ANTI-PROSTATE CANCER ANTIBODIES AND METHODS OF DETECTION AND TREATMENT OF PROSTATE CANCER USING THE SAME

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Disclosed herein are antibodies that detect a lipid-like antigen on prostate cancer cells and methods of detecting and treating prostate cancer using the same.
FIGURE 1
### Reactivity of F77 with human tissue and cancer cell lines

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Immunohistology</th>
<th>Cell Line</th>
<th>Flow cytometry (fluorescent intensity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate</td>
<td>Carcinoma</td>
<td>PC3</td>
<td>118.7 ± 2.99</td>
</tr>
<tr>
<td></td>
<td>Strong stain</td>
<td>PC3-MM2</td>
<td>140.5 ± 3.33</td>
</tr>
<tr>
<td></td>
<td>Normal duct</td>
<td>Du145</td>
<td>117.6 ± 3.73</td>
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<tr>
<td></td>
<td>Unseen stain</td>
<td>LNCaP</td>
<td>92.9 ± 4.03</td>
</tr>
<tr>
<td>Breast</td>
<td>Carcinoma</td>
<td>SKBR3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Slight background</td>
<td>BT474</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Normal duct</td>
<td>MCF7</td>
<td>15.0 ± 2.36</td>
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<tr>
<td></td>
<td>Slight background</td>
<td>MB231</td>
<td>14.9 ± 3.52</td>
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<tr>
<td></td>
<td></td>
<td>HS0-584</td>
<td></td>
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<tr>
<td>Colon</td>
<td>Negative</td>
<td>SW260</td>
<td>12.2 ± 2.7</td>
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<td></td>
<td></td>
<td>HI-135</td>
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<td>Kidney</td>
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<td>Pasteur</td>
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<td>HEK293T</td>
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<td>Cervix</td>
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<td>AtPC1</td>
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<td>BxPC3</td>
<td>13.9 ± 2.88</td>
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<tr>
<td>Bone</td>
<td>Negative</td>
<td>MG63</td>
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<td>Brain</td>
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<td>Retina</td>
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<tr>
<td>Blood</td>
<td>Negative</td>
<td>CEM (T cell)</td>
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<td></td>
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<td>Jurkat (T cell)</td>
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<td></td>
<td></td>
<td>HL-60 (AML)</td>
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<td></td>
<td></td>
<td>U937(Monocyte)</td>
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<td></td>
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<td>SB (B cell)</td>
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<tr>
<td>Ovary</td>
<td>Negative</td>
<td>McDonald</td>
<td>14.9 ± 2.62</td>
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<tr>
<td></td>
<td></td>
<td>OVCA4</td>
<td>16.3 ± 3.17</td>
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**FIGURE 2**
<table>
<thead>
<tr>
<th>Cell subpopulation</th>
<th>No. of mice with palpable tumors</th>
<th>Average time to tumor formation (days)</th>
<th>Average tumor size (mm³)</th>
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<tr>
<td>F77-/RWPE-1</td>
<td>1/6</td>
<td>40</td>
<td>21.67</td>
</tr>
<tr>
<td>F77+/RWPE-1</td>
<td>4/6</td>
<td>31(±7)</td>
<td>59.51±8.16</td>
</tr>
<tr>
<td>RWPE-2</td>
<td>4/6</td>
<td>23(±8)</td>
<td>49.15±6.18</td>
</tr>
</tbody>
</table>

FIGURE 5
FIG. 6A

![Graph showing cell% distribution for different conditions](image)

FIG. 6B

![Graph showing growth inhibition% for different conditions](image)

FIG. 6C

![Graph showing ADCC% for different conditions](image)
Days post tumor cell injection
FIG. 9A

FIG. 9B

PPMP treatment

Fluorescence intensity

PC3+F77 Du+F77 Du+383

FIG. 9C
FIG. 11A

Fluorescence intensity

Counts

FIG. 11B

O.D. 450

1:1 1:4 1:16 1:64

Dilution
FIGURE 12

B-III dodecanoylceramide

B-IV tetraodoacoctylceramide

\[ \text{Galα}-3\text{Galβ}-4\text{GlcNAcb}-6 \]

\[ \text{Fucα}-2 \]

\[ \text{Galα}-6\text{Galβ}-4\text{GlcNAcb}-3 \]

\[ \text{Fucα}-2 \]

\[ \text{Fucα}-2 \]

\[ \text{Galα}-3\text{Galβ}-4\text{GlcNAcb}-6 \]

\[ \text{Fucα}-2 \]

\[ \text{Galα}-6\text{Galβ}-4\text{GlcNAcb}-3 \]

\[ \text{Fucα}-2 \]

\[ \text{Galα}-3\text{Galβ}-4\text{GlcNAcb}-6 \]

\[ \text{Fucα}-2 \]

\[ \text{Galα}-6\text{Galβ}-4\text{GlcNAcb}-3 \]

\[ \text{Fucα}-2 \]
ANTI-PROSTATE CANCER ANTIBODIES
AND METHODS OF DETECTION AND
TREATMENT OF PROSTATE CANCER
USING THE SAME

CROSS-REFERENCE TO RELATED
APPLICATIONS

[0001] This application claims priority to U.S. Provisional
Application No. 61/287,063, filed Dec. 16, 2009, which is
incorporated herein by reference.

TECHNICAL FIELD

[0002] The subject matter described herein relates to the
field of molecular biology and immunology. More specifi-
cally, the subject matter relates to immunotherapeutics,
including antibodies that detect prostate cancer and methods
for using such antibodies to treat subjects in need of such
treatment.

BACKGROUND

[0003] Prostate cancer is the second leading cause of can-
cer-related death in men in the United States. Studies indicate
that 30-45% of patients with clinically localized disease are
found with Stage T3. These patients have extra-capsular
extension, where prostate cancer cells have extended into or
beyond the outer lining of the prostate gland. In these patients,
cancer may relapse and metastasize after local therapy.

[0004] In fact, a large percentage of androgen-independent
prostatic carcinomas metastasize to bone. These metastases
are difficult to treat and contribute to increased morbidity and
mortality, with a median survival of approximately a year
after diagnosis.

[0005] Despite the effectiveness of hormone therapy, most
patients with metastatic disease eventually progress to an
androgen-independent state at which time the disease is
incureable with a median survival rate of 1 year. Overall, the
5-year survival rate for metastatic prostate cancer is only
34%. New diagnostic, prognostic, and therapeutic
approaches are clearly needed for the treatment of advanced
and metastatic prostate cancer.

[0006] One approach is antibody therapy. However, the
antibodies currently available for detection and treatment of
prostate cancers are limited. The MAb 7E11-05.3, which
binds to Prostate-Specific Membrane Antigen (PSMA), has
been developed for clinical trials. The ProstaScint® scan
(Cytogen, Princeton, N.J.), based on Indium-111 labeled
7E11-05.3, appears superior to the conventional imaging
methods for soft-tissue disease, but has limitations because it
binds to the intracellular domain on PSMA. In addition,
PSMA is not expressed in certain advanced, androgen-inde-
pendent tumor cells such as PC3 and Du145, and therefore
this antibody is not useful for imaging bone metastases.
Recent studies show that the anti-prostate stem cell antigen
(PSCA) MAb1G8 can inhibit tumor growth of androgen-
dependent tumor xenografts. However, anti-PSCA MAbs are
ineffective against androgen-independent PC3 tumors, which
do not express PSCA. An analysis of prostate cancer tissue
sections demonstrated that PSCA is absent in about 20% of
specimens. Therefore, defining new prostate specific markers
is important in order to improve the diagnosis and treatment
of advanced androgen-independent prostate cancer.

SUMMARY

[0007] Described herein are antibodies that detect a novel
glycolipid-like antigen, “PCLA,” or “Prostate Cancer Lipid-
like Antigen.” PCLA is highly restricted to prostate cancer
cell and prostatic surfaces. In one embodiment, the anti-
PCLA antibody is the F77 MAb, a mouse monoclonal
antibody as described in Carroll, A M., et al., 1984, Sury Synth
Immunol Immunopath, 33, 268-281, each of which is
incorporated by reference herein.

[0008] Described herein are the amino acid sequences of
the variable regions of the MAbs, a light chain variable region
having the amino acid sequence of SEQ ID NO:1 or a heavy
chain variable region having the amino acid sequence of SEQ
ID NO:4.

[0009] Disclosed herein are novel antibodies. Included are
antibody or antibody functional fragments (other than F77
MAb) that bind to B-III dodecaasoylcervamide or B-IV
dodecaasoylcervamide. In further embodiments are included
antibody or antibody functional fragments that compete for
binding with F77 MAb. In further embodiments are included
antibody or antibody functional fragments that compete for
binding with F77 MAb to B-III dodecaasoylcervamide and/or
B-IV dodecaasoylcervamide. In some embodiments the anti-
body or antibody functional fragments comprise CDRs of the
heavy chain or light chain variable region, Fab, F(ab')2, Fd,
Fabc, Fv or Sc. In some embodiments the antibody or anti-
body functional fragments are humanized. In some embo-
liments the antibody or antibody functional fragment is conju-
gated to a toxin or cytotoxic agent. Also provided are methods
of detecting the degree of differentiation of prostate cancer
comprising contacting a sample of prostate tissue with the
antibody or antibody functional fragment of any one of the
preceding embodiments, and correlating the amount of bind-
ing of the antibody to the sample with the degree of differen-
tiation. Also provided are methods of determining the degree
of differentiation of prostate cancer comprising contacting
prostastomes with the antibody or antibody functional frag-
ment of any one of the preceding embodiments and correlat-
ing the amount of binding of the antibody to the prostastomes
with the degree of differentiation. Also provided are methods
of treating a subject having prostate cancer comprising assay-
ing the degree of differentiation of the cancer and in response
treating with an effective amount of the antibody or antibody
functional fragment of any one of the preceding embed-
ments. Also provided are methods of preferentially inducing
cell death in primary or metastatic prostate cancer cells over
non-cancerous prostate cells in a human, comprising admin-
istering the antibody or antibody functional fragment of any
one of the preceding embodiments. Also provided are meth-
ods of inhibiting prostate tumor growth in a patient having
suffered prostate cancer comprising administering the antibody or
antibody functional fragment of any one of the preceding
embodiments. Also provided are methods of treating a subject
having prostate cancer comprising administering the antibody
or antibody functional fragment of any one of the preceding
embodiments in combination with additional anti-
cancer treatments, including chemotherapy, androgen
depression therapy, and radiation therapy, alone or in com-

[0010] Included are antibodies that compete for binding
with an antibody comprising a light chain variable region
comprising the amino acid sequence of, or substantially simi-
lar to, SEQ ID NO:1, or the CDRs thereof, or a heavy chain
variable region comprising the amino acid sequence of, or substantially similar to, SEQ ID NO:4, or the CDRs thereof. Further embodiments include antibodies that compete for binding with an antibody comprising a light chain variable region having the amino acid sequence of, or substantially similar to, SEQ ID NO:1, or the CDRs thereof, or comprising a heavy chain variable region having the amino acid sequence of, or substantially similar to, SEQ ID NO:4, or the CDRs thereof.

[0011] Described herein are novel antibodies comprising any combination of the following CDR sequences:

- **Light chain CDR1**: C-R-S-S-O-T-L-Y-H-S-N-G-N-T-F-L-(H/A/V) (i.e. the last residue can be either H, A or V)
- **Light chain CDR2**: K-V-S-N-R-F-S
- **Light chain CDR3**: S-Q-G-T-H-A-P-F-T
- **Heavy chain CDR1**: Y-Y-G-V-H
- **Heavy chain CDR2**: I-I-(W/F)-A-G-G-N-T-N-(Y/V/L/I)-N-S-T-(L/Q/A/S/T)-K-S

[0012] Described herein is an antibody comprising a light chain variable region having the amino acid sequence of, or substantially similar to, SEQ ID NO:2 or 3. A further embodiment is an antibody comprising a heavy chain variable region having the amino acid sequence of, or substantially similar to, SEQ ID NOs: 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, or 43. A further embodiment is an antibody comprising a light chain variable region having the amino acid sequence of, or substantially similar to, SEQ ID NO: 2 or 3 and a heavy chain variable region having the amino acid sequence of, or substantially similar to, SEQ ID NOs: 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, or 43.

[0013] Described herein are antibodies or functional fragments thereof that are derived from any of the anti-PCLA antibodies described above. A further embodiment is a functional fragment of an antibody that comprises an amino acid sequence that is identical to, or substantially similar to, SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, or 43, or the CDRs thereof. A further embodiment is a humanized antibody or functional fragment of any of the anti-PCLA antibodies described above, comprising one or more amino acid sequences as set forth in SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, or 43, or the CDRs thereof. A further embodiment is an antibody or functional fragment of any of the anti-PCLA antibodies described above, having one or more amino acid sequences as set forth in SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, or 43, or the CDRs thereof. A further embodiment is an antibody or functional fragment of any of the anti-PCLA antibodies described above, having one or more amino acid sequences as set forth in SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, or 43, or the CDRs thereof.

[0014] Other embodiments include the polynucleotide sequences encoding an anti-PCLA antibody or functional fragment thereof that comprises at least one of the amino acid sequences as set forth in SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, or 43, or a humanized antibody or CDR derived therefrom, or polynucleotide or amino acid sequences that are substantially similar thereto. Other embodiments include the polynucleotide sequences encoding an anti-PCLA antibody or functional fragment thereof that have at least one of the amino acid sequences as set forth in SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, or 43, or a humanized antibody or CDR derived therefrom, or polynucleotide or amino acid sequences that are substantially similar thereto.
or identical to IIWAGGNTYNSTTKS (SEQ ID NO: 53). In some embodiments, antibodies or antigen-binding fragments can include a heavy chain CDR2 amino acid sequence substantially the same as, or identical to IIWAGGNTNVESTGKS (SEQ ID NO: 54). In some embodiments, antibodies or antigen-binding fragments can include a heavy chain CDR2 amino acid sequence substantially the same as, or identical to IIWAGGNTNVE STGKS (SEQ ID NO: 55). In some embodiments, antibodies or antigen-binding fragments can include a heavy chain CDR2 amino acid sequence substantially the same as, or identical to IIWAGGNTNVE STKSN (SEQ ID NO: 56). In some embodiments, antibodies or antigen-binding fragments can include a heavy chain CDR2 amino acid sequence substantially the same as, or identical to IIWAGGNTNVE STKSN (SEQ ID NO: 57). In some embodiments, antibodies or antigen-binding fragments can include a heavy chain CDR2 amino acid sequence substantially the same as, or identical to IIWAGGNTNVLIVGKLS (SEQ ID NO: 58). In some embodiments, antibodies or antigen-binding fragments can include a heavy chain CDR2 amino acid sequence substantially the same as, or identical to IIWAGGNTNVLIVGKLS (SEQ ID NO: 59). In some embodiments, antibodies or antigen-binding fragments can include a heavy chain CDR2 amino acid sequence substantially the same as, or identical to IIWAGGNTNVLIVGKLS (SEQ ID NO: 60). In some embodiments, antibodies or antigen-binding fragments can include a heavy chain CDR2 amino acid sequence substantially the same as, or identical to IIWAGGNTNVLIVGKLS (SEQ ID NO: 61). In some embodiments, antibodies or antigen-binding fragments can include a heavy chain CDR2 amino acid sequence substantially the same as, or identical to IIWAGGNTNVLIVGKLS (SEQ ID NO: 62). In some embodiments, antibodies or antigen-binding fragments can include a heavy chain CDR2 amino acid sequence substantially the same as, or identical to IIWAGGNTNVLIVGKLS (SEQ ID NO: 63). In some embodiments, antibodies or antigen-binding fragments can include a heavy chain CDR2 amino acid sequence substantially the same as, or identical to IIWAGGNTNVLIVGKLS (SEQ ID NO: 64). In some embodiments, antibodies or antigen-binding fragments can include a heavy chain CDR2 amino acid sequence substantially the same as, or identical to IIWAGGNTNVLIVGKLS (SEQ ID NO: 65). In some embodiments, antibodies or antigen-binding fragments can include a heavy chain CDR2 amino acid sequence substantially the same as, or identical to IIWAGGNTNVLIVGKLS (SEQ ID NO: 66). In some embodiments, antibodies or antigen-binding fragments can include a heavy chain CDR2 amino acid sequence substantially the same as, or identical to IIWAGGNTNVLIVGKLS (SEQ ID NO: 67). In some embodiments, antibodies or antigen-binding fragments can include a heavy chain CDR2 amino acid sequence substantially the same as, or identical to IIWAGGNTNVLIVGKLS (SEQ ID NO: 68). In some embodiments, antibodies or antigen-binding fragments can include a heavy chain CDR2 amino acid sequence substantially the same as, or identical to IIWAGGNTNVLIVGKLS (SEQ ID NO: 69). In some embodiments, antibodies or antigen-binding fragments can include a heavy chain CDR2 amino acid sequence substantially the same as, or identical to IIWAGGNTNVLIVGKLS (SEQ ID NO: 70). In some embodiments, antibodies or antigen-binding fragments can include a heavy chain CDR2 amino acid sequence substantially the same as, or identical to IIWAGGNTNVLIVGKLS (SEQ ID NO: 71). In some embodiments, antibodies or antigen-binding fragments can include a heavy chain CDR2 amino acid sequence substantially the same as, or identical to IIWAGGNTNVLIVGKLS (SEQ ID NO: 72). In some embodiments, antibodies or antigen-binding fragments can include a heavy chain CDR2 amino acid sequence substantially the same as, or identical to IIWAGGNTNVLIVGKLS (SEQ ID NO: 73). In some embodiments, antibodies or antigen-binding fragments can include a heavy chain CDR2 amino acid sequence substantially the same as, or identical to IIWAGGNTNVLIVGKLS (SEQ ID NO: 74). In some embodiments, antibodies or antigen-binding fragments can include a heavy chain CDR2 amino acid sequence substantially the same as, or identical to IIWAGGNTNVLIVGKLS (SEQ ID NO: 75).

[0016] In some embodiments, antibodies or antigen-binding fragments can include a heavy chain CDR2 amino acid sequence substantially the same as, or identical to IIWAGGNTNVLIVGKLS (SEQ ID NO: 91). In some embodiments, antibodies or antigen-binding fragments can include a heavy chain CDR2 amino acid sequence substantially the same as, or identical to IIWAGGNTNVLIVGKLS (SEQ ID NO: 92).

[0017] In some embodiments, antibodies or antigen-binding fragments can include a heavy chain CDR2 amino acid sequence substantially the same as, or identical to IIWAGGNTNVLIVGKLS (SEQ ID NO: 76). In some embodiments, antibodies or antigen-binding fragments can include a heavy chain CDR2 amino acid sequence substantially the same as, or identical to IIWAGGNTNVLIVGKLS (SEQ ID NO: 77). In some embodiments, antibodies or antigen-binding fragments can include a heavy chain CDR2 amino acid sequence substantially the same as, or identical to IIWAGGNTNVLIVGKLS (SEQ ID NO: 78). In some embodiments, antibodies or antigen-binding fragments can include a heavy chain CDR2 amino acid sequence substantially the same as, or identical to IIWAGGNTNVLIVGKLS (SEQ ID NO: 79). In some embodiments, antibodies or antigen-binding fragments can include a heavy chain CDR2 amino acid sequence substantially the same as, or identical to IIWAGGNTNVLIVGKLS (SEQ ID NO: 80). In some embodiments, antibodies or antigen-binding fragments can include a heavy chain CDR2 amino acid sequence substantially the same as, or identical to IIWAGGNTNVLIVGKLS (SEQ ID NO: 81).

[0018] In some embodiments, antibodies or antigen-binding fragments can include a heavy chain CDR3 amino acid sequence substantially the same as, or identical to DDYAAAMDY (SEQ ID NO: 46).

[0019] In some embodiments, antibodies or antigen-binding fragments can include a light chain CDR1 amino acid sequence substantially the same as, or identical to CRSSQTLVHSGNFTL1I (SEQ ID NO: 84).

[0020] In some embodiments, antibodies or antigen-binding fragments can include a light chain CDR1 amino acid sequence substantially the same as, or identical to CRSSQTLVHSGNFTL1A (SEQ ID NO: 85). In some embodiments, antibodies or antigen-binding fragments can include a light chain CDR1 amino acid sequence substantially the same as, or identical to CRSSQTLVHSGNFTL1 (SEQ ID NO: 86). In some embodiments, antibodies or anti-
gen-binding fragments can include a light chain CDR2 amino acid sequence substantially the same as, or identical to KVS-NRFS (SEQ ID NO: 82). In some embodiments, antibodies or antigen-binding fragments can include a light chain CDR2 amino acid sequence substantially the same as, or identical to SQGTHAPFT (SEQ ID NO: 83).

**[0021]** Described herein are complexes comprising any of the anti-prostate cancer antibodies described above, including antibodies and functional fragments derived therefrom, bound to PCLA. Further embodiments include complexes wherein the PCLA is on metastatic or androgen independent human prostate cancer tissue.

**[0022]** Described herein are vectors or cells comprising one or more such polynucleotide sequences.

**[0023]** Described herein are kits comprising the antibodies, functional fragments, or humanized or derived antibodies. Further embodiments include kits comprising the polynucleotides encoding the antibodies, functional fragments, or humanized or derived antibodies.

**[0024]** Described herein are novel methods of detecting and treating prostate cancer with any of the anti-prostate cancer antibodies described above, including MAb F77 and the novel antibodies and/or novel functional fragments derived therefrom, either as a sole treatment or in combination with additional anti-cancer treatments, including chemotherapy, androgen deprivation therapy, and radiation therapy, alone or in combination. See Dal Pena, A. et al. Current Oncology 17:28-38 (2010); Foley, R. et al. Prostate (Oct. 13, 2010); Sweeney, C. et al. Expert Opinion Pharmacother. 12:73-84 (2011), which are hereby incorporated by reference in their entirety and for all purposes. Described herein are methods of detecting and treating prostate cancer with any of the anti-PCLA antibodies described above, including antibodies and functional fragments derived therefrom. Described herein are methods of detecting and treating prostate cancer with any of the anti-F77 cancer antibodies described above, including antibodies and functional fragments derived therefrom.

**[0025]** Described herein are methods of detecting the degree of differentiation of prostate cancer comprising contacting a sample of prostate tissue with an anti-PCLA antibody and correlating the amount of binding of said antibody to said sample with said degree of differentiation. Further embodiments include the method of detecting the degree of differentiation of prostate cancer wherein the cancer is androgen independent. Further embodiments include the method of detecting the degree of differentiation of prostate cancer wherein the cancer is metastatic. Further embodiments include the method of detecting the degree of differentiation of prostate cancer in a prostatic tissue sample wherein the sample is human. Further embodiments include the method of detecting wherein the absence or degree of differentiation of prostate cancer is measured using a CDR, Fab, ScFv, or functional fragment of an anti-PCLA antibody. Further embodiments include the method of detecting the degree of differentiation of prostate cancer wherein the anti-PCLA antibody comprises at least one of a variable light chain nucleic acid sequence as set forth in SEQ ID NO:1 or a variable light chain nucleic acid sequence as set forth in SEQ ID NO:4.

**[0026]** Described herein are methods of determining the degree of differentiation of prostate cancer comprising contacting prostasomes with an anti-PCLA antibody and correlating the amount of binding of said antibody to said prostasomes with said degree of differentiation. Further embodiments include the methods of determining the degree of differentiation of prostate cancer comprising contacting prostasomes with an anti-PCLA antibody and correlating the amount of binding of said antibody to said prostasomes with said degree of differentiation wherein the prostasomes comprise semen or serum.

**[0027]** Described herein are methods for treating or preventing a PCLA associated disease a subject in need of such treatment. In one embodiment, the PCLA associated disease is prostate cancer. The methods comprise administering to the subject an antibody or antigen-binding fragment that specifically binds to PCLA in an amount effective to treat or prevent the PCLA associated disease. In some embodiments the methods comprise administering a pharmaceutical composition including an antibody or antigen-binding fragment and a pharmaceutically acceptable carrier.

**[0028]** Described herein are methods of treating a subject having prostate cancer comprising assaying the degree of differentiation of said cancer in response treating with an effective amount of an anti-PCLA antibody.

**[0029]** Embodiments of each of the methods described herein include methods wherein the antibody is a humanized antibody, CDR, Fab, ScFv, or functional fragment thereof. Embodiments of each of the methods described herein include methods wherein the prostate cancer is androgen independent. Embodiments of each of the methods described herein include methods wherein the prostate cancer is androgen dependent. Embodiments of each of the methods described herein include methods wherein the prostate cancer is metastatic. Embodiments of each of the methods described herein include methods wherein the prostate cancer is primary.

**[0030]** Embodiments of each of the methods comprising detecting or treating metastatic prostate cancer include methods wherein the metastasis is present in bone. Further embodiments of each of the methods comprising detecting or treating metastatic prostate cancer include methods wherein the metastatic prostate cancer is present in the brain.

**[0031]** Described herein are methods of preferentially inducing cell death in primary or metastatic prostate cancer cells over non-cancerous prostate cells in a human, comprising administering a humanized antibody CDR, Fab, ScFv, or functional fragment thereof derived from the antibody having a variable light chain sequence as set forth in SEQ ID NO:1, or the CDRs thereof, and a variable heavy chain sequence as set forth in SEQ ID NO:4, or the CDRs thereof. Further embodiments include methods of preferentially inducing cell death in primary or metastatic prostate cancer cells over non-cancerous prostate cells in a human, comprising administering a humanized antibody, or an Fab, ScFv, or functional fragment thereof comprising a variable light chain sequence as set forth in SEQ ID NOs: 1, 2, or 3, or the CDRs thereof. Further embodiments include methods of preferentially inducing cell death in primary or metastatic prostate cancer cells over non-cancerous prostate cells in a human, comprising administering a humanized antibody, Fab, ScFv, or functional fragment thereof comprising a variable heavy chain sequence as set forth in one of SEQ ID NOs: 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, or 43, or the CDRs thereof. Further embodiments include methods of preferentially inducing cell death in primary or metastatic
prostate cancer cells over non-cancerous prostate cells in a human, comprising administering a humanized CDR derived from an antibody comprising a sequence as set forth in SEQ ID NOs: 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, or 43, or the CDRs thereof. Further embodiments include methods of preferentially inducing cell death in primary or metastatic prostate cancer cells over non-cancerous prostate cells in a human, comprising administering a humanized CDR derived from an antibody having a sequence as set forth in SEQ ID NOs: 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, or 43, or the CDRs thereof. Further embodiments include methods of preferentially inducing cell death in primary or metastatic prostate cancer cells over non-cancerous prostate cells in a human, wherein said cell death is induced via antibody dependent cellular cytotoxicity or complement-dependent cytotoxicity.

[0032] Described herein are methods of inhibiting prostate tumor growth in a patient having said tumor comprising administering a humanized antibody CDR, Fab, ScFv, or functional fragment thereof derived from the antibody having a variable light chain sequence as set forth in SEQ ID NO:1, or the CDRs thereof, and a variable heavy chain sequence as set forth in SEQ ID NO:4, or the CDRs thereof.

[0033] Described herein are any of the antibodies described herein further bound to PCLA. Further embodiments include any of the antibodies described herein bound to PCLA on human prostatesomes. Further embodiments include any of the antibodies described herein bound to, wherein the PCLA is on metastatic or androgen independent human prostate cancer tissue.

BRIEF DESCRIPTION OF THE DRAWINGS

[0034] FIG. 1 depicts the binding of monoclonal antibody F77 to various prostate cancer cells using flow cytometry analysis: androgen-dependent prostate cancer cells of the LNCaP cell line; androgen-independent prostate cancer cells of the Du145 and PC3 cell line; high metastatic prostate cancer cells of the PC3-MM2 cell line which are derived from PC3 cells; normal human prostate epithelial cells of the RWPE-1 cell line, which are immortalized by HPV-18; and tumorigenic prostate epithelial cells of the RWPE-2 cell line, which were was derived from RWPE-1 cells via Ki-ras onco-gene transfection. (MAB F77 staining, bold line; negative control (irrelevant) mouse IgG3 staining, dashed line).

[0035] FIG. 2 is a table listing the relative reactivity of F77 MAB with human tissue and cancer cell lines as determined by immunohistochemical staining and fluorescence of cells bound by F77 MAB using flow cytometry, respectively.

[0036] FIG. 3A is a photographic image depicting immunohistochemical staining of benign prostate glands tissue with monoclonal antibody F77, demonstrating a mosaic staining pattern of some benign prostate glands. (Arrow indicates F77-positive prostate gland; scale bar=100 μm).

[0037] FIG. 3B is a photographic image depicting immunohistochemical staining of benign and cancerous prostate gland tissue showing the typical result that benign prostate gland tissue is not bound by MAB F77 (arrow), whereas cancerous prostate gland tissue is bound (arrow). (Scale bar=200 μm).

[0038] FIG. 3C is a photographic image depicting immunohistochemical staining of high grade prostate cancer tissue, demonstrating that poorly differentiated prostate cancer tissue exhibits diffuse staining with MAB F77. (scale bar=50 μm).

[0039] FIG. 3D is a photographic image depicting immunohistochemical staining of bone metastatic prostate cancer tissue, demonstrating that cancerous glandular tissue in a bone metastasis also stain diffusely positive with MAB F77. (scale bar=25 μm).

[0040] FIG. 3E is a bar graph showing quantification of the immunohistochemical staining in 116 specimens stained with MAB F77 (1 μg/ml) (staining intensity grading: 0 (negative), 1+ (weak), 2+ (moderate) and 3+ (strong)).

[0041] FIG. 4A depicts a graph plotting cell number, as measured by absorbance readings in a colorimetric MTT assay, at specific time points, of two subpopulations of RWPE-1 cells sorted by flow cytometry: one that bound F77 MAB antibody (F77+/RWPE-1) and the other that did not (F77−/RWPE-1). The graph demonstrates that the RWPE-1 subpopulation of cells that express the F77 antigen, to which the f77 MAB binds, grow twice as fast as the RWPE-1 cells that do not express the F77 antigen.

[0042] FIG. 4B shows the results of flow cytometry analysis of the F77-positive and F77-negative RWPE-1 subpopulations after 7 days of in vitro cell culture and quantification of staining with F77 MAB, demonstrating that the subpopulation of RWPE-1 prostate cancer cells that bind F77 MAB (F77+/RWPE-1) maintained a high level of expression of the F77 antigen on their surfaces, and exhibit comparable levels of staining to that exhibited by RWPE-2.

[0043] FIG. 5 is a table summarizing the results of tumor formation of the RWPE prostate cancer subpopulations in nude mice, showing tumor formation in mice injected with the RWPE-1 prostate cancer cell subpopulation that binds F77 MAB (F77+/RWPE-1), and a very low incidence of small tumor formation in mice injected with the RWPE-1 prostate cancer cell subpopulation that does not bind F77 MAB (F77−/RWPE-1).

[0044] FIG. 6A is a bar graph plotting the results of Annexin V and propidium iodide staining demonstrating that MAb F77 induces apoptosis in a percentage of A431 and PC3 cells treated with 3 μg/ml or 30 μg/ml MAB F77 for 4 hours at 37°C. Negative controls (neg) are cells treated with a negative control (irrelevant) murine IgG3 MAB. Data expressed as mean±SD of triplicate measurements.

[0045] FIG. 6B is a bar graph plotting percent growth inhibition of F77 MAB exposed, as determined using an MTT assay, indicating that the F77 MAB initiates complement dependent cytolysis in prostate cancer cells in the presence of serum. (H1+77: 25 μg/ml MAB F77 in 100 μl medium containing 1% human serum; M1+77: 25 μg/ml MAB F77 in 100 μl medium containing 1% mouse serum; F77: 25 μg/ml F77 MAB in 100 μl serum free medium; Data expressed as mean±SD of triplicate measurements.)

[0046] FIG. 6C is a bar graph depicting the results of an ADCC assay wherein PC3 or Du145 target cells were treated with negative control (irrelevant) murine IgG3 MAB (0) or MAB F77 (2 and 10 μg/ml, respectively) and exposed to effector cells (monocyte-like U937 cells) at an effector:target cell ratio of 2:1. A431 cells were used as control cells. ADCC % was calculated following the manufacturer’s instructions.
for the CytoTox 96® Non-Radioactive Cytotoxicity Assay (Promega). Data expressed as mean±SD of triplicate measurements.

[0047] FIG. 7A is a graph demonstrating that F77 MAb inhibits growth of prostate tumors in vivo. The graph plots tumor volume versus time for response to treatment with F77 MAb or control (PBS) in male nude mice 7 days following subcutaneous (s.c.) injection with 1×10⁶ PC3 cells in the right flank. Treatment of MAB F77 or control corresponds to i.p. injection of 200 μg/dose at Day 7 and 9, 100 μg/dose at Day 11 and 13.

[0048] FIG. 7B is a graph demonstrating that F77 MAb does not inhibit growth of tumors of A431 cells (human epithelial carcinoma cell line which do not bind F77 MAb). The graph plots tumor volume versus time for response to treatment with F77 MAb or control (PBS) in male nude mice 7 days following subcutaneous (s.c.) injection with 0.5×10⁶ A431 cells in the left flank. Treatment of MAB F77 or control corresponds to i.p. injection of 200 μg/dose at Day 7 and 9, 100 μg/dose at Day 11 and 13. (Mean tumor volume (mm³) ±SEM. P<0.01).

[0049] FIG. 7C is a graph demonstrating that F77 MAb prevents growth of Du145 prostate tumors in vivo. The graph plots tumor volume versus time for response to treatment with F77 MAb or control (PBS) in male nude mice 7 days following subcutaneous (s.c.) injection with 2×10⁵ Du145 cells. Treatment of MAB F77 or control corresponds to i.p. injection of 200 μg/dose at Day 7 and 9, 100 μg/dose at Day 11 and 13. (Mean tumor volume (mm³) ±SEM. P<0.01).

[0050] FIG. 7D is a graph demonstrating that F77 MAb inhibits growth of larger, established Du145 prostate tumors in vivo. The graph plots tumor volume versus time for response to treatment with F77 MAb or control (PBS) in male nude mice with tumors with a mean size of 135 mm³, at 11 days following subcutaneous (s.c.) injection with 2×10⁵ Du145 cells. Treatment of MAB F77 or control corresponds to i.p. injection of 200 μg/dose at Day 7 and 9, 100 μg/dose at Day 11 and 13. (Mean tumor volume (mm³) ±SEM P<0.01).

[0051] FIG. 8 is a photographic image of mice depicting typical results of the experiment of FIG. 7A, demonstrating that F77 MAB treatment shrinks PC3 tumors but not A431 tumors.

[0052] FIG. 9A depicts two images of an electrophoretic gel resolving immunoprecipitates of PC3 and A431 cells lysed in RIPA buffer (10⁵ cells/1 mL RIPA buffer) using F77 MAB or control IgG. The left image shows initial staining of the electrophoretic gel with Pro-Q Emerald 300 carboxylic dye fluorescent stain visualized with a transilluminator and the right image shows the subsequent staining of the same gel with the Coomassie blue protein stain. Lane 1: lysates of PC3 cells bound and precipitated with 10 μg of monoclonal F77 antibody; Lane 2: lysates of A431 cells bound and precipitated with 10 μg of monoclonal F77 antibody; Lane 3: lysates of control PC3 cells bound and precipitated with 5 μg murine IgG.

[0053] FIG. 9B is a bar graph demonstrating that treatment (48 hours) with the glycosphingolipid inhibitor PMP induces a dose-dependent decrease in the level of the antigen recognized by the F77 MAB on both PC3 and Du145 (Du) cell surfaces as analyzed by flow cytometry. (33B3—control anti-CD147 monoclonal antibody).

[0054] FIG. 9C is a graph depicting the specific binding of F77 MAB to glycolipids extracted from PC3 and Du145, and the lack of binding of F77 MAB to glycolipids extracted from control cell lines HEK293 and A431, as measured in an ELISA with F77 antibody (1 μg/ml or control anti-CD147 monoclonal antibody 3B3 (murine IgG3).

[0055] FIG. 10A is an image of an electrophoretic gel resolving F77 MAb immunoprecipitates (50 μg MAB F77 per 5×10⁵ cells for 30 minutes) of lysed Du145, LNCaP, and PC3 prostate cells subjected to a lipid raft isolation procedure. (Lane 1: Du145+F77/CHAPS; Lane 2: LNCaP+F77/CHAPS; Lane 3: PC3+F77/CHAPS; Lane 4: Du145+F77/RIPA; Lane 5: LNCaP+F77/RIPA; Lane 6: PC3+F77/RIPA; Lane 7: the total amount of MAb F77 added in each sample; for each Lane: 1, heavy chain; L, light chain)

[0056] FIG. 10B is a bar graph of the relative levels of F77 antigen soluble in CHAPS buffer and RIPA buffer as measured by the relative intensity of Coomassie blue stained bands on SDS-PAGE. The graph demonstrates that when cells are treated with 0.5% CHAPS on ice, only 4% of PC3 and Du145 cell surface stained with F77 MAB.

[0057] FIG. 11A is an graph of the results of F77 MAB binding to prostate tissues using flow cytometry analysis using a BD FACS Calibur© instrument; prior to analysis, prostate samples were incubated with 1 μg/100 μl DyLight 488 labeled MAB F77 (bold line) or mouse IgG3 control (dashed line) for 30 minutes.

[0058] FIG. 11B is a graph plotting the absorbance readings in an ELISA assay of prostate tissues isolated from one 150 mm cell culture dish of PC3 or A431 cells that were resuspended in 100 μl PBS for subsequent studies. Plates were coated overnight with 5 μg/ml F77 MAB in coating buffer (0.1 M NaHCO₃, pH 9.6) and blocked by incubation with 200 μl/well of 1% BSA/PBS, followed by addition of Prostate samples diluted with PBS (1:1-1:64), 2 hours of incubation at room temperature, and subsequent addition of 1-5 μg/ml biotinylated-F77 MAB or control mouse IgG was added to wells. After washing, streptavidin-HRP (GE healthcare) was then added for 1 hour incubation and absorbance measurements taken.

[0059] FIG. 12 depicts the two epitopes recognized by F77 MAB in a glycolipid (NGL)-based microarrays, namely B-III dodecaasylcarbamide and B-IV tetraasylcarbamide.

**DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS**

[0060] Various terms relating to aspects of the description are used throughout the specification and claims. Such terms are to be given their ordinary meaning in the art unless otherwise indicated. Other specifically defined terms are to be construed in a manner consistent with the definitions provided herein.

[0061] As used in this specification and the appended claims, the singular forms “a,” “an,” and “the” include plural refers when the context clearly dictates otherwise. Thus, for example, reference to “a cell” includes a combination of two or more cells, and the like.

[0062] The term “about” as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of ±20% or ±10%, more preferably ±5%, even more preferably ±1%, and still more preferably ±0.1% from the specified value, as such variations are appropriate to perform the disclosed methods.

[0063] “Polynucleotide,” synonymously referred to as “nucleic acid molecule” or “nucleic acids,” refers to any polynucleoside or polynucleoside precursor, which may be unmodified RNA or DNA or modified RNA or DNA.
“Polynucleotides” include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, double-stranded, or a mixture of single- and double-stranded regions. In addition, “polynucleotide” refers to triplex-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes a DNA or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. “Modified” bases include, for example, triplylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, “polynucleotide” embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. “Polynucleotide” also embraces relatively short nucleic acid chains, often referred to as oligonucleotides.

[0064] “Polypeptide” refers to any peptide or protein comprising amino acids joined by peptide bonds or modified peptide bonds. “Polypeptide” refers to short chains, including peptides, oligopeptides or oligomers, and to longer chains, including proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. “Polypeptides” include amino acid sequences modified either by natural processes, such as post-translational processing, or by chemical modification or other synthetic techniques well known in the art. Such modifications are well described in the art. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino terminus or the carboxy terminus. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from natural posttranslational processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphodiesterositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination.

[0065] “Substantially similar” with respect to nucleic acid or amino acid sequences, means at least about 65% identity between two or more sequences. Preferably, the term refers to at least about 70% identity between two or more sequences, more preferably at least about 75% identity, more preferably at least about 80% identity, more preferably at least about 85% identity, more preferably at least about 90% identity, more preferably at least about 91% identity, more preferably at least about 92% identity, more preferably at least about 93% identity, more preferably at least about 94% identity, more preferably at least about 95% identity, more preferably at least about 96% identity, more preferably at least about 97% identity, more preferably at least about 98% identity, and more preferably at least about 99% or greater identity. Such identity can be determined using algorithms known in the art, such as the mBLAST algorithm.

[0066] “Antibody” refers to all isotypes of immunoglobulins (IgG, IgA, IgE, IgM, IgD, and IgY) including various monomeric and polymeric forms of each isotype, unless otherwise specified.

[0067] “Functional fragments” of such antibodies comprise portions of intact antibodies that retain antigen-binding specificity of the parent antibody molecule. For example, functional fragments can comprise at least the CDRs of either the heavy chain or light chain variable region. Functional fragments can also comprise the heavy chain or light chain variable region, or sequences that are substantially similar to the heavy or light chain variable region. Further suitable functional fragments include, without limitation, antibodies with multiple epitope specificity, bispecific antibodies, diabodies, and single-chain molecules, as well as Fab, Fab(ab)2, Fd, Fdabc, and Fv molecules, single chain (SeFv), individual antibody light chains, individual antibody heavy chains, chimeric fusions between antibody chains and other molecules, heavy chain monomers or dimers, light chain monomers or dimers, dimers consisting of one heavy and one light chain, and the like. All antibody isotypes can be used to produce functional fragments of the antibodies herein. Functional fragments can be recombinantly or synthetically produced, with natural or unnatural nucleic acid or amino acid molecules.

[0068] The antibodies or functional fragments thereof of the disclosed subject matter can be generated from any species. The antibodies or functional fragments thereof described herein can be labeled or otherwise conjugated to various chemical or biomolecule moieties, for example, for therapeutic or diagnostic or detection or treatment applications. The moieties can be cytotoxic, for example, bacterial toxins, viral toxins, radioisotopes, and the like. The moieties can be detectable labels, for example, fluorescent labels, radionuclides, proteins, and the like, which are known in the art.

[0069] “Derived from” can mean any method of derivation and does not require possession of an isolated nucleotide or polypeptide molecule. Rather, “derived from” includes the use of algorithms for designing and theoretically testing antigen binding, creating a functionally equivalent antibody or fragment thereof that retains the binding and/or detection specificity of the parent antibody. “Derived from” contemplates the use of antibodies having substantially similar amino acid or nucleotide sequences to a parent antibody, such as F77 MAb.

[0070] The antibodies and functional fragments thereof described herein bind “PCLA,” Prostate Cancer Lipid-like Antigen, a novel glycolipid-like antigen highly restricted to the prostate cancer cell or prostatic surfaces, as described below in Examples 15 and 16. PCLA is bound by the F77 MAb.

[0071] An “anti-PCLA antibody” described herein detects a glycolipid-like antigen as described below in Examples 15 and 16. An “anti-PCLA antibody” includes the F77 MAb, an antibody comprising a light chain variable region as set forth in SEQ ID NO:1 and a heavy chain variable region as set forth in SEQ ID NO:4. An “anti-PCLA antibody” includes an antibody comprising a light chain variable region as set forth
in SEQ ID NO:1 or a heavy chain variable region as set forth in SEQ ID NO:4. An "anti-PCLA antibody" may compete for binding with an antibody comprising a light chain variable region as set forth in SEQ ID NO:1 or a heavy chain variable region as set forth in SEQ ID NO:4. An "anti-PCLA antibody" includes an antibody that competes for binding with an antibody comprising a light chain variable region as set forth in SEQ ID NO:1 and a heavy chain variable region as set forth in SEQ ID NO:4. An "anti-PCLA antibody" includes an antibody that competes for binding with an antibody comprising a light chain variable region as set forth in SEQ ID NO:1, or the CDRs identified therein, or a heavy chain variable region as set forth in SEQ ID NO:4, or the CDRs identified therein. An "anti-PCLA antibody" includes an antibody comprising a light chain variable region as set forth in SEQ ID NO:2 or 3, or the CDRs identified therein. An "anti-PCLA antibody" includes an antibody comprising a heavy chain variable region as set forth in SEQ ID NOs: 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, or 43, or the CDRs identified therein. An "anti-PCLA antibody" includes an antibody comprising a light chain variable region as set forth in SEQ ID NO:2 or 3, or the CDRs identified therein, and a heavy chain variable region as set forth in SEQ ID NOs: 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, or 43, or the CDRs identified therein.

[0072] The antibodies or functional fragments thereof described herein have binding affinities (in M) for PCLA that include a dissociation constant (KD) of less than 10^-3. In some embodiments, the KD is less than 10^-4. In other embodiments, the KD is less than 10^-5. In still other embodiments, the KD is less than 10^-6. In some embodiments, the KD is less than 10^-7, 2x10^-8, 3x10^-9, 4x10^-9, 5x10^-9, 6x10^-9, 7x10^-9, 8x10^-9, or 9x10^-9. In other embodiments, the KD is less than 10^-7, 2x10^-8, 3x10^-9, 4x10^-9, 5x10^-9, 6x10^-9, 7x10^-9, 8x10^-9, or 9x10^-9. In other embodiments, the KD is less than 10^-8, 2x10^-9, 3x10^-9, 4x10^-9, 5x10^-9, 6x10^-9, 7x10^-9, 8x10^-9, or 9x10^-9. In other embodiments, the KD is less than 10^-9, 2x10^-10, 3x10^-10, 4x10^-10, 5x10^-10, 6x10^-10, 7x10^-10, 8x10^-10, 9x10^-10. In other embodiments, the KD is less than 10^-9, 2x10^-10, 3x10^-10, 4x10^-10, 5x10^-10, 6x10^-10, 7x10^-10, 8x10^-10, 9x10^-10.

[0073] The anti-PCLA antibody antibodies described herein are useful for determining the "degree of differentiation" of prostate cancer in a sample or subject. The phrase "degree of differentiation" refers to the stage of progression of cancer, including tumor grade. One skilled in the art understands that as cancers and/or tumors progress, the cancer cells become less differentiated. One skilled in the art understands that some less differentiated cells can become anchorage independent and/or metastatic. One skilled in the art understands that as prostate cancer progresses, the prostate cancer cells may become androgen independent, and such cells may grow or multiply in the absence of androgen. The degree of differentiation may be quantitatively or qualitatively determined. For example, the degree of differentiation of a prostate tumor may be highly differentiated or completely undifferentiated. As a further example, the degree of differentiation may be zero, low, or high.

[0074] Whether an antibody or functional fragment thereof "competes for binding" with an antibody from which it is derived can be tested in any number of competitive or comparative assays, some of which are exemplified below. These assays include sandwich ELISA, immunoprecipitation, immunohistochemistry, in vivo imaging, flow cytometry, FACS analysis, and other assays known in the art. Competing for binding can be determined by separate assays in which the antibodies exhibit similar detection or binding characteristics, and need not be used in the same assay to be deemed to compete for binding. Traditional competitive assays in which the antibodies are used in the same assay are also contemplated for a determination of whether an antibody competes for binding with another.

[0075] The terms "treating" or "treatment" refer to any success or indicia of success in the attenuation or amelioration of an injury, pathology or condition, including any objective or subjective parameter such as abatement, remission, diminishing of symptoms or making the injury, pathology, or condition more tolerable to the patient, slowing in the rate of degeneration or decline, making the final point of degeneration less debilitating, improving a subject's physical or mental well-being, or prolonging the length of survival. The treatment or amelioration of symptoms can be based on objective or subjective parameters; including the results of a physical examination, neurological examination, and/or psychiatric evaluations.

[0076] "Effective amount" and "therapeutically effective amount" are used interchangeably herein, and refer to an amount of an antibody or functional fragment thereof, as described herein, effective to achieve a particular biological or therapeutic result such as, but not limited to, the biological or therapeutic results disclosed herein. A therapeutically effective amount of the antibody or antigen-binding fragment thereof may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody or functional fragment thereof to elicit a desired response in the individual. Such results may include, but are not limited to, the treatment of cancer, as determined by any means suitable in the art.

[0077] It is to be understood that the embodiments described herein are not limited to particular methods, reagents, compounds, compositions or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing the antibodies and functional fragments thereof, and methods of detecting and/or diagnosing and/or treating, and is not intended to be limiting.

Example 1

Statistical Analysis

[0078] All of the experiments were repeated at least three times unless otherwise indicated. The data were expressed as mean±SD. Statistical analysis was performed using Student’s t test. The criterion for statistical significance used was P<0.05.

Example 2

Cell Lines

[0079] PC3, Ds145, LNCaP, RWPE-1, and RWPE-2 cells were obtained from the American Type Culture Collection (Manassas, Va.). PC3-MM2 cells were purchased from Dr. Isaiah J. Fidler at U.T.M.D. Anderson Cancer Center.
The RWPE-1 cell line of non-tumorigenic human prostatic epithelial cells was developed by immortalization of epithelial cells that were derived from the peripheral zone of a normal human prostate. The RWPE-2 human prostatic carcinoma cell line was derived from RWPE-1 by transformation with Ki-ras.

**Example 3**

**Immunohistochemistry Staining**

All the cases were retrieved from the surgical pathology files at the University of Pennsylvania Medical Center. Immunohistochemical staining with the F77 MAb was performed on 5 μm paraffin embedded tissues including TMA (tissue microarray) sections. Briefly, sections were deparaffinized in xylene and rehydrated in graded alcohols. A heat-based antigen retrieval method was used in citrate buffer (pH 6.0) in a microwave oven. F77 MAb (5 μg/ml diluted in DakoCytomation Antibody Diluent) was added and incubated for 30 minutes at room temperature. Slides were washed 5 times with Tris-buffered saline containing Tween 20% reagent (TBST, pH 7.6; DAKOCytomation, Carpinteria, Calif.) and incubated for 30 minutes at room temperature with horseradish peroxidase-labeled dextran polymer coupled to anti-mouse (DAKOCytomation EnVision™ System HRP; DAKO). Slides were then washed 3 times with TBST, developed with diaminobenzidine, and counterstained with hematoxylin. The negative control lacked the primary antibody. Immunohistochemical staining of F77 MAb was interpreted semi-quantitatively by assessing the intensity of staining on the entire tissue sections or TMA cores by two board certified pathologists.

**Example 4**

**Flow Cytometry Assay**

Cells (0.5–1×10⁶) were resuspended in 100 μl FACS buffer (1% BSA/PBS) containing 1 μg F77 MAb and incubated on ice for 20 minutes. After being washed twice with FACS buffer, cells were incubated with FITC labeled goat anti-mouse antibody (1:100; Jackson ImmunoResearch) for 30 minutes on ice before analysis on a BD FACS Calibur using CellQuest Pro software. Cell sorting was carried out on FACSVantage SE with FACSDiVa Option-DiVa software in the Flow Cytometry and Cell Sorting Resource Laboratory, Penn Medicine Path BioResource.

**Example 5**

**MTT Assay**

Prostate cell lines PC3 and Du145 were aliquoted (1000 cells/well) into 96-well flat-bottom plates. The next day, the cells were treated with 1, 5, and 25 μg/ml F77 MAb or a murine IgG3 (negative control) in the presence of 1% or 10% mouse serum or human serum. Cells were incubated for 3 days at 37°C before the addition of 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) to each well, and absorbance was determined at 570 nm.

**Example 6**

**ADCC Assay**

Cytotoxicity was assayed by using the CytoTox 96® Non-Radioactive Cytotoxicity Assay (Promega). Effector U937 cells were treated with IFN-γ (100 unit/ml) for 12-24 hours and then washed 3 times with serum-free medium and incubated for 2 hours to allow detachment of IgG possibly absorbed from bovine serum, as described in Akiyama, Y., et al., 1984, Cancer Res., 44, 5127-5131, incorporated by reference herein. Prostate cancer cell lines PC3 and Du145 were target cells, while A431 was the control cell line. For ADCC assays, F77 MAb was incubated with target cells (10⁴/well) for 20 minutes before addition of effector cells. After 8-16 hours incubation at 37°C, 50 μl supernatant was removed from each well, transferred to an enzymatic assay plate, mixed with 50 μl reconstituted substrate mix, and incubated for 30 minutes at room temperature (protected from light). Stop solution (50 μl) was added to each well and absorbance at 490 nm recorded. Each test was performed in triplicate. The results are expressed as the percentage of lysis (ADCC%).

**Example 7**

Monoclonal Antibody F77 MAb Specifically Recognizes Prostate Cancer Cells and Tissues

**Flow cytometry analysis, performed as described in Example 3, of the murine IgG3 F77 MAb binding reveals that its targeting antigen is expressed at a high level on androgen-independent prostate cancer cell surfaces, but is undetectable on normal prostate epithelial cells or on epithelial cells derived from other tissues, including breast and skin.**

F77 MAb immunohistochemistry was performed, as described in Example 2, on a wide range of prostate normal and cancerous tissues. F77 MAb staining was significantly more intense in prostate cancerous tissues than in benign tissues, whereas F77 MAb only showed focal staining on a subpopulation of prostate glandular cells (Fig. 3A). Using primary prostate cancer tissue microarrays, we found that 112 of 116 (96.9%) prostate tissue cores were positive for F77 MAb staining (Fig. 3B). The negative cases were well-differentiated prostate carcinomas. Some minimal staining was observed in a small fraction of small blood vessels in human brain. In addition, 29 of 34 (85.3%) of metastatic prostate cancers stained positively with F77 MAb. No specific staining was found in normal or tumor tissues of human colon, kidney, cervix, pancreas, lung, skin and bladder (Fig. 2). These results indicate that the antigen of the F77 MAb is highly restricted to prostate and over-expressed in prostate tumors.
Example 8

The F77 MAb Positive Subpopulation of RWPE-1 Cells Displays Tumorigenic Phenotype

F77 MAb binds to a small population (<10%) of the non-tumorigenic human prostate epithelial cell line RWPE-1, but binds with greater intensity to >80% of tumorigenic RWPE-2 cells that were derived from RWPE-1 after transfection with the constitutively active Ki-ras oncogene (Fig. 1). RWPE-2 cells that express the antigen recognized by the F77 MAb grow faster and display enhanced colony-forming activity when compared to RWPE-1 cells which are not recognized by F77 MAb. Most importantly, the parent RWPE-1 cells do not form tumors when injected into nude mice, whereas RWPE-2 cells do. These data show that the F77 MAb and or an antibody that would compete for binding with F77 MAb, detects a cell surface biomarker indicative of malignant transformation of prostate cells such as RWPE-1.

Example 9

Cell Apoptosis Assay (Annexin V/Propidium Iodide)

To assay for apoptosis, tumor cells were first detached by trypsin/EDTA and then washed twice with RPMI medium. Cells were exposed to 3 or 30 μg/ml F77 MAb or appropriate controls in RPMI medium for 2-6 hours at 37°C, in a humidified incubator, 5% CO2. The cells (1x10^6) were then washed with cold PBS, stained for 15 minutes at room temperature in the dark with 5 μl Alexa Fluor® 488-Annexin V and 1 μl propidium iodide (Vybrant® Apoptosis Assay Kit #2, Invitrogen, V13241), and analyzed by dual color flow cytometry.

Example 10

The F77 Monoclonal Antibody Induces Apoptosis in Prostate Cancer Cells

PC3 cells exposed to F77 MAb for 4 hours were prepared counterstained with Annexin V and propidium iodide as described in Example 5 (Fig. 6A). Cells stained with Annexin V alone represent cells at an early-stage of apoptosis, whereas cells stained with both Annexin V and propidium iodide represent cells at a more advanced stage of apoptosis/necrosis. PC3 cells exposed to 3 μg/ml F77 MAb displayed 4% staining with Annexin V alone and 8% staining with Annexin V and propidium iodide, indicating that the interaction of F77 MAb with its cognate antigen on cells predisposes the cells to modest levels of apoptosis. Less than 1% of PC3 cells exposed to control murine IgG3 displayed staining with Annexin V alone, and there was no difference between F77 MAb and control IgG3 on control cell line A431.

Example 11

Monoclonal Antibody F77 Induces Complement Dependent and Antibody Dependent Cytotoxicity in Prostate Cancer Cells In Vitro and In Vivo

It has been reported that Mouse IgG3 antibodies can mediate both complement dependent cytotoxicity (CDC) and antibody dependent cellular cytotoxicity (ADCC), as described in Carter P J, 2006, Nat Rev Immunol, 6, 343-357, which is hereby incorporated by reference herein. F77 MAb-induced CDC was evaluated using cell viability as measured by MTT assays performed according to Example 5. Fig. 6B shows that the presence of 1% of either mouse or human serum as a source of complement remarkably decreased the number of viable cells. A 32% reduction of viable DU145 cells and 43% reduction of PC3 cells was observed in cells with 25 μg/ml F77 MAb in 1% mouse serum. Cells treated with F77 MAb in the absence of complement, i.e. cells grown in serum-free medium or in heat-inactivated serum, only show a limited decrease in cell viability (about 4%) in a standard MTT assay. Percent growth inhibition ([control wells-treated wells]/control wells)x100 increased in response to the addition of either mouse or human serum, indicating F77 MAb induced CDC.

Example 12

Xenograft Mouse Models for Measuring Prostate Tumors In Vivo

Tumor xenografts were generated by s.c. injection of 0.5x10^6 A431 or 1-2x10^6 PC3 or DU145 cells with or without Matrigel® (BD bioscience) in the flanks of male NCr
athanic-nu/nu mice (Charles River Laboratory, Wilmington, Mass.). Antibody i.p. injection started at day 7 or 11 after tumor cell implantation. Control mice were injected with irrelevant mouse IgG or PBS, which had no effect on tumor growth. Tumor size was determined by vernier caliper measurements, and the tumor volume was calculated as length x width x height (mm$^3$).

Example 13

**F77 MAB Inhibits Growth of Prostate Tumors In Vivo**

To determine if the effects of F77 MAB could inhibit androgen-independent prostate tumor growth in vivo, the antibody was given to mice transplanted with PC3 or Du145 tumor cells. In the first series of experiments, nude mice were injected with PC3 cells, to which F77 MAB binds, on the right flank and A431 cells, to which MAB does not bind, on the left flank. For each injection, PC3 (10$^6$ cells/mouse) and A431 cells (0.5x10$^6$ cells/mouse) were administered. Antibody injection (i.p.) started when tumors were first palpable, i.e. had reached a size of 2-4 mm$^3$, at day 7 after injection of tumor cells. F77 MAB was administrated 4 times (200 µg/dose at days 7 and 9, and 100 µg/dose at days 11 and 13). A mouse IgG negative control (200 µg/dose) or vehicle (PBS) was also used.

**PBS was used as the vehicle control. Mice were sacrificed when tumors were greater than 1 cm in diameter in accordance with the University of Pennsylvania’s IACUC.**

A 1x10$^6$ Du145 (MAB F77 or a control mouse IgG) was administered at Day 7 by i.p. injection of 200 µg/dose every other day for 4 times totally (C). Post-treatment (D) started at Day 11 when tumor volume was greater than 30 mm$^3$. (mean tumor volume (mm$^3$)±SEM. P<0.01)

**Treatment with F77 MAB inhibited PC3 tumor growth.** In fact, PC3 tumor growth was completely suppressed in 5 out of 6 mice in the F77 MAB treatment group. In the one mouse that developed a tumor, only a small tumor was palpated (volume<6 mm$^3$). Mice (n=6) in the vehicle control group all developed tumors with an average volume of 182.5 mm$^3$ at Day 28 (FIG. 7A). A431 tumors were not affected by F77 MAB treatment and grew aggressively with a mean size of 1200 mm$^3$ by day 28 when they had to be sacrificed (FIG. 7B). A photograph of typical results of F77 MAB treatment on PC3 and A431 tumors in vivo is shown in FIG. 8.

**Inhibition of tumor formation was also observed on Du145 tumors treated with F77 MAB. The Du145 group-I was treated following the same protocol as the PC3 group above. Administration of F77 MAB to Du145-injected mice resulted in complete inhibition of tumor growth until day 30 as compared with control mice treated with mouse IgG control (FIG. 7C).**

In Du145 group-II, the effectiveness of F77 MAB on larger established tumors was studied. 2x10$^6$ Du145 cells were injected in each mouse in this group (n=6). F77 MAB (200 µg/dose) was administrated every other day for a total of 4 injections, starting at Day 11 when the mean size of the Du145 tumor was about 35 mm$^3$. A significant reduction (P<0.01) in tumor-growth rate was detected in F77 MAB-treated mice as compared with control mice (FIG. 7D). At day 28, 10 days after the last antibody injection, tumors in the F77 MAB-treated mice reached a mean volume of 71.7 mm$^3$, whereas the control mice developed tumors with mean volume of 180.4 mm$^3$. Thus, these results demonstrate that F77 MAB is able to inhibit androgen-independent prostate tumor formation in both the PC3 and Du145 established tumor models.

**Example 14**

Isolation of Lipid Rafts and Extraction of the Antigen Bound by F77 MAB by Chloroform/Methanol

For the chloroform/methanol extraction, a 2x10$^6$ cell pellet was homogenized with 3 volumes of distilled water. The homogenate was poured into 10.8 volumes of methanol at room temperature and then 5.4 volumes of chloroform were added. The mixture was stirred for 30 minutes and then filtered. 3.5 volumes of water were added. The solvents were carefully mixed by turning the glass tube up and down several times. When the 2 phases were distinctly separated, the upper phase was collected and evaporated to dryness. The residue was resuspended in water or methanol for the subsequent analysis. Such extractions are described in Stevenholm L, et al., 1980, Biochim Biophys Acta, 617, 97-109, which is hereby incorporated by reference.

**Lipid raft microdomains on the cell membrane are resistant to mild detergent such as Triton X100 or CHAPS at 4°C, but are soluble in RIPA buffer.** Thus, to whether F77 MAB binds lipid rafts, the lipid rafts were isolated. To do so, cells (PC3, Du145, LNCAp, and negative control A431) were treated with 0.5% CHAPS buffer (25 mM Tris pH 7.5, 5 mM EDTA, 150 mM NaCl, containing 0.5% CHAPS) on ice for 30 minutes, and then lipid rafts were isolated by ultracentrifugation at 100,000g for 1 hour at 4°C. The CHAPS-insoluble pellets containing the rafts were solubilized in RIPA buffer (25 mM Tris pH 7.5, 5 mM EDTA, 150 mM NaCl, containing 0.1% SDS, 1% Triton X100 detergent, and 0.5% sodium deoxycholate).

**Example 15**

F77 MAB binds Lipid Raft Fractions

**The antigen bound by F77 MAB could not be resolved by conventional protein SDS-PAGE.** The F77 MAB antibody did not detect any protein bands in Western blotting. (Data not shown).

**Thus, detergent characterization was performed.** After immunoprecipitation with F77 MAB, samples were separated by SDS-PAGE (4-16%). A significant band that migrated faster than the dye front (approximately <51D) was detected in the PC3 sample by carbohydrate staining (Pro-Q© Emerald 300 Gel Stain Kit, Invitrogen), but was not stained by Coomassie blue (FIG. 9A). Pro-Q© Emerald 300 staining is based on the principle of periodic acid/Schiff staining as a way to detect carbohydrate chains in glycoproteins or glycolipids, as described in Taittiri R V, et al., 2007, J Biol Chem, 282, 4561-4572, which is incorporated by reference herein.

**Further identify the nature of the antigen recognized by F77 MAB, PPM (1-phenyl-2-palmitolymannosamine-3-morpholinol-1-propanol), a potent inhibitor of glycosphingolipid synthesis, was added to cell cultures at a concentration of 5 µg/ml or 20 µg/ml for 72 hours. To prevent glycosylation of glycoproteins, the cells were cultured for 48-72 hours with 2-4 mM O-glycosylation inhibitor benzyl-N-acetyl-alpha-galactosaminide (Benzyl-α-GalNAc; Sigma B4894) or 0.5-2 µg/ml N-glycosylation inhibitor Tunicamycin.
cin (Sigma), as described in Paul P., et al., 1992, Anal Biochem, 204, 265-272, which is incorporated by reference herein.

PPMP inhibits the synthesis of most glycosphingolipids by blockade of the enzyme glucosylceramide synthase, as described in Abe A., et al., 1992, J Biochem, 111, 191-196, which is hereby incorporated by reference. Glycosphingolipids are the most common glycolipids in mammals, as described in 21. Hakomori, S., 2003, Curr Opin Hema tol, 10, 16-24, which is hereby incorporated by reference. PPMP caused a significant dose-dependent decrease of F77 antigen on both PC3 and Du145 cell surfaces but did not have a specific effect on the glycoprotein CD147 (FIG. 9B). While the raw data appear to show an effect, the modest increase of CD147 levels was due to a decrease in cell viability after PPMP treatment. Two widely used inhibitors of protein glycosylation, Tunicamycin and Benzyl-β-D-galNAc, had no effect on F77 antigen expression (data not shown).

The data indicate that MAb F77 recognizes a unique prostate-specific glycolipid, termed PCLA (prostate cancer lipid-like antigen).

Moreover, glycolipid levels, as extracted in chloroform/methanol/water (1:2:1.4), of different cell lines were analyzed by lipid ELISA. For the assay, a four-fold dilution of the antigen samples was made in methanol. Immediately before addition to microplate wells, sample dilutions were mixed well with an equal volume of water. A 50 μl aliquot was then pipetted into each well of a 96-well flat bottom plate. The solvent was allowed to evaporate at room temperature for 12 hours. The plates were blocked by 1% BSA/PBS for 2 hours at RT. After 3 washes with PBS, F77 antibody or control mouse antibody was diluted with PBS, and 50 μl was added to each well. After 90 minutes of incubation, the plates were washed 3 times with PBS. Secondary antibody (1:5000 diluted HRP-goat anti-mouse, GE Healthcare) was then added and the plates were incubated for 1 hour at RT. The plates were washed 5 times; the color was developed with TMB (Sigma) and measured at 450 nm; such procedures are known in the art. F77 MAb displayed specific binding to the glycolipid extracts from PC3 and Du145 in a concentration-dependent manner, but there was no binding to extracts of control human A431 and HEK293 cells (FIG. 9C).

Recent studies have revealed that glycolipids in cell membranes are preferentially distributed into lipid microdomains, termed lipid rafts as described in Brown D.A., et al., 1998, Annu Rev Cell Dev Biol, 14, 111-136, which is incorporated by reference herein. Thus, the association of the glycolipid-like F77 antigen with lipid rafts on cell surfaces was studied. Lipid rafts can be isolated biochemically as detergent-insoluble fraction of cells which are treated with Triton X100 or CHAPS on ice. 0.5% CHAPS lysis buffer was used to treat prostate cancer cells after the surfaces were saturated with F77 MAb (~8 μg/10^6 cells). Since F77 MAb was pre-bound to the antigen in form antibody-antigen complexes prior to detergent treatment, protein G beads were used to capture the antibody-antigen complexes from the supernatant. The amount of F77 MAb was used as an indicator of detergent-soluble cognate antigens (FIG. 10).

Using this detection system, only 4% of F77 antigen was soluble in 0.5% CHAPS (FIG. 10), demonstrating that the glycolipid-like F77 antigen is in detergent-insoluble fractions (namely lipid rafts). RIPA buffer treatment can disrupt lipid rafts. Indeed, RIPA buffer releases a large percentage of F77 antigens (70%) from PC3 and (60%) from Du145 cell membranes. Taken together, these studies indicate that the F77 antigen is a glycolipid-like small molecule located in lipid raft microdomains on prostate cancer cell surfaces.

Example 16

Isolation and Immunodetection of Prostasomes

The cells were grown in 150 mm plates. When cells reached 80% confluence, they were washed twice with RPMI medium without FBS and then were maintained in serum-free RPMI medium for 24-48 hours. Cell death, as determined by trypan blue exclusion, was not observed under these conditions. Cell culture medium was collected and centrifuged at 10,000 g for 30 min to remove possible cell debris. The supernatant was subsequently subjected to ultracentrifugation using a SW40 rotor for 2 hours at 100,000 g to pellet the prostasomes. The prostasomes were resuspended in PBS.

Example 17

Immunoreactivity of F77 MAb to Prostasomes

PCLA was detected on prostasomes isolated from PC3 medium by a sandwich-ELISA and flow cytometry employing F77 MAb. For both experiments, prostasome pellets were isolated, as described in Example 16, from one 150 mm cell culture dish and resuspended in 100 μl PBS for subsequent studies. For the flow cytometry study, prostasome samples were incubated with 1 μg/100 μl DyLight-488 (Pierce, Rockford, Ill.) labeled F77 MAb (bold line) or mouse IgG3 control (dashed line) for 30 mins and then analyzed using a BD FACSCalibur (FIG. 11A). For the sandwich ELISA, plates were coated overnight with 5 μg/ml F77 MAb in coating buffer (0.1 M NaHCO3, pH 9.6). Additional binding sites were blocked by incubation with 200 μl/well of 1% BSA/PBS. Prostasome samples diluted with PBS (1:1-1:64) were then added. After 2 hours of incubation at room temperature, 1-5 μg/ml biotinylated-F77 MAb or control mouse IgG was added to wells. The plates were incubated for 1 hour at RT and washed 3 times with PBS. 1:3000 diluted streptavidin-HRP (GE Healthcare) was then added for 1 hour incubation (FIG. 11B). Together, these assays demonstrate that the F77 MAb is useful for a non-invasive detection using prostasome containing fluid, including semen or serum.

Example 18

Neoglycolipid (NGL)-Based Microarrays

The NGL technology involves the micro-scale conjugation of oligosaccharides to a lipid tag (Feizi, T., M. S. Stoll, et al. (1994). Methods Enzymol 230: 484-519; Chai, W., M. S. Stoll, et al. (2003), Methods Enzymol 362: 160-95). The resulting NGLs can be readily immobilized on matrices in the clustered state and probed for recognition by carbohydrate-binding proteins. For all intents and purposes NGLs behave like glycolipids. An advantage is that they can be arrayed and analysed side by side with glycosphingolipids. A microarray system based on this principle has been developed (Feizi, T. and W. Chai (2004). Nat Rev Mol Cell Biol 5(7): 582-8; Liu, Y., A. S. Palma, et al. (2009). Bioi Chem 390(7): 647-56). The current microarray encompasses ~500 NGLs derived from glycoproteins and glycosaminoglycans and diverse other mammalian type oligosaccharides. In addition natural and chemically synthesized predominantly mamma-
lian type glycolipids (~160) are included. The probes are robotically arrayed on nitrocellulose-coated glass slides and we have observed that the clustered display with an element of lateral mobility renders their presentation similar to that on cell surfaces.

[F0114] F77 MAb gave exclusive binding to the two blood group B-related probes having branched polyLacNAc backbone sequences, namely B-III dodecaosylceramide (position 226 in the array set) and B-IV tetradecaosylceramide (position 227 in the array set). This is consistent with our earlier findings in the initial screening experiments. The fucosé in these structures may be important, as the ‘B-like’ analogues (e.g. B-like decaosylceramide, position 209 in the array set) which have similar branched polyLacNAc backbones and the same lipid moieties were not recognized by F77 MAb.

[F0115] Each of the references cited herein are incorporated by reference in their entirety.

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35  40  45
Gly Ile Ile Trp Ala Gly Gly Asn Thr Asn Leu Asn Ser Thr Thr Lys
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Ser Arg Ala Thr Met Leu Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
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Gly Val His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile
35   40    45
Gly Ile Ile Trp Ala Gly Gly Asn Thr Aam Ile Aam Ser Thr Ala Lys
50   55    60
Ser Arg Ala Thr Met Leu Val Asp Thr Ser Lys Aam Gln Phe Ser Leu
65   70    75    80
Arg Leu Ser Ser Val Thr Ala Asp Thr Ala Val Tyr Tyr Cys Ala
95   90    95
Arg Asp Asp Tyr Ala Ala Met Asp Tyr Trp Gly Gln Gly Ser Leu Val
100  105   110
Thr Val Ser Ser
115

<210> SEQ ID NO 23
<211> LENGTH: 116
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 23
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1  5  10  15
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Thr Phe Thr Tyr Tyr
20 25 30
Gly Val His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile
35 40 45
Gly Ile Ile Trp Ala Gly Gly Asn Thr Asn Ile Asn Ser Thr Thr Lys
50 55 60
Ser Arg Ala Thr Met Leu Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80
Arg Leu Ser Ser Val Thr Ala Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95
Arg Asp Asp Tyr Ala Ala Met Asp Tyr Trp Gln Gly Gly Ser Leu Val
100 105 110
Thr Val Ser Ser
115

<210> SEQ ID NO 24
<211> LENGTH: 116
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 25
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1  5  10  15
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Thr Phe Thr Tyr Tyr
20 25 30
Gly Val His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile
35 40 45
Gly Ile Ile Phe Ala Gly Gly Asn Thr Asn Ile Asn Ser Thr Leu Lys
50 55 60
Ser Arg Ala Thr Met Leu Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80
Arg Leu Ser Ser Val Thr Ala Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95
Arg Asp Asp Tyr Ala Ala Met Asp Tyr Trp Gln Gly Gly Ser Leu Val
100 105 110
Thr Val Ser Ser
115

<210> SEQ ID NO 25
<211> LENGTH: 116
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 25
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1  5  10  15
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Thr Phe Thr Tyr Tyr
20 25 30
Gly Val His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile
35 40 45
Gly Ile Ile Phe Ala Gly Gly Asn Thr Asn Val Asn Ser Thr Leu Lys
50  55  60
Ser Arg Ala Thr Met Leu Val Asp Thr Ser Lys Asn Gln Phe Ser Leu 65  70  75  80
Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala 85  90  95
Arg Asp Asp Tyr Ala Ala Met Asp Tyr Trp Gly Gln Gly Ser Leu Val 100 105 110
Thr Val Ser Ser
115

<210> SEQ ID NO: 26
<211> LENGTH: 116
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 26
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1  5  10  15
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Thr Phe Thr Tyr Tyr
20  25  30
Gly Val His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Gln Trp Ile
35  40  45
Gly Ile Ile Phe Ala Gly Gly Asn Thr Asn Leu Asn Ser Thr Leu Lys
50  55  60
Ser Arg Ala Thr Met Leu Val Asp Thr Ser Lys Asn Gln Phe Ser Leu 65  70  75  80
Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala 85  90  95
Arg Asp Asp Tyr Ala Ala Met Asp Tyr Trp Gly Gln Gly Ser Leu Val 100 105 110
Thr Val Ser Ser
115

<210> SEQ ID NO: 27
<211> LENGTH: 116
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 27
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1  5  10  15
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Thr Phe Thr Tyr Tyr
20  25  30
Gly Val His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Gln Trp Ile
35  40  45
Gly Ile Ile Phe Ala Gly Gly Asn Thr Asn Leu Ile Asn Ser Thr Leu Lys
50  55  60
Ser Arg Ala Thr Met Leu Val Asp Thr Ser Lys Asn Gln Phe Ser Leu 65  70  75  80
Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala 85  90  95
ARG ASP ASP TYR ALA ALA MET ASP TYR TRP GLY GLN GLY SER LEU VAL 100 105 110

THR VAL SER SER 115

<210> SEQ ID NO 28
<211> LENGTH: 116
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 28

GLN VAL GLN LEU GLN GLU SER GLY PRO GLY LEU VAL ARG PRO SER GLN 1 5 10 15

THR LEU SER LEU THR CYS THR VAL SER GLY PHE THR PHE THR TYR TYR 20 25 30

GLY VAL HIS TRP VAL ARG GLN PRO GLY ARG GLY LEU GLU TRP ILE 35 40 45

GLY ILE ILE PHE ALA GLY ASN THR ASN TYR ASN SER THR GLY LYS 50 55 60

SER ARG ALA THR MET LEU VAL ASP THR SER LYS ASN GLN PHE SER LEU 65 70 75 80

ARG LEU SER SER VAL THR ALA ALA ASP THR ALA VAL TYR CYS ALA 85 90 95

ARG ASP ASP TYR ALA ALA MET ASP TYR TRP GLY GLN GLY SER LEU VAL 100 105 110

THR VAL SER SER 115

<210> SEQ ID NO 29
<211> LENGTH: 116
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 29

GLN VAL GLN LEU GLN GLU SER GLY PRO GLY LEU VAL ARG PRO SER GLN 1 5 10 15

THR LEU SER LEU THR CYS THR VAL SER GLY PHE THR PHE THR TYR TYR 20 25 30

GLY VAL HIS TRP VAL ARG GLN PRO GLY ARG GLY LEU GLU TRP ILE 35 40 45

GLY ILE ILE PHE ALA GLY ASN THR ASN TYR ASN SER THR ALA LYS 50 55 60

SER ARG ALA THR MET LEU VAL ASP THR SER LYS ASN GLN PHE SER LEU 65 70 75 80

ARG LEU SER SER VAL THR ALA ALA ASP THR ALA VAL TYR CYS ALA 85 90 95

ARG ASP ASP TYR ALA ALA MET ASP TYR TRP GLY GLN GLY SER LEU VAL 100 105 110

THR VAL SER SER 115

<210> SEQ ID NO 30
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1 5 10 15
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Thr Phe Thr Tyr Tyr
20 25 30
Gly Val His Trp Val Arg Gln Pro Gly Arg Gly Leu Glu Trp Ile
35 40 45
Gly Ile Ile Phe Ala Gly Gly Asn Tyr Asn Tyr Asn Ser Thr Ser Lys
50 55 60
Ser Arg Ala Thr Met Leu Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80
Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Cys Ala
85 90 95
Arg Asp Asp Tyr Ala Ala Met Asp Tyr Trp Gly Gln Gly Ser Leu Val
100 105 110
Thr Val Ser Ser
115

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1 5 10 15
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Thr Phe Thr Tyr Tyr
20 25 30
Gly Val His Trp Val Arg Gln Pro Gly Arg Gly Leu Glu Trp Ile
35 40 45
Gly Ile Ile Phe Ala Gly Gly Asn Tyr Asn Tyr Asn Ser Thr Ser Lys
50 55 60
Ser Arg Ala Thr Met Leu Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80
Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Cys Ala
85 90 95
Arg Asp Asp Tyr Ala Ala Met Asp Tyr Trp Gly Gln Gly Ser Leu Val
100 105 110
Thr Val Ser Ser
115

Arg Asp Asp Tyr Ala Ala Met Asp Tyr Trp Gly Gln Gly Ser Leu Val
100 105 110
Thr Val Ser Ser
115

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
1 5 10 15
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Thr Phe Thr Tyr Tyr
20 25 30
Gly Val His Trp Val Arg Gln Pro Gly Arg Gly Leu Glu Trp Ile
35 40 45
Gly Ile Ile Phe Ala Gly Gly Asn Tyr Asn Tyr Asn Ser Thr Ser Lys
50 55 60
Ser Arg Ala Thr Met Leu Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80
Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Cys Ala
85 90 95
Arg Asp Asp Tyr Ala Ala Met Asp Tyr Trp Gly Gln Gly Ser Leu Val
100 105 110
Thr Val Ser Ser
115
-continued

1    5    10   15
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Thr Phe Thr Tyr Tyr

20   25   30
Gly Val His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile

35   40   45
Gly Ile Ile Phe Ala Gly Gly Asn Thr Asn Val Asn Ser Thr Gly Lys

50   55   60
Ser Arg Ala Thr Met Leu Val Asp Thr Ser Lys Asn Val Asn Gly Phe Ser Leu

65   70   75   80
Arg Leu Ser Ser Val Thr Ala Asp Thr Ala Val Tyr Tyr Cys Ala

85   90   95
Arg Asp Asp Tyr Ala Ala Met Asp Tyr Trp Gly Gln Gly Ser Leu Val

100  105  110
Thr Val Ser Ser

115

<210> SEQ ID NO 33
<211> LENGTH: 116
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 33
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Glu
1    5    10   15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Thr Phe Thr Tyr Tyr

20   25   30
Gly Val His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile

35   40   45
Gly Ile Ile Phe Ala Gly Gly Asn Thr Asn Val Asn Ser Thr Gly Lys

50   55   60
Ser Arg Ala Thr Met Leu Val Asp Thr Ser Lys Asn Val Asn Gly Phe Ser Leu

65   70   75   80
Arg Leu Ser Ser Val Thr Ala Asp Thr Ala Val Tyr Tyr Cys Ala

85   90   95
Arg Asp Asp Tyr Ala Ala Met Asp Tyr Trp Gly Gln Gly Ser Leu Val

100  105  110
Thr Val Ser Ser

115

<210> SEQ ID NO 34
<211> LENGTH: 116
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 34
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Glu
1    5    10   15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Thr Phe Thr Tyr Tyr

20   25   30
Gly Val His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile

35   40   45
Gly Ile Ile Phe Ala Gly Gly Asn Thr Asn Val Asn Ser Thr Gly Lys
Ser Arg Ala Thr Met Leu Val Asp Thr Ser Lys Asn Gln Phe Ser Leu 65 70 75 80
Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala 85 90 95
Arg Asp Asp Tyr Ala Ala Met Asp Tyr Trp Gly Gln Gly Ser Leu Val 100 105 110
Thr Val Ser Ser 115

<210> SEQ ID NO 35
<211> LENGTH: 116
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 35
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln 1 5 10 15
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Thr Phe Thr Tyr Tyr 20 25 30
Gly Val His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile 35 40 45
Gly Ile Ile Phe Ala Gly Gln Asn Thr Asn Val Asn Ser Thr Thr Lys 50 55 60
Ser Arg Ala Thr Met Leu Val Asp Thr Ser Lys Asn Gln Phe Ser Leu 65 70 75 80
Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala 85 90 95
Arg Asp Asp Tyr Ala Ala Met Asp Tyr Trp Gly Gln Gly Ser Leu Val 100 105 110
Thr Val Ser Ser 115

<210> SEQ ID NO 36
<211> LENGTH: 116
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 36
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln 1 5 10 15
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Thr Phe Thr Tyr Tyr 20 25 30
Gly Val His Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Ile 35 40 45
Gly Ile Ile Phe Ala Gly Gln Asn Thr Asn Val Asn Ser Thr Gly Lys 50 55 60
Ser Arg Ala Thr Met Leu Val Asp Thr Ser Lys Asn Gln Phe Ser Leu 65 70 75 80
Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala 85 90 95
Arg Asp Asp Tyr Ala Ala Met Asp Tyr Trp Gly Gln Gly Ser Leu Val 100 105 110
Thr Val Ser Ser 115
-continued

Thr Val Ser Ser
  115

<210> SEQ ID NO 37
<211> LENGTH: 116
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 37

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
  1   5   10   15
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Thr Phe Thr Tyr Tyr
  20  25   30
Gly Val His Trp Val Arg Gln Pro Gly Arg Gly Leu Glu Trp Ile
  35  40   45
Gly Ile Ile Phe Ala Gly Gly Asn Thr Asn Leu Asn Ser Thr Ala Lys
  50  55   60
Ser Arg Ala Thr Met Leu Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
  65  70   75   80
Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
  85  90   95
Arg Asp Asp Tyr Ala Ala Met Asp Tyr Trp Gly Gln Gly Ser Leu Val
 100 105  110
Thr Val Ser Ser
  115

<210> SEQ ID NO 38
<211> LENGTH: 116
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 38

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln
  1   5   10   15
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Thr Phe Thr Tyr Tyr
  20  25   30
Gly Val His Trp Val Arg Gln Pro Gly Arg Gly Leu Glu Trp Ile
  35  40   45
Gly Ile Ile Phe Ala Gly Gly Asn Thr Asn Leu Asn Ser Thr Ala Lys
  50  55   60
Ser Arg Ala Thr Met Leu Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
  65  70   75   80
Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
  85  90   95
Arg Asp Asp Tyr Ala Ala Met Asp Tyr Trp Gly Gln Gly Ser Leu Val
 100 105  110
Thr Val Ser Ser
  115

<210> SEQ ID NO 39
<211> LENGTH: 116
<212> TYPE: PRT
ORGANISM: Artificial Sequence

FEATURE: OTHER INFORMATION: Synthetic construct

SEQUENCE:

Glin Val Glin Leu Gln Glu Ser Gly Pro Gly Val Val Val Arg Pro Ser Gin

...
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Thr Phe Thr Tyr Tyr
20 25 30
Gly Val His Trp Val Arg Gln Pro Pro Gly Gly Leu Glu Trp Ile
35 40 45
Gly Ile Ile Phe Ala Gly Gly Asn Thr Asn Ile Asn Ser Thr Ala Lys
50 55 60
Ser Arg Ala Thr Met Leu Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80
Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95
Arg Asp Asp Tyr Ala Ala Met Asp Tyr Trp Gly Gln Gly Ser Leu Val
100 105 110
Thr Val Ser Ser 115

SEQ ID NO 42
LENGTH: 116
TYPE: PRT
ORGANISM: Artificial Sequence
FEATURE:
OTHER INFORMATION: Synthetic construct

SEQ ID NO 43
LENGTH: 116
TYPE: PRT
ORGANISM: Artificial Sequence
FEATURE:
OTHER INFORMATION: Synthetic construct
Ser Arg Ala Thr Met Leu Val Asp Thr Ser Lys Asn Gln Phe Ser Leu  
65  70  75  80

Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala  
85  90  95

Arg Asp Asp Tyr Ala Ala Met Asp Tyr Trp Gly Gln Gly Ser Leu Val  
100 105 110

Thr Val Ser Ser  
115

<210> SEQ ID NO 44  
<211> LENGTH: 5  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic construct  

<400> SEQUENCE: 44  
Tyr Tyr Gly Val His  
1  5

<210> SEQ ID NO 45  
<211> LENGTH: 16  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic construct  

<400> SEQUENCE: 45  
Ile Ile Trp Ala Gly Gly Asn Thr Asn Ser Thr Leu Lys Ser  
1  5  10  15

<210> SEQ ID NO 46  
<211> LENGTH: 8  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic construct  

<400> SEQUENCE: 46  
Asp Asp Tyr Ala Ala Met Asp Tyr  
1  5

<210> SEQ ID NO 47  
<211> LENGTH: 16  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic construct  

<400> SEQUENCE: 47  
Ile Ile Trp Ala Gly Gly Asn Thr Asn Val Asn Ser Thr Leu Lys Ser  
1  5  10  15

<210> SEQ ID NO 48  
<211> LENGTH: 16  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetic construct  

<400> SEQUENCE: 48  
Ile Ile Trp Ala Gly Gly Asn Thr Asn Leu Asn Ser Thr Leu Lys Ser  
1  5  10  15
<210> SEQ ID NO 49
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 49

Ile Ile Trp Ala Gly Gly Asn Thr Asn Ile Asn Ser Thr Leu Lys Ser
1 5 10 15

<210> SEQ ID NO 50
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 50

Ile Ile Trp Ala Gly Gly Asn Thr Asn Tyr Asn Ser Thr Gly Lys Ser
1 5 10 15

<210> SEQ ID NO 51
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 51

Ile Ile Trp Ala Gly Gly Asn Thr Asn Tyr Asn Ser Thr Ala Lys Ser
1 5 10 15

<210> SEQ ID NO 52
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 52

Ile Ile Trp Ala Gly Gly Asn Thr Asn Tyr Asn Ser Thr Ser Lys Ser
1 5 10 15

<210> SEQ ID NO 53
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 53

Ile Ile Trp Ala Gly Gly Asn Thr Asn Tyr Asn Ser Thr Thr Lys Ser
1 5 10 15

<210> SEQ ID NO 54
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 54

Ile Ile Trp Ala Gly Gly Asn Thr Asn Val Asn Ser Thr Gly Lys Ser
1 5 10 15
<210> SEQ ID NO 55
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 55

Ile Ile Trp Ala Gly Gly Asn Thr Val Asn Ser Thr Ala Lys Ser
1  5  10  15

<210> SEQ ID NO 56
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 56

Ile Ile Trp Ala Gly Gly Asn Thr Leu Asn Ser Thr Ser Lys Ser
1  5  10  15

<210> SEQ ID NO 57
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 57

Ile Ile Trp Ala Gly Gly Asn Thr Asn Ile Asn Ser Thr Gly Lys Ser
1  5  10  15

<210> SEQ ID NO 58
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 58

Ile Ile Trp Ala Gly Gly Asn Thr Asn Ile Asn Ser Thr Ser Lys Ser
1  5  10  15

<210> SEQ ID NO 59
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 59

Ile Ile Trp Ala Gly Gly Asn Thr Asn Ile Asn Ser Thr Ala Lys Ser
1  5  10  15

<210> SEQ ID NO 60
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 60

Ile Ile Trp Ala Gly Gly Asn Thr Asn Ile Asn Ser Thr Ser Lys Ser
<210> SEQ ID NO 61
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 61
Ile Ile Trp Ala Gly Asn Thr Asn Ser Thr Lys Ser
1      5    10    15

<210> SEQ ID NO 62
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 62
Ile Ile Phe Ala Gly Asn Tyr Asn Ser Thr Leu Lys Ser
1      5    10    15

<210> SEQ ID NO 63
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 63
Ile Ile Phe Ala Gly Asn Val Asn Ser Thr Leu Lys Ser
1      5    10    15

<210> SEQ ID NO 64
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 64
Ile Ile Phe Ala Gly Asn Leu Asn Ser Thr Leu Lys Ser
1      5    10    15

<210> SEQ ID NO 65
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 65
Ile Ile Phe Ala Gly Asn Thr Asn Thr Leu Lys Ser
1      5    10    15

<210> SEQ ID NO 66
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 66
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<thead>
<tr>
<th>Sequence</th>
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<tr>
<td>Ile Ile Phe Ala Gly Gly Asn Thr Val Asn Ser Thr Ser Lys Ser</td>
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<tr>
<td>1 5 10 15</td>
</tr>
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<210> SEQ ID NO 73
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct
<400> SEQUENCE: 73
Ile Ile Phe Ala Gly Gly Asn Thr Val Asn Thr Thr Ser Lys Ser 1 5 10 15

<210> SEQ ID NO 74
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct
<400> SEQUENCE: 74
Ile Ile Phe Ala Gly Gly Asn Thr Val Leu Asn Ser Thr Gly Lys Ser 1 5 10 15

<210> SEQ ID NO 75
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct
<400> SEQUENCE: 75
Ile Ile Phe Ala Gly Gly Asn Thr Val Leu Asn Ser Thr Ala Lys Ser 1 5 10 15

<210> SEQ ID NO 76
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct
<400> SEQUENCE: 76
Ile Ile Phe Ala Gly Gly Asn Thr Val Leu Asn Ser Thr Ser Lys Ser 1 5 10 15

<210> SEQ ID NO 77
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct
<400> SEQUENCE: 77
Ile Ile Phe Ala Gly Gly Asn Thr Val Leu Asn Ser Thr Thr Lys Ser 1 5 10 15

<210> SEQ ID NO 78
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct
<400> SEQUENCE: 78
Ile Ile Phe Ala Gly Gly Asn Thr Asn Ile Asn Ser Thr Gly Lys Ser
1 5 10 15

<210> SEQ ID NO 79
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 79
Ile Ile Phe Ala Gly Gly Asn Thr Asn Ile Asn Ser Thr Ser Lys Ser
1 5 10 15

<210> SEQ ID NO 80
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 80
Ile Ile Phe Ala Gly Gly Asn Thr Asn Ile Asn Ser Thr Thr Lys Ser
1 5 10 15

<210> SEQ ID NO 81
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 81
Ile Ile Phe Ala Gly Gly Asn Thr Asn Ile Asn Ser Thr Thr Lys Ser
1 5 10 15

<210> SEQ ID NO 82
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 82
Lys Val Ser Asn Arg Phe Ser
1 5

<210> SEQ ID NO 83
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 83
Ser Gin Gly Thr His Ala Pro Phe Thr
1 5

<210> SEQ ID NO 84
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct
<400> SEQUENCE: 84
Cys Arg Ser Ser Gln Thr Leu Val His Ser Asn Gly Asn Thr Phe Leu
1 5 10 15

His

<210> SEQ ID NO 85
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 85
Cys Arg Ser Ser Gln Thr Leu Val His Ser Asn Gly Asn Thr Phe Leu
1 5 10 15
 Ala

<210> SEQ ID NO 86
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic construct

<400> SEQUENCE: 86
Cys Arg Ser Ser Gln Thr Leu Val His Ser Asn Gly Asn Thr Phe Leu
1 5 10 15
 Val

<210> SEQ ID NO 87
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: misc_feature
<223> OTHER INFORMATION: Xaa is His, Ala, or Val

<400> SEQUENCE: 87
Cys Arg Ser Ser Gln Thr Leu Val His Ser Asn Gly Asn Thr Phe Leu
1 5 10 15
 Xaa

<210> SEQ ID NO 88
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (3)...(3)
<223> OTHER INFORMATION: Xaa is Trp or Phe

<400> SEQUENCE: 88
Cys Arg Ser Ser Gln Thr Leu Val His Ser Asn Gly Asn Thr Phe Leu
1 5 10 15
 Xaa

<210> SEQ ID NO 89
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (14)...(14)
<223> OTHER INFORMATION: Xaa is Leu, Gly, Ala, Ser or Thr

<400> SEQUENCE: 89
Cys Arg Ser Ser Gln Thr Leu Val His Ser Asn Gly Asn Thr Phe Leu
1 5 10 15
 Xaa
1. A method of detecting the degree of differentiation of prostate cancer comprising contacting a sample of prostatic tissue with an anti-PCLA antibody, or functional fragment thereof, and correlating the amount of binding of said antibody to said sample with said degree of differentiation.

2. The method of claim 1 wherein the cancer is androgen independent.

3. The method of claim 1 wherein the cancer is metastatic.

4. The method of claim 1 wherein the sample is human.

5. The method of claim 1 wherein the degree of differentiation of prostate cancer is measured using a CDR, Fab, ScFv, or functional fragment of an anti-PCLA antibody.

6. The method of claim 1 wherein the antibody comprises at least one of a variable light chain nucleic acid sequence as set forth in SEQ ID NO:1 or a variable light chain nucleic acid sequence as set forth in SEQ ID NO:4.

7. A method of determining the degree of differentiation of prostate cancer comprising contacting prostasomes with an anti-PCLA antibody or functional fragment thereof and correlating the amount of binding of said antibody to said prostasomes with said degree of differentiation.

8. The method of claim 7 wherein the prostasomes are present in semen or serum.

9. A method of treating a subject having prostate cancer comprising assaying the degree of differentiation of said cancer and in response treating with an effective amount of an anti-PCLA antibody or functional fragment thereof.

10. (canceled)

11. (canceled)
12. (canceled)
13. (canceled)
14. (canceled)
15. A method of preferentially inducing cell death in primary or metastatic prostate cancer cells over non-cancerous prostate cells in a human, comprising administering a humanized antibody CDR, Fab, ScFv, or functional fragment thereof derived from the antibody having a variable light chain sequence as set forth in SEQ ID NO:1 and a variable heavy chain sequence as set forth in SEQ ID NO:4.
16. (canceled)
17. A method of inhibiting prostate tumor growth in a patient having said tumor comprising administering a humanized antibody CDR, Fab, ScFv, or functional fragment thereof derived from the antibody having a variable light chain sequence as set forth in SEQ ID NO:1 and a variable heavy chain sequence as set forth in SEQ ID NO:4.
18. An antibody derived from the antibody having a variable light chain sequence as set forth in SEQ ID NO:1 or a variable heavy chain sequence as set forth in SEQ ID NO:4.
19. The antibody of claim 18 further bound to PCLA.
20. The antibody bound to PCLA as set forth in claim 19, wherein the PCLA is on human prostatic cancer tissue.
21. The antibody bound to PCLA as set forth in claim 19, wherein the PCLA is present on metastatic or androgen independent human prostatic cancer tissue.
22. An antibody or antibody functional fragment that binds to B-III dodecaosyleceramide or B-IV tetradecaosyleceramide; provided that the antibody is not F77 MAb.
23. The antibody or antibody functional fragment of claim 22 wherein said antibody or antibody functional fragment competes for binding with F77 MAb.
24. The antibody or antibody functional fragment of claim 23 wherein said antibody or antibody functional fragment competes for binding with F77 MAb to B-III dodecaosyleceramide or B-IV tetradecaosyleceramide.
25. (canceled)
26. (canceled)
27. The antibody or antibody functional fragment of claim 22 wherein said antibody or antibody functional fragment is humanized.
28. The antibody or antibody functional fragment of claim 22 wherein said antibody or antibody functional fragment is conjugated to a toxin or cytotoxic agent.
29. A method of detecting the degree of differentiation of prostate cancer comprising contacting a sample of prostatic tissue with the antibody or antibody functional fragment of claim 22, and correlating the amount of binding of said antibody to said sample with said degree of differentiation.
30. A method of determining the degree of differentiation of prostate cancer comprising contacting prostasomes with the antibody or antibody functional fragment of claim 22 and correlating the amount of binding of said antibody to said prostasomes with said degree of differentiation.
31. A method of treating a subject having prostate cancer comprising assaying the degree of differentiation of said cancer and in response treating with an effective amount of the antibody or antibody functional fragment of claim 22.
32. A method of preferentially inducing cell death in primary or metastatic prostate cancer cells over non-cancerous prostate cells in a human, comprising administering the antibody or antibody functional fragment of claim 22.
33. A method of inhibiting prostate tumor growth in a patient having said tumor comprising administering the antibody or antibody functional fragment of claim 22.

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