

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
1 November 2007 (01.11.2007)

PCT

(10) International Publication Number
WO 2007/124361 A2

(51) International Patent Classification:

G01N 33/533 (2006.01) **G01N 33/487** (2006.01)
G01N 33/53 (2006.01)

(21) International Application Number:

PCT/US2007/066970

(22) International Filing Date: 19 April 2007 (19.04.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/793,437 20 April 2006 (20.04.2006) US

(71) Applicant (for all designated States except US): **MAYO FOUNDATION FOR MEDICAL EDUCATION AND RESEARCH** [US/US]; 200 First Street S.W., Rochester, Minnesota 55905 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **KWON, Eugene D.** [US/US]; 1407 Woodview Lane SW, Rochester, Minnesota 55902 (US). **DONG, Haidong** [CN/US]; 5605 Nancy Lane NW, Rochester, Minnesota 55901 (US). **BARO, Xavier Frigola** [ES/US]; 2011 Viking Drive NW #28, Rochester, Minnesota 55901 (US).

(74) Agents: **KUBE, D., Marie** et al.; Fish & Richardson P.C., P.O. Box 1022, Minneapolis, Minnesota 55440-1022 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

Published:

- without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: SOLUBLE B7-H1

Signal peptide

1 MRIFAVFIFMTYWHLLNAFTVTVPKDLVVEYGSNMTIECKFPVEKQLDL

*

Ig-V-like

51 AALIVYWEMEDKNI IQFVHGEE DLKVQHSSYRQ RARLLKDQLSLGNAALQ

101 ITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVDPVTSE

Ig-C-like

151 HELTCQAEGYPKAEVIWTSSDHQVLSGKTTTNSKREEKLFNVTSTLRIN

*

*

201 TTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLC

*

TM

251 LGVALTFIFRLRKGRMMDVKKCGIQDTNSKKQSDTHLEET

(57) Abstract: This document features methods of evaluating mammals by assessing expression of B7-H4 in the vasculature.



WO 2007/124361 A2

SOLUBLE B7-H1

TECHNICAL FIELD

This document relates to a soluble form of B7-H1, and more particularly, to detecting soluble B7-H1 in body fluids to evaluate mammals.

5

BACKGROUND

The incidence of renal cell carcinoma (RCC) has increased steadily over the last three decades and the mortality rates continue to rise. See Jemal et al. (2005) CA Cancer J. Clin. 55, 10-30. To date, the only acceptable treatment for clinically localized RCC is surgical extirpation. Improvements in imaging technology have led to a stage migration, and with accompanying surgical advancements, improvements in patient survival have been noted. Pantuck et al. (2001) J. Urol. 166, 1611-1623. Regrettably, the five-year survival of RCC patients is still unacceptably low. This low survival rate reflects the 30% of patients who present with metastatic disease and another 25-30% who will subsequently develop disseminated disease after surgical excision of the primary tumor. Motzer et al. (1996) N. Engl. J. Med. 335, 865-875; and Leibovich et al. (2003) Cancer. 97, 1663-1671. Other treatment modalities for advanced disease such as chemotherapy and radiation have not been shown to be effective. Immunotherapy is one adjunct therapy available; however, <10% of patients benefit with durable responses. Fyfe et al. (1995) J. Clin. Oncol. 13, 688-696. Limited therapeutic options have done little to improve the median survival of 6-10 months seen in metastatic disease. Figlin et al. (1997) J. Urol. 158, 740-750. Since a large percent of patients with clinically localized disease subsequently develop metastasis, there is a need for prognostic biomarkers.

10

15

20

SUMMARY

This document is based in part on the discovery that a soluble form of B7-H1 is present in the serum of cancer patients. Identification of a soluble form of B7-H1 allows expression of B7-H1 to be evaluated in patients by a minimally invasive method. B7-H1 is over-expressed by many human cancers, and impairs anti-tumoral responses by inducing T cell apoptosis and by inhibiting T cell cytokine production,

25

proliferation and cytotoxic function. As such, soluble B7-H1 can be used as a biomarker for diagnosis of cancer and prognosis of a patient with cancer.

In general, this document features a method of evaluating a mammal. The method comprises, or consists essentially of, (a) providing a body fluid from the mammal, and (b) detecting the presence or absence of B7-H1 in the body fluid. The mammal can be a human. The B7-H1 can be detected immunologically. The B7-H1 can be detected using a monoclonal antibody. The B7-H1 can be detected using a capture antibody and a reporter antibody, where the reporter antibody comprises a label. The label can be a fluorophore, biotin, an enzyme, or a radioisotope. The fluorophore can be fluorescein, fluorescein isothiocyanate (FITC), phycoerythrin (PE), allophycocyanin (APC), or peridinin chlorophyll protein (PerCP). The capture antibody can be attached to a solid substrate. The solid substrate can be selected from the group consisting of a bead and a microtiter plate. The capture antibody can be a polyclonal antibody. The body fluid can be selected from the group consisting of blood, plasma, serum, urine, cerebrospinal fluid, sputum, tears, and saliva. The body fluid can be serum. The mammal can be suspected of having a cancer. The cancer can be renal cell carcinoma. The presence of B7-H1 in the body fluid can indicate the presence of a cancer in the mammal. The presence of B7-H1 in the body fluid can indicate the mammal is more likely to die of the cancer than if B7-H1 is absent.

In another aspect, this document features a method of evaluating a mammal with renal cell carcinoma. The method comprises, or consists essentially of, (a) providing a body fluid from the mammal, and (b) detecting the presence or absence of B7-H1 in the body fluid. The presence of B7-H1 in the body fluid can indicate the mammal is more likely to die of renal cell carcinoma than if B7-H1 is absent.

In another aspect, this document features a method of detecting B7-H1 in a body fluid. The method comprises, or consists essentially of, (a) providing a solid substrate, the solid substrate coated with capture antibodies having binding affinity for soluble B7-H1; (b) contacting the body fluid with the solid substrate under conditions in which soluble B7-H1, if present, becomes bound to the solid substrate to form a first reacted solid substrate; (c) contacting the first reacted solid substrate with a reporter antibody having binding affinity for soluble B7-H1 to form a second reacted solid substrate; and (d) detecting the presence or absence of the reporter antibody on the second reacted solid substrate, where the presence of reporter antibody indicates

that soluble B7-H1 is present in the body fluid. The reporter antibody can comprise a label selected from the group consisting of a radioisotope, a fluorophore, a luminescent moiety, biotin, and an enzyme. Detecting the presence or absence of the reporter antibody can comprise contacting the second reacted solid substrate with a secondary antibody having binding affinity for the reporter antibody, where the secondary antibody comprises a label. Detecting the presence or absence of the reporter antibody can comprise contacting the second reacted solid substrate with a reagent having binding affinity for the reporter antibody, where the reagent comprises a label. The solid substrate can be a bead or a microtiter plate.

In another aspect, this document features a kit for detecting soluble B7-H1. The kit comprises, or consists essentially of, a pair of antibodies, each antibody of the pair having binding affinity for soluble B7-H1, where each antibody of the pair recognizes a different epitope of soluble B7-H1. The kit can further comprise a solid substrate, a positive control, and/or a negative control.

In another aspect, this document features a method for identifying a mammal as having cancer. The method comprises, or consists essentially of, (a) determining whether or not a mammal has a body fluid containing an elevated level of a B7-H1 polypeptide, and (b) classifying the mammal as having cancer if the mammal has the elevated level and classifying the mammal as not having cancer if the mammal does not have the elevated level. The mammal can be a human. The body fluid can be blood, serum, plasma, or urine. The cancer can be renal cell carcinoma.

In another aspect, this document features a method for assessing the effectiveness of a cancer treatment. The method comprises, or consists essentially of, determining whether or not a mammal having cancer and having received a treatment for the cancer has a level of a B7-H1 polypeptide that is lower than that observed prior to the treatment, where the presence of the level of a B7-H1 polypeptide that is lower than that observed prior to the treatment indicates that the treatment is effective.

In another aspect, this document features a method for identifying a mammal as having cancer that has progressed. The method comprises, or consists essentially of, determining whether or not a level of a B7-H1 polypeptide in a body fluid of a mammal increases over time, where an increase in the level indicates that the mammal has cancer that has progressed, and the absence of an increase in the level indicates that the mammal does not have cancer that has progressed.

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, 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.

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

DESCRIPTION OF DRAWING

FIG. 1 contains the predicted amino-acid sequence of human B7-H1, including the signal peptide, the predicted signal peptide; Ig-V-like, the immunoglobulin V-like domain; Ig- C-like, the immunoglobulin C-like domain; TM (underlined), the transmembrane region; *, the potential N-linked glycosylation site.

FIG. 2 is a graph demonstrating that B7-H1 was detected in the serum of 25% of RCC patients (11/48) and 8% of normal donors (1/12). All samples were diluted 1:20 in PBS. B7-H1 fusion polypeptide and human IgG Fc polypeptide were used as positive and negative controls, respectively.

FIG. 3 is a graph plotting B7-H1 polypeptide levels in serum samples from 14 normal donors (DONOR) and 65 clear cell renal cell carcinoma patients (RCC) analyzed using a sandwich ELISA with anti-B7-H1 antibodies. The horizontal bars represent median values. The p-value of the Wilcoxon rank sum test was 0.0008.

FIG. 4A is histogram plot of flow cytometry data obtained by analyzing cells positive for B7-H1 polypeptide expression that were stained with a biotinylated monoclonal anti-B7-H1 antibody, 2.2B, in the presence (dotted line) or absence (solid line) of an unbiotinylated monoclonal anti-B7-H1 antibody, 5H1-A3. The filled histogram is a plot of data obtained by analyzing unbiotinylated control cells. FIG. 4B is a histogram plot of flow cytometry data obtained by analyzing cells positive for B7-H1 polypeptide expression that were stained with a biotinylated monoclonal anti-B7-H1 antibody, 5H1-A3, in the presence (dotted line) or absence (solid line) of an

unbiotinylated monoclonal anti-B7-H1 antibody, 2.2B. The filled histogram is a plot of data obtained by analyzing unbiotinylated control cells.

FIG. 5 is a histogram plot of flow cytometry data obtained by analyzing cells positive for B7-H1 polypeptide expression that were stained using biotinylated or unbiotinylated 5H1-A3 or 2.2B monoclonal anti-B7-H1 antibody. un-5H1 = unbiotinylated 5H1-A3 antibody, un-2.2B = unbiotinylated 2.2B antibody, b-5H1 = biotinylated 5H1-A3 antibody, and b-2.2B = biotinylated 2.2B antibody.

FIG. 6 is a graph plotting levels of B7-H1 polypeptide in cell culture supernatants incubated with BT10B (BT10B SN), HMCI (HMCI SN), or Caki2 (Caki2 SN) cells for three to four days. The B7-H1 polypeptide levels were analyzed using an ELISA assay. Recombinant human B7-H1 fusion polypeptide (B7H1Fc) was used as a positive control. Phosphate-buffered saline (PBS), recombinant human P-selectin fusion polypeptide (PSelFc), and fresh cell culture media for BT10B and HMCI cells (BT media and HMCI media, respectively), that had not been incubated with cells, were negative controls.

FIG. 7 is a graph plotting levels of B7-H1 polypeptide in cell culture supernatants from 624MEL, B7H1/624MEL, J82, Caki-2, BT10B, and BT10C cells. The B7-H1 polypeptide levels were measured using an ELISA assay. Recombinant human B7-H1 fusion polypeptide (B7-H1Fc) was used as a positive control. PBS and fresh cell culture media for each cell type, which had not been incubated with cells, were analyzed as negative controls.

FIG. 8 is a Western blot analyzing B7-H1 polypeptide expression in extracts from 624MEL, B7-H1/624MEL, BT10B, BT10C, and Caki-2 cells. Immunoblotting was performed using biotinylated monoclonal anti-B7-H1 antibody 5H1-A3. The membrane was stripped and reprobed for actin polypeptide as a loading control.

DETAILED DESCRIPTION

In general, this document provides methods and materials for evaluating mammals for the presence, absence, or amount of B7-H1 in a body fluid (e.g., blood, plasma, serum, urine, cerebrospinal fluid, sputum, tears, or saliva). 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 nucleotide and amino acid sequences of hB7-H1 can be found in GenBank under Accession Nos. AF177937 (GI:6708118) and AAF25807 (GI:6708119), respectively. B7-H1 (also known as PD-L1) is a glycosylated membrane polypeptide of the B7 costimulatory family. The open reading frame of the B7-H1 gene encodes a type I transmembrane polypeptide of 290 amino acids, consisting of immunoglobulin V-like and C-like domains, a hydrophobic transmembrane domain and a cytoplasmic tail of 30 amino acids (FIG. 1). The sequence reveals four structural cysteines (FIG. 1, stars), which are involved in the formation of disulfide bonds of the immunoglobulin V-like and C-like domains. As shown herein, however, a soluble form of B7-H1 (e.g., B7-H1 lacking all or part of the transmembrane domain and/or all or part of the cytoplasmic tail) can be detected in body fluids such as serum.

B7-H1 is a negative regulator of T cell-mediated immunity. See, Dong et al. (1999) Nat. Med. 5, 1365-1369; Dong et al. (2002) Nat. Med. 8, 793-800; and Thompson et al. (2004) Proc. Natl. Acad. Sci. USA 101, 17174-17179. This molecule is constitutively expressed on macrophage-lineage cell surfaces and is expressed in multiple human malignancies. B7-H1 is normally expressed in very limited amounts by monocyte-lineage cells within the liver, lung and tonsils. B7-H1 is markedly over-expressed by many human cancers and has been shown to impair anti-tumoral responses by inducing T cell apoptosis and by inhibiting T cell cytokine production, proliferation and cytotoxic function. As such, B7-H1 expression by tumor cells may be a potent contributor to the immunosuppressive profile that is typically exhibited by advanced cancer patients.

Methods of Evaluating Mammals

In general, methods of the invention include detecting the presence, absence, or amount of B7-H1 in a body fluid of a subject. In some embodiments, the amount of B7-H1 in a body fluid can be expressed relative to the amount from a control population (e.g., the average amount of B7-H1 from a plurality of subjects without cancer). Suitable subjects can be mammals, including, for example, humans (e.g., patients suspected of having a cancer), non-human primates such as monkeys, baboons, or chimpanzees, horses, cows (or oxen or bulls), pigs, sheep, goats, cats, rabbits, guinea pigs, hamsters, rats, gerbils, and mice.

As described herein, soluble B7-H1 was detected in the serum of at least 25% of the RCC patients examined. In contrast, soluble B7-H1 was detected in the serum of only 8% of normal control subjects. Since B7-H1 is markedly over-expressed by many human cancers, impairs anti-tumoral responses, and in RCC patients, is associated with aggressive tumors and increased risk for succumbing to death due to RCC, detecting soluble B7-H1 in a body fluid can be used as a biomarker for diagnosing cancer, determining prognosis, or assessing risk of cancer progression. For example, the presence of soluble B7-H1 in a body fluid from a mammal (e.g., a patient suspected of having a cancer) can indicate the presence of a cancer in the mammal. The presence of soluble B7-H1 in a mammal diagnosed with cancer also can indicate that the mammal is more likely to die of the cancer than if B7-H1 is absent. Since a number of cancers express B7-H1, the methods of the invention can be used to evaluate mammals that are suspected of having a variety of cancers, including, for example, 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, genitourinary cancer, bone cancer, or vascular cancer. Methods of the invention are particularly useful for evaluating mammals with RCC, lung, ovarian, and colon cancer. Additional factors that can be considered when evaluating a mammal can include, for example, patient history, family history, genetic factors, overall health of the mammal, and/or previous responses to therapy.

Furthermore, detecting the presence, absence, or amount of B7-H1 in a body fluid can be used to provide valuable clues as to the course of action to be undertaken in treatment of the cancer since the presence of B7-H1 can indicate a particularly aggressive course of cancer. Detecting the presence, absence, or amount of B7-H1 in a body fluid also can be used to monitor the response of a mammal to a cancer therapy. In some cases, detecting the presence, absence, or amount of B7-H1 in body fluids can be used in population screening for cancer.

In some cases, a mammal can be classified as having cancer if it is determined that a body fluid from the mammal contains a detectable level of a B7-H1 polypeptide. In some cases, a mammal can be classified as not having cancer if it is determined that a body fluid from the mammal does not contain a detectable level of a B7-H1 polypeptide. In some cases, a mammal can be classified as having cancer if it

is determined that a body fluid (e.g., blood) from the mammal contains an elevated level of a B7-H1 polypeptide. If the level of a B7-H1 polypeptide in a body fluid from a mammal is not elevated, then the mammal can be classified as not having cancer. The term "elevated level" as used herein with respect to a level of a B7-H1 polypeptide is any level that is greater than a reference level for the B7-H1 polypeptide. The term "reference level" as used herein with respect to a B7-H1 polypeptide is the level of the B7-H1 polypeptide typically expressed by mammals free of cancer. For example, a reference level of a B7-H1 polypeptide can be the median level of the B7-H1 polypeptide that is present in samples obtained from a random sampling of humans that are free of cancer. Control samples used to determine a reference level can be obtained from any appropriate number of mammals (e.g., 10, 20, 30, 40, 50, 75, 100, 125, 150, 175, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1000 or more mammals) from the same species as the mammal being evaluated. In some cases, control samples can be obtained from humans of the same race, age group, and/or geographic location as the mammal being evaluated.

It will be appreciated that levels from comparable samples are used when determining whether or not a particular level of a B7-H1 polypeptide is an elevated level. For example, the median level of a B7-H1 polypeptide present in serum from a random sampling of mammals may be X units/g of serum, while the median level of a B7-H1 polypeptide present in urine may be Y units/g of urine. In this case, the reference level for a B7-H1 polypeptide in serum would be X units/g of serum, and the reference level for a B7-H1 polypeptide in urine would be Y units/g of urine. Thus, when determining whether or not the level of a B7-H1 polypeptide in serum is elevated, the measured level would be compared to the reference level in serum. In addition, a level of a B7-H1 polypeptide in a body fluid from a mammal is typically compared to a reference level determined by analyzing samples using a technique comparable to the technique used to measure the B7-H1 level in the mammal being evaluated.

An elevated level of a B7-H1 polypeptide can be any level provided that the level is greater than a corresponding reference level. For example, an elevated level of a B7-H1 polypeptide can be 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.2, 2.4, 2.6, 2.8, 3.0, 3.3, 3.5, 3.7, 4.0, 4.5, 5.0, 6.1, 7.2, 8.0, 9.1, 10.0, 15.5, 20.7, or more times greater than a reference level for the B7-H1 polypeptide. In some cases, an

elevated level of a B7-H1 polypeptide can be a level that is at least 2 percent (e.g., at least 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 300, 400, or 500 percent) greater than a corresponding reference level. In addition, a reference level can be any amount. For example, a reference level for a B7-H1 polypeptide can be zero. In this case, any level of the B7-H1 polypeptide greater than zero would be an elevated level.

This document also provides methods and materials for determining the prognosis of a mammal having cancer (e.g., RCC). For example, the presence of a B7-H1 polypeptide in a body fluid from a mammal having cancer can indicate that the mammal is susceptible to a poor outcome, and the absence of a B7-H1 polypeptide in a body fluid from a mammal having cancer can indicate that the mammal is susceptible to a good outcome. In some cases, the presence of a detectable amount of a B7-H1 polypeptide in a body fluid of a mammal having cancer can indicate that the mammal is susceptible to a poor outcome, and the absence of a detectable amount of a B7-H1 polypeptide in a body fluid from a mammal can indicate that the mammal is susceptible to a good outcome. In some cases, the presence of an elevated level of a B7-H1 polypeptide in a body fluid from a mammal having cancer can indicate that the mammal is susceptible to a poor outcome, and the absence of an elevated level of a B7-H1 polypeptide in a body fluid from a mammal having cancer can indicate that the mammal is susceptible to a good outcome.

The prognosis of a mammal having cancer also can be correlated with the degree of elevation of a B7-H1 polypeptide in a body fluid from a mammal. For example, a greater degree of elevation of a B7-H1 polypeptide level in a body fluid from a mammal above a corresponding reference level can indicate that the mammal is more susceptible to a poor outcome, and a lesser degree of elevation of a B7-H1 polypeptide level in a body fluid from a mammal above a corresponding reference level can indicate that the mammal is less susceptible to a poor outcome. In some cases, a level of a B7-H1 polypeptide in a body fluid from a mammal that is at least one standard deviation higher than a reference level can indicate that the mammal is more susceptible to a poor outcome than a mammal having a level of the B7-H1 polypeptide in a corresponding body fluid that is less than one standard deviation higher than the reference level.

In some cases, the presence, absence, or level of a B7-H1 polypeptide in a body fluid from a mammal can be used in combination with other factors to determine the prognosis of a mammal having cancer. For example, the presence, absence, or level of a B7-H1 polypeptide in a body fluid from a mammal having cancer can be used in combination with the clinical stage of the cancer, results of a physical examination, information about a family history of cancer, and/or results from imaging (e.g., magnetic resonance imaging) to determine whether or not the mammal is likely to have a poor outcome. A mammal that is susceptible to a poor outcome can have a more aggressive cancer, experience more rapid cancer progression, and/or die sooner of cancer than a mammal that is susceptible to a good outcome. Information about the prognosis of a mammal having cancer can be used to guide treatment selection. For example, a mammal identified as being susceptible to a poor prognosis can be treated earlier and more aggressively than a mammal identified as being susceptible to a good outcome.

Once a mammal has been identified as having cancer, the mammal can be subsequently evaluated or monitored over time for progression of the cancer. For example, a mammal can be classified as having a cancer that has progressed if it is determined that a body fluid from the mammal contains a B7-H1 polypeptide at a level that is greater than the level of the B7-H1 polypeptide observed in a corresponding body fluid obtained previously from the mammal. In some cases, a mammal can be classified as having a cancer that has not progressed if it is determined that a body fluid from the mammal contains a B7-H1 polypeptide at a level that is equal to or less than the level of the B7-H1 polypeptide observed in a corresponding body fluid obtained previously from the mammal.

A mammal that has been treated for cancer can be monitored for recurrence of the cancer. For example, a mammal that has been treated for cancer can be classified as having a recurring cancer if it is determined that a body fluid taken from the mammal after treatment (e.g., surgical resection of a tumor) contains a B7-H1 polypeptide at a level that is greater than the level of the B7-H1 polypeptide observed in a corresponding body fluid obtained from the mammal at an earlier time point after treatment. In some cases, a mammal can be classified as not having a recurring cancer if it is determined that a body fluid taken from the mammal after treatment with a cancer therapy contains a B7-H1 polypeptide at a level that is equal to or less

than the level of the B7-H1 polypeptide observed in a corresponding body fluid obtained from the mammal at an earlier time point after treatment. A mammal can be monitored for progression or recurrence of a cancer over any period of time with any frequency. For example, a mammal can be monitored once a year, twice a year, three
5 times a year, or more frequently. In some cases, a mammal can be monitored every three months for five years, or once a year for as long as the mammal is alive.

Methods and materials provided herein also can be used to determine whether or not a cancer therapy is effective. For example, a level of a B7-H1 polypeptide can be determined in a body fluid taken from a mammal prior to treatment with a cancer
10 therapy, and the level can be compared to a level of the B7-H2 polypeptide in a corresponding body fluid taken from a mammal during or after treatment. A decrease in the level of the B7-H1 polypeptide in the fluid taken during or after treatment as compared to the level in the fluid taken before treatment can indicate that the treatment is effective. In some cases, an increase or no change in the level of the B7-
15 H1 polypeptide in a body fluid taken during or after a cancer treatment as compared to the level in a corresponding fluid taken before treatment can indicate that the treatment is not effective. In some cases, a decrease in a level of a B7-H1 polypeptide in a body fluid taken from a mammal during or after a cancer treatment as compared to the level in a corresponding fluid taken at an earlier time point during treatment can
20 indicate that the treatment is effective. In some cases, an increase or no change in a level of a B7-H1 polypeptide in a body fluid taken from a mammal during or after a cancer treatment as compared to the level in a corresponding fluid taken at an earlier time point during treatment can indicate that the treatment is not effective.

Any appropriate method can be used to obtain a body fluid from a mammal for
25 analysis of the presence, absence, or amount of a B7-H1 polypeptide. For example, a blood sample can be obtained by peripheral venipuncture, and urine samples can be obtained using standard urine collection techniques. Once obtained, a body fluid can be manipulated prior to being analyzed. For example, a body fluid can be centrifuged prior to being analyzed for a B7-H1 polypeptide. In some cases, a blood sample can
30 be allowed to clot and can be centrifuged prior to being analyzed. In some cases, a body fluid can be stored (e.g., at 4°C) prior to being analyzed for a B7-H1 polypeptide. In addition, polypeptides can be extracted from a body fluid and can be

fractionated (e.g., on a column or in a gel) prior to being analyzed for a B7-H1 polypeptide.

Typically, the presence, absence, or amount of B7-H1 in the body fluid is determined by detecting soluble B7-H1 polypeptide. Methods of detecting polypeptides in body fluids are known in the art. For example, antibodies that bind to an epitope specific for soluble B7-H1 can be used to detect B7-H1 in body fluid. As used herein, the terms “antibody” or “antibodies” include intact molecules (e.g., polyclonal antibodies, monoclonal antibodies, humanized antibodies, or chimeric antibodies) as well as fragments thereof (e.g., single chain Fv antibody fragments, Fab fragments, and F(ab)₂ fragments) that are capable of binding to an epitopic determinant of B7-H1 (e.g., hB7-H1). The term “epitope” refers to an antigenic determinant on an antigen to which the paratope of an antibody binds. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains, and typically have specific three-dimensional structural characteristics, as well as specific charge characteristics. Epitopes generally have at least five contiguous amino acids (a continuous epitope), or alternatively can be a set of noncontiguous amino acids that define a particular structure (e.g., a conformational epitope). Polyclonal antibodies are heterogeneous populations of antibody molecules that are contained in the sera of the immunized animals. Monoclonal antibodies are homogeneous populations of antibodies to a particular epitope of an antigen. An antibody directed against a B7-H1 polypeptide can bind the polypeptide at an affinity of at least 10^4 mol^{-1} (e.g., at least 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} , 10^{11} , or 10^{12} mol^{-1}).

Antibody fragments that can bind to B7-H1 can be generated by known techniques. For example, F(ab')₂ fragments can be produced by pepsin digestion of the antibody molecule; Fab fragments can be generated by reducing the disulfide bridges of F(ab')₂ fragments. Alternatively, Fab expression libraries can be constructed. See, for example, Huse et al., Science, 246:1275 (1989). Once produced, antibodies or fragments thereof are tested for recognition of B7-H1 by standard immunoassay methods including ELISA techniques, radioimmunoassays, and Western blotting. See, Short Protocols in Molecular Biology, Chapter 11, Green Publishing Associates and John Wiley & Sons, Edited by Ausubel, F.M et al., 1992.

Antibodies having specific binding affinity for B7-H1 can be produced through standard methods. See, for example, Dong et al. (2002) Nature Med. 8:793-800. In general, a B7-H1 polypeptide (e.g., B7-H1 comprising or consisting of the extracellular domain of B7-H1) can be recombinantly produced, or can be purified from a biological sample, and used to immunize animals. As used herein, the term “polypeptide” refers to a polypeptide of at least five amino acids in length. To produce a recombinant B7-H1 polypeptide, a nucleic acid sequence encoding the appropriate polypeptide can be ligated into an expression vector and used to transform a bacterial or eukaryotic host cell. Nucleic acid constructs typically include a regulatory sequence operably linked to a B7-H1 nucleic acid sequence. Regulatory sequences do not typically encode a gene product, but instead affect the expression of the nucleic acid sequence. In bacterial systems, a strain of *Escherichia coli* such as BL-21 can be used. Suitable *E. coli* vectors include without limitation the pGEX series of vectors that produce fusion polypeptides with glutathione S-transferase (GST). Transformed *E. coli* are typically grown exponentially, then stimulated with isopropylthiogalactopyranoside (IPTG) prior to harvesting. In general, such fusion polypeptides are soluble and can be purified easily from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

Mammalian cell lines that stably express a B7-H1 polypeptide can be produced by using expression vectors with the appropriate control elements and a selectable marker. For example, the eukaryotic expression vector pCDNA.3.1+ (Invitrogen, San Diego, CA) can be used to express a B7-H1 polypeptide in, for example, COS cells, Chinese hamster ovary (CHO), human melanoma cells, or HEK293 cells. Following introduction of the expression vector by electroporation, DEAE dextran, or other suitable method, stable cell lines can be selected. Alternatively, B7-H1 can be transcribed and translated *in vitro* using wheat germ extract or rabbit reticulocyte lysase.

In eukaryotic host cells, a number of viral-based expression systems also can be utilized to express a B7-H1 polypeptide. A nucleic acid encoding a B7-H1 polypeptide can be introduced into a SV40, retroviral or vaccinia based viral vector and used to infect host cells. Alternatively, a nucleic acid encoding a B7-H1

polypeptide can be cloned into, for example, a baculoviral vector and then used to transfect insect cells.

Various host animals can be immunized by injection of the B7-H1 polypeptide. Host animals include rabbits, chickens, mice, guinea pigs, and rats.

5 Various adjuvants that can be used to increase the immunological response depend on the host species and include Freund's adjuvant (complete and incomplete), mineral gels such as aluminum hydroxide, surface-active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin and dinitrophenol. Monoclonal antibodies can be prepared using a B7-H1 polypeptide
10 and standard hybridoma technology. In particular, monoclonal antibodies can be obtained by any technique that provides for the production of antibody molecules by continuous cell lines in culture such as described by Kohler et al., Nature, 256:495 (1975), the human B-cell hybridoma technique (Kosbor et al., Immunology Today, 4:72 (1983); Cole et al., Proc. Natl. Acad. Sci USA, 80:2026 (1983)), and the EBV-
15 hybridoma technique (Cole et al., "Monoclonal Antibodies and Cancer Therapy", Alan R. Liss, Inc., pp. 77-96 (1983)). Such antibodies can be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD, and any subclass thereof. The hybridoma producing the monoclonal antibodies of the invention can be cultivated *in vitro* and *in vivo*.

20 In immunological assays, an antibody having specific binding affinity for B7-H1 or a secondary antibody that binds to such an antibody can be labeled, either directly or indirectly. Suitable labels include, without limitation, radioisotopes (e.g., ^{125}I , ^{131}I , ^{35}S , ^3H , ^{32}P , ^{33}P , or ^{14}C), fluorophores (e.g., fluorescein, fluorescein-5-isothiocyanate (FITC), PerCP, rhodamine, or phycoerythrin), luminescent moieties
25 (e.g., QdotTM nanoparticles supplied by the Quantum Dot Corporation, Palo Alto, CA), compounds that absorb light of a defined wavelength, or enzymes (e.g., alkaline phosphatase or horseradish peroxidase). Antibodies can be indirectly labeled by conjugation with biotin then detected with avidin or streptavidin labeled with a molecule described above. Methods of detecting or quantifying a label depend on the
30 nature of the label and are known in the art. Examples of detectors include, without limitation, x-ray film, radioactivity counters, scintillation counters, spectrophotometers, colorimeters, fluorometers, luminometers, and densitometers.

Combinations of these approaches (including “multi-layer” assays) familiar to those in the art can be used to enhance the sensitivity of assays.

Immunological assays for detecting B7-H1 can be performed in a variety of known formats, including sandwich assays (e.g., ELISA assays, sandwich Western blotting assays, or sandwich immunomagnetic detection assays), competition assays (competitive RIA), or bridge immunoassays. See, for example, U.S. Patent Nos. 5,296,347; 4,233,402; 4,098,876; and 4,034,074. Methods of detecting B7-H1 generally include contacting a body fluid with an antibody that binds to B7-H1 and detecting or quantifying binding of B7-H1 to the antibody. For example, an antibody having specific binding affinity for B7-H1 can be immobilized on a solid substrate by any of a variety of methods known in the art and then exposed to the biological sample. Binding of B7-H1 to the antibody on the solid substrate can be detected by exploiting the phenomenon of surface plasmon resonance, which results in a change in the intensity of surface plasmon resonance upon binding that can be detected qualitatively or quantitatively by an appropriate instrument, e.g., a Biacore apparatus (Biacore International AB, Rapskatan, Sweden). Alternatively, the antibody can be labeled and detected as described above. A standard curve using known quantities of B7-H1 can be generated to aid in the quantitation of B7-H1 levels.

In other embodiments, a “sandwich” assay in which a capture antibody is immobilized on a solid substrate is used to detect the presence, absence, or amount of soluble B7-H1. The solid substrate can be contacted with the biological sample such that any B7-H1 in the sample can bind to the immobilized antibody. The presence of B7-H1 bound to the antibody can be determined using a “reporter” antibody having specific binding affinity for B7-H1 and the methods described above. It is understood that in these sandwich assays, the capture antibody should not bind to the same epitope (or range of epitopes in the case of a polyclonal antibody) as the reporter antibody. Thus, if a monoclonal antibody is used as a capture antibody, the reporter antibody can be another monoclonal antibody that binds to an epitope that is either completely physically separated from or only partially overlaps with the epitope to which the capture monoclonal antibody binds, or a polyclonal antibody that binds to epitopes other than or in addition to that to which the capture monoclonal antibody binds. If a polyclonal antibody is used as a capture antibody, the reporter antibody can be either a monoclonal antibody that binds to an epitope that is either completely

physically separated from or partially overlaps with any of the epitopes to which the capture polyclonal antibody binds, or a polyclonal antibody that binds to epitopes other than or in addition to that to which the capture polyclonal antibody binds.

Suitable solid substrates to which an antibody (e.g., a capture antibody) can be bound include, without limitation, microtiter plates, tubes, membranes such as nylon or nitrocellulose membranes, and beads or particles (e.g., agarose, cellulose, glass, polystyrene, polyacrylamide, magnetic, or magnetizable beads or particles). Magnetic or magnetizable particles can be particularly useful when an automated immunoassay system is used.

Alternative techniques for detecting soluble B7-H1 include mass-spectrophotometric techniques such as electrospray ionization (ESI), liquid chromatography-mass spectrometry (LC-MS), and matrix-assisted laser desorption-ionization (MALDI). See, for example, Gevaert et al., Electrophoresis 22(9):1645-51, 2001; Chaurand et al., J Am Soc Mass Spectrom 10(2):91-103, 1999. Mass spectrometers useful for such applications are available from Applied Biosystems (Foster City, CA); Bruker Daltronics (Billerica, MA) and Amersham Pharmacia (Sunnyvale, CA). Arrays for detecting polypeptides, two-dimensional gel analysis, and chromatographic separation techniques also can be used to detect soluble B7-H1 polypeptide.

This document also provides methods and materials to assist medical or research professionals in determining whether or not a mammal has cancer, and whether or not a mammal having cancer is susceptible to a poor outcome. Medical professionals can be, for example, doctors, nurses, medical laboratory technologists, and pharmacists. Research professionals can be, for example, principle investigators, research technicians, postdoctoral trainees, and graduate students. A professional can be assisted by (1) determining the presence, absence, or level of a B7-H1 polypeptide in a body fluid from a mammal, and (2) communicating information about that level to that professional.

Any method can be used to communicate information to another person (e.g., a professional). For example, information can be given directly or indirectly to a professional. In addition, any type of communication can be used to communicate the information. For example, mail, e-mail, telephone, and face-to-face interactions can be used. The information also can be communicated to a professional by making that

information electronically available to the professional. For example, the information can be communicated to a professional by placing the information on a computer database such that the professional can access the information. In addition, the information can be communicated to a hospital, clinic, or research facility serving as an agent for the professional.

Articles of Manufacture

Antibodies that can bind to a soluble B7-H1 polypeptide (e.g., soluble hB7-H1) can be combined with packaging material and sold as a kit for detecting B7-H1 from body fluid, diagnosing a cancer, determining prognosis of a subject with cancer, or determining risk of cancer progression in a subject. For example, a kit can include a pair of antibodies, where each antibody of the pair has binding affinity for B7-H1 and where each antibody recognizes a different epitope of soluble B7-H1.

Components and methods for producing articles of manufactures are well known. In addition, the articles of manufacture may further include reagents such as secondary antibodies, sterile water, pharmaceutical carriers, buffers, indicator molecules, solid substrates (e.g., beads, microtiter plate), and/or other useful reagents (e.g., a positive control such as B7-H1 fusion polypeptide in which the extracellular domain of B7-H1 is fused to the CH2-CH3 domain of mouse immunoglobulin G2a and/or a negative control such as human IgG Fc polypeptide) for detecting B7-H1 from body fluids, diagnosing a cancer, determining prognosis of a subject with cancer, or determining risk of cancer progression in a subject. The antibodies can be in a container, such as a plastic, polyethylene, polypropylene, ethylene, or propylene vessel that is either a capped tube or a bottle. In some embodiments, the antibodies can be included on a solid substrate such as bead, microtiter plate, or a handheld device for bedside testing. Instructions describing how the various reagents are effective for detecting B7-H1 from body fluids, diagnosing a cancer, determining prognosis of a subject with cancer, or determining risk of cancer progression in a subject also may be included in such kits.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

EXAMPLE 1

Generation of Anti-B7-H1 Monoclonal Antibodies for ELISA

A plasmid containing a full-length B7-H1 coding sequence (pcDNA3-B7-H1) was transfected into human melanoma 624mel cells by the calcium phosphate method, and cells were selected for resistance to G418. BALB/c mice that were seven to eight weeks old were immunized with human melanoma cells transduced with the plasmid containing a full-length B7-H1 coding sequence (5×10^6 cells per mouse, i.p.). The mice were immunized twice weekly over a five to six week period. Lymphocytes were subsequently isolated and fused with A38 cells to form a hybridoma using standard techniques. Hybridoma supernatants were screened by ELISA for reactivity against a B7-H1.huFc fusion polypeptide. The B7-H1.huFc fusion polypeptide, also referred to as B7-H1hIgG, was prepared by fusing a nucleic acid encoding the extracellular domain of a human B7-H1 polypeptide to a nucleic acid encoding the CH2-CH3 domain of a human immunoglobulin G in an expression plasmid. Hybridoma supernatants also were screened for the absence of cross-reactivity to an irrelevant polypeptide. B7-H1 polypeptide-specific hybridoma cells were cloned and hybridoma clone 2.2B was selected.

Seven to eight week old BALB/c mice were immunized with a human B7-H1mIgG2A fusion polypeptide. The human B7-H1mIgG2A fusion polypeptide was prepared by fusing a nucleic acid encoding the extracellular domain of a human B7-H1 polypeptide to a nucleic acid encoding the CH2-CH3 domain of a mouse immunoglobulin G2a in an expression plasmid. The mice were injected subcutaneously with 100 μ g of the B7-H1mIgG2A fusion polypeptide mixed with Freund's adjuvant. Injections were administered twice weekly over a five to six week period.

Hybridoma supernatants were screened by ELISA for reactivity against B7-H1.huFc polypeptide and for the absence of cross-reactivity to an irrelevant polypeptide. B7-H1 polypeptide-specific hybridoma cells were cloned, and clone 5H1-A3 was selected. The two clones of monoclonal antibody (mAb), 2.2B and 5H1-A3, recognize different antigen epitopes (see Example 4). In ELISA assays, mAb 2.2B was used as a capture antibody and mAb 5H1-A3 was used as a detecting antibody.

EXAMPLE 2

Sandwich ELISA Detection for B7-H1

A sandwich ELISA assay was created using mAb 2.2B and 5H1-A3 to
5 determine if a soluble form of B7-H1 polypeptide was present in serum. Monoclonal
antibody 2.2B was used as a coating antibody, whereas biotinylated mAb 5H1-A3
was used as a detection antibody. After each step, assay plates were washed three
times with Washing Buffer (PBS with 0.05% Tween-20) using a microplate washer
(Bio-Tek, Winooski, VT). High-binding polystyrene plates (Corning Life Sciences,
10 Bedford, MA) were coated overnight at 4°C with 0.1 µg/well of anti-B7-H1 mAb.
The coating solution was aspirated off, the plates were washed, and free binding sites
were blocked with 200 µL/well of Blocking Buffer (PBS and 10% FBS; Invitrogen,
Carlsbad, CA) for two hours at room temperature. After washing, 75 µL of Assay
Buffer (PBS, 10% FBS, 0.05% Tween-20, and HBR II) were added to each well
15 followed by 25 µL of sample. The plates were incubated overnight at 4°C and
washed. One hundred µL of biotinylated mAb (1 µg/mL diluted in Blocking Buffer)
were added to each well, and the plates were incubated for one hour at room
temperature. After washing, 100 µL of horseradish peroxidase-conjugated
streptavidin (BD) at a 1:1,000 dilution were added to each well, and the plates were
20 incubated for 30 minutes at room temperature. The plates were washed and
developed with TMB (Pierce Biotechnology, Rockford, IL). The reaction was
stopped using 100 µL/well of 0.5 N H₂SO₄ and the plates were read at 450 nm using a
Benchmark Plus plate reader (Biorad, Hercules, CA). For calibration of each
sandwich ELISA, standards of 100 to 0.8 ng/mL of recombinant B7-H1 fusion
25 polypeptide were analyzed in parallel with the test samples. The minimal detectable
concentration (MDC) for the assay was determined to be 1 ng/mL.

EXAMPLE 3

Detection of B7-H1 Polypeptide in the Serum of Patients with RCC

30 All serum samples were collected by venipuncture technique from patients and
donors with appropriate informed consent. In general, blood collected by
venipuncture was allowed to clot for 20 minutes at room temperature and then
centrifuged for 15 minutes at 3,200 rpm. RCC cancer serum samples were collected

before the surgical removal of the cancer, and stage and histology of the cancer were determined by pathologists. Normal controls were collected from healthy volunteers undergoing blood donation at blood transfusion center. The serum samples for the preliminary validation study included 12 normal controls (male and female) and 48 samples from patients with RCC. As shown in FIG. 2, eleven of 48 sera specimens from RCC patients demonstrated positive reactivity, while only one in 12 sera specimens from normal donors was positive.

In another experiment, serum samples from 14 normal donors and 65 clear cell renal cell carcinoma patients were analyzed by sandwich ELISA with anti-B7-H1 polypeptide antibodies. The data were analyzed using the Wilcoxon rank sum test, and the p-value was 0.0008 (FIG. 3).

EXAMPLE 4

Evaluation of Anti-B7-H1 Monoclonal Antibodies

Two clones of monoclonal anti-B7-H1 antibodies, 2.2B and 5H1-A3, were created as described in Example 1. Experiments were performed to determine whether the 2.2B and 5H1-A3 antibodies recognize different epitopes of the same polypeptide. Cells expressing B7-H1 polypeptide were stained with biotinylated 2.2B antibody alone, or incubated with unbiotinylated 5H1-A3 antibody and then stained with biotinylated 2.2B antibody. In addition, cells expressing B7-H1 polypeptide were stained with biotinylated 5H1-A3 antibody alone, or incubated with unbiotinylated 2.2B antibody and stained with biotinylated 5H1-A3 antibody. The stained cells along with unbiotinylated control cells were analyzed using flow cytometry. Staining of cells with biotinylated 2.2B antibody in the absence of unbiotinylated 5H1-A3 antibody was comparable to staining in the presence of unbiotinylated 5H1-A3 antibody (FIG. 4A). Likewise, staining of cells with biotinylated 5H1-A3 antibody was comparable in the presence and absence of unbiotinylated 2.2B antibody (FIG. 4B). Results of these experiments indicate that the 2.2B and 5H1-A3 monoclonal anti-B7-H1 antibodies are directed against distinct epitopes of B7-H1 polypeptide.

The intensity of staining produced by the 5H1-A3 antibody was compared to the intensity of staining produced by the 2.2B antibody. Cells expressing B7-H1 polypeptide were stained using biotinylated 5H1-A3 or 2.2B antibody. The stained

cells, along with unbiotinylated control cells, were analyzed using flow cytometry. The intensity of staining produced using the 5H1-A3 antibody was observed to be comparable to the intensity of staining produced using the 2.2B antibody (FIG. 5).

EXAMPLE 5

Detection of B7-H1 Polypeptide in Cell Culture Supernatants

Cell culture supernatants were analyzed for soluble B7-H1 polypeptide levels using an ELISA assay (see Example 2). Monoclonal anti-B7-H1 antibody 2.2B (2 µg/mL) was used as a capture antibody, and biotinylated monoclonal anti-B7-H1 antibody 5H1-A3 was used as a detection antibody. The supernatants consisted of cell culture media that had been incubated with cells for three to four days. Supernatants from the following cell lines were analyzed: a primary human bladder tumor cell line (BT10B), a human mastocyte cell line (HMC1), and a human kidney cancer cell line (Caki-2). Recombinant human B7-H1 fusion polypeptide, B7-H1.huFc, also referred to as B7H1Fc, was prepared as described in Example 1 and used as a positive control at a concentration of 1 µg/mL. A fusion polypeptide comprising the extracellular domain of a human or mouse P-selectin polypeptide fused to the CH2-CH3 domain of a human immunoglobulin G (PSelFc; see, e.g., catalog number 555294, BD Pharmingen™, BD, Franklin Lakes, NJ) was used as a negative control at a concentration of 1 µg/mL. Phosphate-buffered saline (PBS) and cell culture media for BT10B and HMC1 cells, which had not been contacted with cells, served as additional negative controls.

Results of these experiments indicated that BT10B cell supernatant was positive for B7-H1 polypeptide, whereas BT10B cell culture medium that had not been incubated with cells was negative (FIG. 6).

Additional experiments were performed to analyze B7-H1 polypeptide levels in cell culture supernatants from 624MEL, B7-H1/624MEL, J82, Caki-2, BT10B, and BT10C cells. 624MEL is a human melanoma cell line, B7H1/624MEL is a human melanoma cell line (624MEL) transfected with a B7-H1 polypeptide expression vector, J82 is a human bladder carcinoma cell line, Caki-2 is a human kidney cancer cell line, and BT10B and BT10C are primary human bladder tumor cell lines. B7-H1 polypeptide levels were analyzed using an ELISA assay, as described above. Recombinant human B7-H1 fusion polypeptide (B7H1Fc; 1 µg/mL) was used as a

positive control, and the cell culture media for each cell line, along with PBS, were analyzed as negative controls.

Results of these experiments indicated that supernatants from BT10B and BT10C cells were positive for B7-H1 polypeptide, whereas BT10B and BT10C cell culture media that had not been incubated with cells were negative (FIG. 7).

Extracts from 624MEL, B7-H1/624MEL, BT10B, BT10C, and Caki-2 cells were analyzed for B7-H1 polypeptide levels by Western blotting. Polypeptide extracts (50 µg) from each cell line were separated using gel electrophoresis, transferred to a membrane, and immunoblotted with a biotinylated 5H1-A3 antibody. The membrane was stripped and reprobed for actin polypeptide as a loading control. B7-H1 polypeptide was detected in extracts from B7-H1/624MEL, BT10B, and BT10C cells, but not in extracts from 624MEL or Caki-2 cells (FIG. 8).

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A method of evaluating a mammal, said method comprising (a) providing a body fluid from said mammal, and (b) detecting the presence or absence of B7-H1 in said body fluid.

5

2. The method of claim 1, wherein said mammal is a human.

3. The method of claim 1, wherein B7-H1 is detected immunologically.

10

4. The method of claim 1, wherein B7-H1 is detected using a monoclonal antibody.

5. The method of claim 3, wherein B7-H1 is detected using a capture antibody and a reporter antibody, wherein said reporter antibody comprises a label.

15

6. The method of claim 5, wherein said label is a fluorophore, biotin, an enzyme, or a radioisotope.

20

7. The method of claim 6, wherein said fluorophore is fluorescein, fluorescein isothiocyanate (FITC), phycoerythrin (PE), allophycocyanin (APC), or peridinin chlorophyll protein (PerCP).

8. The method of claim 5, wherein said capture antibody is attached to a solid substrate.

25

9. The method of claim 8, wherein said solid substrate is selected from the group consisting of a bead and a microtiter plate.

30

10. The method of claim 5, wherein said capture antibody is a polyclonal antibody.

11. The method of claim 1, wherein said body fluid is selected from the group consisting of blood, plasma, serum, urine, cerebrospinal fluid, sputum, tears, and saliva.

5 12. The method of claim 11, wherein said body fluid is serum.

13. The method of claim 1, wherein said mammal is suspected of having a cancer.

14. The method of claim 13, wherein said cancer is renal cell carcinoma.

10

15. The method of claim 1, wherein the presence of B7-H1 in said body fluid indicates the presence of a cancer in said mammal.

15

16. The method of claim 15, wherein the presence of B7-H1 in said body fluid indicates said mammal is more likely to die of said cancer than if B7-H1 is absent.

17. A method of evaluating a mammal with renal cell carcinoma, said method comprising (a) providing a body fluid from said mammal, and (b) detecting the presence or absence of B7-H1 in said body fluid.

20

18. The method of claim 17, wherein the presence of B7-H1 in said body fluid indicates said mammal is more likely to die of renal cell carcinoma than if B7-H1 is absent.

25

19. A method of detecting B7-H1 in a body fluid, said method comprising:

(a) providing a solid substrate, said solid substrate coated with capture antibodies having binding affinity for soluble B7-H1;

30

(b) contacting said body fluid with said solid substrate under conditions in which soluble B7-H1, if present, becomes bound to said solid substrate to form a first reacted solid substrate;

(c) contacting said first reacted solid substrate with a reporter antibody having binding affinity for soluble B7-H1 to form a second reacted solid substrate; and

(d) detecting the presence or absence of said reporter antibody on said second reacted solid substrate, wherein the presence of reporter antibody indicates that soluble B7-H1 is present in said body fluid.

5 20. The method of claim 19, wherein said reporter antibody comprises a label selected from the group consisting of a radioisotope, a fluorophore, a luminescent moiety, biotin, and an enzyme.

10 21. The method of claim 19, wherein detecting the presence or absence of said reporter antibody comprises contacting said second reacted solid substrate with a secondary antibody having binding affinity for said reporter antibody, wherein said secondary antibody comprises a label.

15 22. The method of claim 19, wherein detecting the presence or absence of said reporter antibody comprises contacting said second reacted solid substrate with a reagent having binding affinity for said reporter antibody, wherein said reagent comprises a label.

20 23. The method of claim 19, wherein said solid substrate is a bead or a microtiter plate.

25 24. A kit for detecting soluble B7-H1, said kit comprising a pair of antibodies, each antibody of said pair having binding affinity for soluble B7-H1, wherein each antibody of said pair recognizes a different epitope of soluble B7-H1.

25 25. The kit of claim 24, said kit further comprising a solid substrate.

26. The kit of claim 24, said kit further comprising a positive control.

30 27. The kit of claim 24, said kit further comprising a negative control.

28. The kit of claim 24, said kit further comprising a solid substrate, a positive control, and a negative control.

29. A method for identifying a mammal as having cancer, said method comprising (a) determining whether or not a mammal has a body fluid containing an elevated level of a B7-H1 polypeptide, and (b) classifying said mammal as having cancer if
5 said mammal has said elevated level and classifying said mammal as not having cancer if said mammal does not have said elevated level.

30. The method of claim 29, wherein said mammal is a human.

10 31. The method of claim 29, wherein said body fluid is blood, serum, plasma, or urine.

32. The method of claim 29, wherein said cancer is renal cell carcinoma.

15 33. A method for assessing the effectiveness of a cancer treatment, said method comprising determining whether or not a mammal having cancer and having received a treatment for said cancer has a level of a B7-H1 polypeptide that is lower than that observed prior to said treatment, wherein the presence of said level of a B7-H1 polypeptide that is lower than that observed prior to said treatment indicates that said
20 treatment is effective.

34. A method for identifying a mammal as having cancer that has progressed, said method comprising determining whether or not a level of a B7-H1 polypeptide in a body fluid of a mammal increases over time, wherein an increase in said level
25 indicates that said mammal has cancer that has progressed, and the absence of an increase in said level indicates that said mammal does not have cancer that has progressed.

Figure 1

Signal peptide
1 MRIFAVFIFMTYWHLNNAFTVTVPKDLYVVEYGSNM^{*}TIECKFPVEKQLDL

Ig-V-like
51 AALIVYWEMEDKNIIQFVHGEEDLKVQHSSYRQRARLLKDQLSLGNAALQ

Ig-C-like
101 ITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVD^{*}DPVTSE^{*}

151 HELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRIN^{*}

201 TTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPNERTHLVILGAILLC^{*}
TM

251 LGVALTFIFRLRKGRMMDVKKCGIQDTNSKKQSDTHLEET

Figure 3

Serum B7-H1

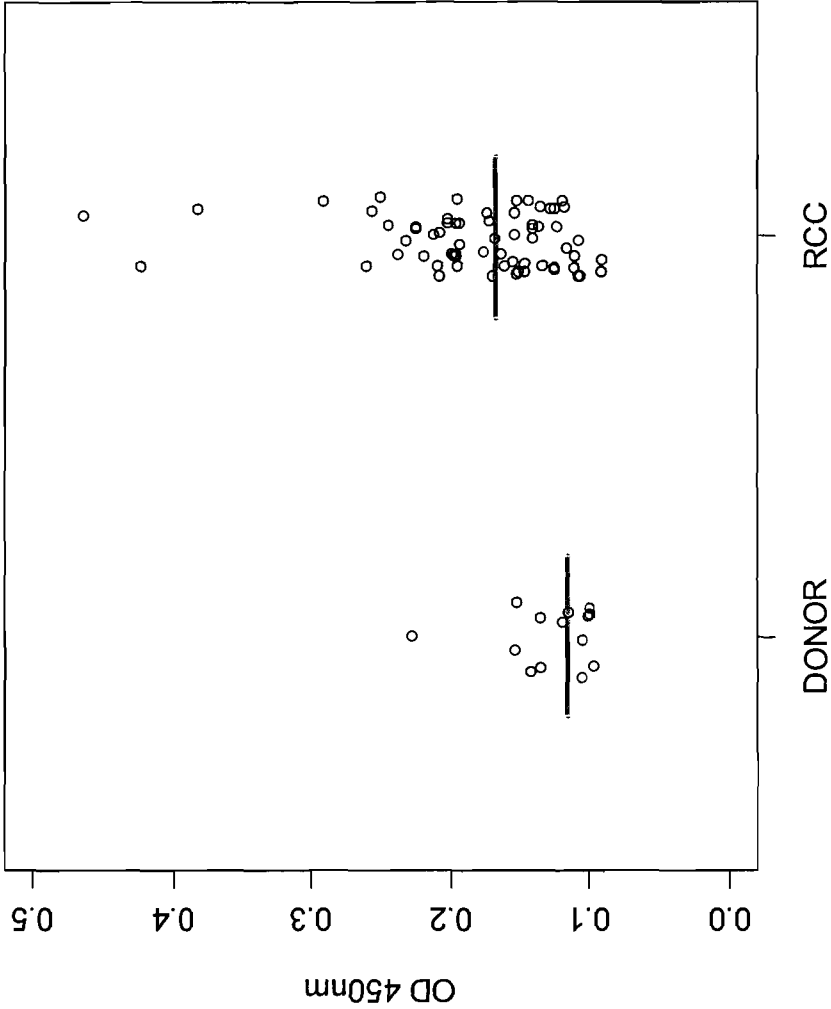


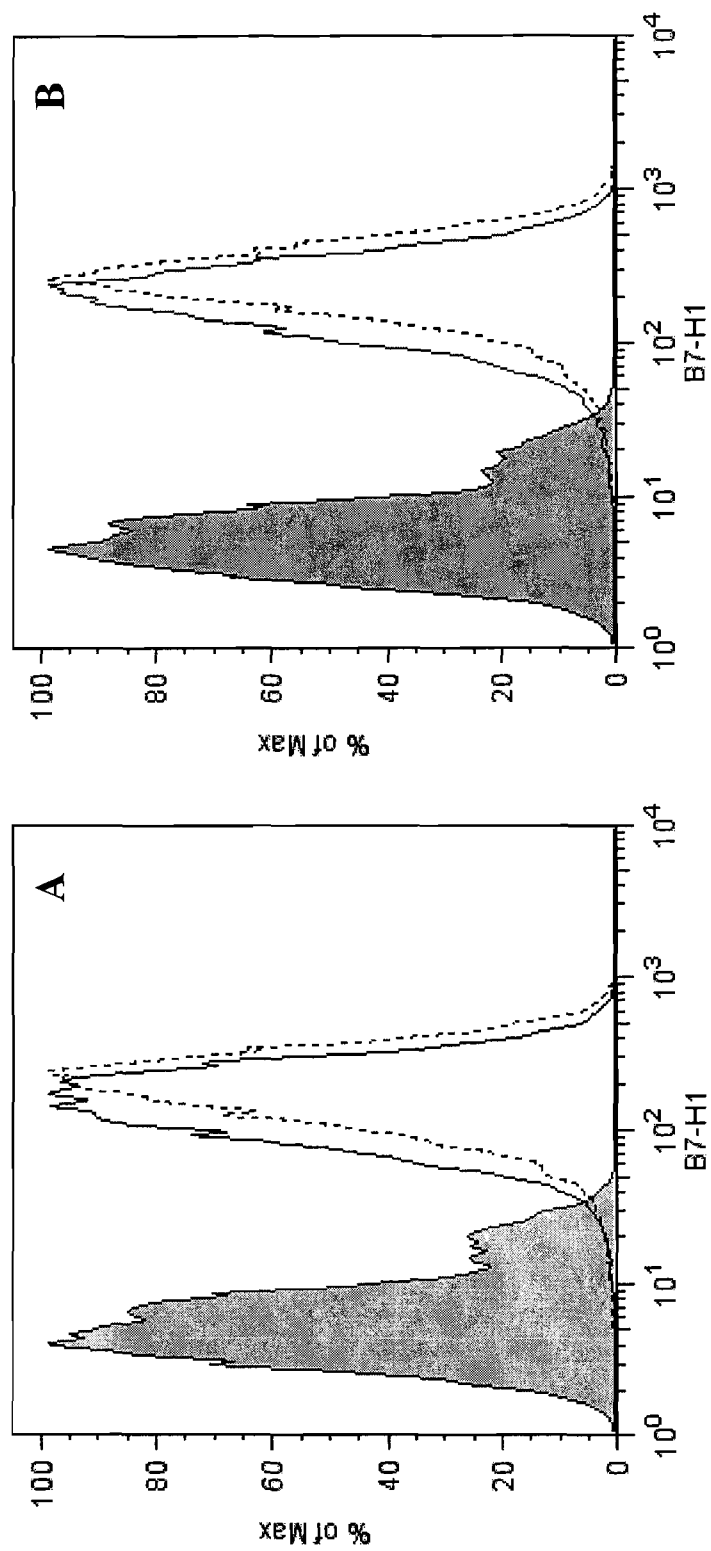
Figure 4

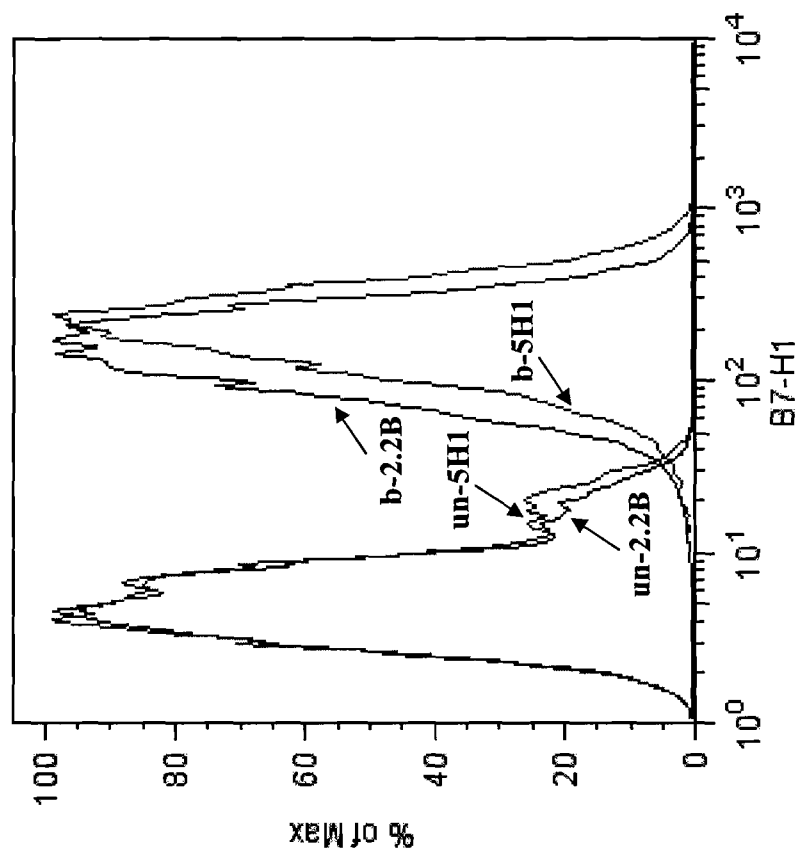
Figure 5

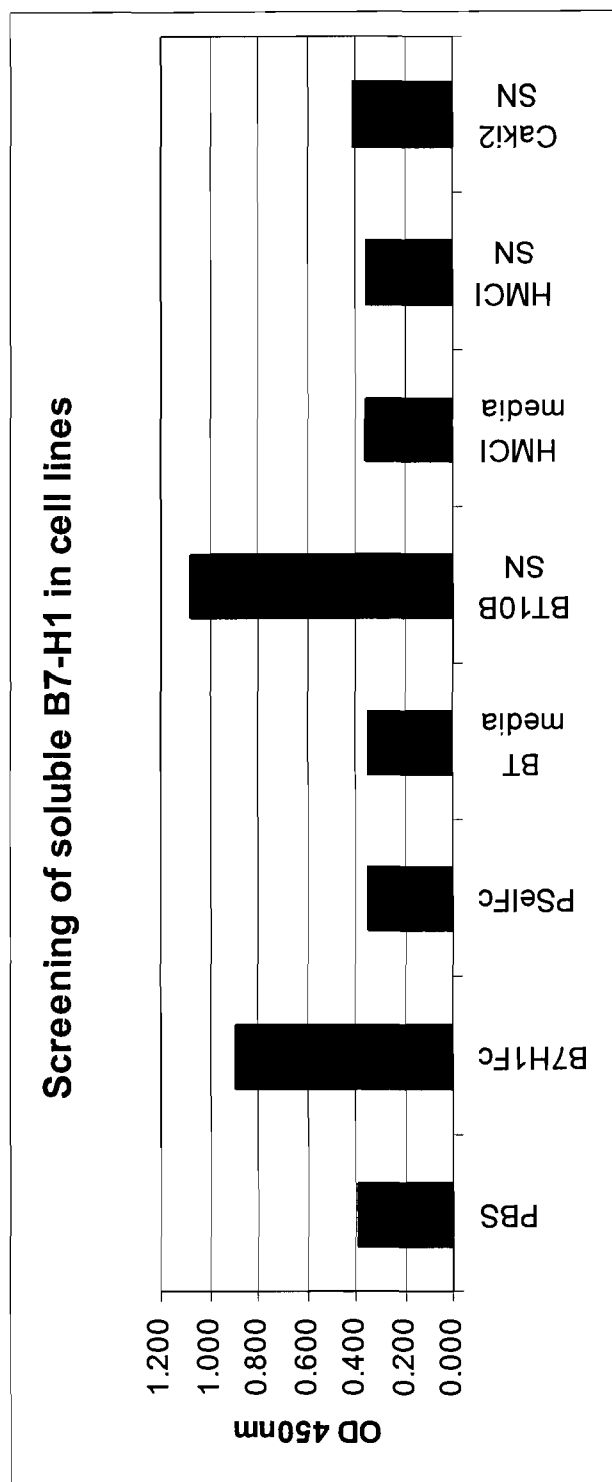
Figure 6

Figure 7

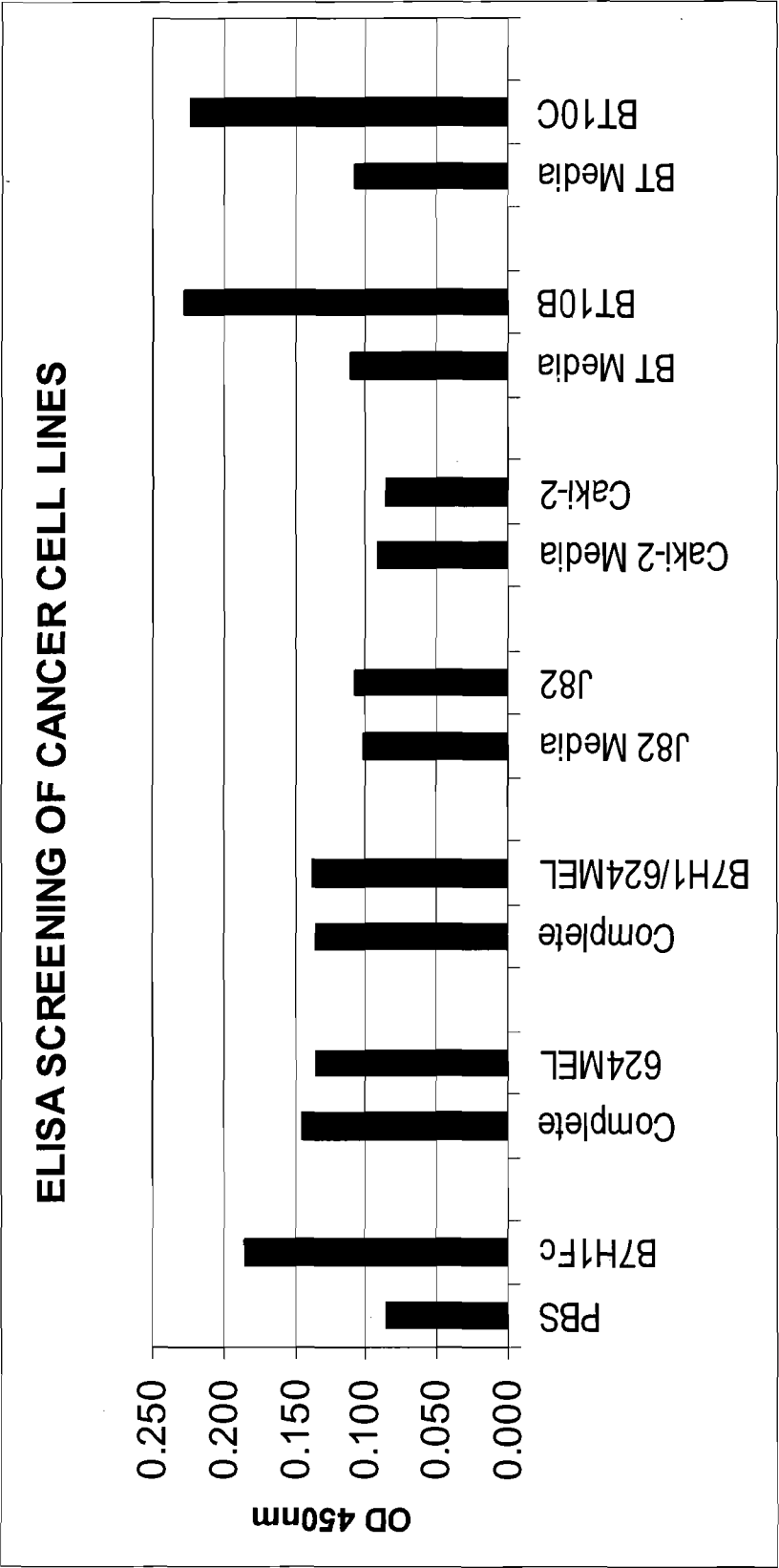


Figure 8

