | 27.12 |  | 71 (50) | 38 (53.52) | 22.03 |  | 0.79(0.64-0.97) | 0.027 |  | 0.77(0.62-0.95) | 0.017 |  |
|  | LGALS3 |  | 71 (50) | 31 (43.66) | 26.76 |  | 71 (50) | 38 (53.52) | 23.44 |  | 0.82(0.65-1.04) | 0.106 |  | 0.85(0.67-1.08) | 0.192 |  |
|  | **Week 22** | PA2024 |  | 30 (50) | 9 (30) | NA |  | 30 (50) | 14 (46.67) | 27.12 |  | 0.93(0.78-1.12) | 0.460 |  | 0.93(0.78-1.11) | 0.412 |  |
|  | PAP |  | 30 (50) | 6 (20) | NA |  | 30 (50) | 17 (56.67) | 27.12 |  | 0.84(0.65-1.08) | 0.164 |  | 0.83(0.64-1.08) | 0.160 |  |
|  | PSA |  | 30 (50) | 9 (30) | NA |  | 30 (50) | 14 (46.67) | 27.45 |  | 0.64(0.46-0.89) | 0.009 |  | 0.63(0.46-0.87) | 0.005 |  |
|  | LGALS3 |  | 30 (50) | 9 (30) | NA |  | 30 (50) | 14 (46.67) | 27.45 |  | 0.74(0.48-1.13) | 0.158 |  | 0.72(0.48-1.09) | 0.119 |  |
|  |  |  |

**Table ST12: Association between OS and IgG responses at week 2 or 22 after treatment in the sipuleucel-T arm in IMPACT.**

|  |  |  |
| --- | --- | --- |
|  | **Table ST12B. Association IgG response (≥2-fold increase in serum IgG level post-treatment) with OS.** |  |
|  | **Time point** | **Antigen** |  | **Change in IgG Level** |  | **HR and P-value** |  |
| **IgG Responder** |  | **IgG Non-responder** | **Univariate Cox Model** |  | **Multivariate Cox Model** |
|  |  | **n (% of total)** | **Deaths, n (%)** | **Median OS (mo)** |  | **n (% of total)** | **Deaths, n (%)** | **Median OS(mo)** |  | **HR (95% CI)** | **P-value** |  | **HR (95% CI)** | **P-value** |  |
|  | **Week 2** | PA2024 |  | 119 (83.8) | 58 (48.74) | 25.38 |  | 23 (16.2) | 11 (47.83) | 21.27 |  | 0.79(0.41-1.51) | 0.473 |  | 0.86(0.44-1.66) | 0.653 |  |
|  | PAP |  | 92 (64.79) | 40 (43.48) | 26.3 |  | 50 (35.21) | 29 (58) | 22.03 |  | 0.72(0.44-1.16) | 0.173 |  | 0.66(0.4-1.07) | 0.091 |  |
|  | PSA |  | 35 (24.65) | 12 (34.29) | NA |  | 107 (75.35) | 57 (53.27) | 22.03 |  | 0.45(0.24-0.83) | 0.012 |  | 0.42(0.22-0.79) | 0.007 |  |
|  | LGALS3 |  | 41 (28.87) | 14 (34.15) | NA |  | 101 (71.13) | 55 (54.46) | 22.98 |  | 0.51(0.28-0.92) | 0.026 |  | 0.57(0.31-1.02) | 0.060 |  |
|  | **Week 22** | PA2024 |  | 52 (86.67) | 19 (36.54) | 27.45 |  | 8 (13.33) | 4 (50) | 28.9 |  | 0.96(0.32-2.86) | 0.937 |  | 1.03(0.34-3.17) | 0.958 |  |
|  | PAP |  | 35 (58.33) | 11 (31.43) | 26.76 |  | 25 (41.67) | 12 (48) | 28.04 |  | 0.99(0.43-2.32) | 0.987 |  | 0.97(0.41-2.29) | 0.937 |  |
|  | PSA |  | 18 (30) | 4 (22.22) | NA |  | 42 (70) | 19 (45.24) | 27.45 |  | 0.4(0.13-1.17) | 0.095 |  | 0.35(0.12-1.05) | 0.060 |  |
|  | LGALS3 |  | 8 (13.33) | 2 (25) | NA |  | 52 (86.67) | 21 (40.38) | 27.45 |  | 0.52(0.12-2.24) | 0.384 |  | 0.39(0.08-1.82) | 0.230 |  |
|  |  |  |

|  |  |  |
| --- | --- | --- |
|  | **Table ST13. Comparison of OS in sipuleucel-T-treated IgG responders and IgG non-responders at weeks 2 and 22 with that in control patients in IMPACT.** |  |
|  |  |  |  | **Control** |  | **Sipuleucel-T** |  | **HR and P-value** |  |
| **IgG Responder** |  | **IgG Non-responder** |  | **Univariate Cox Model** |  | **Multivariate Cox Model** |
|  | **Time point** | **Antigen** |  | **n (% of total)** | **Deaths,n (%)** | **Median OS (mo)** |  | **n (% of total)** | **Deaths, n (%)** | **Median OS (mo)** |  | **n (% of total)** | **Deaths, n (%)** | **Median OS (mo)** |  | **Control vs IgG Responder** |  | **Control vs IgG Non-responder** |  | **Control vs IgG Responder** |  | **Control vs IgG Non-responder** |  |
|  | **HR (95% CI)** | **P-value** |  | **HR (95% CI)** | **P-value** |  | **HR (95% CI)** | **P-value** |  | **HR (95% CI)** | **P-value** |  |
|  | **Week 2** | PA2024 |  | 62(30.39) | 39(62.9) | 21.4 |  | 119(58.33) | 58(48.74) | 25.38 |  | 23(11.27) | 11(47.83) | 21.27 |  | 0.66(0.44-1) | 0.049 |  | 0.86(0.44-1.67) | 0.652 |  | 0.64(0.42-0.96) | 0.030 |  | 0.74(0.38-1.47) | 0.394 |  |
|  | PAP |  | 62(30.39) | 39(62.9) | 21.4 |  | 92(45.1) | 40(43.48) | 26.3 |  | 50(24.51) | 29(58) | 22.03 |  | 0.6(0.39-0.94) | 0.026 |  | 0.86(0.53-1.39) | 0.534 |  | 0.56(0.36-0.87) | 0.011 |  | 0.84(0.52-1.36) | 0.473 |  |
|  | PSA |  | 62(30.39) | 39(62.9) | 21.4 |  | 35(17.16) | 12(34.29) | NA |  | 107(52.45) | 57(53.27) | 22.03 |  | 0.37(0.19-0.71) | 0.003 |  | 0.84(0.56-1.26) | 0.394 |  | 0.33(0.17-0.64) | 9.55E-04 |  | 0.8(0.53-1.21) | 0.298 |  |
|  | LGALS3 |  | 62(30.39) | 39(62.9) | 21.4 |  | 41(20.1) | 14(34.15) | NA |  | 101(49.51) | 55(54.46) | 22.98 |  | 0.42(0.23-0.77) | 0.005 |  | 0.83(0.55-1.25) | 0.363 |  | 0.42(0.23-0.77) | 0.005 |  | 0.76(0.5-1.14) | 0.187 |  |
|  | **Week 22** | PA2024 |  | 16(21.05) | 7(43.75) | 28.34 |  | 52(68.42) | 19(36.54) | 27.45 |  | 8(10.53) | 4(50) | 28.9 |  | 0.73(0.31-1.74) | 0.480 |  | 0.75(0.22-2.6) | 0.649 |  | 0.69(0.28-1.7) | 0.417 |  | 0.63(0.17-2.38) | 0.494 |  |
|  | PAP |  | 16(21.05) | 7(43.75) | 28.34 |  | 35(46.05) | 11(31.43) | 26.76 |  | 25(32.89) | 12(48) | 28.04 |  | 0.73(0.28-1.89) | 0.519 |  | 0.74(0.29-1.89) | 0.525 |  | 0.69(0.26-1.83) | 0.459 |  | 0.67(0.24-1.82) | 0.431 |  |
|  | PSA |  | 16(21.05) | 7(43.75) | 28.34 |  | 18(23.68) | 4(22.22) | NA |  | 42(55.26) | 19(45.24) | 27.45 |  | 0.37(0.11-1.28) | 0.118 |  | 0.92(0.39-2.2) | 0.855 |  | 0.32(0.09-1.15) | 0.081 |  | 0.88(0.35-2.17) | 0.775 |  |
|  | LGALS3 |  | 16(21.05) | 7(43.75) | 28.34 |  | 8(10.53) | 2(25) | NA |  | 52(68.42) | 21(40.38) | 27.45 |  | 0.42(0.09-2.02) | 0.279 |  | 0.79(0.34-1.86) | 0.589 |  | 0.29(0.05-1.59) | 0.154 |  | 0.73(0.3-1.78) | 0.491 |  |
|  | NA, not applicable. |  |

# SUPPLEMENTARY REFERENCES

1. Sheikh NA, Petrylak D, Kantoff PW, Dela Rosa C, Stewart FP, Kuan LY, et al. Sipuleucel-T immune parameters correlate with survival: an analysis of the randomized phase 3 clinical trials in men with castration-resistant prostate cancer. Cancer immunology, immunotherapy : CII. 2013;62:137-47.

2. Schweitzer B, Meng L, Mattoon D, Rai AJ. Immune response biomarker profiling application on ProtoArray protein microarrays. Methods Mol Biol. 2010;641:243-52.

3. Gnjatic S, Ritter E, Buchler MW, Giese NA, Brors B, Frei C, et al. Seromic profiling of ovarian and pancreatic cancer. Proc Natl Acad Sci U S A. 2010;107:5088-93.

4. Kwek SS, Dao V, Roy R, Hou Y, Alajajian D, Simko JP, et al. Diversity of antigen-specific responses induced in vivo with CTLA-4 blockade in prostate cancer patients. J Immunol. 2012;189:3759-66.

5. Nguyen MC, Tu GH, Koprivnikar KE, Gonzalez-Edick M, Jooss KU, Harding TC. Antibody responses to galectin-8, TARP and TRAP1 in prostate cancer patients treated with a GM-CSF-secreting cellular immunotherapy. Cancer immunology, immunotherapy : CII. 2010;59:1313-23.

6. Hudson ME, Pozdnyakova I, Haines K, Mor G, Snyder M. Identification of differentially expressed proteins in ovarian cancer using high-density protein microarrays. Proc Natl Acad Sci U S A. 2007;104:17494-9.

7. Sboner A, Karpikov A, Chen G, Smith M, Mattoon D, Freeman-Cook L, et al. Robust-linear-model normalization to reduce technical variability in functional protein microarrays. J Proteome Res. 2009;8:5451-64.

8. Pickering JW, Martins TB, Schroder MC, Hill HR. Comparison of a multiplex flow cytometric assay with enzyme-linked immunosorbent assay for auantitation of antibodies to tetanus, diphtheria, and Haemophilus influenzae Type b. Clinical and diagnostic laboratory immunology. 2002;9:872-6.

9. Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS, et al. Integrative genomic profiling of human prostate cancer. Cancer cell. 2010;18:11-22.

10. <http://www.cbioportal.org/public-portal/?cancer_type_id=pca>.

11. Newlaczyl AU, Yu LG. Galectin-3--a jack-of-all-trades in cancer. Cancer letters. 2011;313:123-8.

12. Perillo NL, Marcus ME, Baum LG. Galectins: versatile modulators of cell adhesion, cell proliferation, and cell death. J Mol Med (Berl). 1998;76:402-12.

13. Sano H, Hsu DK, Yu L, Apgar JR, Kuwabara I, Yamanaka T, et al. Human galectin-3 is a novel chemoattractant for monocytes and macrophages. J Immunol. 2000;165:2156-64.

14. Califice S, Castronovo V, Bracke M, van den Brule F. Dual activities of galectin-3 in human prostate cancer: tumor suppression of nuclear galectin-3 vs tumor promotion of cytoplasmic galectin-3. Oncogene. 2004;23:7527-36.

15. Laderach DJ, Gentilini LD, Giribaldi L, Delgado VC, Nugnes L, Croci DO, et al. A unique galectin signature in human prostate cancer progression suggests galectin-1 as a key target for treatment of advanced disease. Cancer research. 2013;73:86-96.

16. van den Brule FA, Waltregny D, Liu FT, Castronovo V. Alteration of the cytoplasmic/nuclear expression pattern of galectin-3 correlates with prostate carcinoma progression. International journal of cancer Journal international du cancer. 2000;89:361-7.

17. Wang Y, Nangia-Makker P, Tait L, Balan V, Hogan V, Pienta KJ, et al. Regulation of prostate cancer progression by galectin-3. The American journal of pathology. 2009;174:1515-23.

18. Markowska AI, Liu FT, Panjwani N. Galectin-3 is an important mediator of VEGF- and bFGF-mediated angiogenic response. The Journal of experimental medicine. 2010;207:1981-93.

19. Elad-Sfadia G, Haklai R, Balan E, Kloog Y. Galectin-3 augments K-Ras activation and triggers a Ras signal that attenuates ERK but not phosphoinositide 3-kinase activity. The Journal of biological chemistry. 2004;279:34922-30.

20. Shalom-Feuerstein R, Cooks T, Raz A, Kloog Y. Galectin-3 regulates a molecular switch from N-Ras to K-Ras usage in human breast carcinoma cells. Cancer research. 2005;65:7292-300.

21. Balan V, Nangia-Makker P, Kho DH, Wang Y, Raz A. Tyrosine-phosphorylated galectin-3 protein is resistant to prostate-specific antigen (PSA) cleavage. The Journal of biological chemistry. 2012;287:5192-8.

22. Larsen SL, Pedersen LO, Buus S, Stryhn A. T cell responses affected by aminopeptidase N (CD13)-mediated trimming of major histocompatibility complex class II-bound peptides. The Journal of experimental medicine. 1996;184:183-9.

23. Fukasawa K, Fujii H, Saitoh Y, Koizumi K, Aozuka Y, Sekine K, et al. Aminopeptidase N (APN/CD13) is selectively expressed in vascular endothelial cells and plays multiple roles in angiogenesis. Cancer letters. 2006;243:135-43.

24. Yang E, Shim JS, Woo HJ, Kim KW, Kwon HJ. Aminopeptidase N/CD13 induces angiogenesis through interaction with a pro-angiogenic protein, galectin-3. Biochemical and biophysical research communications. 2007;363:336-41.

25. Guzman-Rojas L, Rangel R, Salameh A, Edwards JK, Dondossola E, Kim YG, et al. Cooperative effects of aminopeptidase N (CD13) expressed by nonmalignant and cancer cells within the tumor microenvironment. Proc Natl Acad Sci U S A. 2012;109:1637-42.

26. Larkin SE, Holmes S, Cree IA, Walker T, Basketter V, Bickers B, et al. Identification of markers of prostate cancer progression using candidate gene expression. Br J Cancer. 2012;106:157-65.

27. Sorensen KD, Abildgaard MO, Haldrup C, Ulhoi BP, Kristensen H, Strand S, et al. Prognostic significance of aberrantly silenced ANPEP expression in prostate cancer. Br J Cancer. 2013;108:420-8.

28. Catterall WA. Structure and function of voltage-sensitive ion channels. Science. 1988;242:50-61.

29. Powers PA, Liu S, Hogan K, Gregg RG. Molecular characterization of the gene encoding the gamma subunit of the human skeletal muscle 1,4-dihydropyridine-sensitive Ca2+ channel (CACNLG), cDNA sequence, gene structure, and chromosomal location. The Journal of biological chemistry. 1993;268:9275-9.

30. Cardozo T, Pagano M. The SCF ubiquitin ligase: insights into a molecular machine. Nature reviews Molecular cell biology. 2004;5:739-51.

31. Zhang YW, Brognard J, Coughlin C, You Z, Dolled-Filhart M, Aslanian A, et al. The F box protein Fbx6 regulates Chk1 stability and cellular sensitivity to replication stress. Molecular cell. 2009;35:442-53.

32. Lambert LA, Whyteside AR, Turner AJ, Usmani BA. Isoforms of endothelin-converting enzyme-1 (ECE-1) have opposing effects on prostate cancer cell invasion. Br J Cancer. 2008;99:1114-20.

33. Herrmann E, Bogemann M, Bierer S, Eltze E, Hertle L, Wulfing C. The endothelin axis in urologic tumors: mechanisms of tumor biology and therapeutic implications. Expert review of anticancer therapy. 2006;6:73-81.

34. Nelson JB, Hedican SP, George DJ, Reddi AH, Piantadosi S, Eisenberger MA, et al. Identification of endothelin-1 in the pathophysiology of metastatic adenocarcinoma of the prostate. Nature medicine. 1995;1:944-9.

35. Nelson JB, Udan MS, Guruli G, Pflug BR. Endothelin-1 inhibits apoptosis in prostate cancer. Neoplasia. 2005;7:631-7.

36. James ND, Caty A, Payne H, Borre M, Zonnenberg BA, Beuzeboc P, et al. Final safety and efficacy analysis of the specific endothelin A receptor antagonist zibotentan (ZD4054) in patients with metastatic castration-resistant prostate cancer and bone metastases who were pain-free or mildly symptomatic for pain: a double-blind, placebo-controlled, randomized Phase II trial. BJU international. 2010;106:966-73.

37. Carducci MA, Padley RJ, Breul J, Vogelzang NJ, Zonnenberg BA, Daliani DD, et al. Effect of endothelin-A receptor blockade with atrasentan on tumor progression in men with hormone-refractory prostate cancer: a randomized, phase II, placebo-controlled trial. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2003;21:679-89.

38. Thakkar SG, Choueiri TK, Garcia JA. Endothelin receptor antagonists: rationale, clinical development, and role in prostate cancer therapeutics. Current oncology reports. 2006;8:108-13.

39. Takahashi K, Mitsui K, Yamanaka S. Role of ERas in promoting tumour-like properties in mouse embryonic stem cells. Nature. 2003;423:541-5.

40. Kubota E, Kataoka H, Aoyama M, Mizoshita T, Mori Y, Shimura T, et al. Role of ES cell-expressed Ras (ERas) in tumorigenicity of gastric cancer. The American journal of pathology. 2010;177:955-63.

41. Darson MF, Pacelli A, Roche P, Rittenhouse HG, Wolfert RL, Saeid MS, et al. Human glandular kallikrein 2 expression in prostate adenocarcinoma and lymph node metastases. Urology. 1999;53:939-44.

42. Williams SA, Xu Y, De Marzo AM, Isaacs JT, Denmeade SR. Prostate-specific antigen (PSA) is activated by KLK2 in prostate cancer ex vivo models and in prostate-targeted PSA/KLK2 double transgenic mice. The Prostate. 2010;70:788-96.

43. Nam RK, Zhang WW, Klotz LH, Trachtenberg J, Jewett MA, Sweet J, et al. Variants of the hK2 protein gene (KLK2) are associated with serum hK2 levels and predict the presence of prostate cancer at biopsy. Clin Cancer Res. 2006;12:6452-8.

44. Nam RK, Zhang WW, Trachtenberg J, Diamandis E, Toi A, Emami M, et al. Single nucleotide polymorphism of the human kallikrein-2 gene highly correlates with serum human kallikrein-2 levels and in combination enhances prostate cancer detection. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2003;21:2312-9.

45. Magklara A, Scorilas A, Stephan C, Kristiansen GO, Hauptmann S, Jung K, et al. Decreased concentrations of prostate-specific antigen and human glandular kallikrein 2 in malignant versus nonmalignant prostatic tissue. Urology. 2000;56:527-32.

46. Helo P, Cronin AM, Danila DC, Wenske S, Gonzalez-Espinoza R, Anand A, et al. Circulating prostate tumor cells detected by reverse transcription-PCR in men with localized or castration-refractory prostate cancer: concordance with CellSearch assay and association with bone metastases and with survival. Clinical chemistry. 2009;55:765-73.

47. Raaijmakers R, de Vries SH, Blijenberg BG, Wildhagen MF, Postma R, Bangma CH, et al. hK2 and free PSA, a prognostic combination in predicting minimal prostate cancer in screen-detected men within the PSA range 4-10 ng/ml. European urology. 2007;52:1358-64.

48. Rittenhouse HG, Finlay JA, Mikolajczyk SD, Partin AW. Human Kallikrein 2 (hK2) and prostate-specific antigen (PSA): two closely related, but distinct, kallikreins in the prostate. Critical reviews in clinical laboratory sciences. 1998;35:275-368.

49. Su ZZ, Lin J, Shen R, Fisher PE, Goldstein NI, Fisher PB. Surface-epitope masking and expression cloning identifies the human prostate carcinoma tumor antigen gene PCTA-1 a member of the galectin gene family. Proc Natl Acad Sci U S A. 1996;93:7252-7.

50. Maecker HT, Todd SC, Levy S. The tetraspanin superfamily: molecular facilitators. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 1997;11:428-42.

51. Arencibia JM, Martin S, Perez-Rodriguez FJ, Bonnin A. Gene expression profiling reveals overexpression of TSPAN13 in prostate cancer. International journal of oncology. 2009;34:457-63.

52. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med. 2010;363:411-22.