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TITRE : COMPOSITIONS COMPRENANT DES DERIVES DE LA DURAMYCINE IMPERMEANTS VIS-A-VIS DES PAROIS CELLULAIRES

TITLE: SELECTED ANTIBODIES AND DURAMYCIN PEPTIDES BINDING TO ANIONIC PHOSPHOLIPIDS AND AMINOPHOSPHOLIPIDS AND THEIR USE IN TREATING VIRAL INFECTIONS AND CANCER

ABRÉGÉ/ABSTRACT:
Disclosed are surprising discoveries concerning the role of anionic phospholipids and aminophospholipids in tumor vasculature and in viral entry and spread, and compositions and methods for utilizing these findings in the treatment of cancer and viral infections. Also disclosed are advantageous antibody, immunoconjugate and duramycin-based compositions and combinations that bind and inhibit anionic phospholipids and aminophospholipids, for use in the safe and effective treatment of cancer, viral infections and related diseases.
Abstract

Disclosed are surprising discoveries concerning the role of anionic phospholipids and aminophospholipids in tumor vasculature and in viral entry and spread, and compositions and methods for utilizing these findings in the treatment of cancer and viral infections. Also disclosed are advantageous antibody, immunoconjugate and duramycin-based compositions and combinations that bind and inhibit anionic phospholipids and aminophospholipids, for use in the safe and effective treatment of cancer, viral infections and related diseases.
CLAIMS

1. A composition comprising a purified antibody, or antigen-binding fragment or immunoonjugate thereof, wherein said antibody binds to phosphatidylserine and effectively competes with the monoclonal antibody 3G4 (deposited as ATCC PTA 4545) for binding to phosphatidylserine.

2. The composition of claim 2, wherein said antibody further binds to phosphatidic acid, phosphatidylinositol, phosphatidylglycerol and cardiolipin and effectively competes with the monoclonal antibody 3G4 (deposited as ATCC PTA 4545) for binding to each of phosphatidic acid, phosphatidylinositol, phosphatidylglycerol and cardiolipin.

3. The composition of claim 1, wherein said antibody further binds to phosphatidylethanolamine.

4. The composition of claim 1, wherein said antibody has substantially the same phospholipid binding profile as the monoclonal antibody 3G4 (deposited as ATCC PTA 4545) as set forth in Table 4.

5. The composition of claim 1, wherein said antibody has an affinity for phosphatidylserine of at least equal to the affinity of the monoclonal antibody 3G4 (deposited as ATCC PTA 4545) for phosphatidylserine as set forth in Table 3.

6. The composition of claim 1, wherein said antibody has substantially the same phospholipid binding profile as the monoclonal antibody 3G4 (deposited as ATCC PTA 4545), as set forth in Table 4, and has an affinity for phosphatidylserine of at least equal to the affinity of the monoclonal antibody 3G4 (deposited as ATCC PTA 4545) for phosphatidylserine, as set forth in Table 3.

7. The composition of claim 1, wherein said antibody is prepared by a process comprising immunizing an animal with activated endothelial cells and selecting from the immunized animal an antibody that binds to phosphatidylserine and effectively competes with the monoclonal antibody 3G4 (deposited as ATCC PTA 4545) for binding to phosphatidylserine.

8. The composition of any one of claims 1 to 7, wherein said antibody is operatively attached to a biological, therapeutic or diagnostic agent.

9. The composition of claim 8, wherein said antibody is operatively attached to a cytotoxin, chemotherapeutic agent, radiotherapeutic agent, anti-angiogenic agent, apoptosis-inducing agent, steroid, antimeatabolite, anti-tubulin drug, combretastatin, antibiotic, cytokine, alkylating agent or coagulant.

10. The composition of claim 9, wherein said antibody is operatively attached to TNFα, IL-12 or LEC.
11. The composition of claim 8, wherein said antibody is operatively attached to an antiviral agent.

12. The composition of any one of claims 1-11, wherein said antibody is a monoclonal antibody or antigen-binding fragment thereof.

13. The composition of any one of claims 1-12, wherein said antibody is an scFv, Fv, Fab', Fab, diabody, linear antibody or F(ab')2 antigen-binding fragment of an antibody or a CDR, univalent fragment, camelized or single domain antibody.

14. The composition of any one of claims 1-13, wherein said antibody is a human, humanized, part-human, chimeric, bispecific, recombinant or engineered antibody or antigen-binding fragment thereof.

15. The composition of any one of claims 1-14, wherein said antibody comprises at least a first variable region that includes an amino acid sequence region having the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, or a variant or mutagenized form of the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, wherein said variant or mutagenized form maintains binding to phosphatidylserine.

16. The composition of any one of claims 1-15, wherein said antibody is the monoclonal antibody 3G4 deposited as ATCC PTA 4545.

17. The composition of any one of claims 1-16, wherein said composition is a stealthed or PEGylated liposome composition that is coated with said antibody or an antigen-binding fragment or immunoconjugate thereof.

18. The composition of claim 17, wherein said stealthed or PEGylated liposome contains an additional therapeutic agent.

19. The composition of any one of claims 1-18, wherein said composition is a pharmaceutically acceptable composition.

20. The composition of any one of claims 1-19, wherein said composition further comprises an additional therapeutic agent.

21. The composition of claim 20, wherein said additional therapeutic agent is an anti-angiogenic agent, anti-cancer agent or anti-viral agent.

22. The composition of claim 21, wherein said additional therapeutic agent is docetaxel.

23. The composition of claim 11 or 21, wherein said anti-viral agent is a nucleoside, reverse transcriptase inhibitor or protease inhibitor.

24. The composition of claim 23, wherein said anti-viral agent is cidofovir or AZT.

25. The composition of any one of claims 1-24, for use in therapy.
26. The composition of any one of claims 1-18, for use in inhibiting angiogenesis.

27. The composition of any one of claims 1-18, for use in treating or preventing cancer.

28. The composition of any one of claims 11, 21 or 23, for use in treating or preventing a viral infection.

29. Use of a composition in accordance with any one of claims 1-18 in the manufacture of a medicament for treating or preventing a disease by inhibiting angiogenesis.

30. Use according to claim 29, wherein said medicament is for treating or preventing macular degeneration, arthritis, arthritis, atherosclerosis, diabetic retinopathy, thyroid hyperplasia, Grave's disease, hemangioma, neovascular glaucoma, psoriasis or cancer.

31. Use of a composition in accordance with any one of claims 1-18 in the manufacture of a medicament for treating or preventing cancer.

32. Use of a composition of any one of claims 11, 21 or 23 in the manufacture of a medicament for treating or preventing a viral infection or disease.

33. Use according to claim 32, wherein said medicament inhibits viral replication.

34. Use according to claim 32, wherein said medicament inhibits viral spread.

35. Use according to any one of claims 32-34, wherein said medicament is for treating or preventing a CMV, RSV, hepatitis, influenza, HIV, herpes, paramyxovirus or arenavirus infection.

36. Use according to any one of claims 32-34, wherein said medicament is for treating or preventing hepatitis, influenza, AIDS, viral pneumonia or respiratory disease or Lassa fever.
FIG. 3
FIG. 5
FIG. 7
FIG. 8G
FIG. 11A

FIG. 11B
**FIG. 13A. DLB**

Duramycin — NH·CO·(CH₂)₅·NH·CO — biotin

\[\begin{align*}
+ & \quad \text{NH₂·Cl} \\
\text{\|} \\
- & 
\end{align*}\]

**FIG. 13B. DIB**

Duramycin — NH·C·(CH₂)₃·S·CH₂·CO·NH·(CH₂)₆·NH·CO — biotin

**FIG. 13C. (DLB)₄NA**

\[\begin{align*}
\text{Dur} & \quad \text{B} \\
\text{B} & \quad \text{Dur} \\
\text{Dur} & \quad \text{B} \\
\text{B} & \quad \text{Dur} \\
\text{Dur} & \quad \text{B} \\
\text{B} & \quad \text{Dur} \\
\text{Dur} & \quad \text{B} \\
\text{B} & \quad \text{Dur} \\
\end{align*}\]

NA = neutravidin

\(\text{B} = \text{biotin}\)

\(\text{Dur} = \text{Duramycin}\)

\(\text{Dur} \quad \text{B} = \text{DLB}\)
FIG. 13D. (DLB)$_4$NA-F

NA = neutravidin  
$\mathbb{B}$ = biotin  
Dur = Duramycin

Dur $\equiv$ $\mathbb{B}$ = DLB  
$\mathbb{F}$ = fluorescein

FIG. 13E. (DIM)$_n$ H1gG

$\varepsilon$-NH$_2$-CO-CH$_2$-N$_2^+$

S. (CH$_2$)$_3$. C. NH — Duramycin

$n = 5$ to 8 Duramycin residues per IgG  
Monomer (150,000 Da) is shown
n = 5 to 8 Duramycin residues per IgG

$\text{F} = \text{fluorescein}$

Monomer (150,000 Da) is shown

$\text{B} = \text{biotin}$

Monomer (150,000 Da) is shown

FIG. 13F. (DIM)$_n$ HlgG-F

FIG. 13G. (DIM)$_n$ HlgG-B
**FIG. 13N. DS-5**

Duramycin $\text{NH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{SO}_3^-$

**FIG. 13O. DC-1**

Duramycin $\text{NH} \cdot \text{C} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{SS}$

- $\text{COO}^-$
- $\text{NO}_2$
Lanthionine
Ala-S-Ala

cystine

β-methylanthionine
Abu-S-Ala

Lysinoalanine
Ala-NH-Lys

FIG. 13Q
FIG. 13R

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{O} \\
\text{N} & \quad \text{CH}_2\text{P} & \text{OH} & \text{CH} & \text{C} & \text{O} & (\text{CH}_2)_n & \text{C} & \text{Duramycin} \\
\text{Cidofovir} & \quad \text{R} & \text{CH}_3
\end{align*}
\]

R = OH, as in cidofovir, or labile hydrophobic group
FIG. 15
SELECTIVE INHIBITION OF DIVIDING ENDOTHELIAL CELLS BY ANTI-PS ANTIBODIES

Confluent
Subconfluent

% Control

anti-PS antibodies

3SB  9D2  3G4

FIG. 16
FIG. 18A

3G4-2BVH original sequence:

```
121
T T G V H S E V Q L Q Q P E L E K P

181
ACT ACA GGT GTC CAC TCT GAG GTC CAG CAG CAG TCT GGA CCT GAG CTG GAG AAG CCT
TGA TGT CCA CAG AGT AGA AGT CTC GAG GTC GAC GTC AGA CCT GGA CTC GAC CTC TGC GGA

241
GAC GGT TCA TCT GAG AGT CTA TCC TCT AGG GCT TCT GCT TCA TCA ACT GGC TCC TCT AGG AAG
CGC TCA TCT GCC AGT AGC TTG AGG AGG AGC AGG ACG CTA CAG AGG TCG ATG TAC TGC

301
AAG TCG GTC AAA CAG AGC CTT GGA AGA ACT GCT AGT TTA GTA CTG ACG TGG ACA ATG

361
TAT GGT GAT ACT TAC TTG ACC AGG GGT ACC AGC AGA GTG GCA TCA ATG AGA TTA CTC
ATC GCA CTA TGA AGG ATG TGG TGC TCT TAC TGA AGC TCC GCC TCG TGG TAC AAG

421
TCC TCC AGC ACA CTA AGG AGG AGC AGC ACT GCT GCT GAG GCT TGC TGT TCA AGA GGT
TGC CAG TGG AAG AGT AGA ACT GCT AGT TGA GAC TCC CTC CTT AGT CTC TGC AGA AGT

481
TAC TGT GTA AAG GCT GGT TAC TGC GGG CAG TGG TAC TCT GAT GTC GGG GCA GGG ACC
ATG ACA CAT TTC CCC CCA ATG ATG CCC GTC ACC ATG AAG GTA CAG CAG CCC GGT CCC TGG

BstEII
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S V T T V S S A T T T A P S V Y F L V P

541
AAG TGC ACC GTC TCC TCA GAC ACA ACA GCC CAC CTC GCT TGC ATC GCC TCT CTC AGC AGC
TGG TCT TGG AGG AGT AGG AGC AGT CTC GTC TCT GCT AGG TCT AGT CTC TGC AGA AGT

601
GGA TCC CCC GGG CTG CAG CAG GTA ATC AAG CTT ATC GAT GGC TCC AGT GAC CTC GAC CTC GAG GGG
```

The RACE product 3G4-2BVH is cloned and grafted onto the human γ1 constant region at the BstEII site. Thus, it contains the mouse leader sequence and its VH is joined with the human CH1 sequence in the following way: leader3G4/VSS-AST...

Mouse Leader

```
1 MGWTWIFILL LSVTGTHSE VQLQSGTFLB EKPGASWKL CDSAGSYFTG
```

BstEII graft site

```
101 QLKLSTDSEDS AVYVCVKGY YGHYYFVWVAG AITTVTIVSS ASTKGPSVFPVL
151 APSSKSTSG 
```

↑mature protein

↓human γ1CH1
FIG. 18B

3G4-2BVL original sequence:

The RACE product 3G4-2BVL is grafted to human κ constant region at the BbsI site. Thus, it contains the mouse leader sequence and its VL is joined with IN the human CL1 sequence in the following way: leader/3G4-VL/TVF-IFP...
FIG. 20
FIG. 21
LOCALIZATION OF ch3G4 TO BLOOD VESSELS IN ORTHOTOPIC MDA-MB-435 TUMORS IN MICE

3G4

Control IgG

Merge

MECA32

Localised antibody

FIG. 22
435s-luc

- 10 nM docetaxel, 24 hr
- untreated

FIG. 25C
Binding of 3G4 to MDA-MB-231 to by FACS

FIG. 26

Human IgG

ch3G4

200

100

10^1

10^2

10^3
FIG. 27
(D-SIAB)_n HlgG effect on MethA tumor growth

mean tumor volume (nl ± S.E)

0 5 10 15 20 25 30
days after injection

DHlgG
HlgG

FIG. 31