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(54) Title: B7-H1 AND METHODS OF DIAGNOSIS, PROGNOSIS, AND TREATMENT OF CANCER

(57) Abstract: The invention features methods of diagnosis by assessing B7-H1 expression in a tissue from a subject that has, or is suspected of having, cancer, methods of treatment with agents that interfere with B7-H1-receptor interaction, methods of selecting candidate subjects likely to benefit from cancer immunotherapy, and methods of inhibiting expression of B7-H1.



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B7-H1 AND METHODS OF DIAGNOSIS, PROGNOSIS, AND  
TREATMENT OF CANCER

This application claims the benefit of U.S. Provisional Application No. 60/616,590, filed October 6, 2004, and U.S. Provisional Application 60/642,794, filed January 11, 2005. The disclosures of U.S. Provisional Application No. 60/616,590 and U.S. Provisional Application 60/642,794 are incorporated herein by reference in their entirety.

**TECHNICAL FIELD**

This invention relates to immune molecules expressed in cancer tissue; and more particularly to evaluating the expression of immune molecules in tumor cells and tumor-infiltrating leukocytes.

**BACKGROUND**

An important determinant for the initiation and progression of cancer is the ability of cancer cells to evade the host's immune system. The presence in cancer tissue of, for example, inadequate, inappropriate, or inhibitory immune molecules can restrict the host's ability to generate immune responses to the cancer.

The disclosures of U.S. Patent No. 6,803,192 and co-pending U.S. Application Serial Nos. 09/649,108; 10/127,282; and 10/719,477; and International Application No. US/02/32364 are incorporated herein by reference in their entirety.

**SUMMARY**

The invention is based in part on the finding that in renal cell carcinoma (RCC) patients the risk of death is proportional to the number of tumor cells, and/or leukocytes in the tumor, expressing the co-stimulatory human glycoprotein B7-H1. As used herein, the term "B7-H1" refers to B7-H1 from any mammalian species and the term "hB7-H1" refers to human B7-H1. Further details on B7-H1 polypeptides and nucleic acids are provided in U.S. Patent No. 6,803,192 and co-pending U.S. Application Serial No. 09/649,108, the disclosures of which are incorporated herein by reference in their entirety.

The invention provides methods of diagnosing subjects having, or that are likely to develop, cancer of a tissue based on the expression of B7-H1 by cells of the cancer tissue, methods of predicting success of immunotherapy, methods of prognosis, and methods of treatment. Leukocytes in a tumor are sometimes referred to herein as “tumor-infiltrating leukocytes” or “leukocytes infiltrating a/the tumor.”

More specifically, the invention provides a method of diagnosis of cancer in a subject. The method involves: (a) providing a tissue sample from a subject suspected of having, or likely to develop, cancer of the tissue, wherein the sample contains test cells, the test cells being cells of the tissue or leukocytes infiltrating the tissue; and (b) assessing whether the test cells express B7-H1, wherein expression by some or all of the test cells is an indication that the subject has cancer.

The assessment of B7-H1 expression can be performed by the detection of B7-H1 polypeptide or mRNA. B7-H1 polypeptide can be detected, for example, by contacting the tissue sample, or test cells contained in the tissue sample, with an antibody that binds to the B7-H1 polypeptide. Suitable methods for detection of B7-H1 polypeptide can include, without limitation, fluorescence flow cytometry (FFC) or immunohistology. B7-H1 mRNA can be detected, for example, by contacting the tissue sample with a nucleic acid probe that hybridizes to the B7-H1 mRNA (e.g., such by *in situ* hybridization) or by reverse transcriptase-polymerase chain reaction. The tissue can be tissue of any organ or anatomical system, and can include, without limitation, lung, epithelial, connective, vascular, muscle, neural, skeletal, lymphatic, prostate, cervical, breast, spleen, gastric, intestinal, oral, esophageal, uterine, ovarian, or testicular tissue. The tissue can also be renal tissue. The subject can be a mammal, such as, for example, a human.

Another aspect of the invention is a method of identifying a candidate for immunotherapy. This method involves: (a) providing a tissue sample from a subject with cancer of the tissue, wherein the tissue sample contains test cells, the test cells being cancer cells or tumor-infiltrating leukocytes; and (b) assessing the level of test cells in the tissue sample that express B7-H1, wherein, if B7-H1 expression is not

detected in the test cells or if less than an immuno-inhibitory threshold level of the test cells express B7-H1, the subject is more likely to benefit from immunotherapy.

The level of B7-H1 can be assessed by detecting B7-H1 polypeptide or mRNA using, for example, any of the methods described above for method of diagnosis. The tissue can be tissue of any organ or anatomical system, and can include, without  
5 limitation, lung, epithelial, connective, vascular, muscle, neural, skeletal, lymphatic, prostate, cervical, breast, spleen, gastric, intestinal, oral, esophageal, uterine, ovarian, or testicular tissue. The tissue can also be renal tissue. The subject can be a mammal, such as, for example, a human. The cancer can be any cancer, and includes, for  
10 example, renal cell carcinoma.

In another embodiment, the invention features a method of determining the prognosis of a subject with cancer. This method involves: (a) providing a tissue sample from a subject with cancer of the tissue, wherein the tissue sample comprises test cells, the test cells being cancer cells or tumor-infiltrating leukocytes; and (b)  
15 assessing the level of test cells in the tissue sample that express B7-H1, wherein, if a prognostic level, or more than a prognostic level, of the test cells express B7-H1, the subject is more likely to die of the cancer than if less than a prognostic level of the test cells express B7-H1. The prognostic level is a predetermined value obtained by performing statistical clinical analyses known in the art, e.g., those described herein.  
20 The assessment of B7-H1 can be performed by detecting B7-H1 polypeptide or B7-H1 mRNA using any of a variety of methods known in the art, including, for example, those listed above for methods of diagnosis and method of immunotherapy. The tissue sample can be of any tissue, and can include, for example, any of those described above. The subject from which the tissue is provided can be a mammal,  
25 e.g., a human.

Yet another aspect of the invention is a method of treatment. The method involves: (a) identifying a subject with cancer, wherein some or all cells of the cancer or some or all tumor-infiltrating leukocytes of the cancer express B7-H1; and (b)  
30 delivering to the subject an agent that interferes with an interaction between B7-H1 and a receptor for B7-H1. The agent can bind to B7-H1 or to a receptor for B7-H1,

e.g., the PD-1 receptor. The agent can be an antibody or an antibody fragment (e.g., Fab', F(ab')<sub>2</sub>, or single chain Fv (scFv) fragment) that binds to B7-H1 or binds to a receptor for B7-H1; soluble B7-H1 or a soluble functional fragment of B7-H1; a soluble receptor for B7-H1 or a soluble functional fragment thereof. Whenever it is  
5 desired, the agent can be administered before, simultaneous with, or after administration of one or more immunomodulatory cytokines, growth factors, or antiangiogenic factors. Examples of such immunomodulatory cytokines, growth factors, and antiangiogenic factors include, without limitation, any of interleukins (IL)-1 to 25, interferon- $\gamma$  (IFN- $\gamma$ ), interferon- $\alpha$  (IFN- $\alpha$ ), interferon- $\beta$  (IFN- $\beta$ ),  
10 interferon- $\gamma$  (IFN- $\gamma$ ), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte macrophage colony stimulating factor (G-CSF), endostatin, angiostatin, and thrombospondin. Administrations of the agent and/or the one or more immunomodulatory cytokines, growth factors, or antiangiogenic factors can be systemic (e.g., intravenous) or local, e.g., during surgery  
15 by direct injection or infusion into the tissue that comprises the cells of the cancer and/or tumor-infiltrating leukocytes. The cancer can be, without limitation, hematological cancer, neurological cancer, melanoma, breast cancer, lung cancer, head and neck cancer, gastrointestinal cancer, liver cancer, pancreatic cancer, renal cancer, genitourinary cancer, bone cancer, or vascular cancer.

20 Yet another aspect of the invention is a method of inhibiting the expression of B7-H1 in a tumor cell or a tumor-infiltrating leukocyte. The method involves: (a) identifying a subject with cancer, the cancer containing a target cell that expresses B7-H1, the target cell being a tumor cell or a tumor-infiltrating leukocyte; and (b) introducing into the target cell: (i) an antisense oligonucleotide that hybridizes to a  
25 B7-H1 transcript, wherein the antisense oligonucleotide inhibits the expression of B7-H1 in the cell; or (ii) a B7-H1 interference RNA (RNAi). The introducing step can involve administration of the antisense oligonucleotide or the RNAi to the subject and uptake of the oligonucleotide or the RNAi by the target cell. Alternatively, the introducing step can involve administering to the subject, and uptake by the cell of, a  
30 nucleic acid comprising a transcriptional regulatory element (TRE) operably linked to a nucleotide sequence complementary to the antisense oligonucleotide, wherein

transcription of the nucleotide sequence inside the cell produces the antisense oligonucleotide. Moreover, the introducing step can include administering to the subject, and uptake by the cell of, a nucleic acid: (a) from which sense and anti-sense strands of the RNAi can be transcribed under the direction of the TREs; or (b) from which both sense and anti-sense strands of the RNAi can be transcribed under the direction of a single TRE.

The tissue sample can be lung, epithelial, connective, vascular, muscle, neural, skeletal, lymphatic, prostate, cervical, breast, spleen, gastric, intestinal, oral, esophageal, dermal, liver, bladder, thyroid, thymic, adrenal, brain, gallbladder, pancreatic, uterine, ovarian, or testicular tissue. The tissue can also be renal tissue. The cancer of the tissue can be any cancer and includes, e.g., renal cell carcinoma.

The subject can be a mammal and includes, for example, a human, a non-human primate (e.g., a monkey), a horse, a cow (or an ox or bull), a pig, a sheep, a goat, a cat, a rabbit, a guinea pig, a hamster, a rat, or a gerbil.

As used herein, "interferes with an interaction between B7-H1 and a receptor for B7-H1" means (a) completely blocks a physical interaction between B7-H1 molecule and a receptor for B7-H1 such that there is substantially no physical interaction between the B7-H1 molecule and the receptor; or (b) modifies the interaction between the B7-H1 molecule and the receptor such that the physical interaction either does not deliver a signal to the cell that comprises B7-H1, and/or the receptor for B7-H1, or delivers a signal that does not substantially affect the antitumoral activity of the cell.

"Polypeptide" and "protein" are used interchangeably and mean any peptide-linked chain of amino acids, regardless of length or post-translational modification. Polypeptides useful for the invention include variant polypeptides that are identical to corresponding wild-type polypeptides but differ by not more than 50 (e.g., not more than: 45; 40; 35; 30; 25; 20; 19; 18; 17; 16; 15; 14; 13; 12; 11; 10; nine; eight; seven; six; five; four; three; two; or one) conservative substitution(s). All that is required is that the variant polypeptide has at least 20% (e.g., at least: 25; 30%; 35%; 40%; 45%; 50%; 60%; 70%; 80%; 85%; 90%; 93%; 95%; 96%; 97%; 98%; 99%; 99.5%; 99.8%;

99.9%; or 100% or more) of the activity of the wild-type polypeptide. Conservative substitutions typically include substitutions within the following groups: glycine and alanine; valine, isoleucine, and leucine; aspartic acid and glutamic acid; asparagine, glutamine, serine, and threonine; lysine, histidine, and arginine; and phenylalanine  
5 and tyrosine.

As used herein, "tumor-infiltrating leukocytes" can be T lymphocytes (such as CD8<sup>+</sup> T lymphocytes and/or CD4<sup>+</sup> T lymphocytes), B lymphocytes, or other bone marrow-lineage cells including granulocytes (neutrophils, eosinophils, basophils), monocytes, macrophages, dendritic cells (i.e., interdigitating dendritic cells),  
10 histiocytes, and natural killer cells.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. In case of conflict, the present document, including definitions, will control. Preferred methods and materials are described below,  
15 although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety. The materials, methods, and examples disclosed herein are illustrative only and not intended to be limiting.

20 Other features and advantages of the invention will be apparent from the following description, from the drawings and from the claims.

### DESCRIPTION OF DRAWINGS

Fig. 1 is a series of photomicrographs (at a magnification of 400X) showing  
25 immunostaining (with an antibody specific for hB7-H1) of: an RCC specimen with high tumor cell hB7-H1 expression (Fig. 1A); an RCC specimen with high leukocyte hB7-H1 expression (Fig. 1B); an RCC specimen with no detectable hB7-H1 expression in either tumor cells or leukocytes (Fig. 1C); and a normal kidney specimen with no detectable hB7-H1 expression in the proximal tubules (Fig. 1D).

Fig. 2 is a series of line graphs showing the associations of hB7-H1 expression with death from RCC in 196 subjects from whom the clear cell RCC specimens were obtained for analysis.

Fig. 2A shows the association of tumor hB7-H1 expression with death from RCC (risk ratio 2.91; 95% CI [Confidence Interval] 1.39 – 6.13; p=0.005). The cancer-specific survival rates (with standard error [SE] and number still at risk indicated in parentheses) at 1, 2, and 3 years following nephrectomy were: 87.8% (4.1%, 53), 72.3% (6.0%, 30), and 63.2% (7.2%, 11), respectively, for patients with specimens that had  $\geq 10\%$  tumor hB7-H1 expression; compared with 93.6% (2.3%, 95), 88.4% (3.4%, 48), and 88.4% (3.4%, 19), respectively, for patients with specimens that had  $< 10\%$  tumor hB7-H1 expression.

Fig. 2B shows the association of adjusted score for leukocyte hB7-H1 expression with death from RCC (risk ratio 3.58; 95% CI 1.74 – 7.37; p<0.001). The cancer-specific survival rates (SE, number still at risk) at 1, 2, and 3 years were: 83.5% (6.2%, 26), 63.9% (9.2%, 13), and 53.6% (10.2%, 5), respectively, for patients with specimens that had a leukocyte hB7-H1 expression score  $\geq 100$ ; compared with 93.5% (2.1%, 122), 86.2% (3.3%, 65), and 84.8% (3.5%, 25), respectively, for patients with specimens that had scores  $< 100$ .

Fig. 2C shows the association of high aggregate intratumoral hB7-H1 expression with death from RCC (risk ratio 4.53; 95% CI 1.94 – 10.56; p<0.001). The cancer-specific survival rates (SE, number still at risk) at 1, 2, and 3 years were: 87.0% (3.8%, 61), 70.0% (5.8%, 32), and 61.9% (6.8%, 13), respectively, for patients with specimens that had high aggregate intratumoral hB7-H1 expression; compared with 94.9% (2.2%, 87), 91.9% (3.1%, 46), and 91.9% (3.1%, 17), respectively, for patients with specimens that had both  $< 10\%$  tumor and  $< 100$  leukocyte (low aggregate intratumoral expression) hB7-H1 expression.

Fig. 3 is a depiction of the amino acid sequence (SEQ ID NO:1) of full-length, immature hB7-H1, i.e., hB7-H1 including a leader peptide of about 22 amino acids.

Fig. 4 is a depiction of the nucleotide sequence (SEQ ID NO:2) of cDNA encoding full-length, immature hB7-H1.



Fig. 5 is a depiction of the amino acid sequence (SEQ ID NO:3) of full-length, immature murine B7-H1.

Fig. 6 is a depiction of the nucleotide sequence (SEQ ID NO:4) of cDNA encoding full-length, immature murine B7-H1.

5

### DETAILED DESCRIPTION

The inventors have discovered that renal cell carcinoma (RCC) patients who have increased levels of tumor cells and/or tumor-infiltrating leukocytes expressing the co-stimulatory glycoprotein hB7-H1 are at an increased risk of death from the RCC. In addition, elevated levels of hB7-H1 expressing tumor cells and/or tumor-infiltrating leukocytes was associated with more aggressive tumors and this association persisted even after controlling for traditional predictors of RCC progression, including, for example, tumor, node, metastasis (TNM) stage; primary tumor size; nuclear grade; and histological tumor necrosis.

Expression of B7-H1 in normal, non-activated mammalian cells is largely, if not exclusively, limited to macrophage-lineage cells and provides a potential costimulatory signal source for regulation of T cell activation. In contrast, aberrant expression of B7-H1 by tumor cells has been implicated in impairment of T cell function and survival, resulting in defective host antitumoral immunity.

The inventors found that human RCC tumors express hB7-H1. In particular, hB7-H1 was found to be expressed by both renal cell carcinoma (RCC) tumors and leukocytes infiltrating RCC tumors. In contrast, proximal tubules of the renal cortex, from which clear cell tumors are believed to arise, failed to express hB7-H1.

Clinical specimens were obtained from 196 patients who were treated with radical nephrectomy or nephron-sparing surgery for unilateral, clear cell RCC between 2000 and 2002 from the Mayo Clinic Nephrectomy Registry. Immunohistological detection and quantification of hB7-H1 expression in the specimens revealed that patients whose tumor specimens exhibited high intratumoral expression levels of hB7-H1 (contributed by tumor cells alone, leukocytes alone, or

tumor and/or leukocytes combined) had aggressive tumors and were at markedly increased risk of death from RCC.

The combination of increased tumor cell hB7-H1 and tumor-infiltrating leukocyte hB7-H1 (high aggregate intratumoral hB7-H1) was an even stronger  
5 predictor of patient outcome than either hB7-H1-expressing tumor cells or tumor-infiltrating leukocytes alone. High aggregate intratumoral hB7-H1 expression levels were also significantly associated with regional lymph node involvement, distant metastases, advanced nuclear grade, and the presence of histologic tumor necrosis.

Based on its ability to impair function and survival of activated tumor-specific  
10 T cells, B7-H1, expressed by either tumor cells (e.g., RCC cells) or infiltrating leukocytes, can contribute to the immunosuppression that is commonly observed in subjects with cancer (e.g., RCC) and can serve as a critical determinant of the subjects' responses to immunotherapy for management of advanced cancer (e.g., IL-2, IL-12, IFN- $\alpha$ , vaccination or T-cell adoptive therapy). This raises the possibility that  
15 administering to cancer patients agents that interfere with the interaction of B7-H1 with its receptor (e.g., PD-1) can serve as a method of immunotherapy, particularly in subjects whose high level of intratumoral B7-H1 expression previously rendered them unresponsive or nearly unresponsive to other modes of immunotherapy.

These findings provide support for the methods of the invention, which are  
20 described below.

#### Methods of Diagnosis

The invention provides a method of diagnosing cancer in a subject. The method involves: (a) providing a tissue sample from a subject suspected of having, or  
25 likely to develop, cancer of the tissue, the sample containing test cells, the test cells being cells of the tissue or leukocytes infiltrating the tissue; and (b) assessing whether the test cells express B7-H1. Expression by some or all of the test cells is an indication that the subject has cancer. Since a wide variety of cancer cells express B7-H1 on their surfaces, the methods of the invention are particularly useful for  
30 diagnosing any such cancer. Test cells can thus be, for example, breast cells, lung

cells, colon cells, pancreatic cells, renal cells, stomach cells, liver cells, bone cells, hematological cells (e.g., lymphoid cells, granulocytic cells, monocytes or macrophages), neural tissue cells, melanocytes, ovarian cells, testicular cells, prostate cells, cervical cells, vaginal cells, bladder cells, or any other cells listed herein.

5 Moreover, test cells can be leukocytes present in relevant tissues containing any of the above-listed test cells. Leukocytes infiltrating the tissue can be T cells ( $CD4^+$  T cells and/or  $CD8^+$  T cells) or B lymphocytes. Such leukocytes can also be neutrophils, eosinophils, basophils, monocytes, macrophages, histiocytes, or natural killer cells. Subjects can be mammals and include, for example, humans, non-human primates  
10 (e.g., monkeys, baboons, or chimpanzees), horses, cows (or oxen or bulls), pigs, sheep, goats, cats, rabbits, guinea pigs, hamsters, rats, gerbils, or mice.

As described herein, the invention provides a number of diagnostic advantages and uses. In the methods of the invention, the level of B7-H1 polypeptide and/or mRNA can be assessed. The level of B7-H1 is assessed in a tissue sample to  
15 diagnose, or to confirm, the presence of cancer in the subject from whom the tissue is obtained.

Methods of detecting a polypeptide in a tissue sample are known in the art. For example, antibodies (or fragments thereof) that bind to an epitope specific for B7-H1 can be used to assess whether test cells from the tissue sample express B7-H1.  
20 Such antibodies can be monoclonal or polyclonal antibodies. In such assays, the antibody itself, or a secondary antibody that binds to it, can be detectably labeled. Alternatively, the antibody can be conjugated with biotin, and detectably labeled avidin (a polypeptide that binds to biotin) can be used to detect the presence of the biotinylated antibody. Combinations of these approaches (including "multi-layer  
25 sandwich" assays) familiar to those in the art can be used to enhance the sensitivity of the methodologies. Some of these protein-detecting assays (e.g., ELISA or Western blot) can be applied to lysates of cells, and others (e.g., immunohistological methods or fluorescence flow cytometry) can be applied to histological sections or unlysed cell suspensions. The tissue sample can be, for example, lung, epithelial, connective,  
30 vascular, muscle, neural, skeletal, lymphatic, prostate, cervical, breast, spleen, gastric,

intestinal, oral, esophageal, dermal, liver, kidney, bladder, thyroid, adrenal, brain, gallbladder, pancreatic, uterine, ovarian, or testicular tissue.

Methods of detecting an mRNA in a tissue sample are known in the art. For example, cells can be lysed and an mRNA in the lysates or in RNA purified or semi-purified from the lysates can be detected by any of a variety of methods including, without limitation, hybridization assays using detectably labeled gene-specific DNA or RNA probes (e.g., Northern Blot assays) and quantitative or semi-quantitative RT-PCR methodologies using appropriate gene-specific oligonucleotide primers. Alternatively, quantitative or semi-quantitative *in situ* hybridization assays can be carried out using, for example, tissue sections or unlysed cell suspensions, and detectably (e.g., fluorescently or enzyme) labeled DNA or RNA probes. Additional methods for quantifying mRNA include RNA protection assay (RPA) and SAGE.

Methods of assessing the level of B7-H1 expression (RNA and/or polypeptide) can be can be quantitative, semi-quantitative, or qualitative. Thus, for example, the level of B7-H1 expression can be determined as a discrete value. For example, where quantitative RT-PCR is used, the level of expression of B7-H1 mRNA can be measured as a numerical value by correlating the detection signal derived from the quantitative assay to the detection signal of a known concentration of: (a) B7-H1 nucleic acid sequence (e.g., B7-H1 cDNA or B7-H1 transcript); or (b) a mixture of RNA or DNA that contains a nucleic acid sequence encoding B7-H1. Alternatively, the level of B7-H1 expression can be assessed using any of a variety of semi-quantitative/qualitative systems known in the art. Thus, the level of expression of B7-H1 in a cell or tissue sample can be expressed as, for example, (a) one or more of "excellent", "good", "satisfactory", "unsatisfactory", and/or "poor"; (b) one or more of "very high", "high", "average", "low", and/or "very low"; or (c) one or more of "++++", "+++", "++", "+", "+/-", and/or "-". Where it is desired, the level of expression of B7-H1 in tissue from a subject can be expressed relative to the expression of B7-H1 from (a) a tissue of a subject known not be cancerous (e.g., a contralateral kidney or lung, or an uninvolved lymph node); or (b) a corresponding tissue from one or more other subjects known not to have the cancer of interest, preferably known not to have any cancer.

Methods of assessing the amount of label depend on the nature of the label and are well known in the art. Appropriate labels include, without limitation, radionuclides (e.g., <sup>125</sup>I, <sup>131</sup>I, <sup>35</sup>S, <sup>3</sup>H, or <sup>32</sup>P), enzymes (e.g., alkaline phosphatase, horseradish peroxidase, luciferase, or  $\beta$ -galactosidase), fluorescent moieties or  
5 proteins (e.g., fluorescein, rhodamine, phycoerythrin, green fluorescent protein (GFP), or blue fluorescent protein (BFP)), or luminescent moieties (e.g., Qdot<sup>TM</sup> nanoparticles supplied by the Quantum Dot Corporation, Palo Alto, CA). Other applicable assays include quantitative immunoprecipitation or complement fixation assays.

10 In the diagnostic assays of the invention, a subject is diagnosed as having cancer if the proportion of test cells from the subject that express B7-H1 is greater than a control value. The control value, can be, for example: (a) the proportion of B7-H1-expressing cells in corresponding tissue of the subject known not to be cancerous (e.g., a contralateral kidney or lung, or an uninvolved lymph node); or (b) the  
15 proportion of B7-H1 expressing cells in a corresponding tissue from one or more other subjects known not to have the cancer of interest, preferably known not to have any cancer.

The method of the invention can be used on its own or in conjunction with other procedures to diagnose cancer. For example, where it is desired or preferred,  
20 the level of B7-H1-expressing test cells in a tissue sample that is, or is suspected of being, cancerous can be assessed before, during, or after assessing the levels of other molecules that are useful diagnostic cancer markers. Such diagnostic markers can be, without limitation, tumor-associated antigens (TAA). Relevant TAA include, without  
25 limitation, carcinoembryonic antigen (CEA), MAGE (melanoma antigen) 1-4, 6, and 12, MUC (mucin) (e.g., MUC-1, MUC-2, etc.), tyrosinase, MART (melanoma antigen), Pmel 17 (gp100), GnT-V intron sequence (N-acetylglucosaminyltransferase V intron V sequence), PSA (prostate-specific antigen), PSMA (prostate-specific membrane antigen), PRAME (melanoma antigen),  $\beta$ -catenin, MUM-1-B (melanoma ubiquitous mutated gene product), GAGE (melanoma antigen) 1, BAGE (melanoma  
30 antigen) 2-10, c-ERB2 (HER2/neu), EBNA (Epstein-Barr Virus nuclear antigen) 1-6,

gp75, human papilloma virus (HPV) E6 and E7, p53m lung resistance protein (LRP), Bcl-2, Ki-67, and VHL (von Hippel-Lindau) gene.

Method of Identifying Cancer Subjects Likely to Benefit from Immunotherapy

5           Another aspect of the invention is a method of identifying a candidate for immunotherapy. This method involves providing a tissue sample from a subject with cancer of the tissue. The tissue sample contains test cells, the test cells being cancer cells or tumor-infiltrating leukocytes. The level of test cells in the tissue sample that express B7-H1 is assessed, such that if B7-H1 expression is not detected in the test  
10   cells, or less than an immuno-inhibitory threshold level of the test cells express B7-H1, the subject is more likely to benefit from immunotherapy.

          The immuno-inhibitory threshold level is a predetermined level of the relevant test cells expressing B7-H1. If the test cells from a cancer subject of interest contain a level of B7-H1-expressing cells that is less than the immuno-inhibitory threshold level  
15   of B7-H1-expressing cells (as predetermined for the relevant cancer), that subject is more likely to benefit from immunotherapy than another subject with the same cancer but whose corresponding test cells contain a level of B7-H1-expressing cells equal to, or greater, than the immuno-inhibitory threshold level. The immuno-inhibitory threshold level can be obtained by performing statistical clinical analyses known in  
20   the art, e.g., those described herein.

          Methods of assessing whether test cells express B7-H1 are the same as those described above for methods of diagnosis. Such methods, also as described above, can be qualitative, semi-quantitative, or qualitative.

          “Immunotherapy” can be active immunotherapy or passive immunotherapy.  
25   For active immunotherapy, treatment relies on the *in vivo* stimulation of the endogenous host immune system to react against tumors with the administration of immune response-modifying agents. These immune-response-modifying agents are described below.

For passive immunotherapy, treatment involves the delivery of agents with established tumor-immune reactivity (such as immune effector cells or antibodies) that can directly, or indirectly mediate, anti-tumor effects and do not necessarily depend on an intact host immune system. Examples of immune effector cells include  
5 leukocytes, e.g., tumor-infiltrating leukocytes as discussed above, T lymphocytes (such as CD8<sup>+</sup> cytotoxic T lymphocytes and/or CD4<sup>+</sup> T-helper lymphocytes), killer cells (such as natural killer cells and lymphokine-activated killer cells), B cells and antigen-presenting cells (such as dendritic cells and macrophages).

Immunotherapy can also be one or more of the methods described below (in  
10 “Methods of Treatment” and “Methods of Inhibiting Expression of B7-H1).

#### Method of Prognosis

In another embodiment, the invention features a method of determining the prognosis of a subject with cancer. This method involves: (a) providing a tissue  
15 sample from a subject with cancer of the tissue, the tissue sample containing test cells, the test cells being cancer cells or tumor infiltrating leukocytes; and (b) assessing the level of test cells in the tissue sample that expresses B7-H1. If a prognostic level, or more than a prognostic level, of the test cells express B7-H1, the subject is more likely to die of the cancer than if less than a prognostic level of the test cells express  
20 B7-H1. The prognostic level is a predetermined value obtained by performing statistical clinical analyses known in the art, e.g., those described herein.

Thus, for example, if test cells from a cancer subject contain a significant level of B7-H1 expressing cells, but less than a prognostic level of B7-H1-expressing cells (as predetermined for the relevant cancer), the cancer subject will be no more likely to  
25 die of the cancer than a subject with the same cancer but whose corresponding test cells contain no detectable B7-H1-expressing cells. On the other hand, if test cells from a cancer subject contain more than a prognostic level of B7-H1-expressing cells, the cancer subject will be more likely to die of the cancer than a subject with the same cancer but whose corresponding test cells contain either no detectable B7-H1-  
30 expressing cells or a level of B7-H1-expressing cells lower than a prognostic level of

B7-H1-expressing cells. Moreover, for subjects with cancer having levels of B7-H1-expressing cells in appropriate test cell populations greater than prognostic levels, the chances of dying from the cancer is likely to be proportional to the level of B7-H1-expressing cells in the test cell population.

5 As used herein, "assessing whether test cells express B7-H1" or "assessing the level of test cells in the tissue sample that express B7-H1" can be determined by any of the methods described above. Methods of prognosis will generally be quantitative or semi-quantitative.

Subjects can be any of those listed for "Methods of Diagnosis" and cancers  
10 can be any of the following: renal cancer, hematological cancer (e.g., leukemia or lymphoma), neurological cancer, melanoma, breast cancer, lung cancer, head and neck cancer, gastrointestinal cancer, liver cancer, pancreatic cancer, pancreatic cancer, genitourinary cancer, bone cancer, or vascular cancer

#### 15 Methods of Treatment

The invention also includes a method of treatment. The method can involve:  
(a) identifying a subject with cancer, some or all cells of the cancer or some or all tumor-infiltrating leukocytes of the cancer expressing B7-H1; and (b) delivering to the subject an agent that interferes with an interaction between B7-H1 and a receptor  
20 for B7-H1. These methods can be performed subsequent to, or without, performing any of the above-described methods. The agent can be an antibody or an antibody fragment, such as, e.g., a Fab', a F(ab')<sub>2</sub>, or a scFv fragment that binds B7-H1. The agent can also be a soluble B7-H1 or a soluble functional fragment of B7-H1; a soluble receptor for B7-H1 or a soluble functional fragment thereof; an antibody, or  
25 an antibody fragment, that binds to a receptor for B7-H1, e.g., the PD-1 receptor. The PD-1 receptor is described in greater detail in U.S. Patent No. 6,808,710, the disclosure of which is incorporated herein by reference in its entirety.

In one embodiment, the agent itself is administered to a subject. Generally, the agent will be suspended in a pharmaceutically-acceptable carrier (e.g.,  
30 physiological saline) and administered orally or by intravenous (i.v.) infusion, or



injected subcutaneously, intramuscularly, intrathecally, intraperitoneally, intrarectally, intravaginally, intranasally, intragastrically, intratracheally, or intrapulmonarily. The agent can, for example, be delivered directly to a site of an immune response. e.g., a lymph node in the region of an affected tissue or organ or spleen. The dosage  
5 required depends on the choice of the route of administration; the nature of the formulation; the nature of the patient's illness; the subject's size, weight, surface area, age, and sex; other drugs being administered; and the judgment of the attending physician. Suitable dosages are in the range of 0.0001-100.0 mg/kg. Wide variations  
10 in the needed dosage are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by i.v. injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization as is well understood in the art. Administrations can be single or multiple (e.g., 2-, 3-, 4-, 6-, 8-, 10-, 20-, 50-, 100-, 150-, or more fold).  
15 Encapsulation of the compound in a suitable delivery vehicle (e.g., polymeric microparticles or implantable devices) may increase the efficiency of delivery, particularly for oral delivery.

Alternatively, where the agent is a polypeptide, a polynucleotide containing a nucleic acid sequence encoding the polypeptide can be delivered to appropriate cells  
20 in a mammal. Expression of the coding sequence can be directed to any cell in the body of the subject. However, expression will preferably be directed to cells in, or close to, lymphoid tissue draining an affected tissue or organ. Expression of the coding sequence can be directed, for example, to cells comprising the cancer tissue (e.g., tumor-infiltrating leukocytes and tumor cells) or immune-related cells, e.g., B  
25 cells, macrophages/monocytes, or interdigitating dendritic cells. This can be achieved by, for example, the use of polymeric, biodegradable microparticle or microcapsule delivery devices known in the art and/or tissue or cell-specific antibodies.

Another way to achieve uptake of the nucleic acid is using liposomes, which can be prepared by standard methods. The vectors can be incorporated alone into  
30 these delivery vehicles or co-incorporated with tissue-specific antibodies.

Alternatively, one can prepare a molecular conjugate composed of a plasmid or other

vector attached to poly-L-lysine by electrostatic or covalent forces. Poly-L-lysine binds to a ligand that can bind to a receptor on target cells [Cristiano et al. (1995), J. Mol. Med. 73:479]. Alternatively, tissue specific targeting can be achieved by the use of tissue-specific transcriptional regulatory elements (TRE) which are known in the art. Delivery of "naked DNA" (i.e., without a delivery vehicle) to an intramuscular, intradermal, or subcutaneous site is another means to achieve *in vivo* expression.

In the relevant polynucleotides (e.g., expression vectors), the nucleic acid sequence encoding the polypeptide of interest with an initiator methionine and optionally a targeting sequence is operatively linked to a promoter or enhancer-promoter combination. Short amino acid sequences can act as signals to direct proteins to specific intracellular compartments. Such signal sequences are described in detail in U.S. Patent No. 5,827,516, the disclosure of which is incorporated herein by reference in its entirety.

Enhancers provide expression specificity in terms of time, location, and level. Unlike a promoter, an enhancer can function when located at variable distances from the transcription initiation site, provided a promoter is present. An enhancer can also be located downstream of the transcription initiation site. To bring a coding sequence under the control of a promoter, it is necessary to position the translation initiation site of the translational reading frame of the peptide or polypeptide between one and about fifty nucleotides downstream (3') of the promoter. The coding sequence of the expression vector is operatively linked to a transcription terminating region.

Suitable expression vectors include plasmids and viral vectors such as herpes viruses, retroviruses, vaccinia viruses, attenuated vaccinia viruses, canary pox viruses, adenoviruses and adeno-associated viruses, among others.

Polynucleotides can be administered in a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are biologically compatible vehicles that are suitable for administration to a human, e.g., physiological saline or liposomes. A therapeutically effective amount is an amount of the polynucleotide that is capable of producing a medically desirable result (e.g., decreased proliferation of cancer cells) in a treated subject. As is well known in the medical arts, the dosage for any one patient

depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Dosages will vary, but a preferred dosage for administration of polynucleotide is from approximately  $10^6$  to approximately  $10^{12}$  copies of the polynucleotide molecule. This dose can be repeatedly administered, as needed. Routes of administration can be any of those listed above.

In addition, the method can be an *ex vivo* procedure that involves providing a recombinant cell which is, or is a progeny of a cell, obtained from a subject and has been transfected or transformed *ex vivo* with one or more nucleic acids encoding one or more agents that interfere with an interaction between B7-H1 and a receptor for B7-H1, so that the cell expresses the agent(s); and administering the cell to the subject. The cells can be cells obtained from a cancer tissue (e.g., tumor cells and/or tumor-infiltrating leukocytes) or from a non-cancerous tissue obtained preferably from a subject to whom these cells are to be administered or from another subject. The donor and recipient of the cells can have identical major histocompatibility complex (MHC; HLA in humans) haplotypes. Optimally, the donor and recipient are homozygotic twins or are the same individual (i.e., are autologous). The recombinant cells can also be administered to recipients that have no, or only one, two, three, or four MHC molecules in common with the recombinant cells, e.g., in situations where the recipient is severely immunocompromised, where only mismatched cells are available, and/or where only short term survival of the recombinant cells is required or desirable.

The efficacy of the agent can be evaluated both *in vitro* and *in vivo*. Briefly, the agent can be tested for its ability, for example, to (a) inhibit the interaction between B7-H1 and a receptor for B7-H1, (b) inhibit growth of cancer cells, (c) induce death of cancer cells, or (d) render the cancer cells more susceptible to cell-mediated immune responses generated by leukocytes (e.g., lymphocytes and/or macrophages). For *in vivo* studies, the agent can, for example, be injected into an animal (e.g., a mouse cancer model) and its effects on cancer are then assessed.

Based on the results, an appropriate dosage range and administration route can be determined.

As used throughout the present application, the term "antibody" refers to a whole antibody (e.g., IgM, IgG, IgA, IgD, or IgE) molecule that is generated by any one of a variety of methods that are known in the art. The antibody can be a polyclonal or a monoclonal antibody. Also useful for the invention are chimeric antibodies and humanized antibodies made from non-human (e.g., mouse, rat, gerbil, or hamster) antibodies. As used herein, the term "antibody fragment" refers to an antigen-binding fragment, e.g., Fab, F(ab')<sub>2</sub>, Fv, and single chain Fv (scFv) fragments. An scFv fragment is a single polypeptide chain that includes both the heavy and light chain variable regions of the antibody from which the scFv is derived.

Antibody fragments that contain the binding domain of the molecule can be generated by known techniques. For example: F(ab')<sub>2</sub> fragments can be produced by pepsin digestion of antibody molecules; and Fab fragments can be generated by reducing the disulfide bridges of F(ab')<sub>2</sub> fragments or by treating antibody molecules with papain and a reducing agent. See, e.g., National Institutes of Health, *1 Current Protocols In Immunology*, Coligan *et al.*, ed. 2.8, 2.10 (Wiley Interscience, 1991). scFv fragments can be produced, for example, as described in U.S. Patent No. 4,642,334, which is incorporated herein by reference in its entirety.

Chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example, using methods described in Robinson *et al.*, International Patent Publication PCT/US86/02269; Akira *et al.*, European Patent Application 184,187; Taniguchi, European Patent Application 171,496; Morrison *et al.*, European Patent Application 173,494; Neuberger *et al.*, PCT Application WO 86/01533; Cabilly *et al.*, U.S. Patent No. 4,816,567; Cabilly *et al.*, European Patent Application 125,023; Better *et al.* (1988) *Science* 240, 1041-43; Liu *et al.* (1987) *J. Immunol.* 139, 3521-26; Sun *et al.* (1987) *PNAS* 84, 214-18; Nishimura *et al.* (1987) *Canc. Res.* 47, 999-1005; Wood *et al.* (1985) *Nature* 314, 446-49; Shaw *et al.* (1988) *J. Natl. Cancer Inst.* 80, 1553-59; Morrison, (1985) *Science* 229, 1202-07; Oi *et al.* (1986) *BioTechniques* 4, 214; Winter, U.S. Patent No.

5,225,539; Jones et al. (1986) Nature 321, 552-25; Veroyan et al. (1988) Science 239, 1534; and Beidler et al. (1988) J. Immunol. 141, 4053-60.

As used herein, a "functional fragment" of a B7-H1 receptor means a fragment of a receptor for B7-H1 that is smaller than the wild-type mature B7-H1 receptor and has at least 10 % (e.g., at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% or more) of the ability of the wild-type mature receptor for B7-H1 to bind to B7-H1. As used herein, a "functional fragment" of B7-H1 means a fragment of the wild-type mature B7-H1 polypeptide that is smaller than the wild-type mature B7-H1 polypeptide and has at least 10 % (e.g., at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% or more) of the ability of the wild-type mature B7-H1 to bind to the B7-H1 receptor. Methods of testing and comparing the ability of molecules to bind to each other are known to those in the art.

15

As used herein, the term "soluble" distinguishes the receptors used in the present invention from their cell membrane-bound counterparts. A soluble receptor, or a soluble functional fragment of a receptor can contain, for example, an extracellular (ligand binding) domain, but lack the transmembrane region that causes retention of a receptor on the cell surface. Methods of producing soluble receptors or fragments thereof are known in the art and include, for example, expressing a DNA fragment encoding an extracellular domain of a receptor in a suitable host cell/expression vector system.

The term "treatment", as used herein, means administration of an agent to a subject, who has cancer (or is suspected of having cancer), with the purpose to cure, alleviate, relieve, remedy, prevent, or ameliorate the disorder, the symptom of the disorder, the disease state secondary to the disorder, or the predisposition toward the disorder. An "effective amount" of a therapeutic agent (or composition) is an amount of the agent (or composition) that is capable of producing a medically desirable result

in a treated subject. The method of the invention can be performed alone or in conjunction with other drugs or therapy.

As used herein, "prophylaxis" can mean complete prevention of the symptoms of a disease, a delay in onset of the symptoms of a disease, or a lessening in the severity of subsequently developed disease symptoms. As used herein, "therapy" can mean a complete abolishment of the symptoms of a disease or a decrease in the severity of the symptoms of the disease.

#### Methods of Inhibiting Expression of B7-H1

Another aspect of the invention is a method of inhibiting the expression of B7-H1 in a tumor cell or a tumor-infiltrating leukocyte. The method involves: (a) identifying a subject with cancer, the cancer containing a target cell that expresses B7-H1, the target cell being a tumor cell or a tumor-infiltrating leukocyte; and (b) introducing into the target cell: (i) an antisense oligonucleotide that hybridizes to a B7-H1 transcript, the antisense oligonucleotide inhibiting the expression of B7-H1 in the cell; or (ii) a B7-H1 interference RNA (RNAi). These methods can be performed subsequent to, or without, performing any of the above-described methods.

Since, as noted above, aberrant B7-H1 expression impairs the function and survival of tumor-specific T cells, it is likely that by inhibiting the cellular expression of B7-H1, as well as by interfering with the interaction between B7-H1 and its receptor, the anti-tumor immune responses can be restored. Thus, the method can be useful for therapy and/or prophylaxis of any cancer recited herein. The method can be used, for example, in the treatment of RCC.

Antisense compounds are generally used to interfere with protein expression either by, for example, interfering directly with translation of a target mRNA molecule, by RNase-H-mediated degradation of the target mRNA, by interference with 5' capping of mRNA, by prevention of translation factor binding to the target mRNA by masking of the 5' cap, or by inhibiting of mRNA polyadenylation. The interference with protein expression arises from the hybridization of the antisense compound with its target mRNA. A specific targeting site on a target mRNA of

interest for interaction with an antisense compound is chosen. Thus, for example, for modulation of polyadenylation, a preferred target site on an mRNA target is a polyadenylation signal or a polyadenylation site. For diminishing mRNA stability or degradation, destabilizing sequences are preferred target sites. Once one or more  
5 target sites have been identified, oligonucleotides are chosen which are sufficiently complementary to the target site (i.e., hybridize sufficiently well under physiological conditions and with sufficient specificity) to give the desired effect.

With respect to this invention, the term "oligonucleotide" refers to an oligomer or polymer of RNA, DNA, or a mimetic of either. The term includes oligonucleotides  
10 composed of naturally-occurring nucleobases, sugars, and covalent internucleoside (backbone) linkages. The normal linkage or backbone of RNA and DNA is a 3' to 5' phosphodiester bond. The term also refers however to oligonucleotides composed entirely of, or having portions containing, non-naturally occurring components which function in a similar manner to the oligonucleotides containing only naturally-  
15 occurring components. Such modified substituted oligonucleotides are often preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for target sequence, and increased stability in the presence of nucleases. In the mimetics, the core base (pyrimidine or purine) structure is generally preserved but (1) the sugars are either modified or  
20 replaced with other components and/or (2) the inter-nucleobase linkages are modified. One class of nucleic acid mimetic that has proven to be very useful is referred to as protein nucleic acid (PNA). In PNA molecules the sugar backbone is replaced with an amide-containing backbone, in particular an aminoethylglycine backbone. The bases are retained and are bound directly to the aza nitrogen atoms of the amide  
25 portion of the backbone. PNA and other mimetics useful in the instant invention are described in detail in U.S. Patent No. 6,210,289, the disclosure of which is incorporated herein by reference in its entirety.

The antisense oligomers to be used in the methods of the invention generally comprise about 8 to about 100 (e.g., about 14 to about 80 or about 14 to about 35)  
30 nucleobases (or nucleosides where the nucleobases are naturally occurring).

The antisense oligonucleotides can themselves be introduced into a cell or an expression vector containing a nucleic sequence (operably linked to a TRE) encoding the antisense oligonucleotide can be introduced into the cell. In the latter case, the oligonucleotide produced by the expression vector is an RNA oligonucleotide and the RNA oligonucleotide will be composed entirely of naturally occurring components.

Where antisense oligonucleotides *per se* are administered, they can be suspended in a pharmaceutically-acceptable carrier (e.g., physiological saline) and administered under the same conditions described above for agents that interfere with an interaction between B7-H1 and a receptor for B7-H1.

Where an expression vector containing a nucleic sequence (operably linked to a TRE) encoding the antisense oligonucleotide is administered to a subject, expression of the coding sequence can be directed to a tumor cell of tumor-infiltrating leukocyte in the body of the subject using any of the cell- or tissue-targeting techniques described above for vectors that express polypeptides that interfere with an interaction between B7-H1 and a receptor for B7-H1.

Double-stranded interfering RNA (RNAi) homologous to B7-H1 DNA can also be used to reduce expression of B7-H1 in tumor cells and/or tumor-infiltrating leukocytes. See, e.g., Fire et al. (1998) *Nature* 391:806-811; Romano and Masino (1992) *Mol. Microbiol.* 6:3343-3353; Cogoni et al. (1996) *EMBO J.* 15:3153-3163; Cogoni and Masino (1999) *Nature* 399:166-169; Misquitta and Paterson (1999) *Proc. Natl. Acad. Sci. USA* 96:1451-1456; and Kennerdell and Carthew (1998) *Cell* 95:1017-1026. The disclosures of all these articles are incorporated herein by reference in their entirety.

The sense and anti-sense RNA strands of RNAi can be individually constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, each strand can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecule or to increase the physical stability of the duplex formed between the sense and anti-sense strands, e.g., phosphorothioate derivatives and acridine substituted nucleotides. The sense or anti-sense strand can



also be produced biologically using an expression vector into which a target B7-H1 sequence (full-length or a fragment) has been subcloned in a sense or anti-sense orientation. The sense and anti-sense RNA strands can be annealed *in vitro* before delivery of the dsRNA to cells. Alternatively, annealing can occur *in vivo* after the sense and anti-sense strands are sequentially delivered to tumor cells and/or tumor-infiltrating leukocytes.

Double-stranded RNAi interference can also be achieved by introducing into tumor cells and/or tumor-infiltrating leukocytes a polynucleotide from which sense and anti-sense RNAs can be transcribed under the direction of separate promoters, or a single RNA molecule containing both sense and anti-sense sequences can be transcribed under the direction of a single promoter.

It will be understood that certain drugs and small molecules can also be used to inhibit expression of B7-H1 in tumor cells and/or tumor-infiltrating leukocytes.

One of skill in the art will appreciate that RNAi, drug, and small molecule methods can be, as for the antisense methods described above, *in vitro* and *in vivo*. Moreover, methods and conditions of delivery are the same as those for antisense oligonucleotides.

In any of the above methods of inhibiting the interaction between B7-H1 and a receptor for B7-H1 and of inhibiting expression of B7-H1, one or more agents (e.g., two, three, four, five, six, seven, eight, nine, ten, 11, 12, 15, 18, 20, 25, 30, 40, 50, 60, 70, 80, 100, or more) including, for example, inhibitory compounds, antisense oligonucleotides, RNAi, drugs, or small molecules (or vectors encoding them), can be used.

Moreover, such agents can be used together with one or more (e.g., two, three, four, five, six, seven, eight, nine, ten, 11, 12, 15, 18, 20, 25, 30, 40, 50, 60, 70, 80, 100, or more) supplementary agents, including immunomodulatory cytokines, growth factors, antiangiogenic factors, immunogenic stimuli, and/or antibodies specific for any of these. Such supplementary agents can be administered before, simultaneously with, or after delivery of any of the above-listed agents.

Examples of immunomodulatory cytokines, growth factors, and antiangiogenic factors include, without limitation, interleukin (IL)-1 to 25 (e.g., IL-2, IL-12, or IL-15), interferon- $\gamma$  (IFN- $\gamma$ ), interferon- $\alpha$  (IFN- $\alpha$ ), interferon- $\beta$  (IFN- $\beta$ ), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte macrophage colony stimulating factor (G-CSF), endostatin, angiostatin, and thrombospondin. Immunomodulatory cytokines, growth factors, antiangiogenic factors include substances that serve, for example, to inhibit infection (e.g., standard anti-microbial antibiotics), inhibit activation of T cells, or inhibit the consequences of T cell activation. For example, where it is desired to decrease a Th1-type immune response (e.g., in a DTH response), a cytokine such as interleukin (IL)-4, IL-10, or IL-13 or an antibody specific for a cytokine such as IL-12 or interferon- $\gamma$  (IFN- $\gamma$ ) can be used. Alternatively, where it is desired to inhibit a Th2-type immune response (e.g., in an immediate type hypersensitivity response), a cytokine such as IL-12 or IFN- $\gamma$  or an antibody specific for IL-4, IL-10, or IL-13 can be used as a supplementary agent. Also of interest are antibodies (or any of the above-described antibody fragments or derivatives) specific for proinflammatory cytokines and chemokines such as IL-1, IL-6, IL-8, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), macrophage inflammatory protein (MIP)-1, MIP-3 $\alpha$ , monocyte chemoattractant protein-1 (MCP-1), epithelial neutrophil activating peptide-78 (ENA-78), interferon- $\gamma$ -inducible protein-10 (IP10), Rantes, and any other appropriate cytokine or chemokine recited herein.

In some instances, it may be desired to increase the immune response in a subject by the administration of one or more immune response modifying-agents. Such immune response-modifying agents include, in addition to any of the immunomodulatory cytokines, growth factors, and angiogenic factors listed above, immunogenic stimuli that can be delivered via the antigen-specific T cell receptor (TCR) expressed on the surface of the T cell. More commonly, but not necessarily, such a stimulus is provided in the form of an antigen for which the TCR is specific. While such antigens will generally be protein, they can also be carbohydrates, lipids, nucleic acids or hybrid molecules having components of two or more of these molecule types, e.g., glycoproteins or lipoproteins. However, the immunogenic

stimulus can also be provided by other agonistic TCR ligands such as antibodies specific for TCR components (e.g., TCR  $\alpha$ -chain or  $\beta$ -chain variable regions) or antibodies specific for the TCR-associated CD3 complex. Antigens useful as immunogenic stimuli include alloantigens (e.g., a MHC alloantigen) on, for example, an antigen presenting cell (APC) (e.g., a dendritic cell (DC), a macrophage, a monocyte, or a B cell). DC of interest are interdigitating DC and not follicular DC; follicular DC present antigen to B cells. For convenience, interdigitating DC are referred to herein as DC. Methods of isolating DC from tissues such as blood, bone marrow, spleen, or lymph node are known in the art, as are methods of generating them *in vitro* from precursor cells in such tissues.

Also useful as immunogenic stimuli are polypeptide antigens and peptide-epitopes derived from them (see below). Unprocessed polypeptides are processed by APC into peptide-epitopes that are presented to responsive T cells in the form of molecular complexes with MHC molecules on the surface of the APC. Useful immunogenic stimuli also include a source of antigen such as a lysate of either tumor cells or cells infected with an infectious microorganism of interest. APC (e.g., DC) pre-exposed (e.g., by coculturing) to antigenic polypeptides, peptide-epitopes of such polypeptides or lysates of tumor (or infected cells) can also be used as immunogenic stimuli. Such APC can also be "primed" with antigen by culture with a cancer cell or infected cell of interest; the cancer or infected cells can optionally be irradiated or heated (e.g., boiled) prior to the priming culture. In addition, APC (especially DC) can be "primed" with either total RNA, mRNA, or isolated TAA-encoding RNA.

Alternatively, an immunogenic stimulus be provided in the form of cells (e.g., tumor cells or infected cells producing the antigen of interest). In addition, immunogenic stimuli can be provided in the form of cell hybrids formed by fusing APC (e.g., DC) with tumor cells [Gong et al. (2000) Proc. Natl. Acad. Sci. USA 97(6):2716-2718; Gong et al. (1997) Nature Medicine 3(5):558-561; Gong et al. (2000) J. Immunol. 165(3):1705-1711] or infected cells of interest.

Also useful as immunogenic stimuli are heat shock proteins bound to antigenic peptide-epitopes derived from antigens (e.g., tumor-associated antigens or antigens

produced by infectious microorganisms) [Srivastava (2000) Nature Immunology 1(5):363-366]. Heat shock proteins of interest include, without limitation, glycoprotein 96 (gp96), heat shock protein (hsp) 90, hsp70, hsp110, glucose-regulated protein 170 (grp170) and calreticulin. Immunogenic stimuli can include one or more  
5 (e.g., one, two, three, four, five, six, seven, eight, nine, ten, more) heat shock proteins isolated from tumor cells. Such tumor are preferably, but not necessarily, from the same subject (i) to whom the agent that interferes with the interaction between B7-H1 and a receptor for B7-H1 is to be delivered or (ii) in whose tumor cells or tumor infiltrating leukocytes the expression of B7-H1 is to be inhibited. The tumor cells can  
10 also be obtained, for example, from another individual having the same as the subject, or a related tumor-type. Alternatively, the heat shock protein can be isolated from mammalian cells expressing a transcriptosome prepared from tumor cells of interest.

As indicated above, immunogenic stimuli useful in the invention can be any of a wide variety of tumor cells, APC "primed" with tumor cells, hybrid cells, or TAA  
15 (see above), peptide-epitopes of such TAA, and APC "primed" with TAA or peptide-epitopes of them. As used herein, a "TAA" is a molecule (e.g., a protein molecule) that is expressed by a tumor cell and either (a) differs qualitatively from its counterpart expressed in normal cells, or (b) is expressed at a higher level in tumor cells than in normal cells. Thus, a TAA can differ (e.g., by one or more amino acid  
20 residues where the molecule is a protein) from, or it can be identical to, its counterpart expressed in normal cells. It is preferably not expressed by normal cells. Alternatively, it is expressed at a level at least two-fold higher (e.g., a two-fold, three-fold, five-fold, ten-fold, 20-fold, 40-fold, 100-fold, 500-fold, 1,000-fold, 5,000-fold, or 15,000-fold higher) in a tumor cell than in the tumor cell's normal counterpart.  
25 Relevant TAA include, without limitation, any of the TAAs listed above.

Administrations of the agents and/or the one or more supplementary agents can be systemic (e.g., intravenous) or local, e.g., during surgery by direct injection or infusion into the tissue that comprises the cells of the cancer and/or tumor-infiltrating leukocytes. The administrations can also be by any of routes, doses, and schedules  
30 recited herein.

In addition, it is understood that the above-described methods can be used in combination with any one of a variety of other therapeutic modalities known in the art, such as, without limitation, chemotherapy, immunotherapy, radiotherapy, or gene therapy.

5           In both of the methods of inhibiting the interaction between B7-H1 and a receptor for B7-H1 and the methods of inhibiting expression of B7-H1, the cancer can be any cancer recited herein and includes, e.g., renal cell carcinoma. Subjects can be mammals and include, for example, humans, non-human primates (e.g., monkeys, baboons, or chimpanzees), horses, cows (or oxen or bulls), pigs, sheep, goats, cats,  
10 rabbits, guinea pigs, hamsters, rats, gerbils, or mice.

The following examples are meant to illustrate, not limit, the invention.

## EXAMPLES

### Example 1. Materials and Methods

#### *Patient selection*

Upon approval from the Mayo Clinic Institutional Review Board, 429 patients  
5 were identified from Mayo Clinic Nephrectomy Registry that were previously treated  
with radical nephrectomy or nephron-sparing surgery for unilateral, sporadic clear cell  
RCC between 2000 and 2002. Since pathologic features and patient outcome differ  
by RCC subtype, all analyses were restricted to patients treated with clear cell RCC  
only, the most common of the RCC subtypes [Cheville et al. (2003) Am. J. Surg.  
10 Pathol. 27:612-624]. Since the hB7-H1-specific monoclonal antibody, 5H1 (see  
below), can reproducibly stain fresh-frozen, but not paraffin-fixed, tissue [Dong et al.  
(2002) Nature Med. 8:793-800], patients were selected based on availability of fresh-  
frozen tissue.

#### *Pathologic features*

15 The pathologic features examined included histologic subtype, tumor size,  
primary tumor stage, regional lymph node involvement, and distant metastases at  
nephrectomy (2002 TNM), nuclear grade, and histologic tumor necrosis. The  
microscopic slides from all specimens were reviewed by a urologic pathologist  
without prior knowledge of patient outcome. Histologic subtype was classified  
20 according to the Union Internationale Contre le Cancer, American Joint Committee on  
Cancer, and Heidelberg guidelines [Storkel et al. (1997) Cancer 80:987-989; Kovacs  
et al. (1997) J. Pathol. 183:131-133]. Nuclear grade was assigned using standardized  
criteria [Lohse et al. (2002) Am. J. Clin. Pathol. 118:877-886]. Histologic tumor  
necrosis was defined as the presence of any microscopic coagulative tumor necrosis.  
25 Degenerative changes such as hyalinization, hemorrhage, and fibrosis were not  
considered necrosis.

#### *Immunohistochemical staining of tumor specimens*

Cryosections generated from RCC tumors and normal renal cortical specimens  
(5  $\mu$ m thickness) were mounted on Superfrost Plus slides, air dried, and fixed in ice-

cold acetone. Sections were stained using the Dako Autostainer and Dako Cytomation Labeled Polymer (EnVision+) HRP detection kit™ (Dako; Carpinteria, California). Slides were blocked with H<sub>2</sub>O<sub>2</sub> for 10 minutes followed by incubation with the primary anti-B7-H1 antibody for 30 minutes at room temperature. A  
5 horseradish peroxidase-conjugated secondary reagent (goat anti-mouse immunoglobulin) was then applied to the slides at room temperature for 15 minutes followed by incubation with chromogen-substrate for 10 minutes. Finally, sections were counter-stained for 3 minutes with modified Schmidt's Hematoxylin. The primary antibody used in this study was 5H1, a mouse anti-hB7-H1 monoclonal  
10 antibody [Dong et al. (2002) Nature Med. 8:793-800). Benign renal tumors and peripheral T cells were not stained in this study. Positive tissues controls for hB7-H1 staining were human tonsillar tissues. Irrelevant isotype-matched antibodies were used to control for non-specific staining.

#### *Quantification of hB7-H1 expression*

15 The percentages of tumor cells and leukocytes that stained positive for hB7-H1 were quantified in 5-10% increments by a urologic pathologist without prior knowledge of patient outcome. The extent of leukocytic infiltration was assessed and recorded as absent, focal (scattered lymphoid aggregates), moderate, or marked. An adjusted score representing leukocytic hB7-H1 expression was calculated as the  
20 percentage of leukocytes that stained positive for hB7-H1 multiplied by the extent of leukocytic infiltration (0=absent, 1=focal, 2=moderate, 3=marked).

#### *Statistical methods*

Comparisons between the pathologic features and hB7-H1 expression were evaluated using chi-square, Fisher's exact and Wilcoxon rank sum tests. Cancer-  
25 specific survival was estimated using the Kaplan-Meier method. The duration of follow-up was calculated from the date of nephrectomy to the date of death or last follow-up. Cause of death was determined from the death certificate or physician correspondence. Scatter plots of the percentage of cells that stained positive for hB7-H1 versus the difference in observed survival and the survival expected from a Cox  
30 proportional hazards regression model (formally known as a Martingale residual)

were used to identify potential cut-off points for hB7-H1 expression [Therneau et al. (2000) Modeling Survival Data: Extending the Cox Model, ed. 1 (Springer-Verlag, Ann Arbor), pp. 87-92]. The associations of these cut points with death from RCC were evaluated using Cox proportional hazards regression models univariately and after adjusting for primary tumor stage, regional lymph node involvement, distant metastases, tumor size, nuclear grade, and histologic tumor necrosis, one feature at a time. The association of hB7-H1 expression with death from RCC was also adjusted for the Mayo Clinic SSIGN (Stage, Size, Grade, and Necrosis) Score, a prognostic composite score specifically developed for patients with clear cell RCC [Frank et al. (2002) J. Urol. 168:2395-2400]. Statistical analyses were performed using the SAS software package (SAS Institute, Cary, North Carolina) and p-values <0.05 were considered statistically significant.

#### Example 2. Survival of RCC Patients with Fresh-Frozen Tissue Samples Available

Of the 429 patients eligible for the study, 196 (46%) had fresh-frozen tissue available for laboratory investigation. Patients with fresh-frozen tissues had larger tumors compared with those who did not (median tumor size 6.0 cm versus 5.0 cm; p=0.008). However, no other feature studied was significantly different between the two groups. Furthermore, there was not a statistically significant difference in cancer-specific survival between patients with and without fresh-frozen tissues (p=0.314).

At last follow-up, 39 of the 196 patients studied had died, including 30 patients who died from clear cell RCC at a median of 1.1 years following nephrectomy (range 0 – 2.5). Among the 157 patients who were still alive at last follow-up, the median duration of follow-up was 2.0 years (range 0 – 4.1). The estimated cancer-specific survival rates (standard error, number still at risk) at 1, 2, and 3 years following nephrectomy were 91.4% (2.1%, 148), 81.8% (3.3%, 78), and 77.9% (3.8%, 30), respectively.



Example 3. Correlation of hB7-H1 Expression in RCC Tumor Cells with Patient Outcome

Immunohistochemical staining of the 196 clear cell RCC specimens revealed either no hB7-H1 expression by RCC tumor cells, or varying degrees of hB7-H1 expressed by either RCC tumor cells and/or RCC tumor-infiltrating leukocytes (Tables 1 and 2 and Fig. 1). In addition, proximal tubules within the renal cortex, from which RCC tumors are believed to arise, exhibited no hB7-H1 expression among the 20 normal renal cortical specimens studied (Fig. 1).

The percentages of tumor cells that stained positive for hB7-H1 for the 196 specimens studied are summarized in Table 1. A scatter plot of tumor hB7-H1 expression versus the expected risk of death for each patient suggested that a cut point of 10% would be appropriate for these data. There were 73 (37.2%) patients with specimens that had  $\geq 10\%$  tumor cell hB7-H1 expression.

Table 1. Percent Tumor hB7-H1 Expression in 196 Clear Cell RCC Specimens

| % hB7-H1 Expression | N (%)     |
|---------------------|-----------|
| 0                   | 66 (33.7) |
| 5                   | 57 (29.1) |
| 10                  | 27 (13.8) |
| 15                  | 4 (2.0)   |
| 20                  | 15 (7.7)  |
| 25                  | 3 (1.5)   |
| 30                  | 6 (3.1)   |
| 40                  | 2 (1.0)   |
| 50                  | 4 (2.0)   |
| 60                  | 3 (1.5)   |
| 70                  | 3 (1.5)   |
| 80                  | 2 (1.0)   |
| 90                  | 3 (1.5)   |
| 100                 | 1 (0.5)   |

Table 2. Adjusted Score for Leukocyte hB7-H1 Expression in 196 Clear Cell RCC Specimens

| Leukocytic Infiltration* | % hB7-H1 Expression | Adjusted Score | N (%)     |
|--------------------------|---------------------|----------------|-----------|
| 0                        | 0                   | 0              | 81 (41.3) |
| 1                        | 5                   | 5              | 4 (2.0)   |
| 1                        | 10                  | 10             | 1 (0.5)   |
| 1                        | 30                  | 30             | 2 (1.0)   |
| 1                        | 50                  | 50             | 4 (2.0)   |
| 1                        | 60                  | 60             | 3 (1.5)   |
| 1                        | 70                  | 70             | 22 (11.2) |
| 1                        | 80                  | 80             | 12 (6.1)  |
| 1                        | 90                  | 90             | 10 (5.1)  |
| 2                        | 5                   | 10             | 3 (1.5)   |
| 2                        | 10                  | 20             | 4 (2.0)   |
| 2                        | 20                  | 40             | 2 (1.0)   |
| 2                        | 30                  | 60             | 2 (1.0)   |
| 2                        | 50                  | 100            | 6 (3.1)   |
| 2                        | 60                  | 120            | 1 (0.5)   |
| 2                        | 70                  | 140            | 9 (4.6)   |
| 2                        | 80                  | 160            | 7 (3.6)   |
| 2                        | 90                  | 180            | 8 (4.1)   |
| 3                        | 5                   | 15             | 1 (0.5)   |
| 3                        | 20                  | 60             | 1 (0.5)   |
| 3                        | 30                  | 90             | 4 (2.0)   |
| 3                        | 70                  | 210            | 2 (1.0)   |
| 3                        | 80                  | 240            | 4 (2.0)   |
| 3                        | 90                  | 270            | 2 (1.0)   |
| 3                        | 100                 | 300            | 1 (0.5)   |

\*The extent of leukocytic infiltration was recorded as 0=absent, 1=focally present, 2=moderately present, or 3=markedly present.

5           The associations of tumor hB7-H1 expression with death from RCC, both univariately and after adjusting for TNM stage, tumor size, nuclear grade, and histologic tumor necrosis are shown in Table 3. Univariately, patients with specimens that had  $\geq 10\%$  tumor hB7-H1 expression were close to 3 times more likely to die from RCC compared with patients with specimens that had  $< 10\%$  expression (risk ratio 2.91; 95% CI 1.39 – 6.13;  $p=0.005$ ; Fig. 2A). In multivariate analyses, patients  
10 with specimens that had  $\geq 10\%$  tumor hB7-H1 expression were significantly more likely to die from RCC, even after adjusting for primary tumor stage, distant

metastases, or primary tumor size.

Table 3. Associations of hB7-H1 Expression with Death from RCC in 196 Clear Cell RCC Specimens

5

| <b>Tumor hB7-H1 Expression <math>\geq 10\%</math></b>     | <b>Risk Ratio (95% CI)*</b> | <b>P-value</b> |
|---|-----------------------------|----------------|
| Univariate Model  | 2.91 (1.39 – 6.13)          | 0.005          |
| Adjusted for:   |                             |                |
| 2002 Primary Tumor Stage (T)                              | 2.83 (1.34 – 5.96)          | 0.006          |
| Regional Lymph Node Involvement (N)                       | 1.97 (0.87 – 4.45)          | 0.103          |
| Distant Metastases (M)                                    | 2.24 (1.06 – 4.73)          | 0.035          |
| Primary Tumor Size  | 2.88 (1.37 – 6.06)          | 0.005          |
| Nuclear Grade   | 1.96 (0.90 – 4.30)          | 0.092          |
| Histologic Tumor Necrosis                                 | 1.69 (0.78 – 3.65)          | 0.183          |
| <b>Leukocytic hB7-H1 Expression <math>\geq 100</math></b> |                             |                |
| Univariate Model  | 3.58 (1.74 – 7.37)          | <0.001         |
| Adjusted for:   |                             |                |
| 2002 Primary Tumor Stage (T)                              | 3.34 (1.62 – 6.90)          | 0.001          |
| Regional Lymph Node Involvement (N)                       | 3.59 (1.74 – 7.41)          | <0.001         |
| Distant Metastases (M)                                    | 2.16 (1.03 – 4.53)          | 0.042          |
| Primary Tumor Size  | 2.64 (1.27 – 5.46)          | 0.009          |
| Nuclear Grade   | 3.03 (1.46 – 6.29)          | 0.003          |
| Histologic Tumor Necrosis                                 | 2.87 (1.39 – 5.95)          | 0.004          |
| <b>High Aggregate Intratumoral hB7-H1 Expression</b>      |                             |                |
| Univariate Model  | 4.53 (1.94 – 10.56)         | <0.001         |
| Adjusted for:   |                             |                |
| 2002 Primary Tumor Stage (T)                              | 4.07 (1.74 – 9.51)          | 0.001          |
| Regional Lymph Node Involvement (N)                       | 3.36 (1.39 – 8.16)          | 0.007          |
| Distant Metastases (M)                                    | 3.12 (1.32 – 7.38)          | 0.009          |
| Primary Tumor Size  | 4.25 (1.82 – 9.91)          | <0.001         |
| Nuclear Grade   | 3.09 (1.28 – 7.50)          | 0.012          |
| Histologic Tumor Necrosis                                 | 2.68 (1.12 – 6.42)          | 0.027          |

\*Risk ratios represent the risk of death from clear cell RCC for the feature listed, either univariately or after multivariate adjustment. For example, patients with specimens that had  $\geq 10\%$  tumor hB7-H1 expression were 2.9 times more likely to die from RCC compared with patients with specimens that had  $< 10\%$  tumor hB7-H1 expression, even after adjusting for primary tumor size ( $p=0.005$ ).

The adjusted scores for leukocytic hB7-H1 expression are summarized in Table 2. There were 40 (20.4%) specimens with an adjusted leukocyte hB7-H1 score of 100 or greater (essentially moderate or marked leukocytic infiltration with at least 50% of the leukocytes staining positive for hB7-H1), which appeared to be a reasonable cut point to examine and illustrate the association of this feature with patient outcome. The associations of leukocyte hB7-H1 expression with death from RCC are summarized in Table 3. Univariately, patients with specimens that had an adjusted leukocyte hB7-H1 score  $\geq 100$  were 3.6 times more likely to die from RCC compared with patients that had specimens with scores  $< 100$  (risk ratio 3.58; 95% CI 1.74 – 7.37;  $p < 0.001$ ; Fig. 2B). Patients with specimens that demonstrated high levels of leukocyte hB7-H1 expression were significantly more likely to die from RCC even after adjusting for TNM stage, primary tumor size, nuclear grade, or histologic tumor necrosis.

Since both tumor and leukocyte hB7-H1 expression were significantly associated with patient outcome both univariately and after multivariate adjustment, the combination of these two features were evaluated. There were 87 (44.4%) specimens that had either  $\geq 10\%$  tumor hB7-H1 expression or an adjusted score for leukocyte hB7-H1 expression  $\geq 100$  (i.e., high aggregate intratumoral hB7-H1 expression). Twenty-six (13.3%) of these specimens had both features. Conversely, 109 (55.6%) specimens had  $< 10\%$  tumor hB7-H1 expression and  $< 100$  leukocyte hB7-H1 expression (i.e., low aggregate intratumoral hB7-H1 expression). The associations of this combined feature with death from RCC are summarized in Table 3. Univariately, patients with specimens that had high aggregate intratumoral hB7-H1 expression were 4.5 times more likely to die from RCC compared with patients with specimens that had both  $< 10\%$  tumor expression and  $< 100$  leukocyte expression (risk ratio 4.53; 95% CI 1.94 – 10.56;  $p < 0.001$ ). After adjusting for the Mayo Clinic SSIGN Score, patients with high aggregate intratumoral hB7-H1 expression remained over twice as likely to die from RCC compared with patients with low aggregate intratumoral hB7-H1, although this difference did not attain statistical significance (risk ratio 2.19; 95% CI 0.91-5.24;  $p = 0.079$ ). However, patients with specimens that had high aggregate intratumoral hB7-H1 expression were significantly more likely to

die from RCC after adjusting for TNM stage, primary tumor size, nuclear grade, and histologic tumor necrosis, one feature at a time. The association of combined tumor and leukocyte hB7-H1 expression with the pathologic features under study were also investigated. High aggregate intratumoral hB7-H1 expression levels were significantly associated with regional lymph node involvement, distant metastases, advanced nuclear grade, and the presence of histologic tumor necrosis (Table 4).

Table 4. Associations of Tumor and Leukocyte hB7-H1 Expression with Pathologic Features in 196 Clear Cell RCC Specimens

10

| Feature                         | High Aggregate<br>Intratumoral hB7-H1 Expression |               | P-value |
|---------------------------------|--|---------------|---------|
|                                 | No   | Yes           |         |
|                                 | N=109<br>N (%)                                   | N=87<br>N (%) |         |
| 2002 Primary Tumor Stage        |  |               |         |
| pT1 and pT2                     | 88 (80.7)  | 62 (71.3)     | 0.120   |
| pT3 and pT4                     | 21 (19.3)  | 25 (28.7)     |         |
| Regional Lymph Node Involvement |  |               |         |
| pNx and pN0                     | 108 (99.1)                                       | 76 (87.4)     | <0.001  |
| pN1 and pN2                     | 1 (0.9)  | 11 (12.6)     |         |
| Distant Metastases              |  |               |         |
| pM0                             | 99 (90.8)  | 69 (79.3)     | 0.022   |
| pM1                             | 10 (9.2)   | 18 (20.7)     |         |
| Primary Tumor Size              |  |               |         |
| <5 cm                           | 46 (42.2)  | 25 (28.7)     | 0.051   |
| ≥5 cm                           | 63 (57.8)  | 62 (71.3)     |         |
| Nuclear Grade                   |  |               |         |
| 1 and 2                         | 69 (63.3)  | 23 (26.4)     | <0.001  |
| 3                               | 36 (33.0)  | 50 (57.5)     |         |
| 4                               | 4 (3.7)  | 14 (16.1)     |         |
| Histologic Tumor Necrosis       |  |               |         |
| Absent                          | 94 (86.2)  | 55 (63.2)     | <0.001  |
| Present                         | 15 (13.8)  | 32 (36.8)     |         |

A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.

15

**WHAT IS CLAIMED IS:**

1. A method of determining the prognosis of a subject with cancer, the method comprising:

5 (a) providing a tissue sample from a subject with cancer of the tissue, wherein the tissue sample comprises test cells, the test cells being cancer cells or tumor-infiltrating leukocytes; and

(b) assessing the level of test cells in the tissue sample that express B7-H1, wherein, if a prognostic level, or more than a prognostic level, of the test cells express B7-H1, the subject is more likely to die of the cancer than if less than a prognostic  
10 level of the test cells express B7-H1.

2. The method of claim 1, wherein the assessing comprises detecting B7-H1 polypeptide.

15 3. The method of claim 2, wherein the detecting comprises contacting the tissue sample with an antibody that binds to the B7-H1 polypeptide.

4. The method of claim 2, wherein the detecting comprises fluorescence flow cytometry (FFC).

20

5. The method of claim 2, wherein the detecting comprises immunohistology.

6. The method of claim 1, wherein the assessing comprises detecting B7-H1 mRNA.

25

7. The method of claim 6, wherein the detecting comprises contacting the sample with a nucleic acid probe that hybridizes to the B7-H1 mRNA.

8. The method of claim 6, wherein the detecting comprises a reverse transcriptase-polymerase chain reaction.

5           9. The method of claim 6, wherein the detecting comprises *in situ* hybridization.

10           10. The method of claim 1, wherein the tissue is selected from the group consisting of lung, epithelial, connective, vascular, muscle, nervous, skeletal, lymphatic, prostate, cervical, breast, spleen, gastric, intestinal, oral, esophageal, dermal, liver, bladder, thyroid, thymic, adrenal, brain, gallbladder, pancreatic, uterine, ovarian, and testicular tissue.

11. The method of claim 1, wherein the tissue is renal tissue.

15

12. The method of claim 1, wherein the cancer of the tissue is renal cell carcinoma.

13. The method of claim 1, wherein the subject is a mammal.

20

14. The method of claim 13, wherein the mammal is a human.

15. A method of diagnosis, the method comprising:

25           (a) providing a tissue sample from a subject suspected of having, or likely to develop, cancer of the tissue, wherein the sample comprises test cells, the test cells being cells of the tissue or leukocytes infiltrating the tissue; and

(b) assessing whether the test cells express B7-H1, wherein expression by some or all of the test cells is an indication that the subject has cancer.

16. The method of claim 15, wherein the assessing comprises detecting B7-  
5 H1 polypeptide.

17. The method of claim 16, wherein the detecting comprises contacting the tissue sample with an antibody that binds to the B7-H1 polypeptide.

10 18. The method of claim 16, wherein the detecting comprises fluorescence flow cytometry (FFC).

19. The method of claim 16, wherein the detecting comprises immunohistology.

15

20. The method of claim 15, wherein the assessing comprises detecting B7-H1 mRNA.

21. The method of claim 16, wherein the detecting comprises contacting the  
20 sample with a nucleic acid probe that hybridizes to the B7-H1 mRNA.

22. The method of claim 16, wherein the detecting comprises a reverse transcriptase-polymerase chain reaction.

25 23. The method of claim 16, wherein the detecting comprises *in situ* hybridization.



24. The method of claim 15, wherein the tissue is selected from the group consisting of lung, epithelial, connective, vascular, muscle, neural, skeletal, lymphatic, prostate, cervical, breast, spleen, gastric, intestinal, oral, esophageal, dermal, liver, bladder, thyroid, thymic, adrenal, brain, gallbladder, pancreatic, uterine,  
5 ovarian, and testicular tissue.

25. The method of claim 15, wherein the tissue is renal tissue.

26. The method of claim 15, wherein the subject is a mammal.

10

27. The method of claim 26, wherein the mammal is a human.

28. A method of identifying a candidate for immunotherapy, the method comprising:

15 (a) providing a tissue sample from a subject with cancer of the tissue, wherein the tissue sample comprises test cells, the test cells being cancer cells or tumor-infiltrating leukocytes; and

(b) assessing the level of test cells in the tissue sample that express B7-H1, wherein, if B7-H1 expression is not detected in the test cells or if less than an  
20 immuno-inhibitory threshold level of the test cells express B7-H1, the subject is more likely to benefit from immunotherapy.

29. The method of claim 28, wherein the assessing comprises detecting B7-H1 polypeptide.

25

30. The method of claim 29, wherein the detecting comprises contacting the tissue sample with an antibody that binds to the B7-H1 polypeptide.

31. The method of claim 29, wherein the detecting comprises fluorescence flow cytometry (FFC).

5 32. The method of claim 29, wherein the detecting comprises immunohistology.

33. The method of claim 28, wherein the assessing comprises detecting B7-H1 mRNA.

10 34. The method of claim 29, wherein the detecting comprises contacting the sample with a nucleic acid probe that hybridizes to the B7-H1 mRNA.

35. The method of claim 29, wherein the detecting comprises a reverse transcriptase-polymerase chain reaction.  
15

36. The method of claim 29, wherein the detecting comprises *in situ* hybridization.

37. The method of claim 28, wherein the tissue is selected from the group  
20 consisting of lung, epithelial, connective, vascular, muscle, nervous, skeletal, lymphatic, prostate, cervical, breast, spleen, gastric, intestinal, oral, esophageal, dermal, liver, bladder, thyroid, thymic, adrenal, brain, gallbladder, pancreatic, uterine, ovarian, and testicular tissue.

25 38. The method of claim 28, wherein the tissue is renal tissue.

39. The method of claim 28, wherein the cancer of the tissue is renal cell carcinoma.

40. The method of claim 28, wherein the subject is a mammal.

5

41. The method of claim 40, wherein the mammal is a human.

42. A method of treatment, the method comprising:

(a) identifying a subject with cancer, wherein some or all cells of the cancer or  
10 some or all tumor-infiltrating leukocytes of the cancer express B7-H1; and

(b) delivering to the subject an agent that interferes with an interaction  
between B7-H1 and a receptor for B7-H1.

43. The method of claim 42, wherein the agent comprises an antibody, or a  
15 fragment thereof.

44. The method of claim 42, wherein the agent binds to B7-H1.

45. The method of claim 42, wherein the agent binds to a receptor for B7-H1.

20

46. The method of claim 45, wherein the receptor is PD-1.

47. The method of claim 44, wherein the agent is a recombinant soluble  
receptor for B7-H1.

25

48. The method of claim 43, wherein the antibody fragment is selected from the group consisting of an Fab' fragment, an F(ab')<sub>2</sub> fragment, or a single chain Fv (sFv) fragment.

5 49. The method of claim 42, further comprising delivering to the subject one or more immunomodulatory cytokines, growth factors, or antiangiogenic factors.

10 50. The method of claim 49, wherein the one or more immunomodulatory cytokines, growth factors, or antiangiogenic factors are selected from the group consisting of interleukin (IL)-1 to 25, interferon- $\gamma$  (IFN- $\gamma$ ), interferon- $\alpha$  (IFN- $\alpha$ ), interferon- $\beta$  (IFN- $\beta$ ), interferon- $\gamma$  (IFN- $\gamma$ ), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte macrophage colony stimulating factor (G-CSF), endostatin, angiostatin, and thrombospondin.

15

51. The method of claim 42, wherein the cancer is selected from the group consisting of hematological cancer, neurological cancer, melanoma, breast cancer, lung cancer, head and neck cancer, gastrointestinal cancer, liver cancer, pancreatic cancer, renal cancer, genitourinary cancer, bone cancer, and vascular cancer.

20

52. The method of claim 42, wherein the cancer is a renal cell carcinoma.

25 53. A method of inhibiting expression of B7-H1 in a tumor cell or a tumor-infiltrating leukocyte, the method comprising: (a) identifying a subject with cancer, the cancer comprising a target cell that expresses B7-H1, the target cell being a tumor cell or a tumor-infiltrating leukocyte; and (b) introducing into the target cell: (i) an antisense oligonucleotide that hybridizes to a B7-H1 transcript, wherein the antisense oligonucleotide inhibits expression of B7-H1 in the cell; or (ii) a B7-H1 interference RNA (RNAi).

54. The method of claim 53, wherein the introducing step comprises administration of the antisense oligonucleotide or the RNAi to the subject and uptake of the antisense oligonucleotide or the RNAi by the target cell.

5 55. The method of claim 53, wherein the introducing step comprises administering to the subject, and uptake by the cell of, a nucleic acid comprising a transcriptional regulatory element (TRE) operably linked to a nucleotide sequence complementary to the antisense oligonucleotide, wherein transcription of the nucleotide sequence inside the cell produces the antisense oligonucleotide.

10

56. The method of claim 53, wherein the introducing step comprises administering to the subject, and uptake by the cell of, a nucleic acid: (a) from which sense and anti-sense strands of the RNAi can be transcribed under the direction of separate TREs; or (b) from which both sense and anti-sense strands of the RNAi can  
15 be transcribed under the direction of a single TRE.

57. The method of claim 53, wherein the subject is a mammal.

58. The method of claim 57, wherein the mammal is a human.

20

59. The method of claim 53, wherein the cancer is a renal cell carcinoma.

60. The method of claim 53, wherein the cancer is of a tissue selected from the group consisting of lung, epithelial, connective, vascular, neural, skeletal,  
25 lymphatic, prostate, cervical, breast, spleen, gastric, intestinal, oral, esophageal, dermal, liver, bladder, thyroid, thymic, adrenal, brain, gallbladder, pancreatic, uterine, ovarian, and testicular tissue.

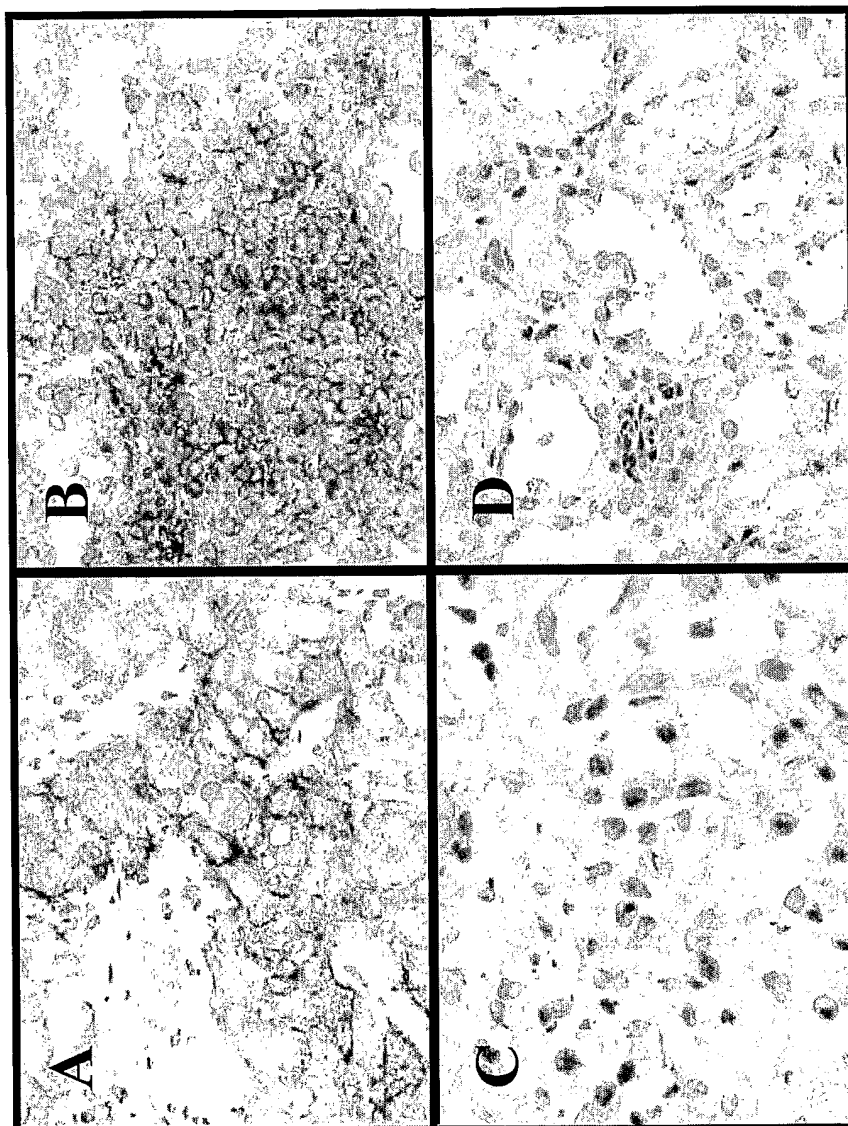


FIG. 1

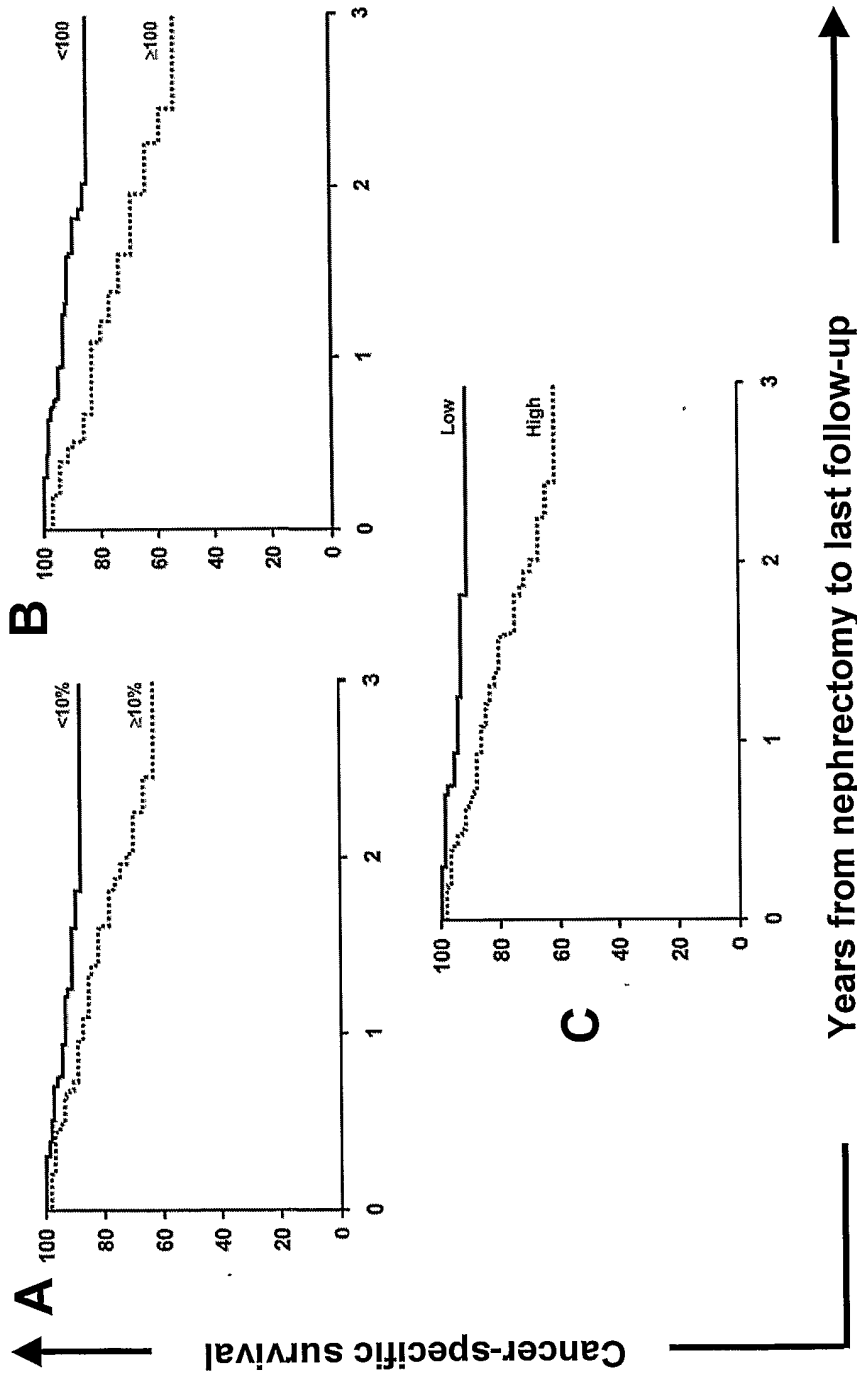


FIG. 2

FIG. 3

MRIFAVFIFMTYWHLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIV  
YWEMEDKNIIQFVHGEECLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQD  
AGVYRCMISYGGADYKRITVKVNAPYNKINQRILVDPVTSEHELTCQAEGYPK  
AEVIWTSSDHQVLSGKTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEE  
NHTAELVIPELPLAHPNERTHLVILGAILLCLGVALTFIFRLRKGRMMDVKKCGI  
QDTNSKKQSDTHLEET



FIG. 4

ATGAGGATATTTGCTGTCTTTATATTCATGACCTACTGGCATTGCTGAACGC  
ATTTACTGTACCGTTCCCAAGGACCTATATGTGGTAGAGTATGGTAGCAATA  
TGACAATTGAATGCAAATCCCAGTAGAAAAACAATTAGACCTGGCTGCACT  
AATTGTCTATTGGGAAATGGAGGATAAGAACATTATTCAATTTGTGCATGGA  
GAGGAAGACCTGAAGGTTTCAGCATAGTAGCTACAGACAGAGGGCCCGGCTG  
TTGAAGGACCAGCTCTCCCTGGGAAATGCTGCACTTCAGATCACAGATGTGA  
AATTGCAGGATGCAGGGGTGTACCGCTGCATGATCAGCTATGGTGGTGCCGA  
CTACAAGCGAATTACTGTGAAAGTCAATGCCCCATACAACAAAATCAACCAA  
AGAATTTTGGTTGTGGATCCAGTCACCTCTGAACATGAACTGACATGTCAGGC  
TGAGGGCTACCCCAAGGCCGAAGTCATCTGGACAAGCAGTGACCATCAAGTC  
CTGAGTGGTAAGACCACCACCAATTCCAAGAGAGAGGAGAAGCTTTTCA  
ATGTGACCAGCACACTGAGAATCAACACAACAATAATGAGATTTTCTACTG  
CACTTTTAGGAGATTAGATCCTGAGGAAAACCATACAGCTGAATTGGTCATC  
CCAGAACTACCTCTGGCACATCCTCCAAATGAAAGGACTCACTGGTAATTCT  
GGGAGCCATCTTATTATGCCTTGGTGTAGCACTGACATTCATCTTCCGTTTAA  
GAAAAGGGAGAATGATGGATGTGAAAAAATGTGGCATCCAAGATACAACT  
CAAAGAAGCAAAGTGATACACATTTGGAGGAGACG

FIG. 5

MRIFAGIIFTACCHLLRAFTITAPKDLYVVEYGSNVTMECRFPVERELDLLALVV  
YWEKEDEQVIQFVAGEEDLKPQHSNFRGRASLPKDQLLKGNAALQITDVKLQDA  
GVYCCIIISYGGADYKRITLKVNPYRKINQRISVDPATSEHELICQAEGYPEAEVI  
WTNSDHQPVSGKRSVTTSRTEGMLLNVTSRLRVNATANDVFYCTFWRSQPGQN  
HTAELIPELPAHPQNRTHWVLLGSILLFLIVVSTVLLFLRKQVRMLDVEKCGV  
EDTSSKNRNDTQFEET

FIG. 6

ATGAGGATATTTGCTGGCATTATATTCACAGCCTGCTGTCACTTGCTACGGGC  
GTTTACTATCACGGCTCCAAAGGACTTGTACGTGGTGGAGTATGGCAGCAAC  
GTCACGATGGAGTGCAGATTCCCTGTAGAACGGGAGCTGGACCTGCTTGCGT  
TAGTGGTGTACTGGGAAAAGGAAGATGAGCAAGTGATTTCAGTTTGTGGCAGG  
AGAGGAGGACCTTAAGCCTCAGCACAGCAACTTCAGGGGGAGAGCCTCGCT  
GCCAAAGGACCAGCTTTTGAAGGGAAATGCTGCCCTTCAGATCACAGACGTC  
AAGCTGCAGGACGCAGGCGTTTACTGCTGCATAATCAGCTACGGTGGTGCGG  
ACTACAAGCGAATCACGCTGAAAGTCAATGCCCCATACCGCAAATCAACCA  
GAGAATTTCCGTGGATCCAGCCACTTCTGAGCATGAACTAATATGTCAGGCC  
GAGGGTTATCCAGAAGCTGAGGTAATCTGGACAAACAGTGACCACCAACCCG  
TGAGTGGGAAGAGAAGTGTCAACCACTTCCCGGACAGAGGGGATGCTTCTCAA  
TGTGACCAGCAGTCTGAGGGTCAACGCCACAGCGAATGATGTTTTCTACTGT  
ACGTTTTGGAGATCACAGCCAGGGCAAACACACAGCGGAGCTGATCATCC  
CAGAACTGCCTGCAACACATCCTCCACAGAACAGGACTCACTGGGTGCTTCT  
GGGATCCATCCTGTTGTTCCCTCATTGTAGTGTCCACGGTCCTCCTCTTCTGAG  
AAAACAAGTGAGAATGCTAGATGTGGAGAAATGTGGCGTTGAAGATACAAG  
CTCAAAAACCGAAATGATACACAATTCGAGGAGACG