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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
62/465,589	03/01/2017		260	1736P		

CONFIRMATION NO. 2950

FILING RECEIPT

125915
Boston Biomedical, Inc.
640 Memorial Drive, 5th floor
Cambridge, MA 02139

OC000000089786394

Date Mailed: 03/09/2017

Receipt is acknowledged of this provisional patent application. It will not be examined for patentability and will become abandoned not later than twelve months after its filing date. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

Chiang Jia Li, Cambridge, MA;
Youzhi LI, Westwood, MA;
Yuan GAO, Belmont, MA;
Wei LI, Wayland, MA;
Xiangao SUN, Chestnut Hill, MA;

Applicant(s)

Boston Biomedical, Inc., Cambridge, MA

Power of Attorney:
Christopher Southgate--54875

Permission to Access Application via Priority Document Exchange: No

Permission to Access Search Results: No

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

If Required, Foreign Filing License Granted: 03/08/2017

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 62/465,589**

Projected Publication Date: None, application is not eligible for pre-grant publication

Non-Publication Request: No

Early Publication Request: No

Title

PDL1-SPECIFIC AND BETA-CATENIN-SPECIFIC ASYMMETRIC INTERFERING RNA COMPOSITIONS, USES OR PREPARATION THEREOF

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as

set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

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The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The U.S. offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to promote and facilitate business investment. SelectUSA provides information assistance to the international investor community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop technology, manufacture products, deliver services, and grow your business, visit http://www.SelectUSA.gov or call +1-202-482-6800.

Doc Code: **TR.PROV**
Document Description: Provisional Cover Sheet (SB16)

PTO/SB/16 (11-08)
Approved for use through 05/31/2015. OMB 0651-0032
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE
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Provisional Application for Patent Cover Sheet

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c)

Inventor(s)

Inventor 1 — Remove

Given Name	Middle Name	Family Name	City	State	Country
Chiang	Jia	Li	Cambridge	MA	US

Inventor 2 — Remove

Given Name	Middle Name	Family Name	City	State	Country
Youzhi		Li	Westwood	MA	US

Inventor 3 — Remove

Given Name	Middle Name	Family Name	City	State	Country
Yuan		Gao	Belmont	MA	US

Inventor 4 — Remove

Given Name	Middle Name	Family Name	City	State	Country
Wei		Li	Wayland	MA	US

Inventor 5 — Remove

Given Name	Middle Name	Family Name	City	State	Country
Xiangao		Sun	Chestnut Hill	MA	US

All Inventors Must Be Listed – Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. — Add

Title of Invention	PDL1-SPECIFIC AND BETA-CATENIN-SPECIFIC ASYMMETRIC INTERFERING RNA COMPOSITIONS, USE, OR PREPARATION THEREOF
Attorney Docket Number (if applicable)	1736P

Correspondence Address

Direct all correspondence to (select one):

◉ The address corresponding to Customer Number	○ Firm or Individual Name
Customer Number	125915

PTO/SB/16 (11-08)
Approved for use through 05/31/2015. OMB 0651-0032
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.
● No.
Yes, the invention was made by an agency of the United States Government. The U.S. Government agency name is:
Yes, the invention was under a contract with an agency of the United States Government. The name of the U.S. Government agency and Government contract number are:

Doc Code: **TR.PROV**
Document Description: Provisional Cover Sheet (SB16)

PTO/SB/16 (11-08)
Approved for use through 05/31/2015. OMB 0651-0032
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

Entity Status
Applicant asserts small entity status under 37 CFR 1.27 or applicant certifies micro entity status under 37 CFR 1.29

○	Applicant asserts small entity status under 37 CFR 1.27
○	Applicant certifies micro entity status under 37 CFR 1.29. Applicant must attach form PTO/SB/15A or B or equivalent.
◉	No

Warning

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

Signature

Please see 37 CFR 1.4(d) for the form of the signature.

Signature	/Christopher Southgate/			Date (YYYY-MM-DD)	2017-03-01
First Name	Christopher	Last Name	Southgate	Registration Number (If appropriate)	54875

This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **This form can only be used when in conjunction with EFS-Web. If this form is mailed to the USPTO, it may cause delays in handling the provisional application.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or paten. Accordingly, pursuant to the requirements of the Act, please be advised that : (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, t o a n other federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

PTO/AIA/14 (12-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	1736P
	Application Number	
Title of Invention	PDL1-SPECIFIC AND BETA-CATENIN-SPECIFIC ASYMMETRIC INTERFERING RNA COMPOSITIONS, USES OR PREPARATION THEREOF	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Chiang	Jia	Li	

Residence Information (Select One) ● US Residency Non US Residency Active US Military Service

City	State/Province	Country of Residence
Cambridge	MA	US

Mailing Address of Inventor:

Address 1	8 Museum Way
Address 2	
City	Cambridge
State/Province	MA
Postal Code	02141
Country	US

Inventor 2 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Youzhi		LI	

Residence Information (Select One) ◉ US Residency Non US Residency Active US Military Service

City	State/Province	Country of Residence
Westwood	MA	US

Mailing Address of Inventor:

Address 1	142 Gay Street
Address 2	
City	Westwood
State/Province	MA
Postal Code	02090
Country	US

Inventor 3 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Yuan		GAO	

Residence Information (Select One) ◉ US Residency Non US Residency Active US Military Service

PTO/AIA/14 (12-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	1736P
	Application Number	
Title of Invention	PDL1-SPECIFIC AND BETA-CATENIN-SPECIFIC ASYMMETRIC INTERFERING RNA COMPOSITIONS, USES OR PREPARATION THEREOF	

City	Belmont	State/Province	MA	Country of Residence	US

Mailing Address of Inventor:

Address 1	23 Cowdin Street
Address 2	

City	Belmont	State/Province	MA
Postal Code	02478	Country	US

Inventor 4 — Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Wei		LI	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	Wayland	State/Province	MA	Country of Residence	US

Mailing Address of Inventor:

Address 1	19 Black Oak street
Address 2	

City	Wayland	State/Province	MA
Postal Code	01778	Country	US

Inventor 5 — Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Xiangao		SUN	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	Chestnut Hill	State/Province	MA	Country of Residence	US

Mailing Address of Inventor:

Address 1	121 Bonad Road
Address 2	

City	Chestnut Hill	State/Province	MA
Postal Code	02467	Country	US

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. — Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

☐ **An Address is being provided for the correspondence Information of this application.**

PTO/AIA/14 (12-13)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	1736P
	Application Number	
Title of Invention	PDL1-SPECIFIC AND BETA-CATENIN-SPECIFIC ASYMMETRIC INTERFERING RNA COMPOSITIONS, USES OR PREPARATION THEREOF	

Customer Number	125915		
Email Address	patent@bostonbiomedical.com	Add Email	Remove Email

Application Information:

Title of the Invention	PDL1-SPECIFIC AND BETA-CATENIN-SPECIFIC ASYMMETRIC INTERFERING RNA COMPOSITIONS, USES OR PREPARATION THEREOF		
Attorney Docket Number	1736P	**Small Entity Status Claimed**	☐
Application Type	Provisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	37	**Suggested Figure for Publication (if any)**	

Filing By Reference :

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	● Customer Number	US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	125915		

PTO/AIA/14 (12-13)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	1736P
	Application Number	
Title of Invention	PDL1-SPECIFIC AND BETA-CATENIN-SPECIFIC ASYMMETRIC INTERFERING RNA COMPOSITIONS, USES OR PREPARATION THEREOF	

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) or indicate National Stage entry from a PCT application. Providing this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the application number blank.

Prior Application Status		Remove	
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button. Add

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55(d). When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(h)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Remove

Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Access Code[i] (if applicable)

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button. Add

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

PTO/AIA/14 (12-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	1736P
	Application Number	
Title of Invention	PDL1-SPECIFIC AND BETA-CATENIN-SPECIFIC ASYMMETRIC INTERFERING RNA COMPOSITIONS, USES OR PREPARATION THEREOF	

Authorization to Permit Access:

☐ Authorization to Permit Access to the Instant Application by the Participating Offices

If checked, the undersigned hereby grants the USPTO authority to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the World Intellectual Property Office (WIPO), and any other intellectual property offices in which a foreign application claiming priority to the instant patent application is filed access to the instant patent application. See 37 CFR 1.14(c) and (h). This box should not be checked if the applicant does not wish the EPO, JPO, KIPO, WIPO, or other intellectual property office in which a foreign application claiming priority to the instant patent application is filed to have access to the instant patent application.

In accordance with 37 CFR 1.14(h)(3), access will be provided to a copy of the instant patent application with respect to: 1) the instant patent application-as-filed; 2) any foreign application to which the instant patent application claims priority under 35 U.S.C. 119(a)-(d) if a copy of the foreign application that satisfies the certified copy requirement of 37 CFR 1.55 has been filed in the instant patent application; and 3) any U.S. application-as-filed from which benefit is sought in the instant patent application.

In accordance with 37 CFR 1.14(c), access may be provided to information concerning the date o f filing this Authorization.

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant 1 Remove

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

Assignee	Legal Representative under 35 U.S.C. 117	Joint Inventor
● Person to whom the inventor is obligated to assign.	Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor :

If the Applicant is an Organization check here. ☒

Organization Name	Boston Biomedical, Inc.

PTO/AIA/14 (12-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	1736P
	Application Number	
Title of Invention	PDL1-SPECIFIC AND BETA-CATENIN-SPECIFIC ASYMMETRIC INTERFERING RNA COMPOSITIONS, USES OR PREPARATION THEREOF	

Mailing Address Information For Applicant:			
Address 1	640 Memorial Drive		
Address 2			
City	Cambridge	**State/Province**	MA
Country i	US	Postal Code	02139
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button. Add

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not subsitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication . An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

Remove

If the Assignee or Non-Applicant Assignee is an Organization check here. ☐

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

Mailing Address Information For Assignee including Non-Applicant Assignee:			
Address 1			
Address 2			
City		**State/Province**	
Country i		Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button. Add

PTO/AIA/14 (12-13)
Approved for use through 01/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	1736P
	Application Number	
Title of Invention	PDL1-SPECIFIC AND BETA-CATENIN-SPECIFIC ASYMMETRIC INTERFERING RNA COMPOSITIONS, USES OR PREPARATION THEREOF	

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ABSTRACT

[000186] The combination of PDL1-specific and β-catenin-specific asymmetric interfering RNAs (aiRNAs) is shown to silence β-Catenin/PD-L1 mRNA and protein in a dose-dependent manner in a wide variety of murine tumor models, including subcutaneous human tumor xenografts, orthotopic human liver and lung tumors, as well as syngeneic mouse colorectal, breast and lung tumors. Biodistribution analysis of fluorescence-labeled PDL1-specific and β-catenin-specific aiRNAs demonstrated that the delivery of aiRNAs to xenograft tumors occurs within 5 minutes of aiRNA administration and lasts at least 8 hours. In all rodent tumor models tested, the administration of PDL1-specific and β-catenin-specific aiRNAs results in a synergistic inhibition of tumor growth not only in β-Catenin over-expressing colorectal tumor models, such as SW480 and APC^{min}, but also in tumor models expressing normal amount of β-Catenin. The administration of PDL1-specific and β-catenin-specific asymmetric interfering RNAs (aiRNAs) is well tolerated and no signs of toxicity are observed even after repeated dosing.

PDL1-SPECIFIC AND β–CATENIN-SPECIFIC ASYMMETRIC INTERFERING RNA COMPOSITIONS, USES, OR PREPARATION THEREOF

BACKGROUND OF THE INVENTION

[0001] Immuno-oncology is a promising new area for cancer therapeutics that targets the specific immune evasion mechanisms that cancer cells use to avoid detection by the host immune system. These evasion mechanisms are the "checkpoints" of the immune system; specific cell-surface molecules that prevent the immune effectors from killing those cells that express them. The PD-1 immune checkpoint pathway is one such example of an immune checkpoint that has emerged as a critical mediator of immunosuppression in the local tumor microenvironment. The inhibitory co-receptor Programmed Death 1 (PD-1; also known as CD279), a member of the extended CD28/CTLA-4 family of T cell regulators, is expressed on immune cells, such as T, B and NK cells, whereas its ligand, the Programmed Cell Death Ligand 1 (PD-L1, also known as CD274 or B7-H1) is a cell surface glycoprotein expressed on the surface of tumor cells of solid tumors as well as on human tumor associated antigen presenting cells (APCs), e.g., dendritic cells and macrophages. The interaction of PD-L1 ligand on tumor cells with the PD-1 receptor on immune cells delivers an inhibitory signal to T lymphocytes that ultimately leads to T cell anergy and immune evasion.

[0002] The development of immune checkpoint inhibitors that prevent the activation of the PD-L1/PD-1 immune checkpoint pathway has resulted in unprecedented and prolonged disease control in about 20-30% of cancer patients with melanoma, non-small cell lung cancer, renal cancer, or head/neck cancer (reviewed by Lipson et al., Semin. Oncol. (2015) 42(4):587-600; Zou et al., Sci. Transl. Med. (2016) vol. 8, issue 328, pp. 328rv4). For example, ipilimumab, first approved in the United States in 2011, targets cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4); while nivolumab and pembrolizumab, both of which were first approved in the United States in 2014, target PD-1.

[0003] Despite this initial success, it remains unclear why 70-80% of cancer patients fail to respond to anti-PD-1 or anti-PD-L1 antibodies or why most patients with colorectal cancer, pancreatic cancer and other non-responsive tumor types are resistant to immune checkpoint

inhibitors. In addition, immune-related adverse, sometimes lethal, events associated with monoclonal antibody therapies have been observed which casts a significant doubt about their safety for long term use (see, for example, Johnson et al. (2016). "Fulminant Myocarditis with Combination Immune Checkpoint Blockade." New England Journal of Medicine 375(18): 1749-1755). Thus, there remains an unmet need for novel safe and effective anticancer compounds, compositions, and uses thereof in treating cancer.

SUMMARY

[0004] The present disclosure addresses such needs by providing a composition that combines a PD-L1-specific and β–catenin-specific asymmetric interfering RNAs (aiRNAs) for inhibiting signaling by the immune checkpoint molecule, PD-1 and Wnt/β-catenin in cells (e.g., tumor cells). In certain embodiments, the aiRNAs are highly effective at silencing PD-L1 and β–catenin mRNA and protein expression *in vivo* for several hours. In certain embodiments, the suppression of PD-L1 expression by tumor cells prevents the activation of the PD-1 immune checkpoint pathway in T cells which is in large part responsible for the down regulation of tumor cell-specific T cell cytotoxicity and the concurrent breakdown of the immune surveillance for oncogenic cells in cancer patients. Thus, silencing of PDL1 in tumor cells and cancer stem cells restores tumor cell-specific immune responses whereas the simultaneous silencing of β-catenin, a key intermediate in stemness signaling pathways, is predicted to prevent proliferation and metastasis of cancer stem cells. Surprisingly, the simultaneous administration of PDL1-specific and β–catenin-specific aiRNAs exhibits therapeutic synergy in the treatment of cancer

[0005] In certain embodiments, the β–catenin-specific asymmetric interfering RNA (aiRNA), comprises an antisense strand having 5'-terminal and 3'-terminal nucleotides that are 17, 18, 19, 20, or 21 nucleotides apart, and a sense strand comprising a 5'-terminal nucleotide that is complementary to a nucleotide of the antisense strand other than its 3'-terminal nucleotide and a 3'-terminal nucleotide that is complementary to a nucleotide of the antisense strand, wherein the antisense strand is at least 70% complementary with the sense strand, and wherein 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 or 23 nucleotides of the antisense strand are colinear with the

corresponding complementary nucleotides in a target nucleotide sequence chosen from SEQ ID NO. 180, 181, 182, 183, 184 or 185.

[0006] In certain embodiments, the PDL1-specific asymmetric interfering RNA (aiRNA) comprises an antisense strand comprising 5'-terminal and 3'-terminal nucleotides that are 17, 18, 19, 20, or 21 nucleotides apart, and a sense strand comprising a 5'-terminal nucleotide that is complementary to a nucleotide of the antisense strand other than its 3'-terminal nucleotide and a 3'-terminal nucleotide that is complementary to a nucleotide of the antisense strand, wherein the antisense strand is at least 70% complementary with the sense strand, and wherein 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 or 23 nucleotides of the antisense strand are colinear with the corresponding complementary nucleotides in a target nucleotide sequence chosen from SEQ ID NO. 167, 168, 169, 170, 171, 172 or 173.

[0007] In certain embodiments, at least 7 nucleotides of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 or 23 nucleotides of the β–catenin-specific aiRNA's antisense strand are contiguous and colinear with the corresponding complementary nucleotides in a target nucleotide sequence chosen from SEQ ID NO. 180, 181, 182, 183, 184 or 185.

[0008] In certain embodiments the 5'-terminal nucleotide of the β–catenin-specific aiRNA's sense strand is complementary to the first, second or third nucleotide adjacent to the 3'-terminal nucleotide of the antisense strand.

[0009] In certain embodiments, the 3'-terminal nucleotide of the β–catenin-specific aiRNA's sense strand is complementary to the first, second or third nucleotide adjacent to the 5'-terminal nucleotide of the antisense strand.

[00010] In certain embodiments, the 5'-terminal and 3'-terminal nucleotides of the β–catenin-specific aiRNA's sense strand are 13 nucleotides apart and the 5'-terminal and 3'-terminal nucleotides of the β–catenin-specific aiRNA's antisense strand are 19 nucleotides apart.

[00011] In certain embodiments, the nucleotide sequence of the antisense strand of the β–catenin-specific asymmetric interfering RNA that is colinear with the corresponding complementary nucleotides in the target nucleotide sequence has a GC content of about 23%,

about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34% or about 35%.

[00012] In certain embodiments, the nucleotide sequence of the antisense strand of the β–catenin-specific asymmetric interfering RNA that is colinear with the corresponding complementary nucleotides in the target nucleotide sequence has a GC content of about 36%, about 37%, about 38%, about 39%, about 40%, about 41%, about 42%, about 43% or about 44%.

[00013] In certain embodiments, the nucleotide sequence of the antisense strand of the β–catenin-specific asymmetric interfering RNA that is colinear with the corresponding complementary nucleotides in the target nucleotide sequence has a GC content of about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55%, about 56%, about 57% or about 58%.

[00014] In certain embodiments, the nucleotide sequence of the antisense strand of the β–catenin-specific asymmetric interfering RNA that is colinear with the corresponding complementary nucleotides in the target nucleotide sequence has a GC content of about 47%.

[00015] In certain embodiments, the present teachings provide a composition comprising PDL1-specific and β–catenin-specific asymmetric interfering RNAs, wherein the β–catenin-specific asymmetric interfering RNA is chosen from aiRNAs 263-305.

[00016] In certain embodiments, at least 7 nucleotides of the 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 or 23 nucleotides of the PDL1-specific aiRNA's antisense strand are contiguous and colinear with the corresponding complementary nucleotides in a target nucleotide sequence chosen from SEQ ID NO. 167, 168, 169, 170, 171, 172 or 173.

[00017] In certain embodiments, the 5'-terminal nucleotide of the PDL1-specific aiRNA's sense strand is complementary to the first, second or third nucleotide adjacent to the 3'-terminal nucleotide of the antisense strand.

[00018] In certain embodiments, the 3'-terminal nucleotide of the PDL1-specific aiRNA's sense strand is complementary to the first, second or third nucleotide adjacent to the 5'-terminal nucleotide of the antisense strand.

[00019] In certain embodiments, the 5'-terminal and 3'-terminal nucleotides of the PDL1-specific aiRNA's sense strand are 13 nucleotides apart. In certain embodiments, the 5'-terminal and 3'-terminal nucleotides of the PDL1-specific aiRNA's antisense strand are 19 nucleotides apart.

[00020] In certain embodiments, the nucleotide sequence of the antisense strand of the PDL1-specific asymmetric interfering RNA that is colinear with the corresponding complementary nucleotides in the target nucleotide sequence has a GC content of about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34% or about 35%.

[00021] In certain embodiments, the nucleotide sequence of the antisense strand of the PDL1-specific asymmetric interfering RNA that is colinear with the corresponding complementary nucleotides in the target nucleotide sequence has a GC content of about 36%, about 37%, about 38%, about 39%, about 40%, about 41%, about 42%, about 43% or about 44%.

[00022] In certain embodiments, the nucleotide sequence of the antisense strand of the PDL1-specific asymmetric interfering RNA that is colinear with the corresponding complementary nucleotides in the target nucleotide sequence has a GC content of about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55%, about 56%, about 57% or about 58%.

[00023] In certain embodiments, the nucleotide sequence of the antisense strand of the PDL1-specific asymmetric interfering RNA that is colinear with the corresponding complementary nucleotides in the target nucleotide sequence has a GC content of about 33%.

[00024] In certain embodiments, the present teachings provide a composition comprising a PDL1-specific and β–catenin-specific asymmetric interfering RNA, wherein the PDL1-specific asymmetric interfering RNA is chosen from aiRNAs 1-46.

[00025] In certain embodiments, the sense or antisense strand of each asymmetric interfering RNA comprises at least one modified nucleotide or its analogue.

[00026] In certain embodiments, a 2'-OH group of the at least one modified ribonucleotide or its analogue is replaced by H or a 2'-O-methyl group.

[00027] In certain embodiments, the at least one modified nucleotide or its analogue is a sugar-, backbone-, and/or base-modified ribonucleotide. In certain embodiments, the backbone-modified ribonucleotide comprises a modification in a phosphodiester linkage with another ribonucleotide, for example, to comprise a nitrogen or a sulfur heteroatom. In certain embodiments, the at least one modified nucleotide or its analogue comprises a phosphothioate group, an inosine or a tritylated base. In certain embodiments, the at least one modified nucleotide or its analogue is a sugar-modified ribonucleotide, wherein a 2'-OH group is replaced by H, OR, R, halo, SH, SR, NH_2, NHR, NR_2, or CN, and wherein each R is independently C_1-C_6 alkyl, alkenyl or alkynyl, and halo is F, Cl, Br, or I.

[00028] In certain embodiments, the PDL1-specific and/or β–catenin-specific aiRNA comprises a deoxyribonucleotide.

[00029] In certain embodiments, the composition comprises nanoparticles having PDL1-specific and β–catenin-specific aiRNAs.

[00030] In certain embodiments, the PDL1-specific and/or β–catenin-specific aiRNA is bound to a peptide, a protein, an antibody, a lipid, a nucleic acid, a carbohydrate, an oligonucleotide, cholesterol, or an aptamer.

[00031] In certain embodiments, the present disclosure provides a pharmaceutical composition comprising a PDL1-specific and β–catenin-specific aiRNAs and a lipid or a cholesterol molecule. In certain embodiments, the lipid or cholesterol molecule is conjugated to the PDL1-specific and/or β–catenin-specific aiRNA.

[00032] In certain embodiments, the PDL1-specific and/or the β–catenin-specific aiRNAs comprise a plurality of modified nucleotides or their analogues, each modified nucleotide or its analogue comprising a 2'-O-methyl or a 2'-fluorine group, and/or a phosphothioate or phosphodiester backbone.

[00033] In certain embodiments, the present disclosure provides a kit for silencing PDL1 and β–catenin gene expression in a cell comprising PDL1-specific and β–catenin-specific aiRNAs, a nanoparticle formulation and instructions for their use.

[00034] In certain embodiments, the present disclosure provides an expression vector comprising a nucleic acid sequence encoding the PDL1-specific and β–catenin-specific aiRNAs. The expression vector can be a viral, a eukaryotic, or a bacterial expression vector.

[00035] The present disclosure further provides an isolated cell comprising the expression vector. In certain embodiments, the present disclosure provides an isolated cell comprising PDL1-specific and β–catenin-specific aiRNAs. The cell can be a mammalian, avian, insect, yeast or bacterial cell.

[00036] In certain embodiments, the present disclosure provides a method for treating cancer in a subject in need thereof comprising administering an effective amount of the PDL1-specific and β–catenin-specific aiRNAs, wherein the combination of PDL1-specific and β–catenin-specific aiRNAs exhibits therapeutic synergy in the treatment of cancer.

[00037] In certain embodiments, the cancer can be an AIDS-Related cancer, a breast cancer, a cancer of the digestive/gastrointestinal tract, an endocrine and neuroendocrine cancer, a cancer of the eye, a genitourinary cancer, a germ cell cancer, a gynecologic cancer, a head and neck cancer, a hematologic cancer, a musculoskeletal cancer, a neurologic cancer, a respiratory/thoracic cancer, a skin cancer, a childhood cancer or a cancer of unknown primary. In other embodiments, the cancer is metastatic, recurrent or resistant to chemotherapy and/or radiation.

[00038] In certain embodiments, the PDL1-specific and β–catenin-specific aiRNAs can be administered systemically or locally. In certain embodiments, the PDL1-specific and β–catenin-specific aiRNAs are effective at inhibiting tumor growth.

[00039] In certain embodiments, the PDL1-specific and β–catenin-specific aiRNAs are effective at inducing cancer stem cell death.

[00040] In certain embodiments, the PDL1-specific and β–catenin-specific aiRNAs are effective at enhancing an immune response against tumor cells.

[00041] In certain embodiments, the cells express nuclear β–catenin. In certain embodiments, the cells do not express nuclear β–catenin.

[00042] In certain embodiments, the PDL1-specific and β–catenin-specific aiRNAs are delivered to tumor cells within 5 minutes of administration and inhibit PDL1 and β–catenin gene expression for at least 8 hours.

BRIEF DESCRIPTION OF THE DRAWINGS

[00043] Without being limited to any particular theory or analysis and with respect to the exemplified RNA molecules, FIG. 1A aligns exemplary β-catenin target nucleotide sequences (e.g. SEQ ID NOs. 186-262) with an exemplary full-length β-catenin mRNA sequence (SEQ ID NO. 179); FIG. 1B shows the exemplary full-length β-catenin mRNA (SEQ ID NO. 179) subdivided into 5 overlapping sequences (SEQ ID NOs.: 180-185), each of which can be targeted by a subset of the exemplary β-catenin-specific RNA molecules. FIG. 1C aligns the sense strands and antisense strands of exemplary β-catenin aiRNAs 263-305 with exemplary β-catenin target mRNA sequences; depicts the length of the sense and antisense strands, the number and % GC content of nucleotides in the antisense strand that are colinear with the corresponding complementary nucleotides of the β-catenin mRNA sequence (shaded in dark grey), and the length of the 5' and 3' overhangs of the antisense strand over the sense strand; and FIG. 1D sorts the exemplary β-catenin target nucleotide sequences (e.g. SEQ ID NOs. 186-262) of the β-catenin aiRNA molecules according to their % GC content.

[00044] Without being limited to any particular theory or analysis and with respect to the exemplified RNA molecules, FIG. 2A aligns exemplary PD-L1 target nucleotide sequences (e.g. SEQ ID NOs. 140, 141, 144, 147, 150, 155, 156, 158, 161 and 164) with an exemplary full-length PD-L1 (SEQ ID NO. 166); FIG. 2B shows the exemplary full-length PD-L1 mRNA (SEQ ID NO. 166) subdivided into 7 overlapping sequences (SEQ ID NOs.: 167-173), each of which can be targeted by a subset of the exemplary RNA molecules. FIG. 2C aligns the sense strands (SEQ ID NOs. 1-46) and antisense strands (SEQ ID NOs. 47-92) with exemplary PD-L1 target mRNA sequences (SEQ ID NOs. 140-164); depicts the length of the sense and antisense strands, the number and % GC content of nucleotides in the antisense strand that are colinear with the

corresponding complementary nucleotides of the PD-L1 mRNA sequence (shaded in dark grey), and the length of the 5' and 3' overhangs of the antisense strand over the sense strand; and portrays the exemplary PD-L1 5'-terminal (SEQ ID NO. 139) and 3' terminal (SEQ ID NO. 165) mRNA sequences, and FIG. 2D sorts the exemplary target nucleotide sequences of PDLI-specific aiRNAs 1-46 according to their % GC content.

[00045] The features and advantages of the present teachings may be more readily understood by those of ordinary skill in the art upon reading the following detailed description. It is to be appreciated that certain features of the present teachings that are, for clarity reasons, described above and below in the context of separate embodiments, may also be combined to form a single embodiment and that various features of the present teachings that are, for brevity reasons, described in the context of a single embodiment, may also be combined so as to form sub-combinations thereof. Embodiments identified herein as exemplary or preferred are intended to be illustrative and not limiting.

[00046] The methods and techniques of the present teachings are generally performed according to conventional methods well known in the art and as described in certain general and more specific references that are cited and discussed throughout the present teachings unless otherwise indicated. See, e.g., M.R. Green and J. Sambrook, Molecular Cloning: A Laboratory Manual, 4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2012), Ausubel *et al.*, Current Protocols, John Wiley & Sons, Inc. (2000-2016), Antibodies: A Laboratory Manual, 2nd edition, edited by Edward A. Greenfield, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2014), and RNA: A Laboratory Manual by Rio *et al.*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2011), all of which are incorporated herein by reference.

[00047] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. In case of conflict, the present teachings, including definitions, will control. Methods and materials are described herein for use in the present teachings; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting.

[00048] As used herein, β-catenin, also referred to in the art as Catenin Beta 1, Catenin, Cadherin-Associated Protein Beta 1 (88kDa), CTNNB, Catenin Beta-1, Beta-Catenin, Armadillo or MRD19, is a structural component of cadherin-based adherens junctions that are necessary for the creation and maintenance of epithelial cell layers by regulating cell growth and adhesion between cells. β-catenin is also a key nuclear effector of canonical Wnt signaling in the nucleus (reviewed in Valenta *et al.* EMBO J. 2012 13;31(12):2714-36). In the absence of Wnt, β-catenin forms a complex with AXIN1, AXIN2, APC, CSNK1A1 and GSK3B that promotes phosphorylation on its N-terminal Ser and Thr residues and the ubiquitination of β-catenin via BTRC which leads to its subsequent degradation by the proteasome. In the presence of Wnt ligand, β-catenin is not ubiquitinated and accumulates in the nucleus, where it acts as a coactivator for transcription factors of the TCF/LEF family, leading to the activation of Wnt responsive genes. β-catenin also blocks apoptosis of malignant kidney and intestinal epithelial cells and promotes their anchorage-independent growth by down-regulating DAPK2. Mutations in β-catenin are a cause of colorectal cancer (CRC), hepatocellular carcinoma, pilomatrixoma (PTR), medulloblastoma (MDB), and ovarian cancer.

[00049] In certain embodiments, a β-catenin expressed nucleotide sequence (e.g., mRNA) refers to a nucleotide sequence comprising at least 25 nucleotides of Homo sapiens catenin beta 1 (CTNNB1) isoform 1, transcript variant 3 having the nucleotide sequence of Accession No. NM_001904.3 or transcript variant 2 having the nucleotide sequence of Accession No: NM_001098209.1 or transcript variant 3 having the nucleotide sequence of Accession No. NM_001098210.1. Transcript variants 1, 2 and 3 encode the same catenin beta-1 isoform 1. Transcript variant 1 represents the longest transcript. Transcript variants 2 and 3 differ in the 3' UTR compared to variant 1.

[00050] In certain embodiments, a β-catenin expressed nucleotide sequence (e.g., mRNA) refers to a nucleotide sequence comprising at least 25 nucleotides of Homo sapiens catenin beta 1 (CTNNB1) isoform 2, transcript variant 4 having the nucleotide sequence of Accession No. NM_001330729.1. Transcript variant 4 differs in the 5' and 3' UTRs and the 5' coding region and initiates translation at a downstream start codon, compared to variant 1. It encodes isoform 2, which has a shorter N-terminus, compared to isoform 1.

[00051] In certain embodiments, a β-catenin expressed nucleotide sequence (e.g., mRNA) refers to a nucleotide sequence comprising at least 25 nucleotides of Homo sapiens catenin beta 1 (CTNNB1) transcript variant X1 having the nucleotide sequence of Accession No. XM_005264886.2, transcript variant X2 having the nucleotide sequence of Accession No. XM_017005738.1, transcript variant X3 having the nucleotide sequence of Accession No. XM_006712983.1, transcript variant X4 having the nucleotide sequence of Accession No. XM_006712984.1 or transcript variant X5 having the nucleotide sequence of Accession No. XM_006712985.1.

[00052] Human β-catenin expressed nucleotide sequences (e.g. mRNAs) are transcribed from the Homo sapiens catenin beta 1 (CTNNB1) gene that is located on chromosome 3 with a nucleotide sequence of NCBI Reference Sequence: NG_013302.2.

[00053] As used herein, PD-L1 refers to a ligand of PD-1, also referred to in the art as CD274 molecule, CD274 antigen, B7 homolog, Programmed Cell Death 1 Ligand 1, PDCD1 ligand, PDCD1LG1, PDCD1L1, PD-L1, B7H1, PDL1, Programmed Death Ligand 1, B7-H1 or B7-H. The PD-L1 gene encodes an immune inhibitory receptor ligand that is expressed by hematopoietic and non-hematopoietic cells, such as T cells and B cells and various types of tumor cells. The encoded protein is a type I transmembrane protein that has immunoglobulin V-like and C-like domains. Interaction of this ligand with its receptor inhibits T-cell activation and cytokine production. During infection or inflammation of normal tissue, this interaction is important for preventing autoimmunity by maintaining homeostasis of the immune response. In tumor microenvironments, this interaction provides an immune escape for tumor cells through cytotoxic T-cell inactivation. Expression of this gene in tumor cells is considered to be prognostic in many types of human malignancies, including colon cancer and renal cell carcinoma. Alternative splicing results in at least 4 transcript variants. Other diseases associated with CD274 include, for example, lymphoepithelioma-like carcinoma and Paget's disease.

[00054] In certain embodiments, a PD-L1 expressed nucleotide sequence refers to a nucleotide sequence comprising at least 25 nucleotides of Homo sapiens CD274 molecule (CD274), transcript variant 1 (3,691 bp linear mRNA; NCBI Reference Sequence:

NM_014143.3) having the sequence of SEQ ID No.: 166. Transcript variant 1. This variant represents the longest transcript and encodes the longest isoform (a).

[00055] In certain embodiments, a PD-L1 expressed nucleotide sequence can refer to a nucleotide sequence comprising at least 25 nucleotides of Homo sapiens CD274 molecule (CD274), transcript variant 2 (3,349 bp linear mRNA; Accession: NM_001267706.1). This variant lacks an alternate in-frame exon in the 5' coding region, compared to variant 1 which results in a shorter protein (isoform b), compared to isoform a.

[00056] In certain embodiments, a PD-L1 expressed nucleotide sequence can refer to a nucleotide sequence comprising at least 25 nucleotides of Homo sapiens CD274 molecule (CD274), transcript variant 3 (3,518 bp linear transcribed-RNA; Accession: NR_052005.1).

[00057] In certain embodiments, a PD-L1 expressed nucleotide sequence can refer to a nucleotide sequence comprising at least 25 nucleotides of Homo sapiens CD274 molecule (CD274), transcript variant 4 (907 bp linear mRNA; Accession: NM_001314029.1).

[00058] Human PD-L1 expressed nucleotide sequences (e.g. mRNAs) are transcribed from the Homo sapiens CD274 gene that is located on chromosome 9 with a nucleotide sequence of NC_000009.12 (REGION: 5450503..5470567 GPC_000001301).

[00059] Unless specifically stated otherwise, references made in the singular may also include the plural. For example, “a” and “an” may refer to either one or one or more.

[00060] The phrase “and/or,” as used herein in the present teachings and in the claims, should be understood to mean “either or both” of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Thus, as a non-limiting example, a reference to “A and/or B”, when used in conjunction with open-ended language such as “comprising” can refer, in one embodiment, to A only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

[00061] When a range of values is listed herein, it is intended to encompass each value and sub-range within that range. For example, “1-5 nucleotides” is intended to encompass 1 nucleotide, 2 nucleotides, 3 nucleotides, 4 nucleotides, 5 nucleotides, 1-2 nucleotides, 1-3

nucleotides, 1-4 nucleotides, 1-5 nucleotides, 2-3 nucleotides, 2-4 nucleotides, 2-5 nucleotides, 3-4 nucleotides, 3-5 nucleotides, or 4-5 nucleotides.

[00062] When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below those numerical values. In general, the term "about" is used herein to modify a numerical value above and below the stated value by a variance of 20%, 10%, 5%, or 1%. In some embodiments, the term "about" is used to modify a numerical value above and below the stated value by a variance of 10%. In certain embodiments, the term "about" is used to modify a numerical value above and below the stated value by a variance of 5%. In certain embodiments, the term "about" is used to modify a numerical value above and below the stated value by a variance of 1%.

[00063] The term "RNA" as used herein refers to its generally accepted meaning in the art. Generally, the term RNA refers to a molecule comprising at least one ribofuranoside moiety. The term can include double-stranded RNA, single-stranded RNA, and isolated RNA such as partially purified RNA, essentially pure RNA, synthetic RNA, recombinantly produced RNA, as well as altered RNA that differs from naturally occurring RNA by the addition, deletion, substitution and/or alteration of one or more nucleotides. Such alterations can include addition of non-nucleotide material, such as to the end(s) of the RNA or internally, for example at one or more nucleotides of the RNA. Nucleotides in the RNA molecules of the present teachings can also comprise non-standard nucleotides, such as non-naturally occurring nucleotides or chemically synthesized nucleotides or deoxynucleotides. These altered RNAs can be referred to as analogs or analogs of naturally-occurring RNA.

[00064] The term "vector" as used herein refers to its meaning as is generally accepted in the art. The term vector generally refers to any nucleic acid- and/or viral-based expression system or technique used to deliver PDL1 and β-catenin aiRNA molecules to a targeted cell or organism.

[00065] The term "RNA interference" or term "RNAi" refer to the biological process of inhibiting or down regulating gene expression in a cell, as is generally known in the art, and which can be mediated by short interfering nucleic acid molecules or asymmetric interfering nucleic acid molecules of the present teachings. Additionally, the term RNAi is meant to be

equivalent to other terms used to describe sequence-specific RNA interference, such as post-transcriptional gene silencing, translational inhibition, transcriptional inhibition, or epigenetics. For example, aiRNA molecules of the present teachings can be used to epigenetically silence genes at either the post-transcriptional level or the pre-transcriptional level. In a non-limiting example, modulation of gene expression by aiRNA molecules of the present teachings can result from aiRNA mediated cleavage of RNA (either coding or non-coding RNA) via RISC, or via translational inhibition, as is known in the art or modulation can result from transcriptional inhibition.

[00066] The term "antisense strand" as used herein refers to what is generally accepted in the art. With reference to exemplary nucleic acid molecules of the present teachings, the term refers to a nucleotide sequence of an aiRNA molecule having at least partial complementarity to an expressed nucleic acid sequence of a target gene. In addition, the antisense region of an aiRNA molecule can optionally comprise a nucleic acid sequence having complementarity to a sense region of the aiRNA molecule. In certain embodiments, the antisense region of the aiRNA molecule is referred to as the antisense strand or guide strand. In certain embodiments, the antisense strand can have 10, 11, 12, 13, 14, 15, 16 or 17 nucleotides that base pair with the sense strand. In certain embodiments, the antisense strand can have 0, 1, 2, 3, 4, 5, 6, 7, 8 or 9 nucleotides that are not contiguous with the corresponding colinear complementary nucleotides in a target nucleotide sequence. In certain embodiments, the antisense strand can have 0, 1, 2, 3, 4, 5, 6, 7, 8 or 9 nucleotides that are not complementary to the corresponding colinear nucleotides in a target nucleotide sequence. In certain embodiments, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, or 23 nucleotides of the antisense strand can be colinear with the corresponding complementary nucleotides in a target nucleotide sequence.

[00067] The terms "short interfering nucleic acid", "siNA", "short interfering RNA", "siRNA", "short interfering nucleic acid molecule", "short interfering oligonucleotide molecule", or "chemically modified short interfering nucleic acid molecule" refer to so-called "canonical" siRNA molecules that are capable of inhibiting or down regulating gene expression or viral replication by mediating RNA interference ("RNAi") or gene silencing in a sequence-specific manner and that includes an antisense strand and a sense strand, and the lengths of the two strands are the same. A canonical siRNAs are double-stranded nucleic acid molecules

comprising self-complementary sense and antisense strands, wherein the antisense strand comprises a nucleotide sequence that is complementary to a nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense strand comprises a nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. Canonical siRNAs are symmetrical interfering double stranded RNAs having a sense and antisense strands of 21 nucleotides each and two 3' overhangs of 2 nucleotides.

[00068] The terms "asymmetric interfering nucleic acid", "aiRNA", "asymmetric interfering oligonucleotide molecule", or "chemically modified asymmetric interfering nucleic acid molecule" refer to any nucleic acid molecule that has an antisense strand and a sense strand, where the lengths of the two strands can be different. These nucleic acid molecules can inhibit or down-regulate gene expression or viral replication by mediating RNA interference ("RNAi") or gene silencing in a sequence-specific manner. These terms can refer to both individual nucleic acid molecules, a plurality of such nucleic acid molecules, or pools of such nucleic acid molecules. The aiRNA can be a double-stranded RNA molecule or RNA duplex comprising at least partially complementary sense and antisense strands, wherein the antisense strand comprises a nucleotide sequence that is at least partially complementary to a nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense strand comprises a nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. The aiRNA can also be a double-stranded nucleic acid molecule comprising complementary sense and antisense strands, wherein the antisense strand comprises a nucleotide sequence that is at least partially complementary to a nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense strand comprises a nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof that may have a nick (missing one nucleotide) or a gap (missing two or more nucleotides). In certain embodiments, an asymmetric interfering RNA as used here can have terminal overhangs on either end or both ends. In certain embodiments, the two strands of the duplex RNA can be linked through a chemical linker. Exemplary aiRNAs are disclosed in U.S. Patent No. 9,328,345, the content of which is hereby incorporated herein in its entirety.

[00069] In certain embodiments, the PDL1 and β-catenin aiRNAs of the present disclosure exhibit significantly (e.g., at least about 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%,

70%, 75%, 80%, 85%, 90% or more) less "off-target" gene silencing when compared to canonical siRNAs targeting the same target sequence.

[00070] As used herein, "off-target" gene silencing refers to unintended gene silencing due to, for example, spurious sequence homology between the antisense (guide) sequence and the unintended target mRNA sequence.

[00071] The term "specific," when used in connection with an aiRNA of the present disclosure, means that the interference or silencing of a target mRNA by such aiRNA is discriminatory. In certain embodiments, there is about 1%, about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, or about 80% off-target interference or silencing. In certain embodiments, there is not greater than about 20%, not greater than about 15%, not greater than about 10%, not greater than about 5%, not greater than about 2%, not greater than about 1%, not greater than about 0.5%, or not greater than about 0.2% of off-target interference or silencing.

[00072] The term "complementarity" or "complementary" as used herein refers to its meaning as is generally accepted in the art. With reference to exemplary nucleic acid molecules of the present teachings, the terms generally refer to the formation or existence of hydrogen bond(s) between one nucleic acid sequence and another nucleic acid sequence by traditional Watson-Crick, forming a base-paired, double-stranded region. In certain embodiments, base pairing i.e. the formation or existence of hydrogen bond(s) between one nucleic acid sequence and another nucleic acid sequence does not include non-traditional base-pairing (e.g. Hoogsteen base pairing) such as between complementary RNA and DNA sequences. In reference to the nucleic molecules of the present teachings, the binding free energy for a nucleic acid molecule with its complementary sequence is sufficient to allow the relevant function of the nucleic acid to proceed, e.g., RNAi activity. "Perfect complementarity" means that all the contiguous residues of a nucleic acid sequence can hydrogen bond with the same number of contiguous residues in a second nucleic acid sequence. "Partial complementarity" can include various mismatches or non-base paired nucleotides (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more mismatches, non-nucleotide linkers, or non-base paired nucleotides) within the nucleic acid molecule, which can result in bulges, loops, or overhangs between the sense strand or sense region and the antisense strand or

antisense region of the nucleic acid molecule or between the antisense strand or antisense region of the nucleic acid molecule and a corresponding target nucleic acid molecule. Such partial complementarity can be represented by a % complementarity that is determined by the number of non-base paired nucleotides the total number of nucleotides. Thus for example, a first RNA sequence can have about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95% or about 99% base pair complementarity with second RNA sequence. Such partial complementarity is permitted to the extent that the nucleic acid molecule (e.g. aiRNA) maintains its function, for example the ability to mediate sequence specific RNAi. "Substantial complementarity" means that the sequences are sufficiently complementary to each other to hybridize under selected reaction conditions. Two substantially complementary strands can be, for example, perfectly complementary or can contain from l to many mismatches so long as the hybridization conditions are sufficient to allow, for example, discrimination between a pairing sequence and a non-pairing sequence. Accordingly, substantially complementary sequences can refer to sequences with base-pair complementarity of about 50%-100% in a double-stranded region. The term "complementarity" in certain embodiments can refer to "perfect complementarity," "partial complementarity," or "substantial complementarity."

[00073] In double-stranded or duplex RNAs, one or more ribonucleotides of one strand stably associates with a complementary ribonucleotide in the other strand. The complementarity between the strands is brought about by the hydrogen bonds or "base pairing" between A and U, and between G and C (see, for example, TABLE IA).

TABLE IA

C	C	U	A	U	A	U	G	U	G	G	U	A	G	A	SEQ ID NO. 174
•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	
G	G	A	U	A	U	A	C	A	C	C	A	U	C	U	SEQ ID NO. 175

TABLE IB

C	C	U	U	U	A	U	U	U	G	G	U	A	G	A	SEQ ID NO. 176
•	•	•		•	•	•		•	•	•	•	•	•	•	
G	G	A	U	A	U	A	C	A	C	C	A	U	C	U	SEQ ID NO. 175

[00074] In certain embodiments, an RNA duplex can have RNA strands that are either perfectly complimentary or partially complimentary, depending on the number of mismatched, i.e., non-base paired nucleotides present in the RNA duplex (see, for example, TABLE IB).

[00075] As used herein, the term "align" refers to the process of comparing the nucleotide sequence of two or more nucleotide sequences to assess their degree of sequence identity. As used herein, a "match" refers to the alignment of two or more nucleotide sequences having 100% sequence identity. Thus, for example, in TABLE II below, ROW 1 is aligned and matches ROW 2.

TABLE II

ROW 1	C	C	U	A	U	A	U	G	U	G	G	U	A	G	A	**SEQ ID NO. 174**
ROW 2	C	C	U	A	U	A	U	G	U	G	G	U	A	G	A	**SEQ ID NO. 174**
	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	
ROW 3	G	G	A	U	A	U	A	C	A	C	C	A	U	C	U	**SEQ ID NO. 175**

[00076] Because the identity of a base on one strand of a RNA duplex can be used to infer the identity of the corresponding base on the other strand, the term "align" can also refer to the comparison between the nucleotide sequence of one strand (sense strand) and its complementary sequence in another RNA strand (antisense strand). Thus, for example, in TABLE II, ROW 2 is aligned with ROW 3 because the complementary sequence of the nucleotide sequence of ROW 3 matches the nucleotide sequence in ROW 2. For example, in certain embodiments, the nucleotide sequence of an aiRNA's antisense strand can be aligned with its perfectly complementary or partially complementary nucleotide sequence in a target nucleotide sequence.

[00077] Generally, the term "contiguous" refers to those nucleotides that are immediately adjacent to each other in a polynucleotide chain. In certain embodiments, the term "contiguous" can refer to those nucleotides that are adjacent to each other in a polynucleotide chain that match the corresponding nucleotides in a second polynucleotide chain. Thus, for example in TABLE III, the nucleotides from position 1 to 11 of RNA strand 2 are contiguous with the corresponding nucleotides 1 to 11 of RNA strand 1 because nucleotides 1-11 of RNA strand 2 match the nucleotides 1-11 of RNA strand 1 without any intervening mismatches.

[00078] In certain embodiments, the term "contiguous" can also refer to those nucleotides that are adjacent to each other in a first polynucleotide chain that align with perfectly complementary nucleotides in a second polynucleotide chain. Thus, for example in TABLE III, the nucleotides of RNA strand 1 are contiguous with the nucleotides in RNA strand 3 because each nucleotide of RNA strand 1 aligns with the corresponding complimentary nucleotide in RNA strand 3 without any intervening mismatches.

TABLE III

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
RNA STRAND 1 (SEQ ID NO. 174)	C	C	U	A	U	A	U	G	U	G	G	U	A	G	A
RNA STRAND 2 (SEQ ID NO. 177)	C	C	U	A	U	A	U	G	U	G	G	G	G	G	A
	•	•	•	•	•	•	•	•	•	•	•			•	•
RNA STRAND 3 (SEQ ID NO. 175)	G	G	A	U	A	U	A	C	A	C	C	A	U	C	U
RNA strand 4 (SEQ ID NO. 178)	G	G	A	A	U	A	U	A	C	A	C	C	A	U	C	U

[00079] Generally, the term "colinear" describes the 1:1 relationship between the linear order of nucleotides in a first RNA strand and the linear order of nucleotides in a second RNA strand. Thus, for example in TABLE III, RNA strand 2 is colinear with RNA strand 1 despite the lack of sequence identity at positions 12 and 13 because the linear order of nucleotides 1-11, 14 and 15 of RNA strand 2 matches the corresponding nucleotides of RNA strand 1. In certain embodiments, the term "colinear" also describes the 1:1 relationship between the linear order of nucleotides in a first RNA strand and the linear order of the corresponding complementary nucleotides in a second RNA strand. Thus, for example in TABLE III, RNA strand 2 is colinear with RNA strand 3 despite the partial complimentary between the two strands because the linear order of nucleotides 1-11, 14 and 15 of RNA strand 2 aligns with the corresponding complementary nucleotides at positions 1-11, 14 and 15 of RNA strand 3. In contrast, RNA strand 3 is not colinear with RNA strand 4 because the linear order of nucleotides in RNA strand 3 does not match the linear order of nucleotides in RNA strand 4 as a result of the insertion of an extra nucleotide at position 3. Similarly, the linear order of the nucleotides in RNA strand 2 is

not colinear with the linear order of the corresponding complementary nucleotides in RNA strand 4 again because of the extra nucleotide at position 3.

[00080] In certain embodiments, the term "colinear" refers to the 1:1 relationship between the linear order of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 or more nucleotides in an aiRNA's antisense strand and the linear order of the corresponding complementary nucleotides in a target nucleotide sequence. In certain embodiments, the aiRNA's antisense strand may have either perfect or partial complementarity with the sense strand.

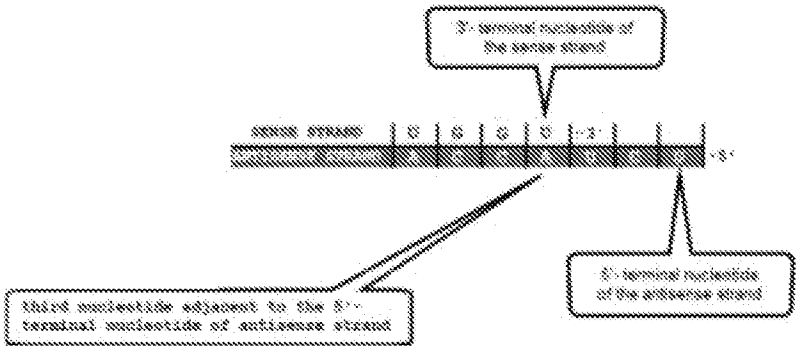
[00081] In certain embodiments, 2 , 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 or more nucleotides in the aiRNA's antisense strand are contiguous with the corresponding complementary nucleotides in a target nucleotide sequence. In certain embodiments, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 or more nucleotides in the aiRNA's antisense strand are not contiguous with the corresponding complementary nucleotides in a target nucleotide sequence.

[00082] With reference to exemplary aiRNA molecules of the present teachings, the term “correspond” or “correspondence” as used herein refers to the relationship between a target nucleotide sequence and a sequence in the antisense strand of an aiRNA molecule of the present teachings. The term can be modified by the word “partially,” “substantially,” or “completely” to indicate the degree of the relationship. In certain embodiments, a partial correspondence means about 20-99% of the ribonucleotides A, U, G, and C in the antisense strand of aiRNA sequence is complementary to its corresponding target nucleotide sequence. In certain embodiments, a substantial correspondence means about 50%-100% of the ribonucleotides A, U, G, and C in the antisense strand of aiRNA sequence are complementary to the corresponding target nucleotide sequence. In certain embodiments, the target nucleotide sequence can comprise PD-L1 or β-catenin mRNA sequences. In certain embodiments, the target nucleotide sequence can be the nucleotide sequence of SEQ ID NO. 166 (PDL1) or SEQ ID NO. 179 (β-catenin). In certain embodiments, the PDL1 target nucleotide sequence comprises a sequence chosen from SEQ ID NOs. 93-138 and the β-catenin target nucleotide sequence comprises a sequence chosen from SEQ ID NOs.186-262.

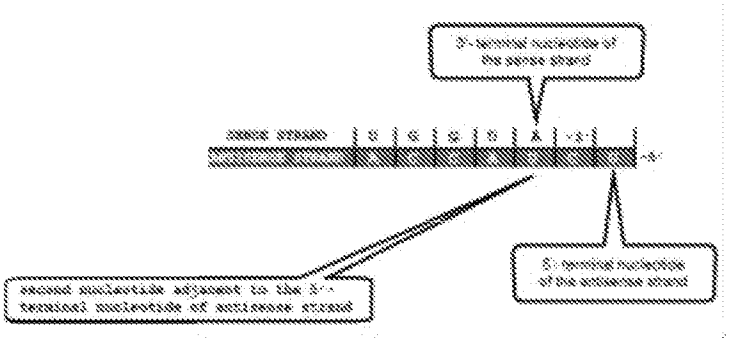
[00083] The term “non-base paired” refers to nucleotides that are not base paired between the sense strand or sense region and the antisense strand or antisense region of a double-stranded

aiRNA molecule; and can include, but is not limited to, for example, mismatches, overhangs, single stranded loops, etc. In certain embodiments, non-Watson Crick base pairing, e.g. Hoogsteen base pairing, for example, as in RNA-DNA base pairing, will be understood to be non-base paired.

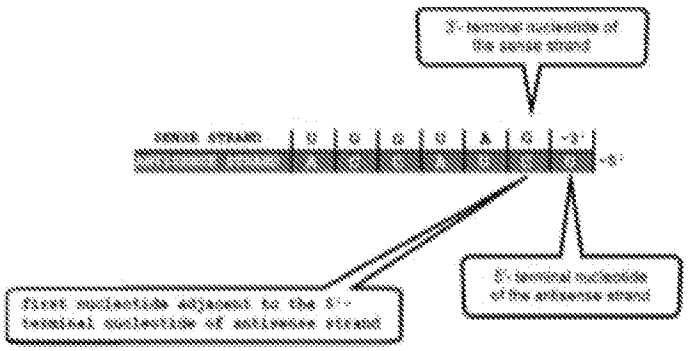
[00084] An exemplary 5' overhang of an aiRNA of the present teachings in which the 3'-terminal nucleotide of the sense strand is complementary to the third nucleotide adjacent to the 5'-terminal nucleotide of the antisense strand is shown below:



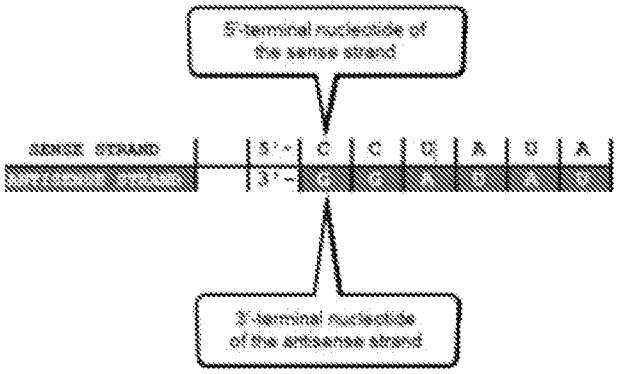
[00085] An exemplary 5' overhang of an aiRNA of the present teachings in which the 3'-terminal nucleotide of the sense strand is complementary to the second nucleotide adjacent to the 5'-terminal nucleotide of the antisense strand is shown below:



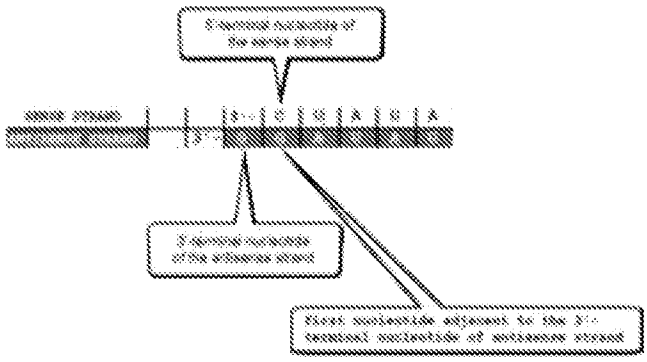
[00086] An exemplary 5' overhang of an aiRNA of the present teachings in which the 3'-terminal nucleotide of the sense strand is complementary to the first nucleotide adjacent to the 5'-terminal nucleotide of the antisense strand is shown below:



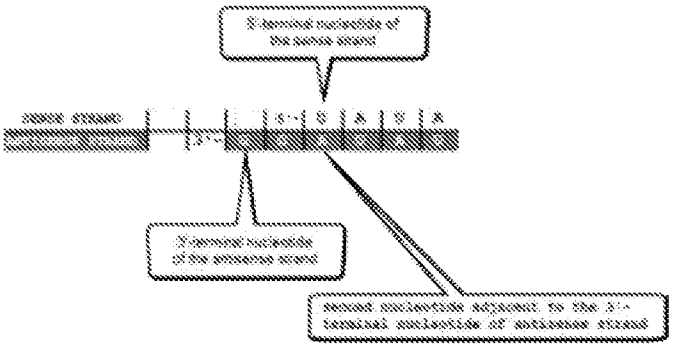
[00087] An exemplary depiction of an aiRNA of the present teachings in which the 5'-terminal nucleotide of the sense strand is complementary to the 3'-terminal nucleotide of the antisense strand is shown below:



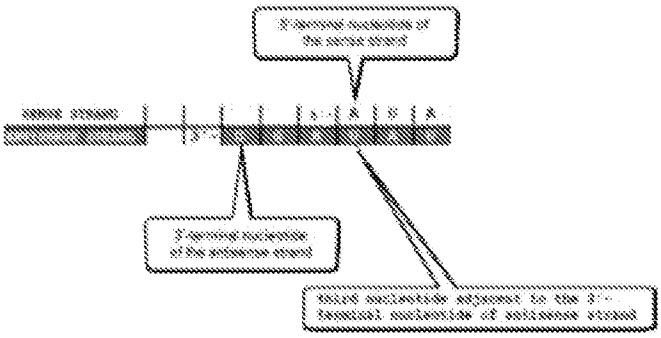
[00088] An exemplary 3' overhang of an aiRNA of the present teachings in which the 5'-terminal nucleotide of the sense strand is complementary to the first nucleotide adjacent to the 3'-terminal nucleotide of the antisense strand is shown below:



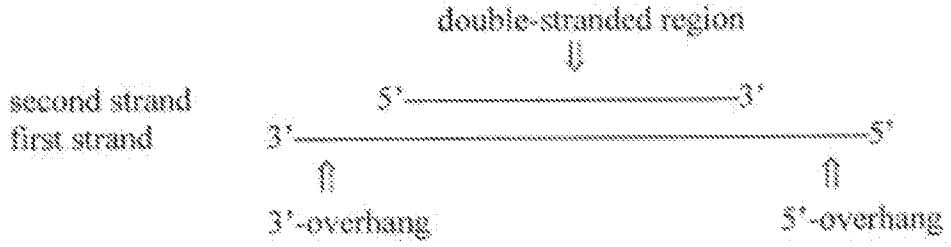
[00089] An exemplary 3' overhang of an aiRNA of the present teachings in which the 5'-terminal nucleotide of the sense strand is complementary to the second nucleotide adjacent to the 3'-terminal nucleotide of the antisense strand is shown below:

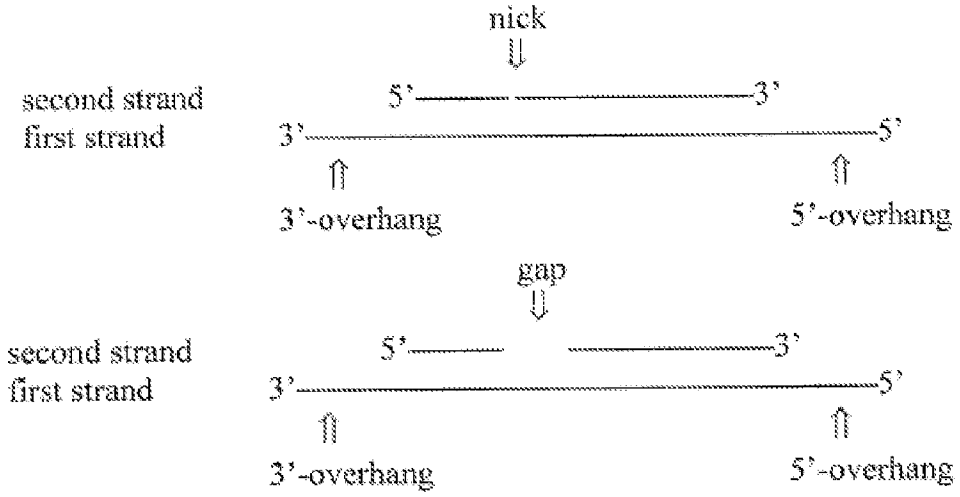


[00090] An exemplary 3' overhang of an aiRNA of the present teachings in which the 5'-terminal nucleotide of the sense strand is complementary to the third nucleotide adjacent to the 3'-terminal nucleotide of the antisense strand is shown below:



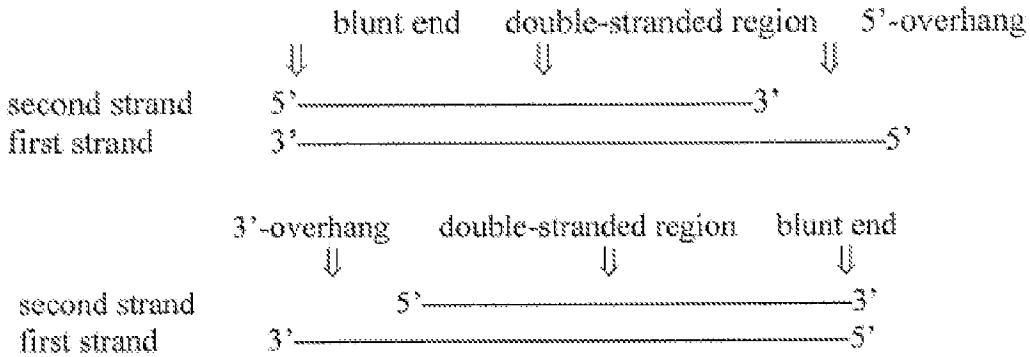
[00091] The term "overhang" as used herein refers to its meaning as is generally accepted in the art. With reference to exemplary double stranded nucleic acid molecules, the term generally refers to the terminal portion of a nucleotide sequence that is not base paired between the two strands of a double-stranded nucleic acid molecule. In certain embodiments, an overhang can be single stranded. In certain embodiments, the nucleic acid molecules of the present teachings include two overhangs at the antisense strand (i.e., 3'- and 5'-overhangs), as exemplified below.





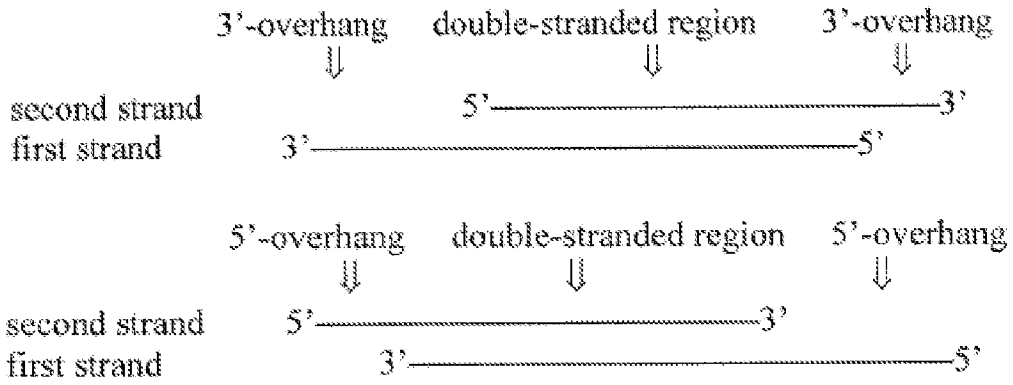
[00092] The term "blunt end" as used herein refers to its meaning as is generally accepted in the art. With reference to exemplary nucleic acid molecules of the present teachings, the term refers to a terminus of a double-stranded aiRNA molecule having no overhanging nucleotides. For example, the two strands of a double-stranded aiRNA molecule having blunt ends align with each other without overhanging nucleotides at the termini. An aiRNA duplex molecule of the present teachings can comprise blunt ends at one or both termini of the duplex, such as the terminus located at the 5'-end of the antisense strand, the 5'-end of the sense strand, or both termini of the duplex. In certain embodiments, a blunt end is formed when the 3'-terminal nucleotide of the sense strand base pairs with the 5'-terminal nucleotide of the antisense strand.

[00093] In certain embodiments, the nucleic acid molecules of the present teachings include one overhang and one blunt end (e.g., a 3'-overhang and a 5'-blunt end; or a 3'-blunt end and a 5'-overhang), as exemplified below.



[00094] In certain embodiments, the 3' overhang is not blunt.

[00095] Exemplary duplex RNAs having either two 3' overhangs or two 5' overhangs are depicted below.



[00096] The term "nucleotide" (or "nt") generally comprises a nucleobase, a sugar, and an internucleoside linkage, e.g., a phosphodiester bond. The base can be a natural base (standard), modified bases, or a base analog. Such bases can be generally located at the 1'-position of a nucleotide sugar moiety. Additionally, the nucleotides can be unmodified or modified at the sugar, internucleoside linkage, and/or base moiety (also referred to interchangeably as nucleotide analogs, modified nucleotides, non-natural nucleotides, non-standard nucleotides and others). In certain embodiments, a nucleotide can be a ribonucleotide (which sometimes refers to as a RNA nucleotide). In certain embodiments, a nucleotide can be a deoxyribonucleotide (which sometimes refers to as a DNA nucleotide). In certain embodiments, the term nucleotide also includes a moiety having a nucleobase and a sugar. For example, when a nucleotide is located at one of the termini of a sense strand or an antisense strand, the nucleotide can include only a nucleobase and a sugar.

[00097] The term "ribonucleotide" as used herein refers to its meaning as is generally accepted in the art. The term generally refers to a nucleotide with a hydroxyl group at the 2′ position of a β-D-ribofuranose moiety. A ribonucleotide consists of a phosphate group, a ribose sugar group, and a nucleobase that can be either adenine (A), guanine (G), cytosine (C), or uracil (U). An RNA strand refers to a chain of ribonucleotides linked together by phosphodiester bonds

between the 5'-phosphate of one nucleotide and the 3' hydroxyl group of the next nucleotide. However, in certain embodiments, the chain of ribonucleotides may comprise bonds other than phosphodiester bonds between the 5'-phosphate of one nucleotide and the 3' hydroxyl group of the next nucleotide.

[00098] The term “deoxyribonucleotide” as used herein refers to its meaning as is generally accepted in the art. The term generally refers to a nucleotide with a proton at the 2’ position of a β-D-deoxyribofuranose moiety. This term also includes any deoxyribonucleotides that are chemically modified. "dT" refers to 2'-deoxythymidine.

[00099] As used herein, a “polynucleotide” refers to a polymeric chain containing two or more nucleotides. “Polynucleotides” includes primers, oligonucleotides, nucleic acid strands, etc. A polynucleotide may contain standard or non-standard nucleotides. A polynucleotide has an end-to-end chemical orientation: the 5' end has a hydroxyl or phosphate group on the 5' carbon of its terminal sugar and the 3' end usually has a hydroxyl group on the 3' carbon of its terminal sugar. This directionality, plus the fact that synthesis proceeds 5' to 3', has given rise to the convention that polynucleotide sequences are written and read in the 5'—>3' direction (from left to right); for example, the sequence AUG is assumed to be (5')AUG(3').” (see Molecular Cell Biology 4th edition by Harvey Lodish et al. © 2000, W. H. Freeman and Company).

[000100] Accordingly, as used herein, the nucleotide or base at the 5' end of a polynucleotide may be referred to herein as the "5'-terminal nucleotide" or "5'-terminal base" respectively. The nucleotide or base at the 3' end of a polynucleotide may be referred to herein as the "3'-terminal nucleotide" or "3'-terminal base" respectively.

[000101] As used herein, the "5'-terminal nucleotide" shall be understood to encompass a 5'-terminal nucleoside having a 5'-terminal hydroxyl group or a 5'-terminal nucleotide having 5'-terminal phosphate.

[000102] The term “modified nucleotide” as used herein refers to its meaning as is generally accepted in the art. The term generally refers a nucleotide, which contains a modification in the chemical structure of the base, sugar and/or phosphate of the unmodified (or natural) nucleotide as is generally known in the art.

[000103] The term "chemical modification" as used herein refers to its meaning as is generally accepted in the art. With reference to the exemplary PDL1 and β-catenin aiRNAs molecules of the present teachings, the term refers to any modification of the chemical structure of the nucleotides that differs from nucleotides of a native nucleic acid in general. The term "chemical modification" encompasses, for example, the addition, substitution, or modification of native RNA at the sugar, base, or internucleotide linkage, as described herein or as is otherwise known in the art. In certain embodiments, the term "chemical modification" can refer to certain forms of RNA that are naturally occurring in certain biological systems, for example 2'-O-methyl or 2'-fluorine modifications or inosine modifications.

[000104] The terms "internucleoside linkage" or "internucleoside linker" or "internucleotide linkage" or "internucleotide linker" can be used herein interchangeably and refer to any linker or linkage between two nucleoside units, as is known in the art, including, for example, but not limited to, phosphate, analogs of phosphate, phosphonate, guanidium, hydroxylamine, hydroxythydrazinyl, amide, carbamate, alkyl, and substituted alkyl linkages. The term "phosphorothioate" refers to an internucleotide phosphate linkage comprising one or more sulfur atoms in place of an oxygen atom. Hence, the term phosphorothioate refers to both phosphorothioate and phosphorodithioate internucleotide linkages.

[000105] In certain embodiments, the PDL1 and β-catenin aiRNAs can be chemically modified to facilitate cellular uptake. For example, an aiRNA can be bound covalently via a linker or non-covalently to a positively-charged molecule, a peptide, a protein, a carbohydrate, a cholesterol, a lipid to improve cellular uptake. For example, conjugation of aiRNAs with cholesterol or octyl, dodecyl, and octadecyl residues or poly-L-lysine facilitates cellular uptake.

[000106] The terms "composition" or "formulation" as used herein generally refer to a composition or formulation, such as in a pharmaceutically acceptable carrier or diluent, in a form suitable for administration, e.g., systemic or local administration, into a cell or subject, including, for example, a human. Suitable forms, in part, depend upon the use or the route of entry, for example, oral, transdermal, inhalation, or by injection. Such forms should not prevent the composition or formulation from reaching a target cell (i.e., a cell to which the negatively charged nucleic acid is desirable for delivery). For example, compositions injected into the blood

stream should be soluble. Other factors are known in the art, and include considerations such as toxicity and forms that prevent the composition or formulation from exerting its effect.

[000107] As used herein, pharmaceutical formulations include formulations for human and veterinary use. Non-limiting examples of agents suitable for formulation with the nucleic acid molecules of the present teachings include: lipid nanoparticles (see for example Semple et al., 2010, *Nat Biotechnol.*, February; 28 (2):172-6); P-glycoprotein inhibitors (such as Pluronic P85); biodegradable polymers, such as poly (DL-lactide-coglycolide) microspheres for sustained release delivery (Emerich, D F et al, 1999, *Cell Transplant*, 8, 47-58); and loaded nanoparticles, such as those made of polybutylcyanoacrylate. Other non-limiting examples of delivery strategies for the nucleic acid molecules of the present teachings include material described in Boado et al., 1998, *J. Pharm Sci.*, 87, 1308-1315; Tyler et al., 1999, *FEBS Lett.*, 421, 280-284; Pardridge et al., 1995, *PNAS USA.*, 92, 5592-5596; Boado, 1995, *Adv. Drug Delivery Rev.*, 15, 73-107; Aldrian-Herrada et al., 1998, *Nucleic Acids Res.*, 26, 4910-4916; and Tyler et al., 1999, *PNAS USA.*, 96, 7053-7058.

[000108] A "pharmaceutically acceptable salt" or "salt" of an RNA interfering agent, e.g. aiRNA, is a product of the disclosed RNA interfering agent that contains an ionic bond, and is typically produced by reacting the disclosed RNA interfering agent with either an acid or a base, suitable for administering to a subject. Pharmaceutically acceptable salt can include, but is not limited to, acid addition salts including hydrochlorides, hydrobromides, phosphates, sulphates, hydrogen sulphates, alkylsulphonates, arylsulphonates, acetates, benzoates, citrates, maleates, fumarates, succinates, lactates, and tartrates; alkali metal cations such as Na, K, Li, alkali earth metal salts such as Mg or Ca, or organic amine salts.

[000109] A "pharmaceutical composition" is a formulation containing the disclosed RNA interfering agent, e.g. aiRNA, in a form suitable for administration to a subject. In one embodiment, the pharmaceutical composition is in bulk or in unit dosage form. The unit dosage form is any of a variety of forms, including, for example, a capsule, an IV bag, a tablet, a single pump on an aerosol inhaler, or a vial. The quantity of active ingredient *(e.g.,* a formulation of the disclosed duplex RNA molecule or salts thereof) in a unit dose of composition is an effective amount and is varied according to the particular treatment involved. One skilled in the art will

appreciate that it is sometimes necessary to make routine variations to the dosage depending on the age and condition of the patient. The dosage will also depend on the route of administration. A variety of routes are contemplated, including oral, pulmonary, rectal, parenteral, transdermal, subcutaneous, intravenous, intramuscular, intraperitoneal, intranasal, and the like. Dosage forms for the topical or transdermal administration of a RNA interfering agent, e.g. aiRNA, of this disclosure include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. In one embodiment, the active RNA interfering agent, e.g. aiRNA, is mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants that are required.

[000110] The present disclosure provides a method of treatment comprising administering an effective amount of the pharmaceutical composition to a subject in need thereof. In some embodiments, the pharmaceutical composition is administered via a route selected from the group consisting of iv, sc, topical, po, and ip. In another embodiment, the effective amount can be 5pM per day, 50pM per day or 500pM per day. In other embodiments, the effective amount can be 1 ng to 1 g per day, 100 ng to 1 g per day, or 1 ug to 1 mg per day.

[000111] The present disclosure also provides pharmaceutical formulations comprising a RNA interfering agent, e.g. aiRNA, of the present disclosure in combination with at least one pharmaceutically acceptable excipient or carrier. As used herein, “pharmaceutically acceptable excipient” or “pharmaceutically acceptable carrier” is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Suitable carriers are described in “Remington: The Science and Practice of Pharmacy, Twentieth Edition,” Lippincott Williams & Wilkins, Philadelphia, PA., which is incorporated by reference herein by reference. Examples of such carriers or diluents include, but are not limited to, water, saline, Ringer's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active RNA interfering agent, e.g. aiRNA, use thereof in the compositions is contemplated. Supplementary active RNA interfering agents, e.g. aiRNAs, can also be incorporated into the compositions.

[000112] An RNA interfering agent, e.g. aiRNA, of the present disclosure is administered in a suitable dosage form prepared by combining a therapeutically effective amount *(e.g.,* an efficacious level sufficient to achieve the desired therapeutic effect through inhibition of tumor growth, killing of tumor cells, treatment or prevention of cell proliferative disorders, etc.) of a RNA interfering agent, e.g. aiRNA, of the present disclosure (as an active ingredient) with standard pharmaceutical carriers or diluents according to conventional procedures (i.e., by producing a pharmaceutical composition of the disclosure). These procedures may involve mixing, granulating, compressing, or dissolving the ingredients as appropriate to attain the desired preparation. In another embodiment, a therapeutically effective amount of a RNA interfering agent, e.g. aiRNA, of the present disclosure is administered in a suitable dosage form without standard pharmaceutical carriers or diluents.

[000113] Pharmaceutically acceptable carriers include solid carriers such as lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid and the like. Exemplary liquid carriers include syrup, peanut oil, olive oil, water and the like. Similarly, the carrier or diluent may include time-delay material known in the art, such as glyceryl monostearate or glyceryl distearate, alone or with a wax, ethylcellulose, hydroxypropylmethylcellulose, methylmethacrylate or the like. Other fillers, excipients, flavorants, and other additives such as are known in the art may also be included in a pharmaceutical composition according to this disclosure.

[000114] The term "solvate" represents an aggregate that comprises one or more molecules of a compound of the present disclosure with one or more molecules of a solvent or solvents. Solvates of the compounds of the present disclosure include, for example, hydrates.

[000115] The pharmaceutical compositions containing active RNA interfering agent, e.g. aiRNA, of the present disclosure may be manufactured in a manner that is generally known, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes. Pharmaceutical compositions may be formulated in a conventional manner using one or more physiologically acceptable carriers comprising excipients and/or auxiliaries which facilitate processing of the active duplex

RNA molecules into preparations that can be used pharmaceutically. Of course, the appropriate formulation is dependent upon the route of administration chosen.

[000116] An RNA interfering agent, e.g. aiRNA, or pharmaceutical composition of the disclosure can be administered to a subject in many of the well-known methods currently used for chemotherapeutic treatment. For example, for treatment of cancers, a RNA interfering agent, e.g. aiRNA, of the present disclosure may be injected directly into tumors, injected into the blood stream or body cavities, taken orally, or applied through the skin with patches.

[000117] In certain embodiments, aiRNA nanoparticles may be formulated in dosage unit form for ease of administration and uniformity of dosage. The expression "dosage unit form" as used herein refers to a physically discrete unit of nanoparticle appropriate for the patient to be treated. It will be understood, however, that the total daily usage of the compositions of the present disclosure will be decided by the attending physician within the scope of sound medical judgment. For any nanoparticle, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models, usually mice, rabbits, dogs, or pigs. The animal model is also used to achieve a desirable concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans. Therapeutic efficacy and toxicity of nanoparticles can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED50 (the dose is therapeutically effective in 50% of the population) and LD50 (the dose is lethal to 50% of the population). The dose ratio of toxic to therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50. Pharmaceutical compositions which exhibit large therapeutic indices may be useful in some embodiments. The data obtained from cell culture assays and animal studies can be used in formulating a range of dosage for human use.

[000118] Compositions suitable for parenteral administration may comprise at least one more pharmaceutically acceptable sterile isotonic aqueous or non-aqueous solutions, dispersions, suspensions, emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

[000119] In various embodiments, a composition described herein includes an aiRNA composition and pharmaceutically acceptable salts and solvates thereof and one or more surfactants. In certain embodiments, the surfactant is sodium lauryl sulfate (SLS), sodium dodecyl sulfate (SDS), or one or more polyoxylglycerides. For example, the polyoxylglyceride can be lauroyl polyoxylglycerides (sometimes referred to as Gelucire™) or linoleoyl polyoxylglycerides (sometimes referred to as Labrafil™). Examples of such compositions are disclosed in PCT Patent Application No. PCT/US2014/033566, the content of which is incorporated by reference herein in its entirety for any purpose.

[000120] In certain embodiments, a kit is disclosed that comprises (1) one or more PDL1 aiRNAs and (2) one or more β-catenin aiRNAs, pharmaceutically acceptable salts of any of the foregoing, together with instructions for administration and/or use.

[000121] In certain embodiments, aiRNAs of the present disclosure conjugated to a natural ligand such as cholesterol, an aptamer, or bound non-covalently to an antibody-protamine fusion proteins can be injected directly into a tumor. Other carrier choices include positive charged carriers (e.g., cationic lipids and polymers) and various protein carriers. In certain embodiments, the delivery of the aiRNA molecules of the present disclosure uses a ligand-targeted delivery system based on the cationic liposome complex or polymer complex systems (Woodle, et al. *J Control Release* 74: 309-311; Song, et al. *Nat. Biotechnol.* 23(6): 709-717 (2005); Morrissey et al. *Nat Biotechnol.* 23(8): 1002-1007 (2005)).

[000122] In other embodiments, the aiRNA molecules of the present disclosure can be formulated with a collagen carrier, e.g., atelocollagen, for *in vivo* delivery. Atelocollagen has been reported to protect siRNA from being digested by RNase and to enable sustained release (Minakuchi, et al. Nucleic Acids Res. 32: e109 (2004); Takei et al. Cancer Res. 64: 3365-3370 (2004)). In other embodiment, the aiRNA molecules of the present disclosure are formulated with nanoparticles or form a nanoemulsion, e.g., RGD peptide ligand targeted nanoparticles. It has been shown that different siRNA oligos can be combined in the same RGD ligand targeted nanoparticle to target several genes at the same time (Woodle et al. Materials Today 8 (suppl 1): 3441 (2005)).

[000123] In certain embodiments, the aiRNAs of the present disclosure are encapsulated in non-liposomal inorganic magnesium nanoparticles as described previously (see the published U.S. Patent Application No. 2016/0046936, the content of which is hereby incorporated herein in its entirety).

[000124] The term "subject" as used herein refers to its meaning as is generally accepted in the art. The term generally refers an organism to which the nucleic acid molecules of the present teachings can be administered. A subject can be a mammal or mammalian cell, including a human or human cell. The term also refers to an organism, which is a donor or recipient of explanted cells or the cells themselves. In certain embodiments, the term "subject" refers to any animal (e.g., a mammal), including, but not limited to humans, mammals and non-mammals, such as a non-human primate, a mouse, a rabbit, sheep, a dog, a cat, a horse, a cow, a chicken, an amphibian, a fish, an insect or a reptile which is to be the recipient of a particular treatment. Under some circumstances, the terms "subject" and "patient" can be used interchangeably herein in reference to a human subject.

[000125] The term "cell" as used herein refers to its meaning as is generally accepted in the art. With reference to exemplary nucleic acid molecules of the present teachings, the term can be used in its usual biological sense, and does not refer to an entire multicellular organism, e.g., specifically does not refer to a human being. The cell can be present in an organism, e.g., birds, plants, and mammals, such as humans, cows, sheep, apes, monkeys, swine, dogs, and cats. The cell can be prokaryotic (e.g., bacterial cell) or eukaryotic (e.g., mammalian or plant cell). The cell can be of somatic or germ line organ, totipotent or pluripotent, dividing or non-dividing. The cell can also be derived from or can comprise a gamete or embryo, a stem cell, or a fully differentiated cell. In certain embodiments, a cell can be a cancer stem cell.

[000126] As used herein, the term "cancer" in a subject refers to cells having uncontrolled proliferation, immortality, metastatic potential, rapid growth and increased proliferation rate, as well as certain morphological features. Cancer cells can aggregate in the form of a tumors or masses and/or circulate in the blood stream or lymphatic system as independent cells.

[000127] The term "cancer" comprises, for example, AIDS-Related cancers, breast cancers, cancers of the digestive/gastrointestinal tract, endocrine and neuroendocrine cancers, cancers of

the eye, genitourinary cancers, germ cell cancers, gynecologic cancers, head and neck cancers, hematologic cancers, musculoskeletal cancers, neurologic cancers, respiratory/thoracic cancers, skin cancers, childhood cancers as well as cancers of unknown primary.

[000128] In certain embodiments, the cancer comprises cancer stem cells that express stemness gene, e.g. activated phosphorylated STAT3.

[000129] Exemplary AIDS-related cancers include, but are not limited to, AIDS-Related Lymphoma, Primary Central Nervous System Lymphoma and Kaposi Sarcoma.

[000130] Exemplary breast cancers include, but are not limited to, ductal carcinomas in situ (DCIS), invasive ductal carcinomas (IDC), invasive lobular carcinoma (ILC), triple negative breast cancers (where the tumor cells are negative for progesterone, estrogen, and her2/neu receptors), inflammatory breast cancers, metastatic breast cancers, breast cancers during pregnancy, Paget disease of the nipple, Phyllodes tumor, adenoid cystic (or adenocystic) carcinoma, low-grade adenosquamous carcinoma, medullary carcinomas, tubular carcinomas, papillary carcinoma, mucinous (colloid) carcinomas, lymphoma of the breast, adenomyoepithelioma, giant cell sarcoma of the breast, leiomyosarcoma of the breast, angiosarcoma of the breast, cystosarcoma phylloides, and liposarcoma of the breast, carcinoid tumors of the breast, acinic cell carcinoma, oncocytic carcinoma (mammary epithelial oncocytoma), mucoepidermoid carcinoma, spindle cell carcinoma of the breast, squamous cell carcinoma of the breast, secretory carcinoma of the breast (juvenile secretory carcinoma), metaplastic carcinoma of the breast, invasive micropapillary carcinoma of the breast, adenoid cystic carcinoma of the breast, cribriform carcinoma, myofibroblastoma of the breast (benign spindle stromal tumor of the breast) and glycogen-rich clear cell carcinoma of the breast.

[000131] Exemplary cancers of the digestive/gastrointestinal tract include, but are not limited to, anal cancer, appendix cancer, gastrointestinal carcinoid tumor, bile duct cancer, carcinoid tumor, gastrointestinal cancer, colon cancer, esophageal cancer, gallbladder cancer, gastrointestinal stromal tumors (GIST), islet cell tumors, pancreatic neuroendocrine tumors, liver cancer, pancreatic cancer, rectal cancer, small intestine cancer, gastro-esophageal junction (GEJ) cancer, and stomach (gastric) cancer.

[000132] Exemplary endocrine and neuroendocrine cancers include, but are not limited to, adrenocortical carcinomas, gastrointestinal carcinoid tumors, islet cell tumors, pancreatic neuroendocrine tumors, Merkel cell carcinomas, non-small cell lung neuroendocrine tumors, small cell lung neuroendocrine tumors, parathyroid cancers, pheochromocytomas, pituitary tumors, and thyroid cancers.

[000133] Exemplary genitourinary cancers include, but are not limited to, bladder cancer, kidney (renal cell) cancer, penile cancer, prostate cancer, renal pelvis and ureter cancer, transitional cell, testicular cancer, urethral cancer, Wilms tumor and other childhood kidney tumors.

[000134] Exemplary gynecologic cancers include, but are not limited to, cervical cancer, endometrial cancer, fallopian tube cancer, gestational trophoblastic tumor, ovarian epithelial cancer, ovarian germ cell tumor, ovarian low malignant potential tumor, primary peritoneal cancer, uterine sarcoma, vaginal cancer and vulvar cancer.

[000135] Exemplary head and neck cancers include, but are not limited to, hypopharyngeal cancer, laryngeal cancer, lip and oral cavity cancer, metastatic squamous neck cancer with occult primary, mouth cancer, nasopharyngeal cancer, oral cavity cancer, lip and oropharyngeal cancer, paranasal sinus and nasal cavity cancer, parathyroid cancer, pharyngeal cancer, salivary gland cancer, throat cancer and thyroid cancer.

[000136] Exemplary hematologic cancers include, but are not limited to, leukemias, acute lymphoblastic leukemia, adult, childhood acute lymphoblastic leukemia, adult acute myeloid leukemia, childhood acute myeloid leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia, hairy cell leukemia, lymphomas, AIDS-related lymphoma, cutaneous T-cell lymphoma, adult Hodgkin lymphoma, childhood Hodgkin lymphoma, Hodgkin lymphoma during pregnancy, mycosis fungoides, childhood Non-Hodgkin lymphoma, adult Non-Hodgkin lymphoma, Non-Hodgkin lymphoma during pregnancy, primary central nervous system lymphoma, Sézary syndrome, cutaneous T-cell lymphoma, Waldenström macroglobulinaemia, chronic myeloproliferative neoplasms, Langerhans cell histiocytosis, multiple myeloma/plasma cell neoplasm, myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms.

[000137] Exemplary musculoskeletal cancers include, but are not limited to, bone cancer, Ewing's sarcoma, osteosarcoma, malignant fibrous histiocytoma of bone, childhood rhabdomyosarcoma and soft tissue sarcoma.

[000138] Exemplary neurologic cancers include, but are not limited to, adult brain tumor, childhood brain tumor, astrocytomas, brain and spinal cord tumors, brain stem glioma, atypical teratoid/rhabdoid central nervous system tumor, embryonal central nervous system tumors, germ cell central nervous system tumors, craniopharyngioma, ependymoma, neuroblastoma, pituitary tumor and primary central nervous system (CNS) lymphoma.

[000139] Exemplary respiratory/thoracic cancers include, but are not limited to, non-small cell lung cancer, small cell lung cancer, malignant mesothelioma, thymoma and thymic carcinoma.

[000140] Exemplary skin cancers include, but are not limited to, cutaneous T-cell lymphoma, Kaposi sarcoma, melanoma, Merkel cell carcinoma, skin cancer, cutaneous T-cell lymphoma, mycosis fungoides and Sézary syndrome.

[000141] Also included within the term “cancer” is “solid tumor.” As used herein, the term “solid tumor” refers to those conditions, such as cancer, that form an abnormal tumor mass, such as sarcomas, carcinomas, and lymphomas. Examples of solid tumors include, but are not limited to, non-small cell lung cancer (NSCLC), neuroendocrine tumors, thyomas, fibrous tumors, metastatic colorectal cancer (mCRC), and the like. In certain embodiments, the solid tumor disease can be an adenocarcinoma, squamous cell carcinoma, large cell carcinoma, and the like.

[000142] The terms “administer,” “administering,” or “administration” are used herein in their broadest sense. These terms refer to any method of delivering the composition described herein to a subject, cell or tumor by, for example, introducing the composition systemically, locally, or in situ to the subject. Thus, a compound of the present teachings produced in a subject from a composition (whether or not it includes the compound) is encompassed by these terms. When these terms are used in connection with the term “systemic” or “systemically,” they generally refer to *in vivo* systemic absorption or accumulation of the compound or composition in the blood stream and its distribution throughout the entire body. In certain embodiments, the terms “administer,” “administering,” or “administration” can refer to, for example, delivering

one or more recombinant vectors to a tumor cell, wherein the vector expresses an RNA interfering agent.

[000143] The term "parenteral" as used herein refers to its meaning as is generally accepted in the art. The term generally refers to methods or techniques of administering the aiRNA composition described herein in a manner other than through the digestive tract, and includes epicutaneous, subcutaneous, intravascular (e.g., intravenous), intramuscular, or intrathecal injection or infusion techniques and the like.

[000144] The term "sensitize" means to alter cancer cells or tumor cells in a way that allows for more effective treatment of the associated cancer with a cancer therapy. In certain embodiments, normal cells are not affected to an extent that causes the normal cells to be unduly injured by the cancer therapy. In certain embodiments, an increased sensitivity or a reduced sensitivity to a therapeutic treatment can be measured according to a known method in the art for the particular treatment and methods described herein below, including, but not limited to, cell proliferative assays (Tanigawa *et al.* Cancer Res 1982; 42: 2159-2164) or cell death assays (Weisenthal *et al.* Cancer Res 1 84; 94: 161 - 173; Weisenthal *et al.* Cancer Treat Rep 1985; 69: 615-632; Weisenthal *et al.* Drug Resistance in Leukemia and Lymphoma. Langhorne, P A: Harwcod Academic Publishers, 1993: 41 -432; Weisenthal L M, Contrib Gynecol Obstet 1994; 19: 82-90). The sensitivity or resistance may also be measured in animals by measuring the tumor size reduction over a period of time, for example, 6 months for humans and 4-6 weeks for mice. A composition or a method sensitizes cancer cells or tumor cells to a therapeutic treatment (e.g. inhibition of PD-1/PDLI signaling) if the increase in treatment sensitivity or the reduction in resistance is about 25% or more, for example, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90% or more, compared to treatment sensitivity or resistance in the absence of such composition or method. In certain embodiments, the increase in treatment sensitivity or the reduction in resistance is about 2-fold, about 3-fold, about 4-fold, about 5-fold, about 10-fold, about 15-fold, about 20-fold or more compared to treatment sensitivity or resistance in the absence of such composition or method. The determination of sensitivity or resistance to a therapeutic treatment is routine in the art and within the skill of an ordinarily skilled clinician. It is to be understood that any method described herein for enhancing the

efficacy of a cancer therapy can be applied to methods for sensitizing hyperproliferative or otherwise cancerous cells (e.g., resistant cells) to the cancer therapy.

[000145] The term “synergy,” "therapeutic synergy," “synergistic,” “synergistically,” or “enhanced” as used herein refers to an effect of the combination of PDL1-specific and β-catenin-specific aiRNAs that is greater than the sum of their separate effects (or “additive effects”). A therapeutic synergistic effect may be attained when the PDL1-specific and β-catenin-specific aiRNAs are: (1) co-formulated and administered or delivered simultaneously in a combined formulation; (2) delivered by alternation or in parallel as separate formulations; or (3) by some other regimen. When delivered in alternation therapy, a therapeutic synergistic effect may be attained when the PDL1-specific and β-catenin-specific aiRNAs are administered or delivered sequentially, e.g. in separate tablets, pills or capsules, or by different injections in separate syringes. A therapeutic synergistic anticancer effect denotes an anticancer effect which is greater than the predicted purely additive effects of the PDL1-specific and β-catenin-specific aiRNAs administered separately.

[000146] A “therapeutically effective amount” of a combination of PDL1-specific and β-catenin-specific aiRNAs in reference to the treatment of cancer, means an amount capable of invoking one or more of the following effects: (1) inhibition, to some extent, of cancer or tumor growth, including slowing down growth or complete growth arrest; (2) reduction in the number of cancer or tumor cells; (3) reduction in tumor size; (4) inhibition (i.e., reduction, slowing down, or complete stopping) of cancer or tumor cell infiltration into peripheral organs; (5) inhibition (i.e., reduction, slowing down, or complete stopping) of metastasis; (6) enhancement of anti-tumor immune response, which may, but is not required to, result in the regression or rejection of the tumor, or (7) relief, to some extent, of one or more measurable symptoms associated with the cancer or tumor. The therapeutically effective amount may vary according to factors such as the disease state, age, sex, and weight of the individual and the ability of one or more anti-cancer agents to elicit a desired response in the individual. A “therapeutically effective amount” is also one in which any toxic or detrimental effects are outweighed by the therapeutically beneficial effects.

[000147] In certain embodiments, a subject is successfully "treated" according to the methods of the present teachings if the patient shows one or more of the following: a reduction in the number of or complete absence of cancer cells; a reduction in the tumor size; inhibition of or an absence of cancer cell infiltration into peripheral organs including the spread of cancer into soft tissue and bone; inhibition of or an absence of tumor metastasis; inhibition or an absence of tumor growth reduced morbidity and mortality. In certain embodiments, the term "treating cancer," "treatment of cancer," or an equivalent thereof, means to decrease, reduce, or inhibit the replication of cancer cells; decrease, reduce or inhibit the spread (formation of metastases) of cancer; decrease tumor size; decrease the number of tumors (i.e. reduce tumor burden); lessen or reduce the number of cancerous cells in the body; prevent recurrence of cancer after surgical removal or other anti-cancer therapies; or ameliorate measurable treatment endpoints (i.e., outcomes). "Treatment" can also mean prolonging survival as compared to expected survival in the absence of treatment.

[000148] As used herein, the terms "inhibiting", "to inhibit" and their grammatical equivalents, when used in the context of a bioactivity, refer to a down-regulation of the bioactivity, which may reduce or eliminate the targeted function, such as the production of a protein or the phosphorylation of a molecule. When used in the context of an organism (including a cell), the terms refer to a down-regulation of a bioactivity of the organism, which may reduce or eliminate a targeted function, such as the production of a protein or the phosphorylation of a molecule. In particular embodiments, inhibition may refer to a reduction, e.g., of about 10%, of about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 99%, or about 100% of the targeted activity. When used in the context of a disorder or disease, the terms refer to success at preventing the onset of measurable symptoms, alleviating measurable symptoms, or eliminating the disease, condition or disorder.

[000149] For example, the administration of PDL1-specific and β-catenin-specific aiRNAs to a tumor cell results in the simultaneous inhibition of PDL1 and β-catenin gene expression in the tumor cell including the level of PDL1 and β-catenin mRNA molecules or the level of PDL1 and β-catenin proteins or the activity of PDL1 and β-catenin proteins, below that observed in the

absence of the nucleic acid molecules (e.g., aiRNA) of the present teachings or in the presence of an aiRNA having a random 'scrambled' nucleotide sequence.

[000150] In certain embodiments, the exemplary PDL1-specific and β-catenin-specific aiRNAs of the present teachings silence or inhibit the expression of PDL1 and β-catenin mRNAs by about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90% or about 95% of the targeted gene.

[000151] In certain embodiments, the combination of the exemplary PDL1-specific and β-catenin-specific aiRNAs of the present teachings inhibit tumor growth by about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 99% or about 100%.

[000152] The present disclosure provides compositions comprising PDL1-specific and β-catenin-specific asymmetrical interfering RNAs (aiRNA), that can induce potent gene silencing of PDL1- and β-catenin gene expression in tumor cells. In one aspect, PDL1-specific and β-catenin-specific asymmetrical interfering RNAs are characterized in the length asymmetry of the two RNA strands. This structural design can not only be functionally potent in effecting gene silencing but offer several advantages over the current state-of-art siRNAs. Among the advantages, the PDL1-specific and β-catenin-specific aiRNAs can have RNA duplex structure of much shorter length than previously reported siRNA designs, which reduces the cost of synthesis and abrogate/reduce the length-dependent triggering of nonspecific interferon-like responses. In addition, the asymmetry of the aiRNA structure abrogates and/or otherwise reduces the sense-strand mediated off-target effects. PDL1-specific and β-catenin-specific aiRNA are therefore, in certain embodiments, more efficacious, more potent, with a more rapid-onset, and more durable at inducing gene silencing than any of the other RNA interfering agents.

[000153] In certain embodiments, PDL1-specific and β-catenin-specific aiRNAs disclosed herein each comprises an antisense strand with a length from 18-23 nucleotides (nt) and a sense strand with a length from 12-17 nucleotides. In certain embodiments, the sense strand is substantially complementary to the antisense strand. In certain embodiments, the sense strand forms a double-stranded region with the antisense strand. In certain embodiments, the antisense

strand can have a 3'-overhang of 1, 2, 3, 4, 5, 6, 7, 8 or 9 nucleotides. In certain embodiments, the antisense strand can have a 5'-overhang from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 nucleotides.

[000154] In certain embodiments, the antisense strand of the PDL1-specific or β-catenin-specific aiRNA can be 18, 19, 20, 22, or 23 nucleotides long.

[000155] In certain embodiments, the sense strand of the PDL1-specific or β-catenin-specific aiRNA can be 12, 13, 14, 15, 16, or 17 nucleotides long.

[000156] In certain embodiments, the 3'-overhang of the antisense strand is greater than 0 nucleotides in length.

[000157] In certain embodiments, the antisense strand comprises a sequence being substantially complementary to a target PDL1 and β-catenin mRNA sequences. In certain embodiments, the antisense strand of the PDL1-specific and β-catenin-specific aiRNAs comprise a sequence that is at least about 50%, about 60%, about 70 %, about 80%, about 90%, about 95%, about 99% or about 100% complementary to their corresponding target mRNA sequences.

[000158] In certain embodiments, the present disclosure provides a method of treating cancer comprising administering PDL1 and β-catenin aiRNAs, wherein the β-catenin aiRNA acts in synergy with the co-administered PDL1 aiRNA to inhibit tumor growth.

[000159] In certain embodiments, the change in the efficacy of a PDL1-specific aiRNA. as a result of the co-administration of a β-catenin-specific aiRNA, can be evaluated in subcutaneous tumor animal models at endpoints such as the percent test/control (%T/C) tumor weights calculated on each day that tumors are measured, tumor growth delay, net log cell kill, median days to a defined tumor weight or to a specified number of tumor doublings, and tumor regression. In certain embodiments, the lowest calculated %T/C seen over time can be defined as the optimal %T/C because it defines the greatest level of activity seen with β-catenin-specific aiRNA. The rate and duration of partial and complete tumor regressions can also be considered to be clinically relevant endpoints.

[000160] For example, a T/C = 0% means no tumor growth. A T/C = 100% means no antitumor activity, i.e., the treated and control tumors grew equally. A T/C equal to or less than 42% is considered significant antitumor activity by the Drug Evaluation Branch of the Division

of Cancer Treatment (NCI). A T/C value < 10% is considered to indicate highly significant antitumor activity, and is the level used by NCI to justify a clinical trial if toxicity, formulation, and certain other requirements are met (termed DN- 2 level activity).

[000161] The present disclosure reports on the surprising discovery that a treatment combination of PDL1-specific and β-catenin-specific aiRNAs have a greater effect in inhibiting tumor cell growth than the added effects of the PDL1-specific aiRNA and β-catenin-specific aiRNAs acting alone.

EXAMPLES

Example 1: Preparation of PDL1-specific and β–catenin-specific aiRNAs

[000162] PDL1-specific or β-catenin-specific aiRNAs are synthesized in DMT-on mode. Following completion of the synthesis, the solid support is suspended in 600 µl EtOH/NH_4OH solution (prepared by mixing 1 volume of 200 proof ethanol with 3 volumes of 28% NH_4OH) and heated at 55 °C for 2 hours. After primary de-protection, EtOH/NH_4OH is evaporated and the RNA oligo is dried to a pellet. 100µl of RNA de-protection solution (NMP/TEA.3HF (3:2)) is added and the solution is heated at 65 °C for 1.5 hours. The reaction is then quenched with 400 µl of 1.5 M ammonium bicarbonate. Purification is performed with Clarity® QSP Cartridges (Phenomenex, USA). The annealing of the resulting duplexes is confirmed on 15% PAGE gel. The location of mRNA sequences targeted by exemplary β-catenin-specific aiRNAs is shown in FIG. 1C. The location of mRNA sequences targeted by exemplary PDL1-specific aiRNAs is shown in FIG. 2C. For certain applications, selected nucleotides in the aiRNAs are modified with a 2'-OH methyl and/or 2'-OH fluorine group.

Example 2: Transfection of GFP, PDL1-specific and β-catenin-specific aiRNAs into luciferase-MDA-MB-231 cells

[000163] Luciferase expressing MDA-MB-231 cells (Luc-MDA-MB-231) are purchased from Cell Biolabs, Inc. and grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% inactivated fetal calf serum (FCS). 24 h before transfection, the cells are seeded into 6-well plates (5 x 10^4 cells/2 mL/well). The cells are then transfected with PD-L1-

specific aiRNA (aiPD-L1 #22) and β-catenin-specific aiRNA (aiβ-cat #57) or GFP aiRNA using Lipofectamine® RNAiMAX (Thermo Fisher, USA) at 1 nM final concentration according to the manufacturer's instructions. The aiRNAs and RNAiMAX are incubated for 20 minutes in serum free OPTI-MEM (Thermo Fisher, USA), before being added to the cells with culture medium. 48 hours after transfection, the cells are harvested using Accutase® cell detachment solution (Sigma Aldrich, USA). GFP aiRNA is used as a control. PD-L1 and β-catenin mRNA levels are measured by RT-PCR and PD-L1 protein expression was determined by flow cytometry. 1 nM of aiPD-L1 #22 decreases PD-L1 and β-catenin mRNA levels in Luc-MDA-MB-231 cell line by at least 75% and aiPD-L1 #22 reduces the surface PD-L1 expression on Luc-MDA-MB-231 cell.

Example 3: Potency of 2'-OMe modified β-Catenin-specific and aiPDL1-specific aiRNAS

[000164] An unmodified PD-L1-specific aiRNA (aiPD-L1 #22) and β-catenin-specific aiRNA (aiβ-cat #57) or a 2'-OMe modified PD-L1-specific aiRNA (aiPD-L1 #22) and β-catenin-specific aiRNA (aiβ-cat #57) (with 10 modifications at both 3'- and 5'-termini in the first strand and 5 modifications at both 3'- and 5'-termini in the second strand) are incubated in 100% human serum for 3 hours. aiRNA degradation in each sample is then assessed by running a 15% PAGE gel. The 2'-OMe modified PD-L1-specific and β-catenin-specific aiRNAs are significantly more stable than the unmodified PD-L1-specific and β-catenin-specific aiRNAs.

[000165] The 2'-OMe modified PD-L1-specific and β-catenin-specific aiRNAs are transfected into RKO cells at a concentration of 50 pM, 250 pM, and 500 pM. Western blot analysis of PD-L1 and β-catenin expression is conducted two days post transfection. Immunoblot analysis of total cell lysates is then performed using anti-PD-L1, anti-β-catenin and anti-β-actin antibodies. The 2'-OMe modified aiPD-L1 and aiβ-catenin are effective at inhibiting PD-L1 protein expression even at pM concentrations.

Example 4: Preparation of PDL1-specific or β–catenin-specific aiRNA nanoparticles (NPs)

[000166] Nanoparticles (NPs) loaded with PD-L1-specific and β-catenin-specific aiRNAs are prepared using the double-emulsion process described in WO2014123935, the content of which is hereby incorporated by reference herein in its entirety.

[000167] Specifically, in each of vials A and B, 5 mL of cyclohexane/Igepal CO-520 solution (71/29 v/v) is prepared from reagents respectively available from EMD and Sigma.

[000168] PD-L1-specific and β-catenin-specific aiRNAs are dispersed in 1x RNAse-free buffer to make the desired concentration (e.g., about 5 μg/μl). A volume of PD-L1-specific and β-catenin-specific aiRNAs (e.g., about 50 μg) is mixed with 100 μL of 500 mM $MgCl_2$. Then, the $MgCl_2$-aiRNA solution is added drop-wise to the oil/surfactant solution in vial A to form a well-dispersed emulsion without reverse micro-emulsion.

[000169] In vial B, 100 μL of 25 mM Na_2HPO_4 (pH=9) is added drop-wise to the oil/surfactant solution. The contents of vials A and B are then mixed and stirred for 30 minutes at room temperature. Afterwards, the contents are transferred into 10 centrifuge tubes (1.5 mL) and centrifuged for 30 minutes at 13,000g. The supernatant is discarded and the pellet is washed with absolute ethanol (1 mL) twice. After the alcohol is removed, the resulting pellet is air-dried for 3-4 hours.

[000170] A polymer-based shell is coated onto the MgP nanoparticle cores already loaded with aiPD-L1 and aiβ-catenin. Specifically, biodegradable polymers, PEG(5k)-Poly-L-Lysine (10U), Poly-L-Arginine (50U) are coated onto the cores at a polymer ratio of 2.5:1 (PLL: PLR) & a complex ratio of 2.5:1 (polymer: aiRNA).

[000171] Measurement for size/PdI determination of liposomes is then performed. The average size of the nanoparticles is about 70 nm and the surface charge is about +25 mV. The nanoparticles exhibit good plasma stability, cellular uptake and endosomal escape.

Example 5: Inhibition of PDL1 and β-catenin exhibits therapeutic synergy in the treatment of human breast xenografted tumors

[000172] Approximately, 6 X 10^6 human breast adenocarcinoma cells (MDA-MB-231 (ATCC® CRM-HTB-26™)) /mouse are inoculated subcutaneously into female athymic nude mice. Treatment starts when the tumor burden (mouse weight) reaches approximately 20 grams (~21 days after injection of cells). Xenografted mice are randomized into the following treatment groups:

- Group 1: Control aiRNA NP

- Group 2: aiPDL-1 NP
- Group 3: aiβ-catenin NP
- Group 4: aiPDL-1 + aiβ-catenin NP

[000173] Nanoparticle (NP) preparations comprising either an aiRNA control, PDL1-specific aiRNA, β-catenin-specific aiRNA or PDL1-specific and β-catenin-specific aiRNAs are administered to Groups 1-4 by intravenous injection starting on day 1. The aiRNA injections are then repeated every other day for 12 days, i.e., at a dose of 2.5 mg/kg/NP preparation on day 1, 3, 5, 8, and 10).

[000174] Xenografted animals are periodically weighed to assess the effect of treatment on tumor burden. The treatment is well tolerated with minimal adverse side effects. Experimental groups contain 10 mice/group and are representative of at least 2 independent experiments.

[000175] Administration of either ai-PDL1 NP or aiβ-catenin NP results in a delay in tumor progression as compared to tumor progression in subjects treated with the aiRNA control NP. However, the administration of ai-PDL1 NP and aiβ-catenin NP together to the xenografted mice of Group 4 results in a synergistic anti-tumor response with a T/C value < 10% when compared to time-matched, xenografted mice receiving either ai-PDL1 NP or aiβ-catenin NP.

Example 6: Inhibition of PDL1 and β-catenin gene expression exhibits therapeutic synergy in the treatment of human colon xenografted tumors

[000176] SW480 colon cancer cells are inoculated subcutaneously into male athymic nude mice (8 x 10^6 cells/mouse) and allowed to form palpable tumors. Once the tumors reached approximately 200 mm^3, the xenografted mice are randomized into the following treatment groups (10 mice/group):

- Group 1: Control aiRNA NP
- Group 2: aiPDL-1 NP
- Group 3: aiβ-catenin NP
- Group 4: aiPDL-1 + aiβ-catenin NP

[000177] Nanoparticle (NP) preparations comprising either an aiRNA control, PDL1-specific aiRNA, β-catenin-specific aiRNA or PDL1-specific and β-catenin-specific aiRNAs are

administered to Groups 1-4 by intravenous injection starting on day 1. The aiRNA injections are then repeated every other day for 12 days, i.e., at a dose of 2.5 mg/kg/NP preparation on day 1, 3, 5, 8, and 10).

[000178] Xenografted animals are periodically weighed to assess the effect of treatment on tumor burden. The treatment is well tolerated with minimal adverse side effects. Experimental groups contain 10 mice/group and are representative of at least 2 independent experiments.

[000179] Administration of either ai-PDL1 NP or aiβ-catenin NP results in a delay in tumor progression as compared to tumor progression in subjects treated with the aiRNA control NP. However, the administration of ai-PDL1 NP and aiβ-catenin NP together to the xenografted mice of Group 4 results in a synergistic anti-tumor response with a T/C value < 10% when compared to time-matched, xenografted mice receiving either ai-PDL1 NP or aiβ-catenin NP.

[000180] Significant tumor growth inhibition (e.g. from about 30 to 95%) by ai-PDL1 NP and aiβ-catenin NP is achieved not only in β-Catenin over-expressed colorectal tumor models, SW480 and APC^{min}, but also in the rest of β-Catenin normal-expressed tumor models.

Example 6: Inhibition of PDL1 and β-catenin gene expression exhibits therapeutic synergy in the treatment of orthotopic human lung tumors

[000181] A549-Luc NSCLC lung cancer cells are cultured in RPMI 1640 medium supplemented with 10% FBS in an atmosphere of 95% air/5% CO_2 at 37°C. The A549-Luc lung cancer cell line was previously established using a lentivirus encoding the luciferase gene driven by ubiquitin promoter, and stable clones were isolated by G418 selection (Dikmen *et al.* Cancer Res. (2005) 65:7866–7873). A total of 1×10^6 cells are subsequently mixed with matrigel (1:1) in a final volume of 30 µl and injected into the intercostal of anesthetized male nude rats (NIH-RNU, NCI-Frederick, MD) between ribs 5 and 6 of the right lung using a 28G1/2 needle.

[000182] Tumor growth is monitored by routine bioluminescence imaging using an IVIS Lumina Imaging System (Xenogen, Alameda, CA). Anesthetized rats are injected i.p. with D-luciferin (187.5 mg/kg). Bioluminescence images were acquired 10 min after luciferin injection. Once the tumors reached approximately 5 mm in diameter, the xenografted rats are randomized into the following treatment groups (10 rats/group):

- Group 1: Control aiRNA NP
- Group 2: aiPDL-1 NP
- Group 3: aiβ-catenin NP
- Group 4: aiPDL-1 + aiβ-catenin NP

[000183] Nanoparticle (NP) preparations comprising either an aiRNA control, PDL1-specific aiRNA, β-catenin-specific aiRNA or PDL1-specific and β-catenin-specific aiRNAs are administered to Groups 1-4 by intravenous injection starting on day 1. The aiRNA injections are then repeated every other day for 12 days, i.e., at a dose of 2.5 mg/kg/NP preparation on day 1, 3, 5, 8, and 10).

[000184] Administration of either ai-PDL1 NP or aiβ-catenin NP results in a delay in tumor progression as compared to tumor progression in subjects treated with the aiRNA control NP. However, the administration of ai-PDL1 NP and aiβ-catenin NP together to the orthotopic rats of Group 4 results in a synergistic anti-tumor response with a T/C value < 10% when compared to time-matched, rats receiving either ai-PDL1 NP or aiβ-catenin NP.

Example 7: Biodistribution analysis of fluorescence-labeled aiRNA

[000185] Approximately, 6 X 10^6 human breast adenocarcinoma cells (MDA-MB-231 (ATCC® CRM-HTB-26™)) /mouse are inoculated subcutaneously into female athymic nude mice. Once the tumor burden (mouse weight) reaches approximately 20 grams (~21 days after injection of cells), nanoparticle (NP) preparations comprising fluorescently labeled PDL1-specific and β-catenin-specific aiRNAs are administered to the xenografted mice by intravenous injection starting at a dose of 2.5 mg/kg/NP preparation. Delivery of the fluorescently labeled aiPDL-1 and aiβ-catenin to xenograft tumors occurs within 5 minutes of aiRNA administration and lasts at least 8 hours without any apparent adverse side effects.

CLAIMS

1. A composition comprising

a therapeutically effective amount of a β–catenin-specific asymmetric interfering RNA (aiRNA), and

a therapeutically effective amount of a PDL1-specific asymmetric interfering RNA (aiRNA),

wherein the combination of the PDL1-specific and β–catenin-specific aiRNAs exhibits therapeutic synergy in the treatment of cancer

2. The composition of claim 1, wherein the β–catenin-specific asymmetric interfering RNA (aiRNA) comprises

an antisense strand comprising 5'-terminal and 3'-terminal nucleotides that are 17, 18, 19, 20, or 21 nucleotides apart, and

a sense strand comprising a 5'-terminal nucleotide that is complementary to a nucleotide of the antisense strand other than its 3'-terminal nucleotide and a 3'-terminal nucleotide that is complementary to a nucleotide of the antisense strand,

wherein the antisense strand is at least 70% complementary with the sense strand, and

wherein 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 or 23 nucleotides of the antisense strand are colinear with the corresponding complementary nucleotides in a target nucleotide sequence chosen from SEQ ID NO. 180, 181, 182, 183, 184 or 185.

3. The composition any one of claims 1 or 2, wherein the PDL1-specific asymmetric interfering RNA (aiRNA) comprises

an antisense strand comprising 5'-terminal and 3'-terminal nucleotides that are 17, 18, 19, 20, or 21 nucleotides apart, and

a sense strand comprising a 5'-terminal nucleotide that is complementary to a nucleotide of the antisense strand other than its 3'-terminal nucleotide and a 3'-terminal nucleotide that is complementary to a nucleotide of the antisense strand,

wherein the antisense strand is at least 70% complementary with the sense strand, and

wherein 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 or 23 nucleotides of the antisense strand are colinear with the corresponding complementary nucleotides in a target nucleotide sequence chosen from SEQ ID NO. 167, 168, 169, 170, 171, 172 or 173.

4. The composition of claim 2, wherein at least 7 of the 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 or 23 nucleotides of the antisense strand of the β–catenin-specific asymmetric interfering RNA (aiRNA) are contiguous and colinear with the corresponding complementary nucleotides in a target nucleotide sequence chosen from SEQ ID NO. 180, 181, 182, 183, 184 or 185.

5. The composition of claim 2, wherein the 5'-terminal nucleotide of the sense strand of the β–catenin-specific asymmetric interfering RNA (aiRNA) is complementary to the first, second or third nucleotide adjacent to the 3'-terminal nucleotide of the antisense strand.

6. The composition of claim 2, wherein the 3'-terminal nucleotide of the sense strand of the β–catenin-specific asymmetric interfering RNA (aiRNA) is complementary to the first, second or third nucleotide adjacent to the 5'-terminal nucleotide of the antisense strand.

7. The composition of claim 2, wherein the 5'-terminal and 3'-terminal nucleotides of the sense strand of the β–catenin-specific asymmetric interfering RNA (aiRNA) are 13 nucleotides apart.

8. The composition of claim 7, wherein the 5'-terminal and 3'-terminal nucleotides of the antisense strand of the β–catenin-specific asymmetric interfering RNA (aiRNA) are 19 nucleotides apart.

9. The composition of claim 2, wherein the nucleotide sequence of the antisense strand of the β–catenin-specific asymmetric interfering RNA that is colinear with the corresponding complementary nucleotides in the target nucleotide sequence has a GC content of about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34% or about 35%.

10. The composition of claim 2, wherein the nucleotide sequence of the antisense strand of the β–catenin-specific asymmetric interfering RNA that is colinear with the corresponding complementary nucleotides in the target nucleotide sequence has a GC content of about 36%, about 37%, about 38%, about 39%, about 40%, about 41%, about 42%, about 43% or about 44%.

11. The composition of claim 2, wherein the nucleotide sequence of the antisense strand of the β–catenin-specific asymmetric interfering RNA that is colinear with the corresponding complementary nucleotides in the target nucleotide sequence has a GC content of about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55%, about 56%, about 57% or about 58%.

12. The composition of claim 2, wherein the nucleotide sequence of the antisense strand of the β–catenin-specific asymmetric interfering RNA that is colinear with the corresponding complementary nucleotides in the target nucleotide sequence has a GC content of about 47%.

13. The composition of claim 3, wherein at least 7 of the 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 or 23 nucleotides of the antisense strand of the PDL1-specific asymmetric interfering RNA (aiRNA) are contiguous and colinear with the corresponding complementary nucleotides in a target nucleotide sequence chosen from SEQ ID NO. 167, 168, 169, 170, 171, 172 or 173.

14. The composition of claim 3, wherein the 5'-terminal nucleotide of the sense strand of the PDL1-specific asymmetric interfering RNA (aiRNA) is complementary to the first, second or third nucleotide adjacent to the 3'-terminal nucleotide of the antisense strand.

15. The composition of claim 3, wherein the 3'-terminal nucleotide of the sense strand of the PDL1-specific asymmetric interfering RNA (aiRNA) is complementary to the first, second or third nucleotide adjacent to the 5'-terminal nucleotide of the antisense strand.

16. The composition of claim 3, wherein the 5'-terminal and 3'-terminal nucleotides of the sense strand of the PDL1-specific asymmetric interfering RNA (aiRNA) are 13 nucleotides apart.

17. The composition of claim 16, wherein the 5'-terminal and 3'-terminal nucleotides of the antisense strand of the PDL1-specific asymmetric interfering RNA (aiRNA) are 19 nucleotides apart.

18. The composition of claim 3, wherein the nucleotide sequence of the antisense strand of the PDL1-specific asymmetric interfering RNA that is colinear with the corresponding complementary nucleotides in the target nucleotide sequence has a GC content of about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34% or about 35%.

19. The composition of claim 3, wherein the nucleotide sequence of the antisense strand of the PDL1-specific asymmetric interfering RNA that is colinear with the corresponding complementary nucleotides in the target nucleotide sequence has a GC content of about 36%, about 37%, about 38%, about 39%, about 40%, about 41%, about 42%, about 43% or about 44%.

20. The composition of claim 3, wherein the nucleotide sequence of the antisense strand of the PDL1-specific asymmetric interfering RNA that is colinear with the corresponding complementary nucleotides in the target nucleotide sequence has a GC content of about 45%, about 46%, about 47%, about 48%, about 49%, about 50%, about 51%, about 52%, about 53%, about 54%, about 55%, about 56%, about 57% or about 58%.

21. The composition of claim 3, wherein the nucleotide sequence of the antisense strand of the PDL1-specific asymmetric interfering RNA that is colinear with the corresponding complementary nucleotides in the target nucleotide sequence has a GC content of about 33%.

22. The composition of any one of claims 2-21, wherein the sense or antisense strand of each asymmetric interfering RNA comprises at least one modified nucleotide or its analogue.

23. The composition of claim 22, wherein a 2'-OH group of the at least one modified ribonucleotide or its analogue is replaced by H or a 2'-O-methyl group.

24. The composition of claim 23, wherein the at least one modified nucleotide or its analogue is a sugar-, backbone-, and/or base-modified ribonucleotide.

25. The composition of claim 24, wherein the backbone-modified ribonucleotide comprises a modification in a phosphodiester linkage with another ribonucleotide.

26. The composition of claim 25, wherein the phosphodiester linkage is modified to comprise a nitrogen or a sulfur heteroatom.

27. The composition of claim 22, wherein the at least one modified nucleotide or its analogue comprises a phosphothioate group.

28. The composition of claim 22, wherein the at least one modified nucleotide or its analogue comprises inosine or a tritylated base.

29. The composition of claim 22, wherein the at least one modified nucleotide or its analogue is a sugar-modified ribonucleotide, wherein a 2'-OH group is replaced by H, OR, R, halo, SH, SR, NH2, NHR, NR2, or CN, and wherein each R is independently C1-C6 alkyl, alkenyl or alkynyl, and halo is F, Cl, Br, or I.

30. The composition of claim 1, wherein the PDL1-specific and/or β–catenin-specific aiRNA comprises a deoxyribonucleotide.

31. The composition of any one of claims 1-3, wherein the composition comprises nanoparticles having PDL1-specific and β–catenin-specific aiRNAs.

32. The composition of any one of claims 1-31, wherein the PDL1-specific and/or β–catenin-specific aiRNA is bound to a peptide, an antibody, a polymer, a lipid, an oligonucleotide, cholesterol, or an aptamer.

33. A pharmaceutical composition comprising the PDL1-specific and β–catenin-specific aiRNAs of the compositions of any one of claims 1-29 and a lipid or a cholesterol molecule.

34. The pharmaceutical composition of claim 33, wherein the lipid or cholesterol molecule is conjugated to the PDL1-specific and/or β–catenin-specific aiRNA.

35. The pharmaceutical composition of claim 33, wherein the PDL1-specific and β–catenin-specific aiRNAs comprise a plurality of modified nucleotides or their analogues, each modified nucleotide or its analogue comprising:

a 2'-O-methyl or a 2'-fluorine group, and/or

a phosphothioate or phosphodiester backbone.

36. A kit for silencing PDL1 and β–catenin gene expression in tumor cells comprising PDL1-specific and β–catenin-specific aiRNAs of the compositions of any one of claims 1-35, a nanoparticle formulation and instructions for their use.

37. An expression vector comprising a nucleic acid sequence encoding the PDL1-specific and/or β–catenin-specific aiRNAs of the compositions of any one of claims 1-21.

38. The expression vector of claim 37, wherein the expression vector is a viral, a eukaryotic, or a bacterial expression vector.

39. An isolated cell comprising the expression vector of claim 37.

40. An isolated cell comprising the PDL1-specific and β–catenin-specific aiRNAs of the compositions of any one of claims 1-35.

41. The cell of claim 40, wherein the cell is a mammalian, avian, insect, yeast or bacterial cell.

42. A method for treating cancer in a subject in need thereof comprising administering an effective amount of the PDL1-specific and β–catenin-specific aiRNAs of the

compositions of any one of claims 1-35, wherein the combination of PDL1-specific and β–catenin-specific aiRNAs exhibits therapeutic synergy in the treatment of cancer.

43. The method of claim 42, wherein the cancer is an AIDS-Related cancer, a breast cancer, a cancer of the digestive/gastrointestinal tract, an endocrine and neuroendocrine cancer, a cancer of the eye, a genitourinary cancer, a germ cell cancer, a gynecologic cancer, a head and neck cancer, a hematologic cancer, a musculoskeletal cancer, a neurologic cancer, a respiratory/thoracic cancer, a skin cancer, a childhood cancer or a cancer of unknown primary.

44. The method of claim 42, wherein the cancer is metastatic, recurrent or resistant to chemotherapy and/or radiation.

45. The method of claim 42, wherein the PDL1-specific and β–catenin-specific aiRNAs of the compositions of any one of claims 1-35 are administered systemically or locally.

46. The method of claim 42, wherein the PDL1-specific and β–catenin-specific aiRNAs of the compositions of any one of claims 1-35 are effective silencing PDL1 and β–catenin gene expression.

47. The method of claim 42, wherein the PDL1-specific and β–catenin-specific aiRNAs of the compositions of any one of claims 1-35 are effective at inhibiting tumor growth.

48. The method of claim 42, wherein the PDL1-specific and β–catenin-specific aiRNAs of the compositions of any one of claims 1-35 are effective at inducing cancer stem cell death.

49. The method of claim 42, wherein the PDL1-specific and β–catenin-specific aiRNAs of the compositions of any one of claims 1-35 are effective at enhancing an immune response against tumor cells.

50. The method of claim 47, wherein the tumor comprises cells that express nuclear β–catenin.

51. The method of claim 47, wherein the tumor comprises cells that do not express nuclear β–catenin.

52. The method of claim 44, wherein the PDL1-specific and β–catenin-specific aiRNAs of the compositions of any one of claims 1-35 silence PDL1 and β–catenin gene expression for at least 8 hours.

53. The method of claim 44, wherein the PDL1-specific and β–catenin-specific aiRNAs of the compositions of any one of claims 1-35 are delivered to tumors within 5 minutes of administration.

FIG. 1A


Homo sapiens catenin beta 1 (CTNNB1),
transcript variant 2, mRNA
(ACCESSION No.: NM_001098209.1; SEQ ID NO: 179)
1 AGGATACAGC GGCTTCTGCG CGACTTATAA GAGCTCCTTG TGCGGCGCCA TTTTAAGCCT
61 CTCGGTCTGT GGCAGCAGCG TTGGCCCGGC CCCGGGAGCG GAGAGCGAGG GGAGGCGGAG
121 ACGGAGGAAG GTCTGAGGAG CAGCTTCAGT CCCCGCCGAG CCGCCACCGC AGGTCGAGGA
181 CGGTCGGACT CCCGCGGCGG GAGGAGCCTG TTCCCCTGAG GGTATTTGAA GTATACCATA
(aiRNA#180)
(aiRNA#519)
241 CAACTGTTTT GAAAATCCAG CGTGGACAAT GGCTACTCAA GCTGATTTGA TGGAGTTGGA
(aiRNA#181)
(aiRNA#205)
301 CATGGCCATG GAACCAGACA GAAAAGCGGC TGTTAGTCAC TGGCAGCAAC AGTCTTACCT
361 GGACTCTGGA ATCCATTCTG GTGCCACTAC CACAGCTCCT TCTCTGAGTG GTAAAGGCAA
(aiRNA#206)
(aiRNA#508)
421 TCCTGAGGAA GAGGATGTGG ATACCTCCCA AGTCCTGTAT GAGTGGGAAC AGGGATTTTC
481 TCAGTCCTTC ACTCAAGAAC AAGTAGCTGA TATTGATGGA CAGTATGCAA TGACTCGAGC
(aiRNA#210)
541 TCAGAGGGTA CGAGCTGCTA TGTTCCCTGA GACATTAGAT GAGGGCATGC AGATCCCATC
(aiRNA#532)
(aiRNA#216)
601 TACACAGTTT GATGCTGCTC ATCCCACTAA TGTCCAGCGT TTGGCTGAAC CATCACAGAT
(aiRNA#528)
(aiRNA#182)
(aiRNA#220)
661 GCTGAAACAT GCAGTTGTAA ACTTGATTAA CTATCAAGAT GATGCAGAAC TTGCCACACG
(aiRNA#545)
(aiRNA#183)
(aiRNA#302)
(aiRNA#509)
721 TGCAATCCCT GAACTGACAA AACTGCTAAA TGACGAGGAC CAGGTGGTGG TTAATAAGGC
(aiRNA#546)
781 TGCAGTTATG GTCCATCAGC TTTCTAAAAA GGAAGCTTCC AGACACGCTA TCATGCGTTC
841 TCCTCAGATG GTGTCTGCTA TTGTACGTAC CATGCAGAAT ACAAATGATG TAGAAACAGC
(aiRNA#224)
(aiRNA#185)
901 TCGTTGTACC GCTGGGACCT TGCATAACCT TTCCCATCAT CGTGAGGGCT TACTGGCCAT
(aiRNA#534)
(aiRNA#546)
961 CTTTAAGTCT GGAGGCATTC CTGCCCTGGT GAAAATGCTT GGTTCACCAG TGGATTCTGT
(aiRNA#57)

FIG. 1A (ctd.)


1021 GTTGTTTTAT GCCATTACAA CTCTCCACAA CCTTTTATTA CATCAAGAAG GAGCTAAAAT
1081 GGCAGTGCGT TTAGCTGGTG GGCTGCAGAA AATGGTTGCC TTGCTCAACA AAACAAATGT
1141 TAAATTCTTG GCTATTACGA CAGACTGCCT TCAAATTTTA GCTTATGGCA ACCAAGAAAG
CTTG GCTATTACGA C (aiRNA#235)
A CAGACTGCCT TCAAATTT (aiRNA#511)
GACC CCAAGCTTTA GTAAA (aiRNA#535)
1201 CAAGCTCATC ATACTGGCTA GTGGTGGACC CCAAGCTTTA GTAAATATAA TGAGGACCTA
1261 TACTTACGAA AAACTACTGT GGACCACAAG CAGAGTGCTG AAGGTGCTAT CTGTCTGCTC
1321 TAGTAATAAG CCGGCTATTG TAGAAGCTGG TGGAATGCAA GCTTTAGGAC TTCACCTGAC
G TGGAATGCAA GCTTTAGG (aiRNA#241)
GGAATGCAA GCTTTAGGAC (aiRNA#520)
AC (aiRNA#68)
AGATCGAAGT GAACGTC
1381 AGATCCAAGT CAACGTCTTG TTCAGAACTG TCTTTGGACT CTCAGGAATC TTTCAGATGC
AGATGC (aiRNA#245)
TGCAACTAAA CAGG
CAGGAATC TTTCAGATGC (aiRNA#190)
G
1441 TGCAACTAAA CAGGAAGGGA TGGAAGGTCT CCTTGGGACT CTTGTTCAGC TTCTGGGTTC
CTTGTTCAGC TTCTGGGTT (aiRNA#537)
C TTCTGGGTTC (aiRNA#154)
AGNTGATA
1501 AGATGATATA AATGTGGTCA CCTGTGCAGC TGGAATTCTT TCTAACCTCA CTTGCAATAA
C TGGAATTCTT TCTAACCT (aiRNA#77)
GGAATTCTT TCTAACCTCA (aiRNA#109)
TTCTT TCTAACCTCA CTTG (aiRNA#480)
1561 TTATAAGAAC AAGATGATGG TCTGCCAAGT GGGTGGTATA GAGGCTCTTG TGCGTACTGT
TAAGAAC AAGATGATGG TC (aiRNA#440)
GGCTGGTATA GAGGCTCTT (aiRNA#538)
1621 CCTTCGGGCT GGTGACAGGG AAGACATCAC TGAGCCTGCC ATCTGTGCTC TTCGTCATCT
1681 GACCAGCCGA CACCAAGAAG CAGAGATGGC CCAGAATGCA GTTCGCCTTC ACTATGGACT
1741 ACCAGTTGTG GTTAAGCTCT TACACCCACC ATCCCACTGG CCTCTGATAA AGGCTACTGT
ATAA AGGCTACTGT (aiRNA#513)
TGGAT
CTGT (aiRNA#158)
TGGATTGATT CGAAA
1801 TGGATTGATT CGAAATCTTG CCCTTTGTCC CGCAAATCAT GCACCTTTGC GTGAGCAGGG
CTTG CCCTTTGTCC CGCAA (aiRNA#539)
1861 TGCCATTCCA CGACTAGTTC AGTTGCTTGT TCGTGCACAT CAGGATACCC AGCGCCGTAC
CA CGACTAGTTC AGTTGCT (aiRNA#159)
GACTAGTTC AGTTGCTTGT (aiRNA#191)
1921 GTCCATGGGT GGGACACAGC AGCAATTTGT GGAGGGGGTC CGCATGGAAG AAATAGTTGA
GGAAG AAATAGTTGA (aiRNA#521)
AGGT
1981 AGGTTGTACC GGAGCCCTTC ACATCCTAGC TCGGGATGTT CACAACCGAA TTGTTATCAG
2041 AGGACTAAAT ACCATTCCAT TGTTTGTGCA GCTGCTTTAT TCTCCCATTG AAAACATCCA
CTAAAT ACCATTCCAT TGT (aiRNA#162)

FIG. 1A (ctd.)

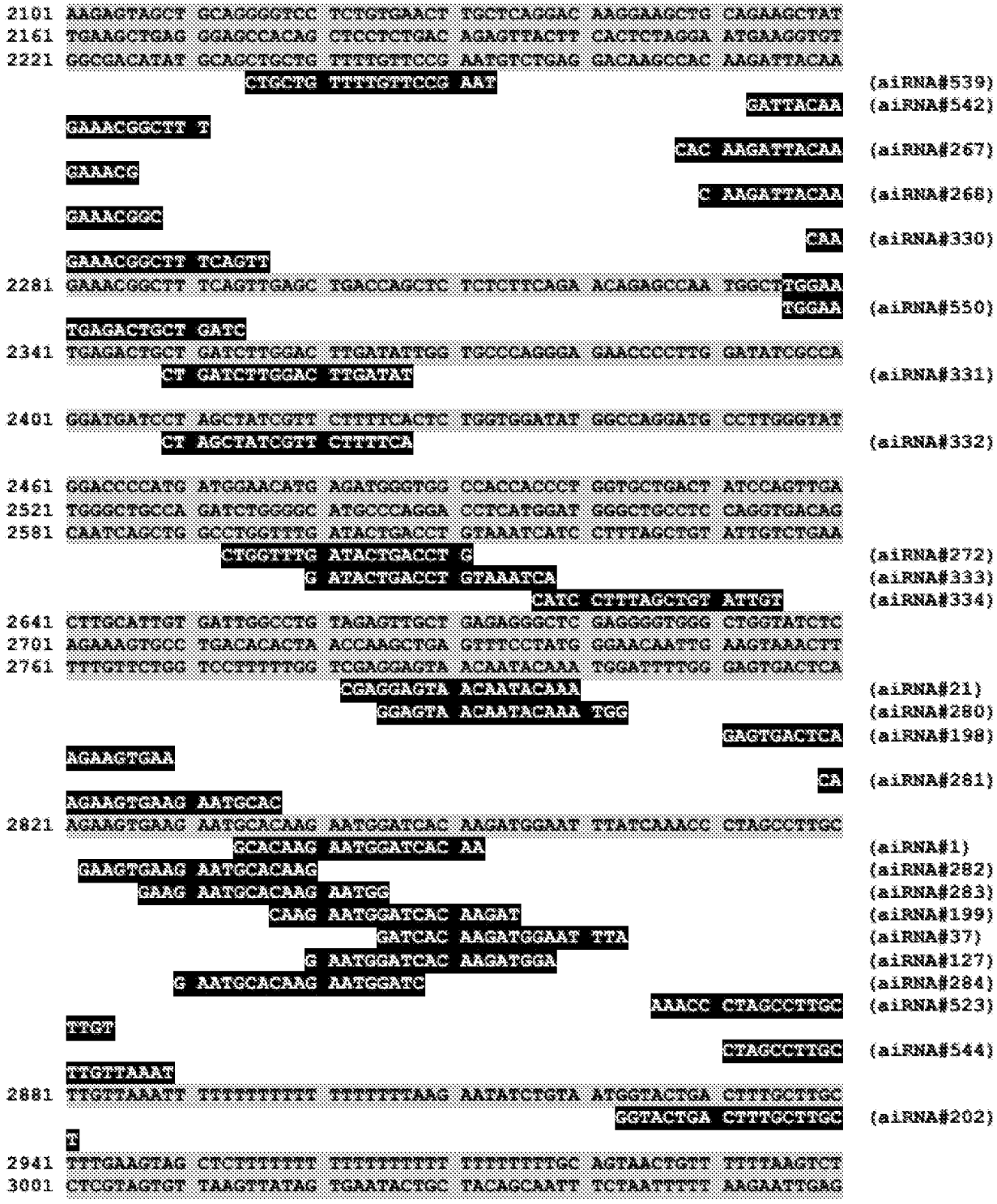

2101 AAGACTAGCT GCAGGGGTCC TCTGTGAACT TGCTCAGGAC AAGGAAGCTG CAGAAGCTAT
2161 TGAAGCTGAG GGAGCCACAG CTCCTCTGAC AGAGTTACTT CACTCTAGGA ATGAAGGTGT
2221 GGCCACATAT GCAGCTGCTG TTTTGTTCCG AATGTCTGAG GACAAGCCAC AAGATTACAA
CTGCTG TTTTGTTCCG AAT (aiRNA#539)
GATTACAA (aiRNA#542)
GAAACGGCTT T
CAC AAGATTACAA (aiRNA#267)
GAAACG
C AAGATTACAA (aiRNA#268)
GAAACGGC
CAA (aiRNA#330)
GAAACGGCTT TCAGTT
2281 GAAACGGCTT TCAGTTGAGC TGACCAGCTC TCTCTTCAGA ACAGAGCCAA TGGCTGGAA
TGGAA (aiRNA#550)
TGAGACTGCT GATC
2341 TGAGACTGCT GATCTTGGAC TTGATATTGG TGCCCAGGGA GAACCCCTTG GATATCGCCA
CT GATCTTGGAC TTGATAT (aiRNA#331)
2401 GGATGATCCT AGCTATCGTT CTTTTCACTC TGGTGGATAT GGCCAGGATG CCTTGGGTAT
CT AGCTATCGTT CTTTTCA (aiRNA#332)
2461 GGACCCCATG ATGGAACATG AGATGGGTGG CCACCACCCT GGTGCTGACT ATCCAGTTGA
2521 TGGGCTGCCA GATCTGGGGC ATGCCCAGGA CCTCATGGAT GGGCTGCCTC CAGGTGACAG
2581 CAATCAGCTG GCCTGGTTTG ATACTGACCT GTAAATCATC CTTTAGCTGT ATTGTCTGAA
CTGGTTTG ATACTGACCT G (aiRNA#272)
G ATACTGACCT GTAAATCA (aiRNA#333)
CATC CTTTAGCTGT ATTGT (aiRNA#334)
2641 CTTGCATTGT GATTGGCCTG TAGAGTTGCT GAGAGGGCTC GAGGGGTGGG CTGGTATCTC
2701 AGAAAGTGCC TGACACACTA ACCAAGCTGA GTTTCCTATG GGAACAATTG AAGTAAACTT
2761 TTTGTTCTGG TCCTTTTTGG TCGAGGAGTA ACAATACAAA TGGATTTTGG GAGTGACTCA
CGAGGAGTA ACAATACAAA (aiRNA#21)
GGAGTA ACAATACAAA TGG (aiRNA#280)
GAGTGACTCA (aiRNA#198)
AGAAGTGAA
CA (aiRNA#281)
AGAAGTGAAG AATGCAC
2821 AGAAGTGAAG AATGCACAAG AATGGATCAC AAGATGGAAT TTATCAAACC CTAGCCTTGC
GCACAAG AATGGATCAC AA (aiRNA#1)
GAAGTGAAG AATGCACAAG (aiRNA#282)
GAAG AATGCACAAG AATGG (aiRNA#283)
CAAG AATGGATCAC AAGAT (aiRNA#199)
GATCAC AAGATGGAAT TTA (aiRNA#37)
G AATGGATCAC AAGATGGA (aiRNA#127)
G AATGCACAAG AATGGATC (aiRNA#284)
AAACC CTAGCCTTGC (aiRNA#523)
TTGT
CTAGCCTTGC (aiRNA#544)
TTGTTAAAT
2881 TTGTTAAATT TTTTTTTTTT TTTTTTTAAG AATATCTGTA ATGGTACTGA CTTTGCTTGC
GGTACTGA CTTTGCTTGC (aiRNA#202)
T
2941 TTTGAAGTAG CTCTTTTTTT TTTTTTTTTT TTTTTTTTGC AGTAACTGTT TTTTAAGTCT
3001 CTCGTAGTGT TAAGTTATAG TGAATACTGC TACAGCAATT TCTAATTTTT AAGAATTGAG

FIG. 1A (ctd.)

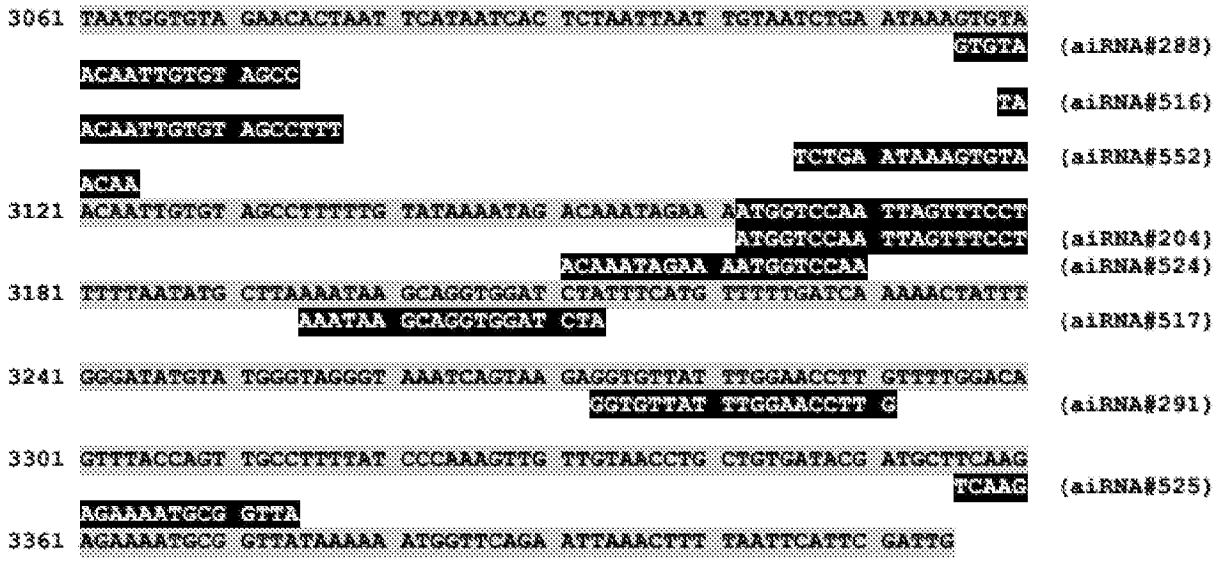

3061 TAATGGTGTA GAACACTAAT TCATAATCAC TCTAATTAAT TGTAATCTGA ATAAAGTGTA
GTGTA (aiRNA#288)
ACAATTGTGT AGCC
TA (aiRNA#516)
ACAATTGTGT AGCCTTT
TCTGA ATAAAGTGTA (aiRNA#552)
ACAA
3121 ACAATTGTGT AGCCTTTTTG TATAAAATAG ACAAATAGAA AATGGTCCAA TTAGTTTCCT
ATGGTCCAA TTAGTTTCCT (aiRNA#204)
ACAAATAGAA AATGGTCCAA (aiRNA#524)
3181 TTTTAATATG CTTAAAATAA GCAGGTGGAT CTATTTCATG TTTTTGATCA AAAACTATTT
AAATAA GCAGGTGGAT CTA (aiRNA#517)
3241 GGCATATGTA TGGGTAGGGT AAATCAGTAA GAGGTGTTAT TTGGAACCTT GTTTTGGACA
GGTGTTAT TTGGAACCTT G (aiRNA#291)
3301 GTTTACCAGT TGCCTTTTAT CCCAAAGTTG TTGTAACCTG CTGTGATACG ATGCTTCAAG
TCAAG (aiRNA#525)
AGAAAATGCG GTTA
3361 AGAAAATGCG GTTATAAAAA ATGGTTCAGA ATTAAACTTT TAATTCATTC GATTG

FIG. 1B

Homo sapiens catenin beta 1 (CTNNB1), transcript variant 2, mRNA (ACCESSION No.: NM_001098209.1; SEQ ID NO: 179)	**β–catenin mRNA fragments**		**β–catenin aiRNA #**
	Location	**SEQ ID NO.**	
1 AGGATACAGC GGCTTCTGCG CGACTTATAA GAGCTCCTTG TGCGGCGCCA TTTTAAGCCT	**1-526**	**180**	**β–cat aiRNA#180**
61 CTCGGTCTGT GGCAGCAGCG TTGGCCCGGC CCCGGGAGCG GAGAGCGAGG GGAGGCGGAG			**β–cat aiRNA#181**
121 ACGGAGGAAG GTCTGAGGAG CAGCTTCAGT CCCCGCCGAG CCGCCACCGC AGGTCGAGGA			**β–cat aiRNA#205**
181 CGGTCGGACT CCCGCGGCGG GAGGAGCCTG TTCCCCTGAG GGTATTTGAA GTATACCATA			**β–cat aiRNA#206**
241 CAACTGTTTT GAAAATCCAG CGTGGACAAT GGCTACTCAA GCTGATTTGA TGGAGTTGGA			**β–cat aiRNA#210**
301 CATGGCCATG GAACCAGACA GAAAAGCGGC TGTTAGTCAC TGGCAGCAAC AGTCTTACCT			
361 GGACTCTGGA ATCCATTCTG GTGCCACTAC CACAGCTCCT TCTCTGAGTG GTAAAGGCAA			
421 TCCTGAGGAA GAGGATGTGG ATACCTCCCA AGTCCTGTAT GAGTGGGAAC AGGGATTTTC			
(SEQ ID NO: 210) 481 TCAGTCCTTC ACTCAAGAAC AA**GTAGCTGA TATTGATGGA C**			
(SEQ ID NO: 210) GTAGCTGA TATTGATGGA CAGTATGCAA TGACTCGAGC	**5500-1140**	**1181**	**β–cat aiRNA#180**
541 TCAGAGGGTA CGAGCTGCTA TGTTCCCTGA GACATTAGAT GAGGGCATGC AGATCCCATC			**β–cat aiRNA#181**
601 TACACAGTTT GATGCTGCTC ATCCCACTAA TGTCCAGCGT TTGGCTGAAC CATCACAGAT			**β–cat aiRNA#205**
661 GCTGAAACAT GCAGTTGTAA ACTTGATTAA CTATCAAGAT GATGCAGAAC TTGCCACACG			**β–cat aiRNA#206**
721 TGCAATCCCT GAACTGACAA AACTGCTAAA TGACGAGGAC CAGGTGGTGG TTAATAAGGC			**β–cat aiRNA#210**
781 TGCAGTTATG GTCCATCAGC TTTCTAAAAA GGAAGCTTCC AGACACGCTA TCATGCGTTC			**β–cat aiRNA#216**
841 TCCTCAGATG GTGTCTGCTA TTGTACGTAC CATGCAGAAT ACAAATGATG TAGAAACAGC			**β–cat aiRNA#182**
901 TCGTTGTACC GCTGGGACCT TGCATAACCT TTCCCATCAT CGTGAGGGCT TACTGGCCAT			**β–cat aiRNA#220**
961 CTTTAAGTCT GGAGGCATTC CTGCCCTGGT GAAAATGCTT GGTTCACCAG TGGATTCTGT			**β–cat aiRNA#183**
1021 GTTGTTTTAT GCCATTACAA CTCTCCACAA CCTTTTATTA CATCAAGAAG GAGCTAAAAT			**β–cat aiRNA#302**
1081 GGCAGTGCGT TTAGCTGGTG GGCTGCAGAA AATGGTTGCC TTGCTCAACA AAACAAATGT			**β–cat aiRNA#224**
			β–cat aiRNA#185
			β–cat aiRNA#57

(SEQ ID NO:210) **GTAGCTGA TATTGATGGA C**AGTATGCAA TGACTCGAGC	**5500-1560**	**1182**	**β–cat aiRNA#180**
541 TCAGAGGGTA CGAGCTGCTA TGTTCCCTGA GACATTAGAT GAGGGCATGC AGATCCCATC			**β–cat aiRNA#181**
601 TACACAGTTT GATGCTGCTC ATCCCACTAA TGTCCAGCGT TTGGCTGAAC CATCACAGAT			**β–cat aiRNA#205**
661 GCTGAAACAT GCAGTTGTAA ACTTGATTAA CTATCAAGAT GATGCAGAAC TTGCCACACG			**β–cat aiRNA#206**
721 TGCAATCCCT GAACTGACAA AACTGCTAAA TGACGAGGAC CAGGTGGTGG TTAATAAGGC			**β–cat aiRNA#210**
781 TGCAGTTATG GTCCATCAGC TTTCTAAAAA GGAAGCTTCC AGACACGCTA TCATGCGTTC			**β–cat aiRNA#216**
841 TCCTCAGATG GTGTCTGCTA TTGTACGTAC CATGCAGAAT ACAAATGATG TAGAAACAGC			**β–cat aiRNA#182**
901 TCGTTGTACC GCTGGGACCT TGCATAACCT TTCCCATCAT CGTGAGGGCT TACTGGCCAT			**β–cat aiRNA#220**
961 CTTTAAGTCT GGAGGCATTC CTGCCCTGGT GAAAATGCTT GGTTCACCAG TGGATTCTGT			**β–cat aiRNA#183**
1021 GTTGTTTTAT GCCATTACAA CTCTCCACAA CCTTTTATTA CATCAAGAAG GAGCTAAAAT			**β–cat aiRNA#302**
1081 GGCAGTGCGT TTAGCTGGTG GGCTGCAGAA AATGGTTGCC TTGCTCAACA AAACAAATGT			**β–cat aiRNA#224**
1141 TAAATTCTTG GCTATTACGA CAGACTGCCT TCAAATTTTA GCTTATGGCA ACCAAGAAAG			**β–cat aiRNA#185**
1201 CAAGCTCATC ATACTGGCTA GTGGTGGACC CCAAGCTTTA GTAAATATAA TGAGGACCTA			**β–cat aiRNA#57**
1261 TACTTACGAA AAACTACTGT GGACCACAAG CAGAGTGCTG AAGGTGCTAT CTGTCTGCTC			**β–cat aiRNA#235**
1321 TAGTAATAAG CCGGCTATTG TAGAAGCTGG TGGAATGCAA GCTTTAGGAC TTCACCTGAC			**β–cat aiRNA#241**
1381 AGATCCAAGT CAACGTCTTG TTCAGAACTG TCTTTGGACT CTCAGGAATC TTTCAGATGC			**β–cat aiRNA#68**
1441 TGCAACTAAA CAGGAAGGGA TGGAAGGTCT CCTTGGGACT CTTGTTCAGC TTCTGGGTTC			**β–cat aiRNA#245**
1501 AGATGATATA AATGTGGTCA CCTGTGCAGC TGGAATTCTT TCTAACCTCA CTTGCAATAA			**β–cat aiRNA#190**
			β–cat aiRNA#154
			β–cat aiRNA#77
			β–cat aiRNA#109
(SEQ ID NO. 210) **GTAGCTGA TATTGATGGA C**AGTATGCAA TGACTCGAGC	**500-2100**	**183**	**β–cat aiRNA#180**
541 TCAGAGGGTA CGAGCTGCTA TGTTCCCTGA GACATTAGAT GAGGGCATGC AGATCCCATC			**β–cat aiRNA#181**
601 TACACAGTTT GATGCTGCTC ATCCCACTAA TGTCCAGCGT TTGGCTGAAC CATCACAGAT			**β–cat aiRNA#205**
661 GCTGAAACAT GCAGTTGTAA ACTTGATTAA CTATCAAGAT GATGCAGAAC TTGCCACACG			**β–cat aiRNA#206**
721 TGCAATCCCT GAACTGACAA AACTGCTAAA TGACGAGGAC CAGGTGGTGG TTAATAAGGC			**β–cat aiRNA#210**
781 TGCAGTTATG GTCCATCAGC TTTCTAAAAA GGAAGCTTCC AGACACGCTA TCATGCGTTC			**β–cat aiRNA#180**

841 TCCTCAGATG GTGTCTGCTA TTGTACGTAC CATGCAGAAT ACAAATGATG TAGAAACAGC			**β–cat aiRNA#181**
901 TCGTTGTACC GCTGGGACCT TGCATAACCT TTCCCATCAT CGTGAGGGCT TACTGGCCAT			**β–cat aiRNA#205**
961 CTTTAAGTCT GGAGGCATTC CTGCCCTGGT GAAAATGCTT GGTTCACCAG TGGATTCTGT			**β–cat aiRNA#206**
1021 GTTGTTTTAT GCCATTACAA CTCTCCACAA CCTTTTATTA CATCAAGAAG GAGCTAAAAT			**β–cat aiRNA#210**
1081 GGCAGTGCGT TTAGCTGGTG GGCTGCAGAA AATGGTTGCC TTGCTCAACA AAACAAATGT			**β–cat aiRNA#216**
1141 TAAATTCTTG GCTATTACGA CAGACTGCCT TCAAATTTTA GCTTATGGCA ACCAAGAAAG			**β–cat aiRNA#182**
1201 CAAGCTCATC ATACTGGCTA GTGGTGGACC CCAAGCTTTA GTAAATATAA TGAGGACCTA			**β–cat aiRNA#220**
1261 TACTTACGAA AAACTACTGT GGACCACAAG CAGAGTGCTG AAGGTGCTAT CTGTCTGCTC			**β–cat aiRNA#183**
1321 TAGTAATAAG CCGGCTATTG TAGAAGCTGG TGGAATGCAA GCTTTAGGAC TTCACCTGAC			**β–cat aiRNA#302**
1381 AGATCCAAGT CAACGTCTTG TTCAGAACTG TCTTTGGACT CTCAGGAATC TTTCAGATGC			**β–cat aiRNA#224**
1441 TGCAACTAAA CAGGAAGGGA TGGAAGGTCT CCTTGGGACT CTTGTTCAGC TTCTGGGTTC			**β–cat aiRNA#185**
1501 AGATGATATA AATGTGGTCA CCTGTGCAGC TGGAATTCTT TCTAACCTCA CTTGCAATAA			**β–cat aiRNA#57**
1561 TTATAAGAAC AAGATGATGG TCTGCCAAGT GGGTGGTATA GAGGCTCTTG TGCGTACTGT			**β–cat aiRNA#235**
1621 CCTTCGGGCT GGTGACAGGG AAGACATCAC TGAGCCTGCC ATCTGTGCTC TTCGTCATCT			**β–cat aiRNA#241**
1681 GACCAGCCGA CACCAAGAAG CAGAGATGGC CCAGAATGCA GTTCGCCTTC ACTATGGACT			**β–cat aiRNA#68**
1741 ACCAGTTGTG GTTAAGCTCT TACACCCACC ATCCCACTGG CCTCTGATAA AGGCTACTGT			**β–cat aiRNA#245**
1801 TGGATTGATT CGAAATCTTG CCCTTTGTCC CGCAAATCAT GCACCTTTGC GTGAGCAGGG			**β–cat aiRNA#190**
1861 TGCCATTCCA CGACTAGTTC AGTTGCTTGT TCGTGCACAT CAGGATACCC AGCGCCGTAC			**β–cat aiRNA#154**
1921 GTCCATGGGT GGGACACAGC AGCAATTTGT GGAGGGGGTC CGCATGGAAG AAATAGTTGA			**β–cat aiRNA#77**
1981 AGGTTGTACC GGAGCCCTTC ACATCCTAGC TCGGGATGTT CACAACCGAA TTGTTATCAG			**β–cat aiRNA#109**
2041 AGGACTAAAT ACCATTCCAT TGTTTGTGCA GCTGCTTTAT TCTCCCATTG AAAACATCCA			**β–cat aiRNA#158**
			β–cat aiRNA#159
			β–cat aiRNA#191
			β–cat aiRNA#162
(SEQ ID NO. 210) **GTAGCTGA TATTGATGGA C**AGTATGCAA TGACTCGAGC	**5500-2700**	**1184**	**β–cat aiRNA#180**
541 TCAGAGGGTA CGAGCTGCTA TGTTCCCTGA GACATTAGAT GAGGGCATGC AGATCCCATC			**β–cat aiRNA#181**
601 TACACAGTTT GATGCTGCTC ATCCCACTAA TGTCCAGCGT TTGGCTGAAC CATCACAGAT			**β–cat aiRNA#205**
661 GCTGAAACAT GCAGTTGTAA ACTTGATTAA CTATCAAGAT GATGCAGAAC TTGCCACACG			**β–cat aiRNA#206**

721 TGCAATCCCT GAACTGACAA AACTGCTAAA TGACGAGGAC CAGGTGGTGG TTAATAAGGC			**β–cat aiRNA#210**
781 TGCAGTTATG GTCCATCAGC TTTCTAAAAA GGAAGCTTCC AGACACGCTA TCATGCGTTC			**β–cat aiRNA#180**
841 TCCTCAGATG GTGTCTGCTA TTGTACGTAC CATGCAGAAT ACAAATGATG TAGAAACAGC			**β–cat aiRNA#181**
901 TCGTTGTACC GCTGGGACCT TGCATAACCT TTCCCATCAT CGTGAGGGCT TACTGGCCAT			**β–cat aiRNA#205**
961 CTTTAAGTCT GGAGGCATTC CTGCCCTGGT GAAAATGCTT GGTTCACCAG TGGATTCTGT			**β–cat aiRNA#206**
1021 GTTGTTTTAT GCCATTACAA CTCTCCACAA CCTTTTATTA CATCAAGAAG GAGCTAAAAT			**β–cat aiRNA#210**
1081 GGCAGTGCGT TTAGCTGGTG GGCTGCAGAA AATGGTTGCC TTGCTCAACA AAACAAATGT			**β–cat aiRNA#216**
1141 TAAATTCTTG GCTATTACGA CAGACTGCCT TCAAATTTTA GCTTATGGCA ACCAAGAAAG			**β–cat aiRNA#182**
1201 CAAGCTCATC ATACTGGCTA GTGGTGGACC CCAAGCTTTA GTAAATATAA TGAGGACCTA			**β–cat aiRNA#220**
1261 TACTTACGAA AAACTACTGT GGACCACAAG CAGAGTGCTG AAGGTGCTAT CTGTCTGCTC			**β–cat aiRNA#183**
1321 TAGTAATAAG CCGGCTATTG TAGAAGCTGG TGGAATGCAA GCTTTAGGAC TTCACCTGAC			**β–cat aiRNA#302**
1381 AGATCCAAGT CAACGTCTTG TTCAGAACTG TCTTTGGACT CTCAGGAATC TTTCAGATGC			**β–cat aiRNA#224**
1441 TGCAACTAAA CAGGAAGGGA TGGAAGGTCT CCTTGGGACT CTTGTTCAGC TTCTGGGTTC			**β–cat aiRNA#185**
1501 AGATGATATA AATGTGGTCA CCTGTGCAGC TGGAATTCTT TCTAACCTCA CTTGCAATAA			**β–cat aiRNA#57**
1561 TTATAAGAAC AAGATGATGG TCTGCCAAGT GGGTGGTATA GAGGCTCTTG TGCGTACTGT			**β–cat aiRNA#235**
1621 CCTTCGGGCT GGTGACAGGG AAGACATCAC TGAGCCTGCC ATCTGTGCTC TTCGTCATCT			**β–cat aiRNA#241**
1681 GACCAGCCGA CACCAAGAAG CAGAGATGGC CCAGAATGCA GTTCGCCTTC ACTATGGACT			**β–cat aiRNA#68**
1741 ACCAGTTGTG GTTAAGCTCT TACACCCACC ATCCCACTGG CCTCTGATAA AGGCTACTGT			**β–cat aiRNA#245**
1801 TGGATTGATT CGAAATCTTG CCCTTTGTCC CGCAAATCAT GCACCTTTGC GTGAGCAGGG			**β–cat aiRNA#190**
1861 TGCCATTCCA CGACTAGTTC AGTTGCTTGT TCGTGCACAT CAGGATACCC AGCGCCGTAC			**β–cat aiRNA#154**
1921 GTCCATGGGT GGGACACAGC AGCAATTTGT GGAGGGGGTC CGCATGGAAG AAATAGTTGA			**β–cat aiRNA#77**
1981 AGGTTGTACC GGAGCCCTTC ACATCCTAGC TCGGGATGTT CACAACCGAA TTGTTATCAG			**β–cat aiRNA#109**
2041 AGGACTAAAT ACCATTCCAT TGTTTGTGCA GCTGCTTTAT TCTCCCATTG AAAACATCCA			**β–cat aiRNA#158**
2101 AAGAGTAGCT GCAGGGGTCC TCTGTGAACT TGCTCAGGAC AAGGAAGCTG CAGAAGCTAT			**β–cat aiRNA#159**
2161 TGAAGCTGAG GGAGCCACAG CTCCTCTGAC AGAGTTACTT CACTCTAGGA ATGAAGGTGT			**β–cat aiRNA#191**
2221 GGCGACATAT GCAGCTGCTG TTTTGTTCCG AATGTCTGAG GACAAGCCAC AAGATTACAA			**β–cat aiRNA#162**
2281 GAAACGGCTT TCAGTTGAGC TGACCAGCTC TCTCTTCAGA ACAGAGCCAA TGGCTTGGAA			**β–cat aiRNA#267**
2341 TGAGACTGCT GATCTTGGAC TTGATATTGG TGCCCAGGGA GAACCCCTTG GATATCGCCA			**β–cat aiRNA#268**
2401 GGATGATCCT AGCTATCGTT CTTTTCACTC TGGTGGATAT GGCCAGGATG CCTTGGGTAT			**β–cat aiRNA#330**

2461 GGACCCCATG ATGGAACATG AGATGGGTGG CCACCACCCT GGTGCTGACT ATCCAGTTGA			**β–cat aiRNA#272**
2521 TGGGCTGCCA GATCTGGGGC ATGCCCAGGA CCTCATGGAT GGGCTGCCTC CAGGTGACAG			
2581 CAATCAGCTG GCCTGGTTTG ATACTGACCT GTAAATCATC CTTTAGGTAA GAAGTTTTAA			
2641 AAAGCCAGTT TGGGTAAAAT ACTTTTACTC TGCCTACAGA ACTTCAGAAA GACTTGGTTG			
(SEQ ID NO.186) **GTAGCTGA TATTGATGGA C**AGTATGCAA TGACTCGAGC	**5500-3415**	**1185**	**β–cat aiRNA#180**
541 TCAGAGGGTA CGAGCTGCTA TGTTCCCTGA GACATTAGAT GAGGGCATGC AGATCCCATC			**β–cat aiRNA#181**
601 TACACAGTTT GATGCTGCTC ATCCCACTAA TGTCCAGCGT TTGGCTGAAC CATCACAGAT			**β–cat aiRNA#205**
661 GCTGAAACAT GCAGTTGTAA ACTTGATTAA CTATCAAGAT GATGCAGAAC TTGCCACACG			**β–cat aiRNA#206**
721 TGCAATCCCT GAACTGACAA AACTGCTAAA TGACGAGGAC CAGGTGGTGG TTAATAAGGC			**β–cat aiRNA#210**
781 TGCAGTTATG GTCCATCAGC TTTCTAAAAA GGAAGCTTCC AGACACGCTA TCATGCGTTC			**β–cat aiRNA#180**
841 TCCTCAGATG GTGTCTGCTA TTGTACGTAC CATGCAGAAT ACAAATGATG TAGAAACAGC			**β–cat aiRNA#181**
901 TCGTTGTACC GCTGGGACCT TGCATAACCT TTCCCATCAT CGTGAGGGCT TACTGGCCAT			**β–cat aiRNA#205**
961 CTTTAAGTCT GGAGGCATTC CTGCCCTGGT GAAAATGCTT GGTTCACCAG TGGATTCTGT			**β–cat aiRNA#206**
1021 GTTGTTTTAT GCCATTACAA CTCTCCACAA CCTTTTATTA CATCAAGAAG GAGCTAAAAT			**β–cat aiRNA#210**
1081 GGCAGTGCGT TTAGCTGGTG GGCTGCAGAA AATGGTTGCC TTGCTCAACA AAACAAATGT			**β–cat aiRNA#216**
1141 TAAATTCTTG GCTATTACGA CAGACTGCCT TCAAATTTTA GCTTATGGCA ACCAAGAAAG			**β–cat aiRNA#182**
1201 CAAGCTCATC ATACTGGCTA GTGGTGGACC CCAAGCTTTA GTAAATATAA TGAGGACCTA			**β–cat aiRNA#220**
1261 TACTTACGAA AAACTACTGT GGACCACAAG CAGAGTGCTG AAGGTGCTAT CTGTCTGCTC			**β–cat aiRNA#183**
1321 TAGTAATAAG CCGGCTATTG TAGAAGCTGG TGGAATGCAA GCTTTAGGAC TTCACCTGAC			**β–cat aiRNA#302**
1381 AGATCCAAGT CAACGTCTTG TTCAGAACTG TCTTTGGACT CTCAGGAATC TTTCAGATGC			**β–cat aiRNA#224**
1441 TGCAACTAAA CAGGAAGGGA TGGAAGGTCT CCTTGGGACT CTTGTTCAGC TTCTGGGTTC			**β–cat aiRNA#185**
1501 AGATGATATA AATGTGGTCA CCTGTGCAGC TGGAATTCTT TCTAACCTCA CTTGCAATAA			**β–cat aiRNA#57**
1561 TTATAAGAAC AAGATGATGG TCTGCCAAGT GGGTGGTATA GAGGCTCTTG TGCGTACTGT			**β–cat aiRNA#235**
1621 CCTTCGGGCT GGTGACAGGG AAGACATCAC TGAGCCTGCC ATCTGTGCTC TTCGTCATCT			**β–cat aiRNA#241**
1681 GACCAGCCGA CACCAAGAAG CAGAGATGGC CCAGAATGCA GTTCGCCTTC ACTATGGACT			**β–cat aiRNA#68**
1741 ACCAGTTGTG GTTAAGCTCT TACACCCACC ATCCCACTGG CCTCTGATAA AGGCTACTGT			**β–cat aiRNA#245**

1801 TGGATTGATT CGAAATCTTG CCCTTTGTCC CGCAAATCAT GCACCTTTGC GTGAGCAGGG			**β–cat aiRNA#190**
1861 TGCCATTCCA CGACTAGTTC AGTTGCTTGT TCGTGCACAT CAGGATACCC AGCGCCGTAC			**β–cat aiRNA#154**
1921 GTCCATGGGT GGGACACAGC AGCAATTTGT GGAGGGGGTC CGCATGGAAG AAATAGTTGA			**β–cat aiRNA#77**
1981 AGGTTGTACC GGAGCCCTTC ACATCCTAGC TCGGGATGTT CACAACCGAA TTGTTATCAG			**β–cat aiRNA#109**
2041 AGGACTAAAT ACCATTCCAT TGTTTGTGCA GCTGCTTTAT TCTCCCATTG AAAACATCCA			**β–cat aiRNA#158**
2101 AAGAGTAGCT GCAGGGGTCC TCTGTGAACT TGCTCAGGAC AAGGAAGCTG CAGAAGCTAT			**β–cat aiRNA#159**
2161 TGAAGCTGAG GGAGCCACAG CTCCTCTGAC AGAGTTACTT CACTCTAGGA ATGAAGGTGT			**β–cat aiRNA#191**
2221 GGCGACATAT GCAGCTGCTG TTTTGTTCCG AATGTCTGAG GACAAGCCAC AAGATTACAA			**β–cat aiRNA#162**
2281 GAAACGGCTT TCAGTTGAGC TGACCAGCTC TCTCTTCAGA ACAGAGCCAA TGGCTTGGAA			**β–cat aiRNA#267**
2341 TGAGACTGCT GATCTTGGAC TTGATATTGG TGCCCAGGGA GAACCCCTTG GATATCGCCA			**β–cat aiRNA#268**
2401 GGATGATCCT AGCTATCGTT CTTTTCACTC TGGTGGATAT GGCCAGGATG CCTTGGGTAT			**β–cat aiRNA#330**
2461 GGACCCCATG ATGGAACATG AGATGGGTGG CCACCACCCT GGTGCTGACT ATCCAGTTGA			**β–cat aiRNA#272**
2521 TGGGCTGCCA GATCTGGGGC ATGCCCAGGA CCTCATGGAT GGGCTGCCTC CAGGTGACAG			**β–cat aiRNA#21**
2581 CAATCAGCTG GCCTGGTTTG ATACTGACCT GTAAATCATC CTTTAGCTGT ATTGTCTGAA			**β–cat aiRNA#280**
2641 CTTGCATTGT GATTGGCCTG TAGAGTTGCT GAGAGGGCTC GAGGGGTGGG CTGGTATCTC			**β–cat aiRNA#198**
2701 AGAAAGTGCC TGACACACTA ACCAAGCTGA GTTTCCTATG GGAACAATTG AAGTAAACTT			**β–cat aiRNA#281**
2761 TTTGTTCTGG TCCTTTTTGG TCGAGGAGTA ACAATACAAA TGGATTTTGG GAGTGACTCA			**β–cat aiRNA#1**
2821 AGAAGTGAAG AATGCACAAG AATGGATCAC AAGATGGAAT TTATCAAACC CTAGCCTTGC			**β–cat aiRNA#282**
2881 TTGTTAAATT TTTTTTTTTT TTTTTTTAAG AATATCTGTA ATGGTACTGA CTTTGCTTGC			**β–cat aiRNA#283**
2941 TTTGAAGTAG CTCTTTTTTT TTTTTTTTTT TTTTTTTTGC AGTAACTGTT TTTTAAGTCT			**β–cat aiRNA#199**
3001 CTCGTAGTGT TAAGTTATAG TGAATACTGC TACAGCAATT TCTAATTTTT AAGAATTGAG			**β–cat aiRNA#37**
3061 TAATGGTGTA GAACACTAAT TCATAATCAC TCTAATTAAT TGTAATCTGA ATAAAGTGTA			**β–cat aiRNA#127**
3121 ACAATTGTGT AGCCTTTTTG TATAAAATAG ACAAATAGAA AATGGTCCAA TTAGTTTCCT			**β–cat aiRNA#284**
3181 TTTTAATATG CTTAAAATAA GCAGGTGGAT CTATTTCATG TTTTTGATCA AAAACTATTT			**β–cat aiRNA#202**
3241 GGGATATGTA TGGGTAGGGT AAATCAGTAA GAGGTGTTAT TTGGAACCTT GTTTTGGACA			**β–cat aiRNA#288**
3301 GTTTACCAGT TGCCTTTTAT CCCAAAGTTG TTGTAACCTG CTGTGATACG ATGCTTCAAG			**β–cat aiRNA#204**
3361 AGAAAATGCG GTTATAAAAA ATGGTTCAGA ATTAAACTTT TAATTCATTC GATTG			**β–cat aiRNA#291**

FIG. 1C

Human β-catenin		NM 001098209 IC50<100pM	SEQ ID NO	%GC
siRNA#	Position	Target Sequence		
1	2675	GCACAAGAATGGATCACAA	186	42.11
21	2782	CGAGGAGTAACAATACAAA	187	36.84
37	2855	GATCACAAGATGGAATTTA	188	31.58
57	1007	CCAGTGGATTCTGTGTTGT	189	47.37
68	1379	ACAGATCCAAGTCAACGTC	190	47.37
77	1530	CTGGAATTCTTTCTAACCT	191	36.84
109	1532	GGAATTCTTTCTAACCTCA	192	36.84
127	2840	GAATGGATCACAAGATGGA	193	42.11
154	1490	CTTCTGGGTTCAGATGATA	194	42.11
158	1797	CTGTTGGATTGATTCGAAA	195	36.84
159	1869	CACGACTAGTTCAGTTGCT	196	47.37
162	2045	CTAAATACCATTCCATTGT	197	31.58
180	220	GGGTATTTGAAGTATACCA	198	36.84
181	271	GGCTACTCAAGCTGATTTG	199	47.37
182	651	CATCACAGATGCTGAAACA	200	42.11
183	668	CATGCAGTTGTAAACTTGA	201	36.84
185	885	ATGATGTAGAAACAGCTCG	202	42.11
190	1423	CAGGAATCTTTCAGATGCT	203	42.11
191	1872	GACTAGTTCAGTTGCTTGT	204	42.11
198	2811	GAGTGACTCAAGAAGTGAA	205	42.11
199	2837	CAAGAATGGATCACAAGAT	206	36.84
202	2923	GGTACTGACTTTGCTTGCT	207	47.37
204	3162	ATGGTCCAATTAGTTTCCT	208	36.84
205	281	GCTGATTTGATGGAGTTGG	209	47.37
206	362	GACTCTGGAATCCATTCTG	210	47.37
210	503	GTAGCTGATATTGATGGAC	211	42.11
216	597	CATCTACACAGTTTGATGC	212	42.11
220	656	CAGATGCTGAAACATGCAG	213	47.37
224	845	CAGATGGTGTCTGCTATTG	214	47.37
235	1147	CTTGGCTATTACGACAGAC	215	47.37
241	1350	GTGGAATGCAAGCTTTAGG	216	47.37
245	1436	GATGCTGCAACTAAACAGG	217	47.37

FIG. 1C (ctd.)

267	2268	CACAAGATTACAAGAAAC	218	38.84
268	2270	CAAGATTACAAGAAACGGC	219	42.11
272	2593	CTGGTTTGATACTGACCTG	220	47.36
280	2785	GGAGTAACAATACAAATGG	221	38.84
281	2819	CAAGAAGTGAAGAATGCAC	222	42.11
282	2822	GAAGTGAAGAATGCACAAG	223	42.11
283	2827	GAAGAATGCACAAGAATGG	224	42.11
284	2830	GAATGCACAAGAATGGATC	225	42.11
288	3116	GTGTAACAATTGTGTAGCC	226	42.11
291	3273	GGTGTTATTTGGAACCTTG	227	42.11
302	662	CTGAAACATGCAGTTGTAA	228	38.84
330	2278	CAAGAAACGGCTTTCAGTT	229	42.11
331	2349	CTGATCTTGGACTTGATAT	230	38.84
332	2409	CTAGCTATCGTTCTTTTCA	231	38.84
333	2600	GATACTGACCTGTAAATCA	232	38.84
334	2617	CATCCTTTAGCTGTATTGT	233	38.84
440	1564	TAAGAACAAGATGATGGTC	234	38.84
480	1536	TTCTTTCTAACCTCACTTG	235	38.84
508	390	CCACAGCTCCTTCCTCTGAG	236	57.89
509	666	AAACATGCAGTTGTAAACT	237	31.58
511	1160	ACAGACTGCCTTCAAATTT	238	38.84
513	1787	ATAAAGGCTACTGTTGGAT	239	38.84
516	3119	TAACAATTGTGTAGCCTTT	240	31.58
517	3195	AAATAAGCAGGTGGATCTA	241	38.84
519	228	GAAGTATACCATACAACTG	242	38.84
520	1352	GGAATGCAAGCTTTAGGAC	243	47.37
521	1966	GGAAGAAATAGTTGAAGCT	244	38.84
523	2866	AAACCCTAGCCTTGCTTGT	245	47.37
524	3152	CAAATAGAAAATGGTCCAA	246	31.58
525	3356	TCAAGAGAAAATGCGGTTA	247	38.84
528	622	TCCCACTAATGTCCAGCGT	248	52.63
532	542	CAGAGGTACGAGCTGCTA	249	57.89
534	914	GGGACCTTGCATAACCTTT	250	47.37
535	1227	GACCCCAAGCTTTAGTAAA	251	42.11
537	1481	CTTGTTCAGCTTCTGGGTT	252	47.37

FIG. 1C (ctd.)

S38	1591	GGGTGGTATAGAGGCTCTT	283	52.63
S39	1917	CTTGCCTTTGTCCCGCAA	284	57.89
S41	2235	CTGCTGTTTTGTTCCGAAT	285	42.11
S42	2273	GATTACAAGAAACGGCTTT	286	36.84
S44	2871	CTAGCCTTGCTTGTTAAAT	287	36.84
S45	692	TATCAAGATGATGCAGAAC	288	36.84
S46	770	GTTAATAAGGCTGCAGTTA	289	36.84
S47	932	TCCCATCATCGTGAGGGCT	290	57.89
S50	2336	TGGAATGAGACTGCTGATC	291	47.37
S52	3106	TCTGAATAAAGTGTAACAA	292	26.32

FIG. 1D

% G+C	Exemplary β–catenin aiRNA #
23%-35%	1, 37, 162, 509, 516, 524, 552
36%-44%	21, 77, 109, 127, 154, 158, 180, 182, 183, 185, 190, 191, 198, 199, 204, 210, 216, 267, 268, 280, 281, 282, 283, 284, 288, 291, 302, 330, 331, 332, 333, 334, 440, 480, 511, 513, 517, 519, 521, 525, 535, 541, 542, 544, 545, 546
45%-57%	57, 68, 159, 181, 202, 205, 206, 220, 224, 235, 241, 245, 272, 508, 520, 523, 528, 532, 534, 537, 538, 539, 547, 550

FIG. 1E

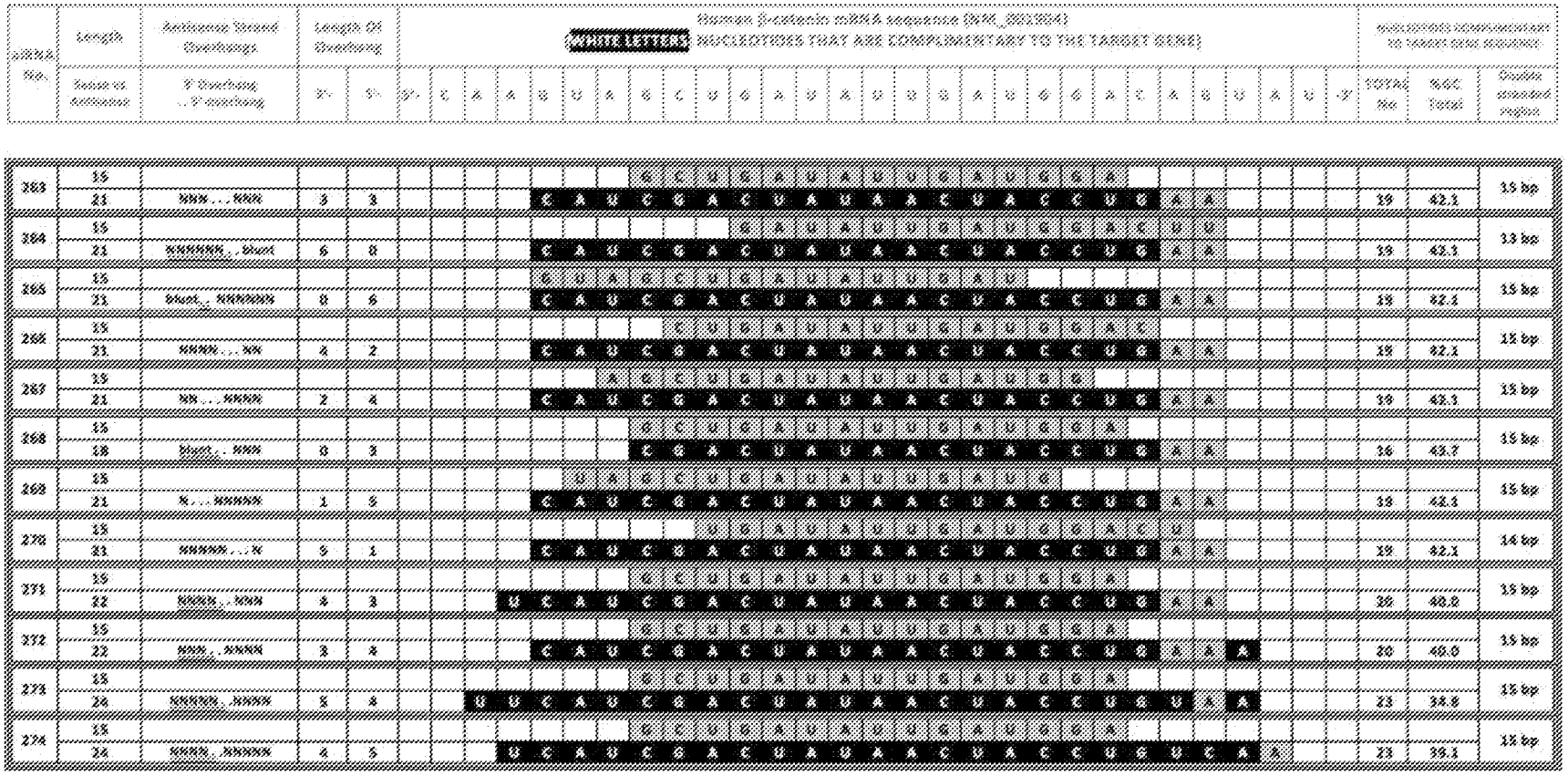


FIG. 1E (ctd.)

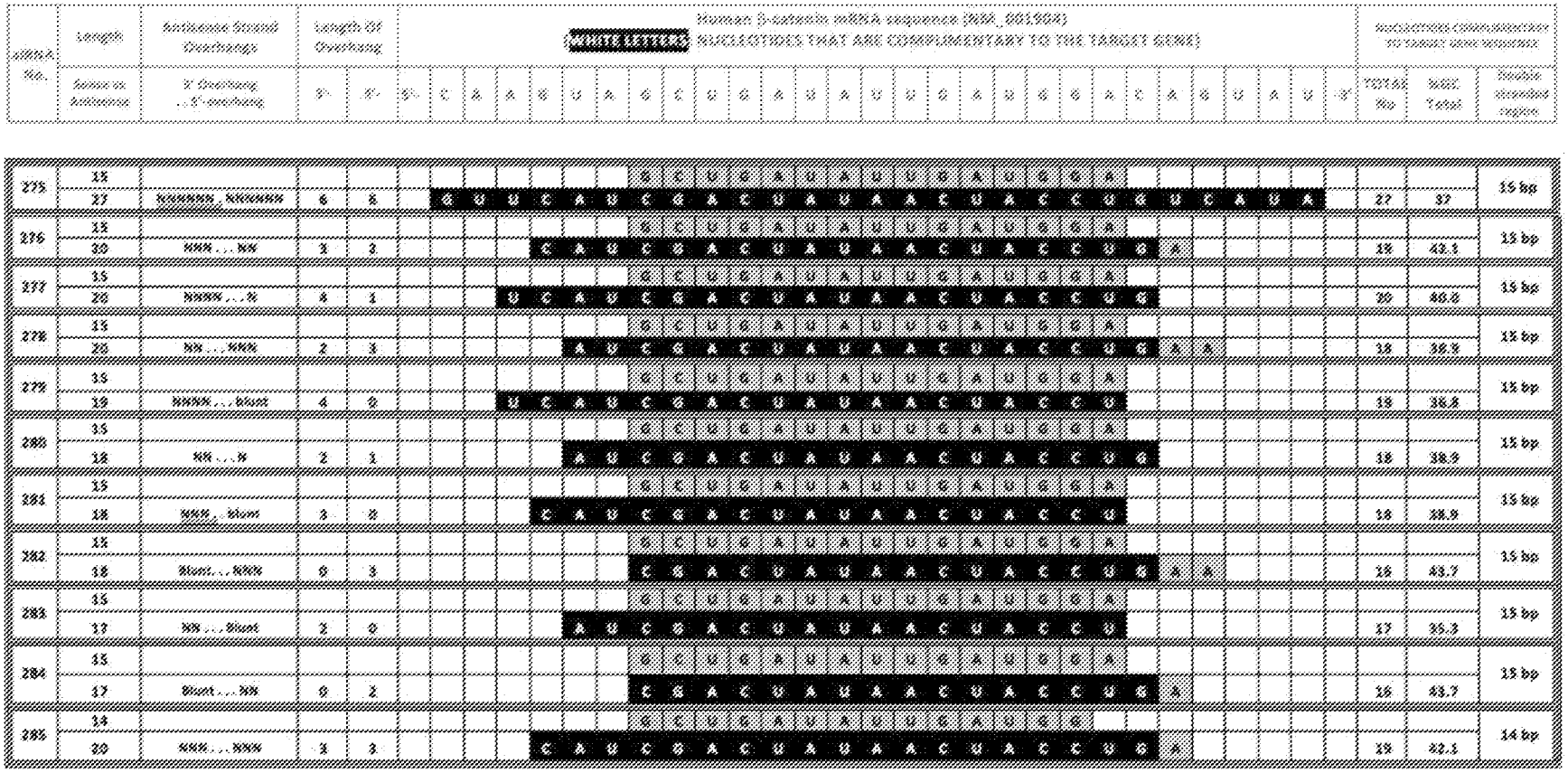


FIG. 1E (ctd.)

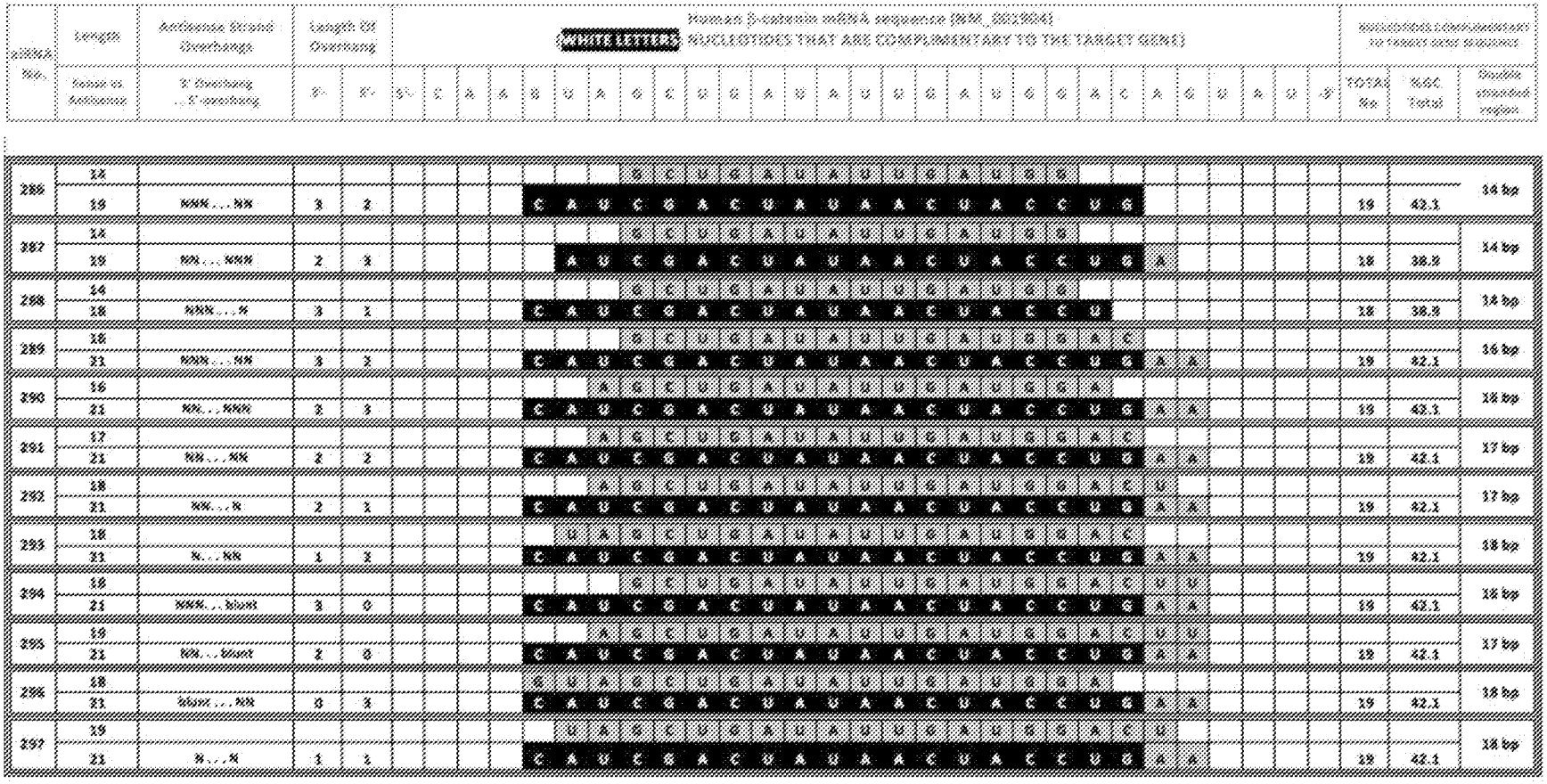


FIG. 1E (ctd.)

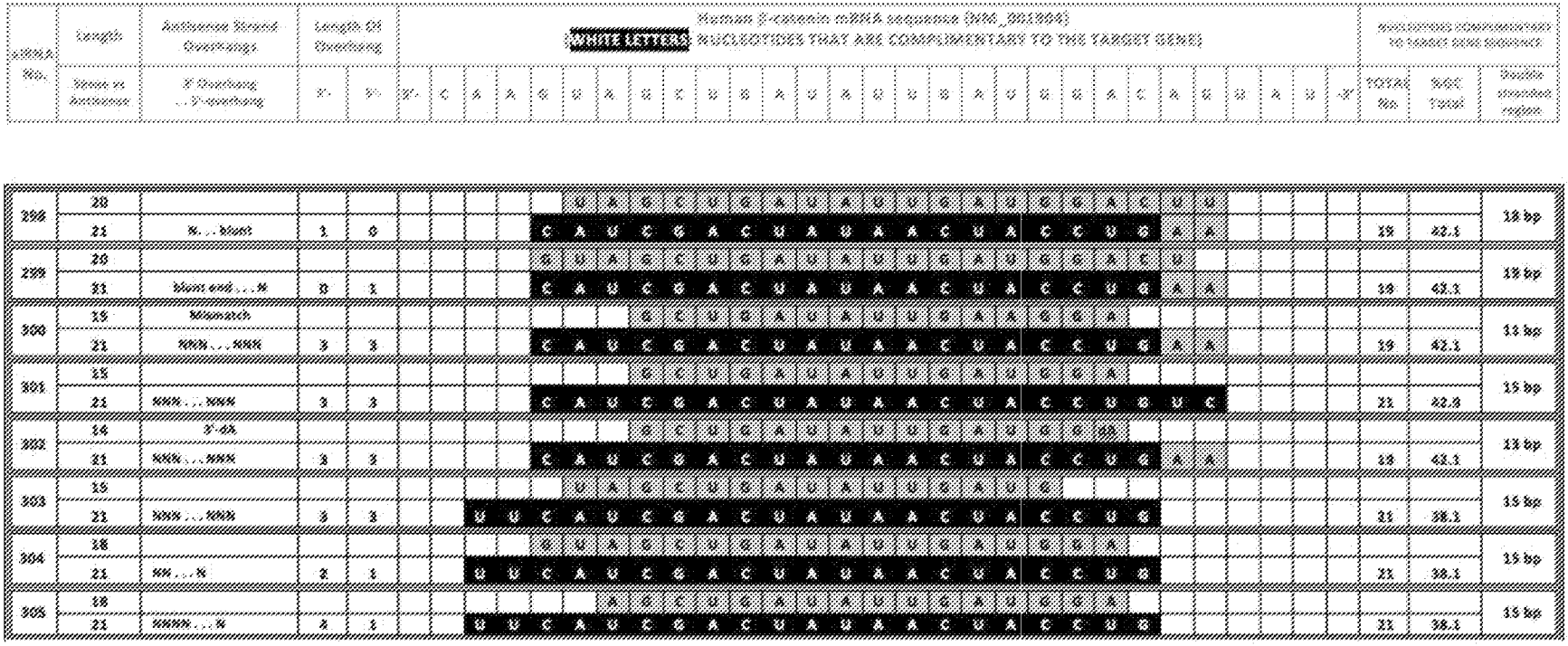


FIG. 2A

PD-L1 mRNA SEQUENCE (Transcript Variant 1: SEQ ID NO.: 166)
(ACCESSION No.: NM 014143)
PD-L1 CDS: 109-981

(SEQ ID NO:139)
1 GGCGCAACGCUGAGCAGCUGGCGCGUCCCGCGCGGCCCCAGUUCUGCGCA 50

51 GCUUCCCGAGGCUCCGCACCAGCCGCGCUUCUGUCCGCCUGCAGGGCAUU 100

101 CCAGAAAGAUGAGGAUAUUUGCUGUCUUUAUAUUCAUGACCUACUGGCAU 150

(SEQ ID NO.: 140)
151 UUGCUGAACGCAUUUACUGUCACGGUUCCCAAGGACCUAUAUGUGGUAGA 200

201 GUAUGGUAGCAAUAUGACAAUUGAAUGCAAAUUCCCAGUAGAAAAACAAU 250

(SEQ ID NO.: 141)
251 UAGACCUGGCUGCACUAAUUGUCUAUUGGGAAAUGGAGGAUAAGAACAUU 300

301 AUUCAAUUUGUGCAUGGAGAGGAAGACCUGAAGGUUCAGCAUAGUAGCUA 350

(SEQ ID NO.: 144)
351 CAGACAGAGGGCCCGGCUGUUGAAGGACCAGCUCUCCCUGGGAAAUGCUG 400

401 CACUUCAGAUCACAGAUGUGAAAUUGCAGGAUGCAGGGGUGUACCGCUGC 450

451 AUGAUCAGCUAUGGUGGUGCCGACUACAAGCGAAUUACUGUGAAAGUCAA 500

(SEQ ID NO.: 147)
501 UGCCCCAUACAACAAAAUCAACCAAAGAAUUUUGGUUGUGGAUCCAGUCA 550

551 CCUCUGAACAUGAACUGACAUGUCAGGCUGAGGGCUACCCCAAGGCCGAA 600

(SEQ ID NO.: 150)
601 GUCAUCUGGACAAGCAGUGACCAUCAAGUCCUGAGUGGUAAGACCACCAC 650

651 CACCAAUUCCAAGAGAGAGGAGAAGCUUUUCAAUGUGACCAGCACACUGA 700

(SEQ ID NO.: 155)
701 GAAUCAACACAACAACCUAAUGAGAUUUUCUACUGCACUUUUAGGAGAUUA 750

751 GAUCCUGAGGAAAACCAUACAGCUGAAUUGGUCAUCCCAGAACUACCUCU 800

801 GGCACAUCCUCCAAAUGAAAGGACUCACUUGGUAAUUCUGGGAGCCAUCU 850

FIG. 2A (ctd.)

PD-L1 mRNA SEQUENCE (Transcript Variant 1: SEQ ID NO.: 166) (ACCESSION No.: NM 014143) PD-L1 CDS: 109-981	
851 UAUUAUGCCUUGGUGUAGCACUGACAUUCAUCUUCCGUUUAAGAAAAGGG	900
(SEQ ID NO.: 156)	
901 AGAAUGAUGGAUGUGAAAAAAUGUGGCAUC**CAAGAUACAAACUCAAAGAA**	950
951 **GCAAAGUGAUACA**CAUUGGAGGAGACG**UAA**UCCAGCAUUGGAACUUCUG [STOP CODON]	1000
1001 AUCUUCAAGCAGGGAUUCUCAACCUGUGGUUUAGGGGUUCAUCGGGGCUG	1050
1051 AGCGUGACAAGAGGAAGGAAUGGGCCCGUGGGAUGCAGGCAAUGUGGGAC	1100
1101 UUAAAAGGCCCAAGCACUGAAAAUGGAACCUGGCGAAAGCAGAGGAGGAG	1150
1151 AAUGAAGAAAGAUGGAGUCAAACAGGGAGCCUGGAGGGAGACCUUGAUAC	1200
1201 UUUCAAAUGCCUGAGGGGCUCAUCGACGCCUGUGACAGGGAGAAAGGAUA	1250
1251 CUUCUGAACAAGGAGCCUCCAAGCAAAUCAUCCAUUGCUCAUCCUAGGAA	1300
1301 GACGGGUUGAGAAUCCCUAAUUUGAGGGUCAGUUCCUGCAGAAGUGCCCU	1350
1351 UUGCCUCCACUCAAUGCCUCAAUUUGUUUUCUGCAUGACUGAGAGUCUCA	1400
(SEQ ID NO.: 158)	
1401 GUGUUC**GAACGGGACAGUAUUUAUGUAUGAGUUUUUCCU**AUUUAUUUUGA	1450
1451 GUCUGUGAGGUCUUCUUGUCAUGUGAGUGUGGUUGUGAAUGAUUUCUUUU	1500
(SEQ ID NO.: 161)	
1501 GAAGAUAUAUUGUAGUAGAUGUUACAAU**UUUGUCGCCAAACUAAACUUGC**	1550
1551 **UGCUUAAUGAU**UUGCUCACAUCUAGUAAAACAUGGAGUAUUUGUAAGGUG	1600
1601 CUUGGUCUCCUCUAUAACUACAAGUAUACAUUGGAAGCAUAAAGAUCAAA	1650
(SEQ ID NO.: 164)	
1651 CCGUUGGUUGCAUAGGAUGUCACCUUUAUUUAACCCAUUAAUA**CUCUGGU**	1700

FIG. 2A (ctd.)

PD-L1 mRNA SEQUENCE (Transcript Variant 1: SEQ ID NO.: 166) (ACCESSION No.: NM 014143) PD-L1 CDS: 109-981		
1701	UGACCUAAUCUUAUUCUCAGACCUCAAGUGUCUGUGGCAGUAUCUGUUCCA	1750
1751	UUUAAAAUAUCAGCUUUACAAUUAUGUGGUAGCCUACACACAUAAUCUCAU	1800
1801	UUCAUCGCUGUAACCACCCUGUUGUGAUAACCACUAUUAUUUUACCCAUC	1850
1851	GUACAGCUGAGGAAGCAAACAGAUUAAGUAACUUGCCCAAACCAGUAAAU	1900
1901	AGCAGACCUCAGACUGCCACCCACUGUCCUUUUAUAAUACAAUUUACAGC	1950
1951	UAUAUUUUACUUUAAGCAAUUCUUUUAUUCAAAAACCAUUUAUUAAGUGC	2000
2001	CCUUGCAAUAUCAAUCGCUGUGCCAGGCAUUGAAUCUACAGAUGUGAGCA	2050
2051	AGACAAAGUACCUGUCCUCAAGGAGCUCAUAGUAUAAUGAGGAGAUUAAC	2100
2101	AAGAAAAUGUAUUAUUACAAUUUAGUCCAGUGUCAUAGCAUAAGGAUGAU	2150
2151	GCGAGGGGAAAACCCGAGCAGUGUUGCCAAGAGGAGGAAAUAGGCCAAUG	2200
2201	UGGUCUGGGACGGUUGGAUAUACUUAAACAUCUUAAUAAUCAGAGUAAUU	2250
2251	UUCAUUUACAAAGAGAGGUCGGUACUUAAAAUAACCCUGAAAAAUAACAC	2300
2301	UGGAAUUCCUUUUCUAGCAUUAUAUUUAUUCCUGAUUUGCCUUUGCCAUA	2350
2351	UAAUCUAAUGCUUGUUUAUAUAGUGUCUGGUAUUGUUUAACAGUUCUGUC	2400
2401	UUUUCUAUUUAAAUGCCACUAAAUUUUAAAUUCAUACCUUUCCAUGAUUC	2450
2451	AAAAUUCAAAAGAUCCCAUGGGAGAUGGUUGGAAAAUCUCCACUUCAUCC	2500
2501	UCCAAGCCAUUCAAGUUUCCUUUCCAGAAGCAACUGCUACUGCCUUUCAU	2550
2551	UCAUAUGUUCUUCUAAAGAUAGUCUACAUUUGGAAAUGUAUGUUAAAAGC	2600
2601	ACGUAUUUUUAAAAUUUUUUUCCUAAAUAGUAACACAUUGUAUGUCUGCU	2650

FIG. 2A (ctd.)

PD-L1 mRNA SEQUENCE (Transcript Variant 1: SEQ ID NO.: 166) (ACCESSION No.: NM 014143) PD-L1 CDS: 109-981		
2651	GUGUACUUUGCUAUUUUUAUUUAUUUUAGUGUUUCUUAUAUAGCAGAUGG	2700
2701	AAUGAAUUUGAAGUUCCCAGGGCUGAGGAUCCAUGCCUUCUUUGUUUCUA	2750
2751	AGUUAUCUUUCCCAUAGCUUUUCAUUAUCUUUCAUAUGAUCCAGUAUAUG	2800
2801	UUAAAUAUGUCCUACAUAUACAUUUAGACAACCACCAUUUGUUAAGUAUU	2850
2851	UGCUCUAGGACAGAGUUUGGAUUUGUUUAUGUUUGCUCAAAAGGAGACCC	2900
2901	AUGGGCUCUCCAGGGUGCACUGAGUCAAUCUAGUCCUAAAAAGCAAUCUU	2950
2951	AUUAUUAACUCUGUAUGACAGAAUCAUGUCUGGAACUUUUGUUUUCUGCU	3000
3001	UUCUGUCAAGUAUAAACUUCACUUUGAUGCUGUACUUGCAAAAUCACAUU	3050
3051	UUCUUUCUGGAAAUUCCGGCAGUGUACCUUGACUGCUAGCUACCCUGUGC	3100
3101	CAGAAAAGCCUCAUUCGUUGUGCUUGAACCCUUGAAUGCCACCAGCUGUC	3150
3151	AUCACUACACAGCCCUCCUAAGAGGCUUCCUGGAGGUUUCGAGAUUCAGA	3200
3201	UGCCCUGGGAGAUCCCAGAGUUUCCUUUCCCUCUUGGCCAUAUUCUGGUG	3250
3251	UCAAUGACAAGGAGUACCUUGGCUUUGCCACAUGUCAAGGCUGAAGAAAC	3300
3301	AGUGUCUCCAACAGAGCUCCUUGUGUUAUCUGUUUGUACAUGUGCAUUUG	3350
3351	UACAGUAAAUUGGUGUGACAGUGUUCUUUGUGUGAAUUACAGGCAAGAAUU	3400
3401	GUGGCUGAGCAAGGCACAUAGUCUACUCAGUCUAUUCCUAAGUCCUAACU	3450
3451	CCUCCUUGUGGGUGUUGGAUUUGUAAGGCACUUUAUCCCUUUUGUCUCAUG	3500
3501	UUUCAUCGUAAAUGGCAUAGGCAGAGAUGAUACCUAAUUCUGCAUUUGAU	3550
3551	UGUCACUUUUUGUACCUGCAUUAAUUUAAUAAAAAUAUUCUUAUUUAUUUU	3600
3601	GUUACUUGGUACACCAGCAUGUCCAUUUUCUUGUUUAUUUUGUGUUUAAU	3650
SEQ ID NO.: 165		
3651	AAAAUGUUCAGUUUAACAUCCCAGUGGAGAAAGUUAAAAAA	3691

FIG. 2B

PD-L1 mRNA SEQUENCES (ACCESSION No.: NM 014143)	PD-L1 mRNA sequence	SEQ ID NO.	Exemplary PD-L1 siRNAs
(SEQ ID NO:139) 1 GGCGCAACGCUGAGCAGCUGGCGCGUCCCGCGCGGCCCCAGUUCUGCGCA 50 51 GCUUCCCGAGGCUCCGCACCAGCCGCGCUUCUGUCCGCCUGCAGGGCAUU 100 101 CCAGAAAGAUGAGGAUAUUUGCUGUCUUUAUAUUCAUGACCUACUGGCAU 150 151 UUGCUGAACGCAUUUACUGUCACGGUUCCCAAGGACCUAUAUGUGGUAGA 200 201 GUAUGGUAGCAAUAUGACAAUUGAAUGCAAAUUCCCAGUAGAAAAACAAU 250 (SEQ ID NO.: 141) 251 UAGACCUGGCUGCACUAAUUGUCUAUUGGGAAAUGGAGGAUAAGAACAUU 300 301 AUUCAAUUUGUGCA 314	1~314	167	1,2, 22,30
(SEQ ID NO.: 141) 282 AAUGGAGGAUAAGAACAUU 300 301 AUUCAAUUUGUGCAUGGAGAGGAAGACCUGAAGGUUCAGCAUAGUAGCUA 350 (SEQ ID NO.: 144) 351 CAGACAGAGGGCCCGGCUGUUGAAGGACCAGCUCUCCCUGGGA 393	282~393	168	3,4,30, 38,25
(SEQ ID NO.: 144) 361 GCCCGGCUGUUGAAGGACCAGCUCUCCCUGGGAAAUGCUG 400 401 CACUUCAGAUCACAGAUGUGAAAUUGCAGGAUGCAGGGGUGUACCGCUGC 450	361~563	169	4,5,6,7, 8,9,26,34 40

FIG. 2B (ctd.)

PD-L1 mRNA SEQUENCES (ACCESSION No.: NM 014143)	PD-L1 mRNA sequence	SEQ ID NO.	Exemplary PD-L1 siRNAs
451 AUGAUCAGCUAUGGUGGUGCCGACUACAAGCGAAUUACUGUGAAAGUCAA 500			
(SEQ ID NO.: 147) 501 UGCCCCAUACAACAAAAUCAACCAAAGAAUUUUGGUUGUGGAUCCAGUCA 550			
551 CCUCUGAACAUGA 563			
(SEQ ID NO.: 147) 531 UUUGGUUGUGGAUCCAGUCA 550	531-649	170	9,10,11, 12,26,32, 33,35,40
551 CCUCUGAACAUGAACUGACAUGUCAGGCUGAGGGCUACCCCAAGGCCGAA 600			
(SEQ ID NO.: 150) 601 GGCAUCUGGACAAGCAGUGACCAUCAAGUCCUGAGUGGUAAGACCACCA 649			
(SEQ ID NO.: 150) 617 GUGACCAUCAAGUCCUGAGUGGUAAGACCACCAC 650	617-963	171	12,39,13, 36,37,27, 14,15,16, 29,31,17, 18,19,20
651 CACCAAUUCCAAGAGAGAGGAGAAGCUUUUCAAUGUGACCAGCACACUGA 700			
701 GAAUCAACACAACAACUAAUGAGAUUUUCUACUGCACUUUUAGGAGAUUA 750			
751 GAUCCUGAGGAAAACCAUACAGCCGAAUUGGUCAUCCCAGAACUACCUCU 800			
801 GGCACAUCCUCCAAAUGAAAGGACUCACUUGGUAAUUCUGGGAGCCAUCU 850			
851 UAUUAUGCCUUGGUGUAGCACUGACAUCAUCUUCCGUUUAAGAAAAGGG 900			
(SEQ ID NO.: 156) 901 AGAAUGAUGGAUGUGAAAAAAUGUGGCAUCCAAGAUACAAACUCAAAGAA 950			

FIG. 2B (ctd.)

PD-L1 mRNA SEQUENCES (ACCESSION No.: NM 014143)	PD-L1 mRNA sequence	SEQ ID NO.	Exemplary PD-L1 aiRNAs
951 GCAAAGUGAUACA 963			
(SEQ ID NO.: 156) 931 CAAGAUACAAACUCAAAGAA 950	931~1439	172	18,19,20, 21,41,43, 28
951 GCAAAGUGAUACACAUUUGGAGGAGACGUAAUCCAGCAUUGGAACUUCUG 1000			
1001 AUCUUCAAGCAGGGAUUCUCAACCUGUGGGUUUAGGGGUUCAUCGGGGCCG 1050			
1051 AGCGUGACAAGAGGAAGGAAUGGGCCCGUGGGAUGCAGGCAAUGUGGGAC 1100			
1101 UUAAAAGCCCCAAGCACUCAAAAUGGAACCUGCGCAAAGCAGAGGAGGAG 1150			
1151 AAUGAAGAAAGAUGGAGUCAAACAGGGAGCCUGGAUGGAGACCUUGAUAC 1200			
1201 UUUCAAAUGCCUGAGGGGCUCAUCGACGCCUGUGACAGGGAGAAAGGAUA 1250			
1251 CUUCUGAACAAGGAGCCUCCAAGCAAAUCAUCCAUUGCUCAUCCUAGGAA 1300			
1301 GACGGGUUGAGAAUCCCUAAUUUGAGGGUCAGUUCCUGCAGAAGUGCCCU 1350			
1351 UUGCCUCCACUCAAUGCCUCAAUUUGUUUUCUGCAUGACUGAGAGUCUCA 1400			
(SEQ ID NO.: 158) 1401 GAACGGGACAGUAUUUAUGUAUGAGUUUUUCCU 1439			
(SEQ ID NO.: 158) 1407 GAACGGGACAGUAUUUAUGUAUGAGUUUUUCCUAUUUAUUUUGA 1450	1407~3689	173	43,28,22, 23,42,45, 44, 46
1451 GUCUGUGAGGUCUUCUUGUCAUGUGAGUGUGGUUGUGAAUGAUUUCUUUU 1500			

FIG. 2B (ctd.)

PD-L1 mRNA SEQUENCES (ACCESSION No.: NM 014143)	PD-L1 mRNA sequence	SEQ ID NO.	Exemplary PD-L1 siRNAs
1501 GAACAUAUAUUGUAGUAGAUGUUACAAUUUUGUCCCCAAACUAAACUUGC 1550			
1551 UGCUUAAUGAUUUGCUCACAUCUAGUAAAACAUGGAGUAUUUGUAAGGUG 1600			
1601 CUUGGUCUCCUCUAUAACUACAAGUAUACAUUGGAAGCAUAAAGAUCAAA 1650			
1651 CCGUUGGUUGCAUAGGAUGUCACCUUUAUUUAACCCAUUAAUACUCUGGU 1700			
1701 UGACCUAAUCUUAUUCUCAGACCUCAACUGUCUGUGCAGUAUCUGUUCCA 1750			
1651 CCGUUGGUUGCAUAGGAUGUCACCUUUAUUUAACCCAUUAAUACUCUGGU 1700			
1701 UGACCUAAUCUUAUUCUCAGACCUCAAGUGUCUGUGCAGUAUCUGUUCCA 1750			
1701 UGACCUAAUCUUAUUCUCAGACCUCAAGUGUCUGUGCAGUAUCUGUUCCA 1750			
1751 UUUAAAUAUCAGCUUUACAAUUAUGUGGUAGCCUACACACAUAAUCUCAU 1800			
1801 UUCAUCCCUGUAACCACCCUGUUGUGAUAACCACUAUUAUUUUACCCAUC 1850			
1851 GUACAGCUGAGGAAGCAAACAGAUUAAGUAACUUGCCCAAACCAGUAAAU 1900			
1901 AGCAGACCUCAGACUGCCACCCACUGUCCUUUUAUAAUACAAUUUACAGC 1950			
1951 UAUAUUUUACUUUAAGCAAUUCUUUUAUUCAAAAACCAUUUAUUAACGUC 2000			
2001 CCUUGCAAGUAUCAAUCCCUGUGCCAGGCAUUGAAUCUACAGAUGUGAGCA 2050			
2051 AGACAAAGUACCUGUCCUCAAGGAGCUCAUAGUAUAAUGAGGAGAUUAAC 2100			
2101 AAGAAAAUGUAUUAUUACAAUUUAGUCCAGUGUCAUAGCAUAAGGAUGAU 2150			
2151 GCGAGGGGAAAACCCGAGCAGUGUUGCCAAGAGGAGGAAAUAGGCCAAUG 2200			

FIG. 2B (ctd.)

PD-L1 mRNA SEQUENCES (ACCESSION No.: NM 014143)			PD-L1 mRNA sequence	SEQ ID NO.	Exemplary PD-L1 siRNAs
2201	UGGUCUGGGACGGUUGGAUAUACUUAAACAUCUUAAUAAUCAGAGUAAUU	2250			
2251	UUCAUUUACAAAGAGAGGUCGGUACUUAAAAUAACCCUGAAAAAUAACAC	2300			
2301	UGGAAUUCCUUUUCUAGCAUUAUAUUUAUUCCUGAUUGGCCUUUGCCAUA	2350			
2351	UAAUCUAAUGCUUGUUUAUAUAGUGUCUGGUAUUGUUUAACAGUUCUGUC	2400			
2401	UUUUCUAUUUAAAUGCCACUAAAUUUUAAAUUCAUACCUUUCCAUGAUUC	2450			
2451	AAAAUUCAAAAGAUCCCAUGGCAGAUGGUUGCAAAAUCUCCACUUCAUCC	2500			
2501	UCCAAGCCAUUCAAUUUUCCUUUCCAGAAGCAACUGCUACUGCCUUUCAU	2550			
2551	UCAUAUGUUCUUCUAAACAUAGUCUACAUUUUGCAAAUGUAUGUUAAAAUC	2600			
2601	ACGUAUUUUUAAAAUUUUUUUCCUAAAUAGUAACACAUUGUAUGUCUGCU	2650			
2651	GUGUACUUUGCUAUUUUUAUUUAUUUUAGUGUUUCUUAUAUAGCAGAUGG	2700			
2701	AAUGAAUUUGAAGUUCCCAGGGCUGAGGAUCCAUGCCUUCUUUGUUUCUA	2750			
2751	AGUUAUCUUUCCCAUAGCUUUUCAUUAUCUUUCAUAUGAUCCAGUAUAUG	2800			
2801	UUAAAUAUGUCCUACAUAUACAUUUAGACAACCACCAUUUGUUAAGUAUU	2850			
2851	UGCUCUAGGACAGAGUUUGGAUUUGUUAUGUUUGCUCAAAAGGAGACCC	2900			
2901	AUGGGCUCUCCAGGGUGCACUGAGUCAAUCUAGUCCUAAAAAGCAAUCUU	2950			
2951	AUUAUUAACUCUGUAUGACAGAAUCAUGUCUGGAACUUUUGUUUCUGCU	3000			

FIG. 2B (ctd.)

PD-L1 mRNA SEQUENCES (ACCESSION No.: NM 014143)	PD-L1 mRNA sequence	SEQ ID NO.	Exemplary PD-L1 aiRNAs
3001 UUCUGUCAAGUAUAAACCUCACUUUGAUGCUGUACUUGCAAAAUCACAUU 3050			
3051 UUCUUUCUGGAAAUUCCGGCAGUGUACCUUGACUGCUAGCUACCCUGUGC 3100			
3101 CAGAAAAGCCCUCAUUCGUUGUGCUUGAACCCUUGAAUGCCACCAGCUGUC 3150			
3151 AUCACUACACAGCCCUCCUAAGAGGCUUCCUGCAGGUUCGAGAUCACA 3200			
3201 UGCCCUGGGAGAUCCCAGAUUUCCUUUCCCUCUUGGCCAUAUUCUGGUG 3250			
3251 UCAAUGACAAGGAGUACCUUGCCUUUGCCACAUGUCAAGCUGAAGAAAC 3300			
3301 AGUGUCUCCAACAGAGCUCCUUGUGUUAUCUGUUUGUACAUGUGCAUUUG 3350			
3351 UACAGUAAUUGGUGUGACAGUGUUCUUUGUGUGAAUUACAGGCAACAAUU 3400			
3401 GUGGCUGAGCAAGCCACAUAGUCUACUCAGUCUAUUCCUAAGUCCUAACU 3450			
3451 CCUCCUUGUGGGUGUUGGAUUUGUAAGGCACUUUAUCCCUUUUGUCUCAUG 3500			
3501 UUUCAUCGUAAAUGGCAUAGGCAGAGAUGAUACCUAAUUCUGCAUUGAU 3550			
3551 UGUCACUUUUUGUACCUGCAUUAAGUUAAUAAAAUAUCUUAUUUAUUU 3600			
3601 GUUACUUGGUACACCAGCAUGUCCAUUUUCUUGUUUAUUUUGUGUUUAAU 3650			
(SEQ ID NO.: 165)			
3651 AAAAUGUUCAGUUUAACAUCCCAGUGGAGAAAGUUAAAA 3689			

FIG. 2C

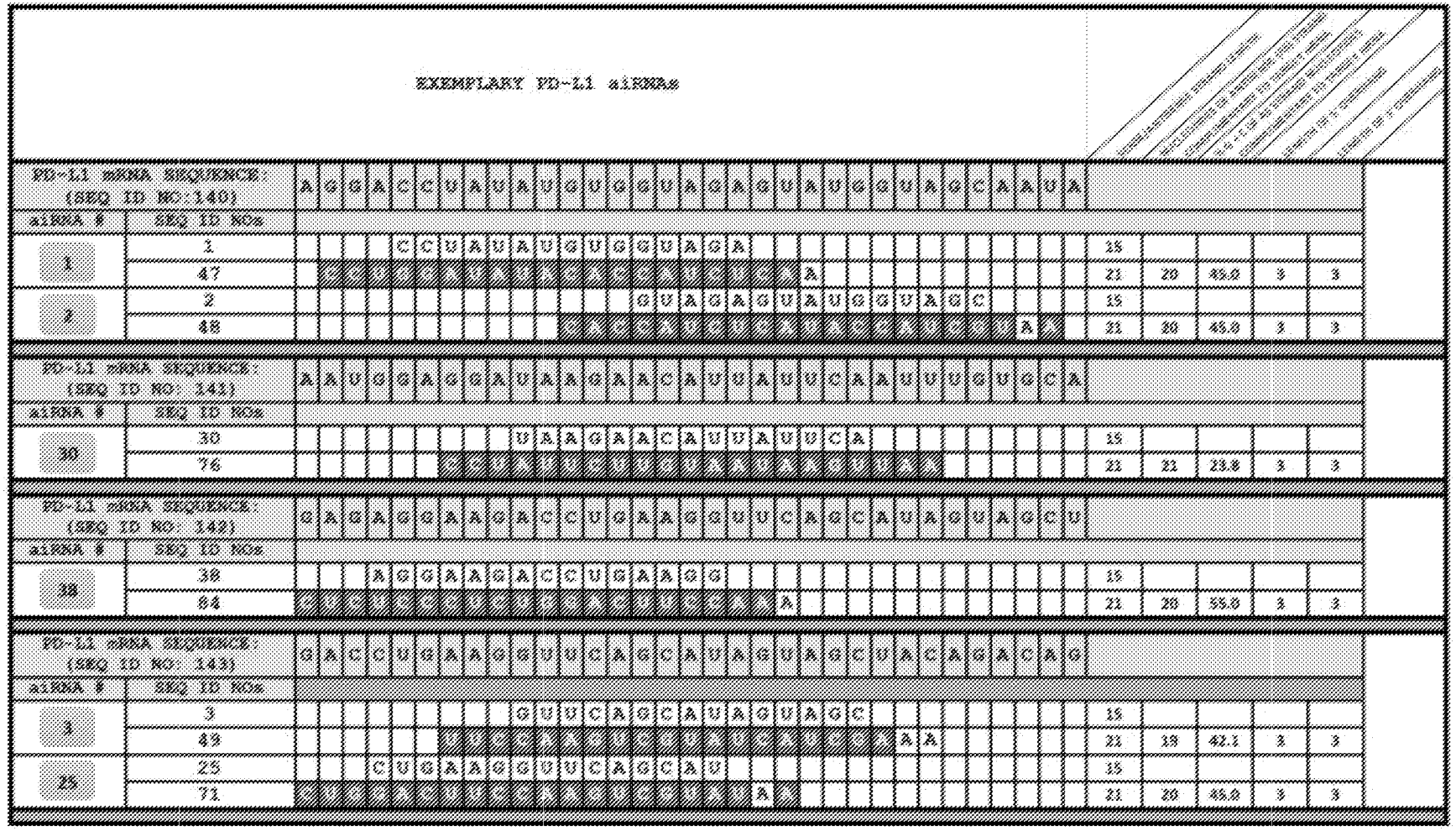

EXEMPLARY PD-L1 siRNAs
PD-L1 mRNA SEQUENCE: (SEQ ID NO:140)
A G G A C C U A U A U G U G G U A G A G U A U G G U A G C A A U A
siRNA # SEQ ID NOs
1
1
47
2
2
48
PD-L1 mRNA SEQUENCE: (SEQ ID NO: 141)
A A U G G A G G A U A A G A A C A U U A U U C A A U U U G U G C A
30
30
76
PD-L1 mRNA SEQUENCE: (SEQ ID NO: 142)
G A G A G G A A G A C C U G A A G G U U C A G C A U A G U A G C U
38
38
84
PD-L1 mRNA SEQUENCE: (SEQ ID NO: 143)
G A C C U G A A G G U U C A G C A U A G U A G C U A C A G A C A G
3
3
49
25
25
71

FIG. 2C (ctd.)

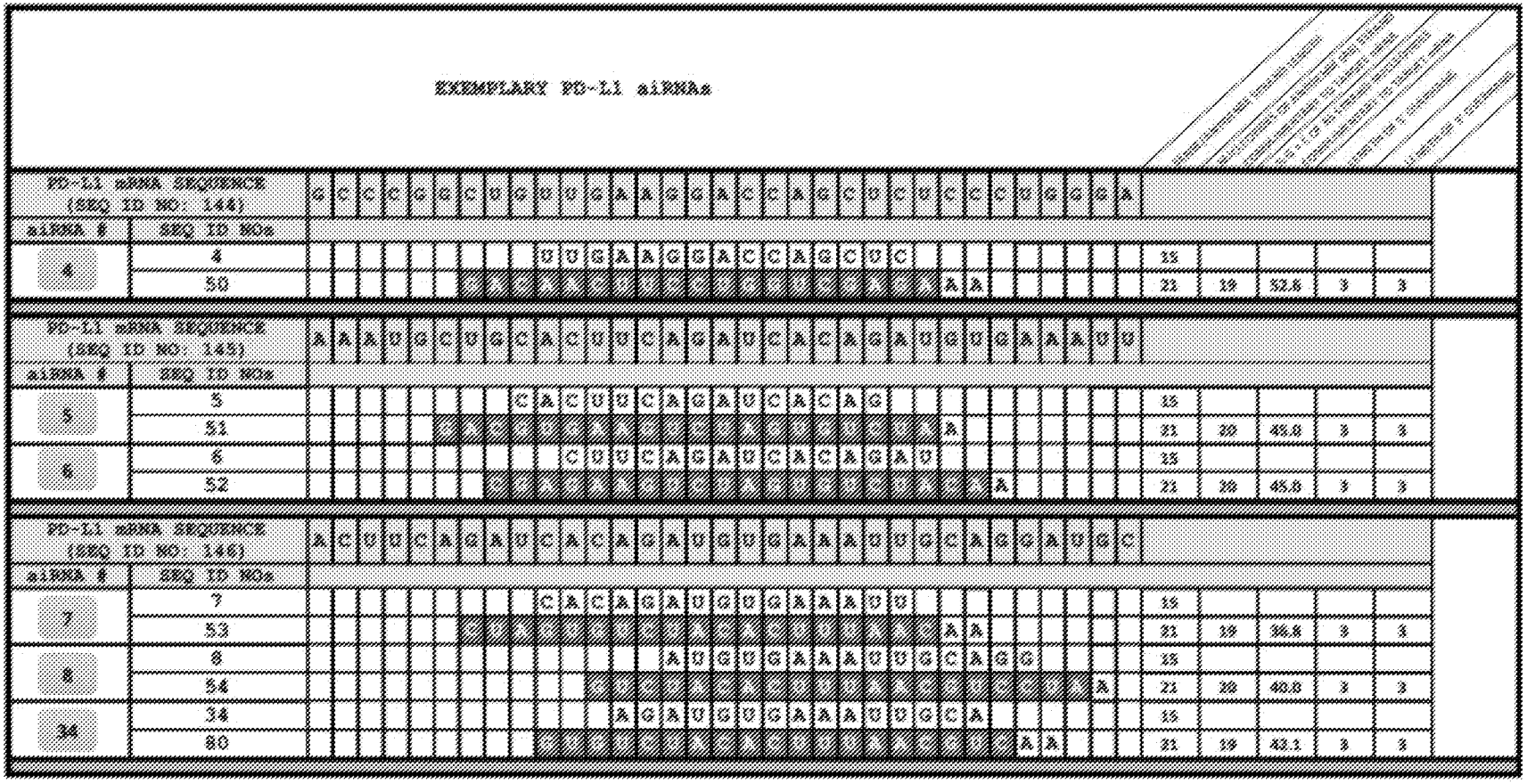

EXEMPLARY PD-L1 siRNAs
PD-L1 mRNA SEQUENCE (SEQ ID NO: 144)
G C C C G G C U G U U G A A G G A C C A G C U C C C U G G G A
siRNA #
SEQ ID NOs
4
4
50
PD-L1 mRNA SEQUENCE (SEQ ID NO: 145)
A A A U G C U G C A C U U C A G A U C A C A G A U G U G A A A U U
5
5
51
6
6
52
PD-L1 mRNA SEQUENCE (SEQ ID NO: 146)
A C U U C A G A U C A C A G A U G U G A A A U U G C A G G A U G C
7
7
53
8
8
54
34
34
80

FIG. 2C (ctd.)

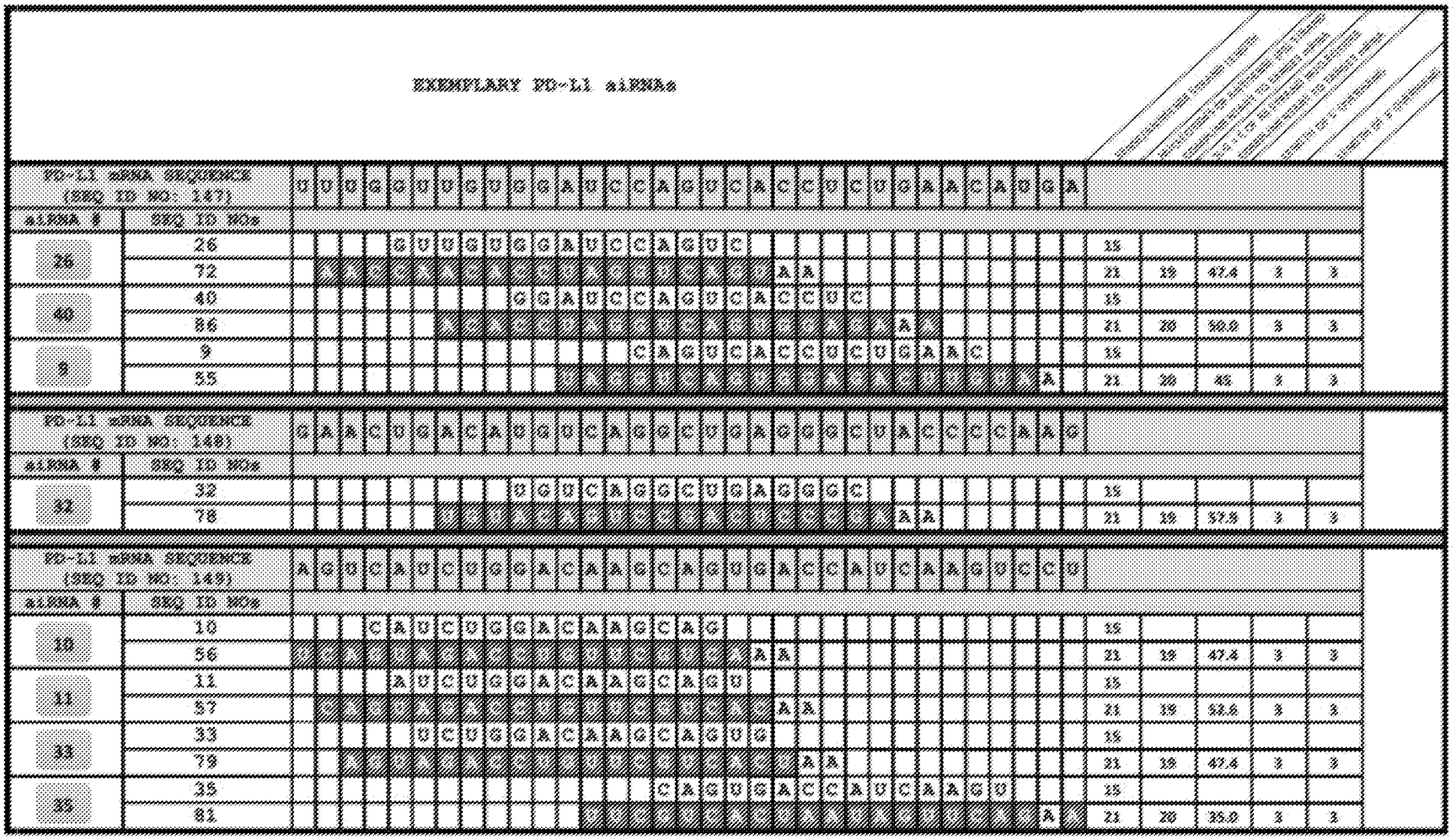

EXEMPLARY PD-L1 siRNAs
PD-L1 mRNA SEQUENCE (SEQ ID NO: 147)
siRNA #
SEQ ID NOs
PD-L1 mRNA SEQUENCE (SEQ ID NO: 148)
PD-L1 mRNA SEQUENCE (SEQ ID NO: 149)

FIG. 2C (ctd.)

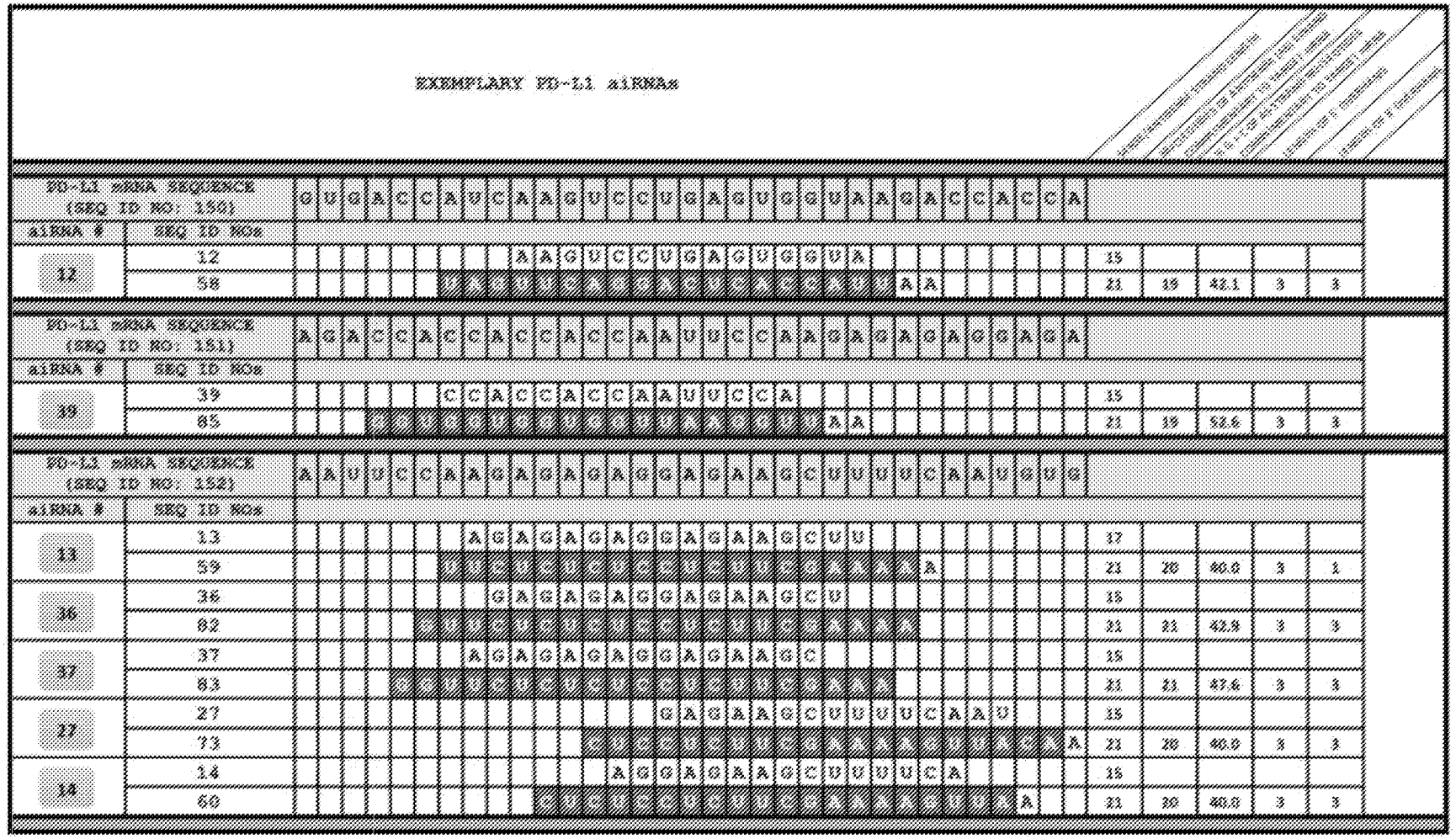

EXEMPLARY PD-L1 aiRNAs
PD-L1 mRNA SEQUENCE (SEQ ID NO: 150)
G U G A C C A U C A A G U C C U G A G U G G U A A G A C C A C C A
aiRNA #
SEQ ID NOs
12
58
PD-L1 mRNA SEQUENCE (SEQ ID NO: 151)
A G A C C A C C A C C A C C A A U U C C A A G A G A G A G A G G A G A
39
85
PD-L1 mRNA SEQUENCE (SEQ ID NO: 152)
A A U U C C A A G A G A G A G G A G A A G C U U U U C A A U G U G
13
59
36
82
37
83
27
73
14
60

FIG. 2C (ctd.)

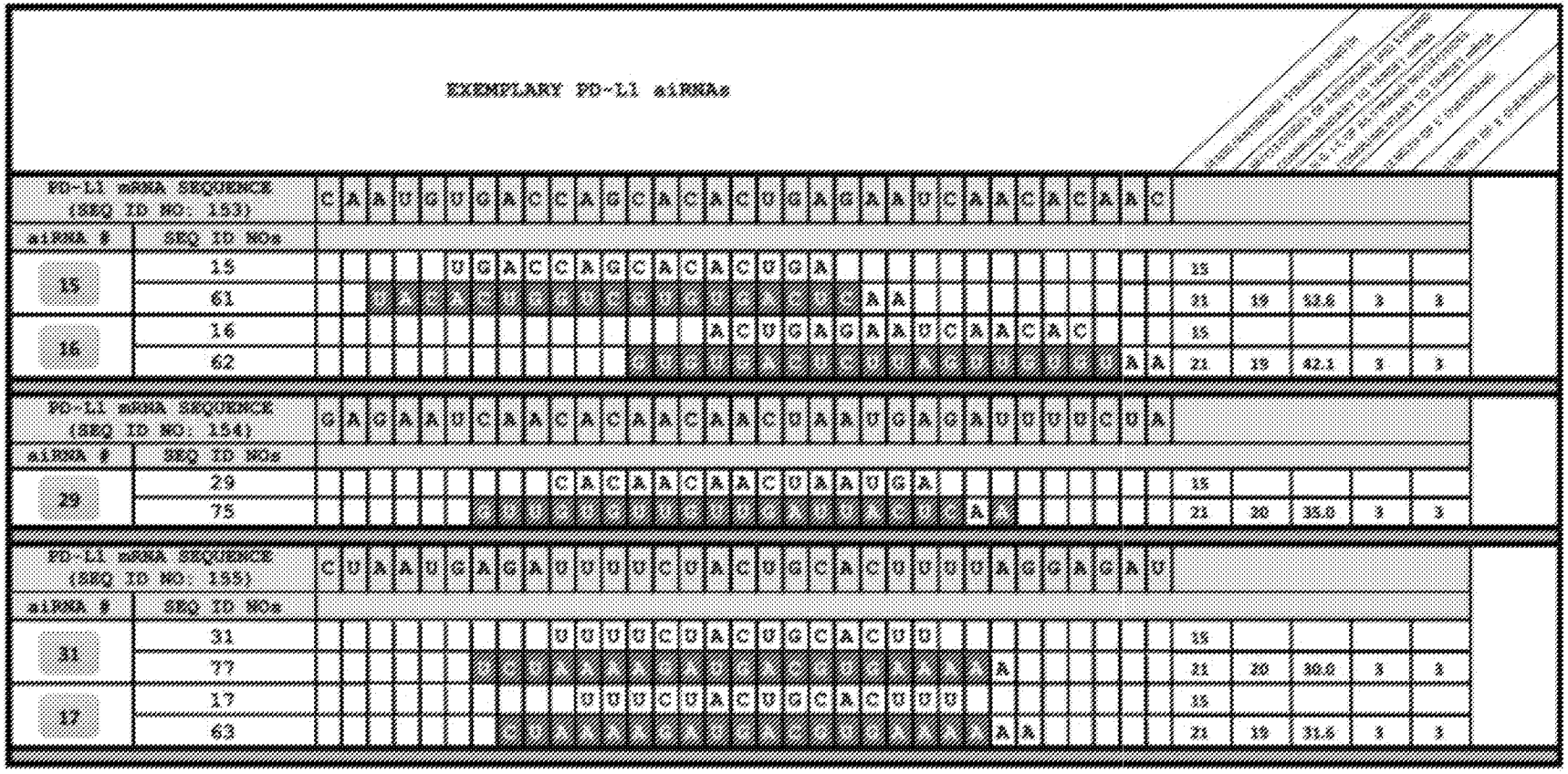

EXEMPLARY PD-L1 siRNAs
PD-L1 mRNA SEQUENCE (SEQ ID NO: 153)
C A A U G U G A C C A G C A C A C U G A G A A U C A A C A C A A C
siRNA #
SEQ ID NOs
15
15
61
16
16
62
PD-L1 mRNA SEQUENCE (SEQ ID NO: 154)
G A G A A U C A A C A C A A C A A C U A A U G A G A U U U U C U A
29
29
75
PD-L1 mRNA SEQUENCE (SEQ ID NO: 155)
C U A A U G A G A U U U U C U A C U G C A C U U U A G G A G A U
31
31
77
17
17
63

FIG. 2C (ctd.)

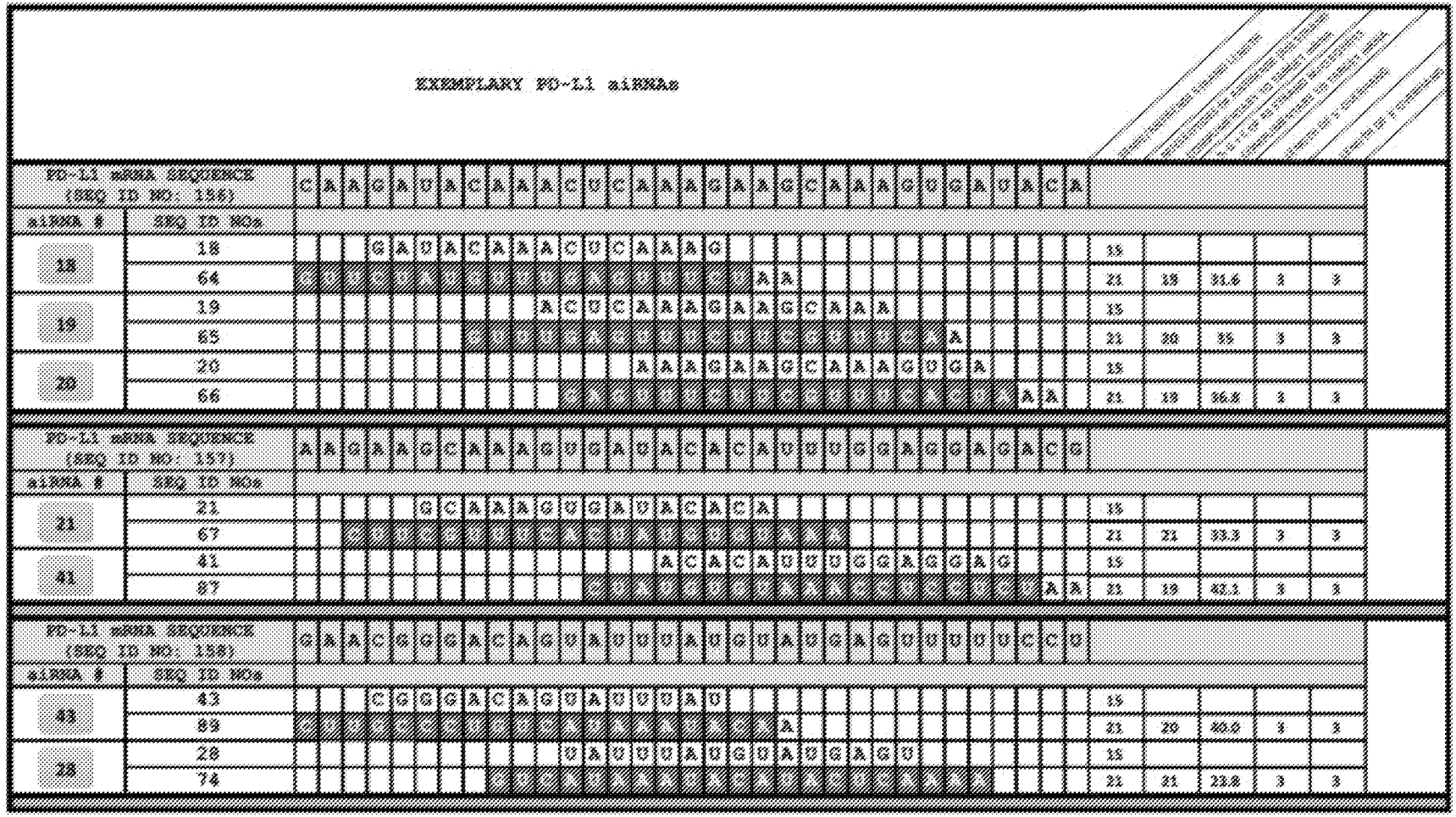

EXEMPLARY PD-L1 aiRNAs
PD-L1 mRNA SEQUENCE (SEQ ID NO: 156)
C A A G A U A C A A A C U C A A A G A A G C A A A G U G A U A C A
aiRNA #
SEQ ID NOs
18
18
64
19
65
20
20
66
PD-L1 mRNA SEQUENCE (SEQ ID NO: 157)
A A G A A G C A A A G U G A U A C A C A U U U G G A G G A G A C G
aiRNA #
SEQ ID NOs
21
21
67
41
41
87
PD-L1 mRNA SEQUENCE (SEQ ID NO: 158)
G A A C G G G A C A G U A U U U A U G U A U G A G U U U U U C C U
aiRNA #
SEQ ID NOs
43
43
89
28
28
74

FIG. 2C (ctd.)

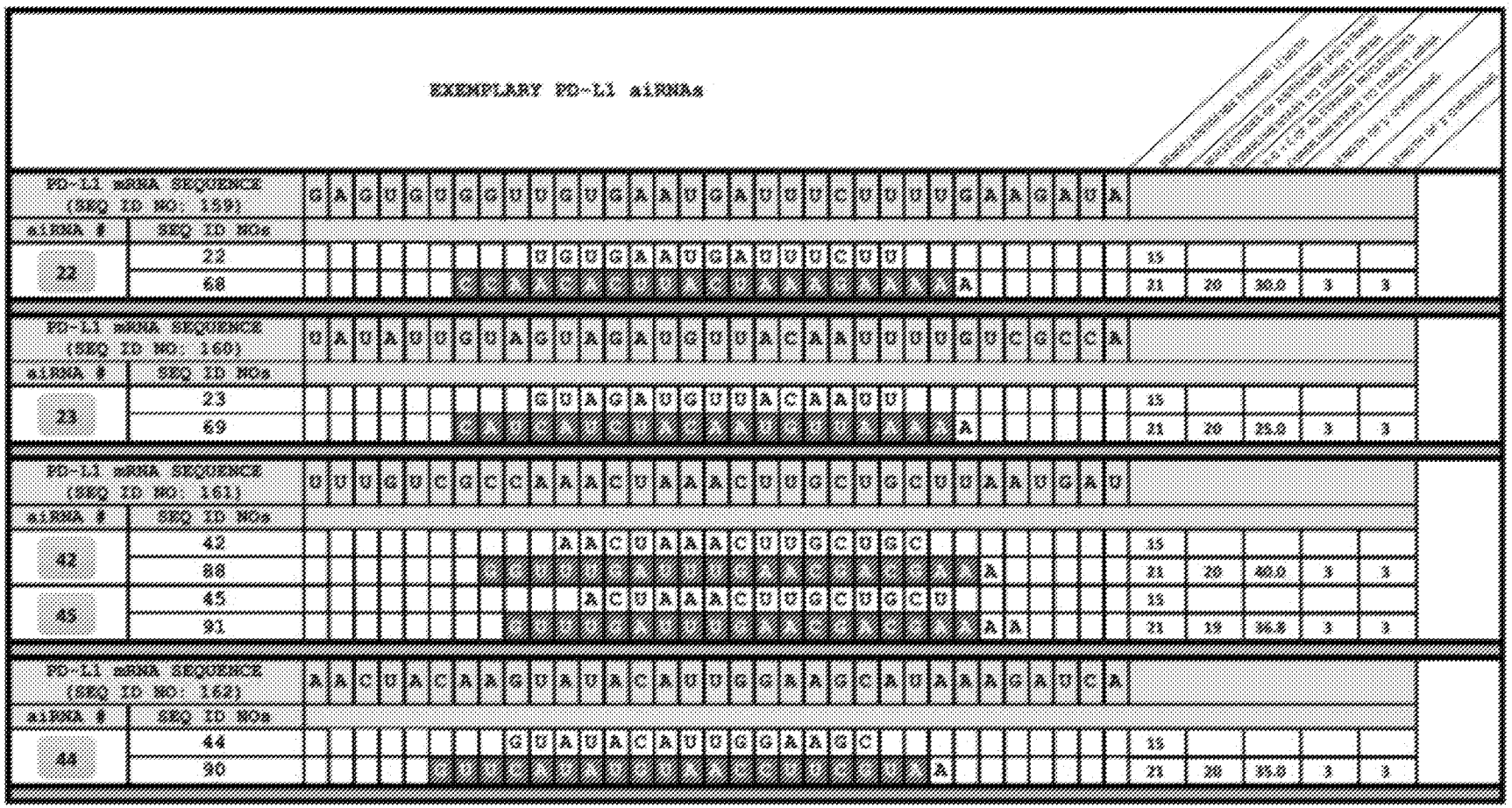

EXEMPLARY PD-L1 siRNAs
PD-L1 mRNA SEQUENCE (SEQ ID NO: 159)
siRNA #
SEQ ID NOs
22
PD-L1 mRNA SEQUENCE (SEQ ID NO: 160)
23
PD-L1 mRNA SEQUENCE (SEQ ID NO: 161)
42
45
PD-L1 mRNA SEQUENCE (SEQ ID NO: 162)
44

FIG. 2C (ctd.)

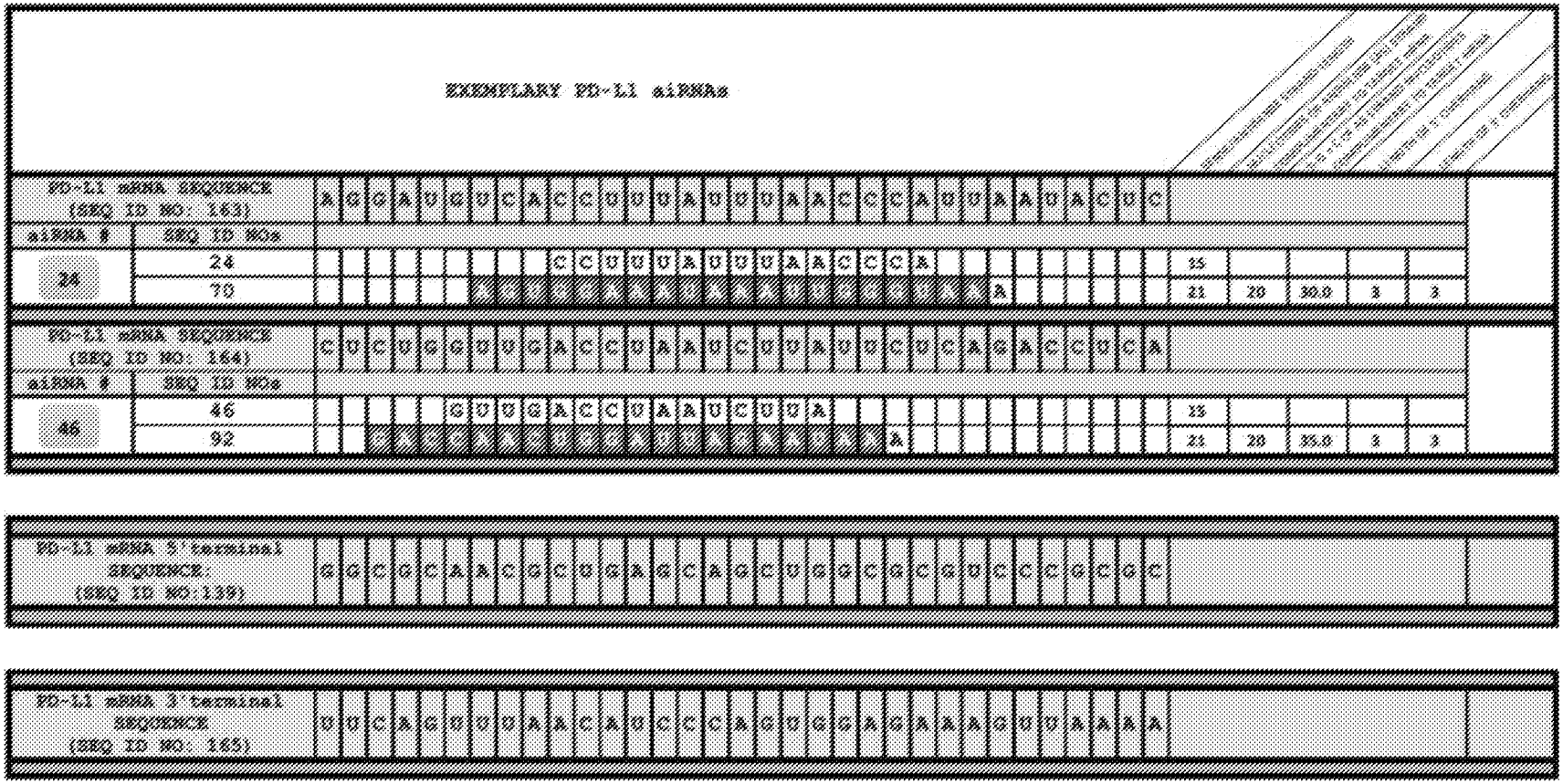

EXEMPLARY PD-L1 siRNAs
PD-L1 mRNA SEQUENCE (SEQ ID NO: 163)
A G G A U G U C A C C U U U A U U U A A C C C A U U A A U A C U C
siRNA #
SEQ ID NOs
24
24
70
PD-L1 mRNA SEQUENCE (SEQ ID NO: 164)
C U C U G G U U G A C C U A A U C U U U A U G C U C A G A C C U C A
siRNA #
SEQ ID NOs
46
46
92
PD-L1 mRNA 5'terminal SEQUENCE: (SEQ ID NO: 139)
G G C G C A A C G C U G A G C A G C U G G C G C G U C C C G C G C
PD-L1 mRNA 3'terminal SEQUENCE (SEQ ID NO: 165)
U U C A G U U U A A C A U C C C A G U G G A G A A A G U U U A A A A

FIG. 2D

% G+C	Exemplary PD-L1 aiRNA #
23%-35%	17, 18, 19, 21, 22, 23, 24, 28, 29, 30, 31, 35, 44, 46
36%-44%	3, 7, 8, 12, 13, 14, 16, 20, 27, 34, 36, 41, 42, 43, 45
45%-57%	1, 2, 4, 5, 6, 9, 10, 11, 15, 25, 26, 38, 40, 32, 33, 37, 39